รายงานวิจัยฉบับสมบูรณ์

ภาคผนวก (การตีพิมพ์เผยแพร่ผลงาน)

โครงการกลไกทางสรีระเชิงนิเวศน์และพันธุกรรมที่ควบคุมการใช้ธาตุอาหารในพืช

(Ecophysiological processes and genetic controls relating to plant nutrition)

โดย เบญจวรรณ ฤกษ์เกษม และคณะ

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คณะผู้วิจัย	สังกัด
1. ศ.ดร. เบญจวรรณ ฤกษ์เกษม	มหาวิทยาลัยเชียงใหม่
2. รศ.ดร. สายสมร ลำยอง	มหาวิทยาลัยเชียงใหม่
3. รศ. ดร. ศันสนีย์ จำจด	มหาวิทยาลัยเชียงใหม่
4. ผศ. ดร.ศักดา จงแก้ววัฒนา	มหาวิทยาลัยเชียงใหม่
5. ดร. กนก ฤกษ์เกษม	มหาวิทยาลัยเชียงใหม่
6. ดร.จรรยา มณีโชติ	กรมวิชาการเกษตร
7. นายสิทธิชัย ลอดแก้ว	มหาวิทยาลัยเชียงใหม่
8. นายพิภพ ลำยอง	มหาวิทยาลัยเชียงใหม่
9. นายนริศ ยิ้มแย้ม	มหาวิทยาลัยเชียงใหม่
10. นางปณิดา บุญสิทธิ	มหาวิทยาลัยเชียงใหม่

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

ชุดโครงการส่งเสริมกลุ่มวิจัย

ภาคผนวก

บทความวิชาการ

1. **Peer-reviewed papers published (ตีพิมพ์เผยแพร่แล้ว หรืออยู่ในระหว่างการตีพิมพ์)**

1.1 Rerkasem B, Jamjod S, Niruntrayagul, S. Boron *In press.* Increasing boron efficiency in many international bread wheat, durum wheat, triticale and barley germplasm will boost production on soils low in boron. Field Crop Research

1.2 Sansanee Jamjod, Sunisa Niruntrayagul & Benjavan Rerkasem. *In press.* Genetic control of boron efficiency in wheat (*Triticum aestivum* L.) Euphyitca

1.3 Wongmo, J., Jamjod, S. and Rerkasem, B. *In press.* Contrasting responses to boron deficiency in barley and wheat. Plant and Soil

1.4 Duangjai Nachiangmai, Bernie Dell, Richard Bell, Longbin Huang and Benjavan Rerkasem. *In press.* Genotypic variation in boron long distance transport into the reproductive organ of wheat. Plant and Soil

1.5 Yimyam, N. Rerkasem, K and Rerkasem, B. 2003. Fallow enrichment with pada (*Macaranga denticulata* (Bl.) Muell. Arg.) trees in rotational shifting cultivation in Northern Thailand Agroforestry Systems 57: 79-86.

1.6 M. Leesawatwong, S. Jamjod and B. Rerkasem. 2003 Determinants of a premium priced special quality rice. International Rice Research Notes. 28: 34

1.7 C. Prom-u-thai and B. Rerkasem. 2003. The effect of nitrogen on rice grain iron. International Rice Research Notes. December 2003.

1.8 Rerkasem K, Korsamphan C, Thong-ngam C, Yimyam N and Rerkasem B. 2002. Agrodiversity lessons in mountain land management. Mt. Res. Dev. 22: 4-9 (บทความได้รับรางวัลที่ 1 จาก United Nations University)

1.9 C. Prom-u-thai, B. Dell, G. Thomson, B. Rerkasem. *In press.* Easy and rapid detection of iron in rice grain. ScienceAsia

1.10 Rerkasem, B. and K. Rerkasem. 2002 Agrodiversity for *in situ* conservation of Thailand's native rice germplasm. CMU J. 1: 129-148.

1.11 Rerkasem B and Jamjod S. 2001. Overcoming wheat sterility problem with boron efficiency. Dev. Plant Soil Sci. 92: 82-83.

1.12 Prom-u-thai, C. and Rerkasem, B. 2001. Iron in Thai rice. Dev. Plant Soil Sci. 92: 350-351.

1.13 Benjavan Rerkasem. 2003. Biotechnology and Agriculture. An invited review, pp. 293-321, *in* Social Challenges for the Mekong Region, Mingsarn Kaosa-ard and John Dore (Eds.). White Lotus, Bangkok.

1.14 Kanok Rerkasem. 2003. Uplands Land Use. An invited review, pp. 323-346, *in* Social Challenges for the Mekong Region, Mingsarn Kaosa-ard and John Dore (Eds.). White Lotus, Bangkok.

1.15 Rerkasem, B. 2002. Crop responses to boron and genotypic variations. An invited review, pp. 269-280, *in* All Aspects of Plant and Animal Boron Nutrition, Eds: H. E. Goldbach, B. Rerkasem, M. A. Wimmer, P. H. Brown, M. Thellier and R.W. Bell. Kluwer and Plenum Academic Publishers

1.16 Ahmed M, Jaihiruddin M, Jamjod S and Rerkasem B. 2002. Boron efficiency in a wheat germplasm from Bangladesh. Pp. 299-303, *in* All Aspects of Plant and Animal Boron Nutrition, Eds: H. E. Goldbach, B. Rerkasem, M. A. Wimmer, P. H. Brown, M. Thellier and R.W. Bell. Kluwer and Plenum Academic Publishers.

1.17 NaChiangmai D, Dell B, Huang L, Bell R and Rerkasem B. 2002. The effect of boron on pollen development in two wheat cultivars (*Triticum aestivum* L.). Pp. 181-185, *in* All Aspects of Plant and Animal Boron Nutrition, Eds: H. E. Goldbach, B. Rerkasem, M. A. Wimmer, P. H. Brown, M. Thellier and R.W. Bell. Kluwer and Plenum Academic Publishers.

1.18 Rerkasem K, Thong-ngam C, Korsamphan C, Yimyam N and Rerkasem B. 2002. Pp. 200-232, *in*: Land Use Changes in the Highlands of Northern Thailand. An invited review paper in 'Cultivating Biodiversity' Eds. H Brookfield, C Padoch, H Parson and M Stocking. ITDG Publishers, London and United Nations University, Tokyo.

1.19 เนตรนภา อินสลุด Richard W. Bell และเบญจวรรณ ฤกษ์เกษม 2546 การตอบสนองของพันธุ์ข้าวไร่และข้าวนาสวนต่อสภาพดินขังน้ำและดินระบายน้ำดี วารสารเกษตร (มช) ACCEPTED

1.20 จำเนียร วงษ์โม้, ศันสนีย์ จำจด และ เบญจวรรณ ฤกษ์เกษม 2546 เปรียบเทียบการตอบสนองต่อการขาดธาตุโบรอนในข้าวบาร์เลย์และข้าวสาลี วารสารเกษตร (มช) ACCEPTED

1.21 ทินกร ศรีวิชัย ศันสนีย์ จำจด และ เบญจวรรรณ ฤกษ์เกษม 2546 การตอบสนองต่อโบรอนในถั่วพุ่ม วารสารเกษตร (มช) ACCEPTED

1.22 นริศ ยิ้มแย้ม สิทธิชัย ลอดแก้ว เบญจวรรณ ฤกษ์เกษม และ กนก ฤกษ์เกษม 2546 การจัดการความหลากหลายของดันปะดะในไร่หมุนเวียนของกะเหรี่ยงโปว์ ในภาคเหนือของประเทศไทย วารสารเกษตร (มช) ACCEPTED

1.23 รัตญา ยานะพันธุ์ และ เบญจวรรณ ฤกษ์เกษม 2546 การคัดเลือกพันธุ์ข้าวไทยภายใต้สภาพขาดธาตุเหล็กโดยวัดปริมาณคลอโรฟิลล์ในใบ วารสารเกษตร (มช) ACCEPTED

1.24 การเคลื่อนย้ายโบรอนในถั่วเขียว 2546 สาวิกา กอนแสง และเบญจวรรณ ฤกษ์เกษม วารสารเกษตร (มช) ACCEPTED

1.25 สุพรรณิการ์ พันชนะ ศันสนีย์ จำจด และเบญจวรรณ ฤกษ์เกษม 2546 การตอบสนองต่อความเป็นพิษของโบรอนในข้าวสาลีสามพันธุ์ที่มีระดับความทนทานต่อการขาดโบรอนแตกต่างกัน วารสารเกษตร (มช) ACCEPTED

1.26 อยุชย์ คงปั้น ศันสนีย์ จำจด และ เบญจวรรณ ฤกษ์เกษม 2546 อิทธิพลของโบรอนต่อคุณภาพเมล็ดในถั่วเขียวต่างพันธุ์ วารสารเกษตร (มช) ACCEPTED

2. **Papers submitted for publication in peer-reviewed journals**

2.1 Somchit Youpensuk, Benjavan Rerkasem, Bernie Dell and Saisamorm Lumyong. Arbuscular mycorrhizal fungi from the rhizosphere of a fallow enriching tree, *Macaranga denticulata* Muell. Arg. and their effect on the host plant. Submitted to Agroforestry Systems (January 2003).

2.2 C. Prom-u-thai, B. Dell, G. Thomson, B. Rerkasem. Distribution and structure of protein and phytin bodies in seed of four rice genotypes. Submitted to Canadian Journal of

3. **Papers in preparation, submission expected by end of 2003**

3.1 Ayut Kongpan, Sansanee Jamjod and Benjavan Rerkasem. Boron efficient germplasm identified in *Vigna mungo* (L.) Hepper and *Vigna radiation* (L.) Wilczek. For submission to Plant and Soil or Field Crop Research.

3.2 Supannika Punchana, Sansanee Jamjod and Benjavan Rerkasem. Are boron efficient wheat always susceptible to boron toxicity? For submission to Euphytica.

3.3 Chanakan Prom-u-thai and Benjavan Rerkasem. Iron in the Grain of High and Low Iron Density Rice Grown in Different Water Regimes.

3.4 Supawadee Ngorian, Sansanee Jamjod and Benjavan Rerkasem. Response of F_2 population derived from boron efficient (Fang 60) x boron inefficiency

(Bonza) wheat (*Triticum aestivum* L.) genotypes to boron levels. For submission to Euphytica or J. Plant Breeding.

3.5 Benjavan Rerkasem and Sansanee Jamjod. Boron Deficiency in Wheat: a Review Submitted to Field Crop Reserch

4. Book edited

4.1 Goldbach, H.E., Rerkasem, B., Wimmer, M.A., Brown, P.H., Thellier M. and Bell, R.W. 2002. All aspects of Plant and Animal Boron Nutrition, Kluwer and Plenum Academic Publishers

Increasing boron efficiency in many international bread wheat, durum wheat, triticale and barley germplasm will boost production on soils low in boron

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Abstract

Boron deficiency causes grain set failure and yield loss in many of the world's wheat growing countries. We suggest growing B efficient genotypes as a means to overcome the problem. This study evaluated an international germplasm of bread wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum* Dest.), triticale (x*Triticosecale* Wittmack) and barley (*Hordeum vulgare* L.) for B efficiency. The first set of germplasm consisted of bread wheat, durum wheat and triticale from CIMMYT 1996/97 nurseries and a B efficient check wheat cv. 'Fang 60'. The lines were grown in the field on soil with 0.1 and 0.2 , and 0.3 mg HWS B kg^{-1} for durum wheat and triticale. The grain set index (GSI, percentage grain set in the first two florets of 10 central spikelets) measured B efficiency in wheat, durum and triticale genotypes without the need for a B sufficiency control. Three quarters of the lines tested

were B inefficient, which included all of the durum wheat, 84% of the triticale and 60% of the bread wheat lines. Six of the bread wheat lines evaluated were in the same B efficient class as Fang 60. The response to low B was confirmed in a sand culture without added B. Similarly high frequency of B inefficiency was found in a second set of germplasm which included bread and durum wheat, barley and triticale lines from CIMMYT and ICARDA 2000/01 international nurseries. Incorporating the B efficiency trait in germplasm such as these, would ensure their adaptation to low B soils, and so enable their genetic potential to be fully realized in some of the world's difficult production areas.

Keywords: Boron deficiency

Abbreviations: B, boron; HWS, hot water soluble; CIMMYT, the International Maize and Wheat Improvement Center; GSI, grain set index; ICARDA, International Center for Agricultural Research in the Dry Areas; 4HTWYT, 4th High Temperature Wheat Yield Trial; 17ESWYT, 17th Elite Selection Wheat Yield Trial; 18SAWSN, 18th Semi-Arid Areas Wheat Screening Nursery; 28IBON, 28th International Barley Observation Nursery; 28ITYN, 28th International Triticale Yield Nursery; 28IDYN, 28th International Durum Yield Nursery; 33IDYN, 33rd International Durum Yield Nursery; 33ITSN, 33rd International Triticale Screening Nursery; 33IBWSN, 33rd International Bread Wheat Screening Nursery.

1. Introduction

In many of the world's wheat growing areas, from Brazil's irrigated flood plains (da Silva and de Andrade, 1980) and Cerrados (da Silva and de Andrade, 1983), China (Li et al., 1978; Liu et al., 1981) to India's northwestern states and along the Indo-Nepal border (Tandon and

Naqvi,1992), Nepal (Misra et al., 1992) and Bangladesh (Reuter, 1987; Rerkasem, 1996), severe yield losses may be caused by B deficiency, through adverse effects on male fertility and grain set. These and later studies (Jamjod et al., 1992; Subedi et al., 1997) also reported large genotypic variation in the response to B, or B efficiency, in bread wheat. The term B efficiency is used here without inferring a mechanism, in the same way as defined for Zn efficiency (Graham, 1984), to designate the ability of a genotype to perform well in soils too deficient in B for other genotypes. Selecting for B efficiency has been suggested as one cost effective solution to the problem of yield loss due to boron deficiency in commercially grown wheat crops (Rerkasem and Jamjod, 1997).

The International Maize and Wheat Improvement Center (CIMMYT) is the world's single most important source of wheat germplasm. Each year thousands of lines and breeding populations from CIMMYT are introduced into countries throughout the world. In the last four years alone, more than 160 bread wheat, durum wheat, triticale, and barley varieties derived from CIMMYT germplasm have been released by more than 30 countries (www.cimmyt.org/Research/wheat). About 55 million hectares of spring bread wheat, nearly 80% of annual spring wheat area in the developing world excluding China, are now sown each year with varieties developed from the CIMMYT germplasm. Information about B efficiency of this major source of bread wheat and durum wheat, triticale and barley germplasm, especially the portion destined for areas with low B soils, could help to prevent crop losses due to B deficiency. This study aimed to evaluate the potential to perform in soils low in B of bread and durum wheat, triticale and barley germplasm.

2. Materials and methods

An international germplasm of bread and durum wheat and triticale from CIMMYT and barley from CIMMYT/ICARDA was grown in the field on a sandy loam Tropaqualf soil of San Sai series and in sand culture in Chiang Mai, Thailand. The first set of germplasm included 98 lines of bread wheat (the 4HTWYT and 17ESWYT) and 49 lines each of durum wheat (28IDYN) and triticale (28ITYN) and a B efficient check wheat, cv 'Fang 60' (Jamjod et al., 1992). The experiment was arranged as a split plot, with soil B levels in duplicated mainplots and lines of wheat and triticale (sown, at the seed rate of 3 g m^{-1}, in single rows 5 m long, 0.25 m between rows) as subplots. Borax at 0, 1 and 1.5 kg B ha^{-1} was applied to create the B levels, giving the soil 0.1 (B0.1), 0.2 (B0.2) and 0.3 (B0.3) mg HWS B kg^{-1}. Bread wheat are generally less sensitive to B deficiency than durum wheat and triticale (Rerkasem, unpublished), the 4HTWYT and 17ESWYT were grown at B0.1 and B0.2 only. At maturity, assessment was made of the number of grains per ear, number of spikelets per ear, and grain set index (GSI, percentage grain set in the first two florets of 10 central spikelets, Rerkasem and Loneragan, 1994) from 20 randomly selected ears, and the number of ears in one m length of row.

The 4HTWYT, 28ITYN and 28IDYN were also evaluated against Fang 60 in a sand culture to which no B was supplied. Entries were grown in duplicated earthenware pots (Ø 30 cm, 30 cm deep) containing washed river quartz sand. The pots were watered twice daily with 1 liter of nutrient solution containing 1000 μM $CaCl_2$, 250 μM $MgSO_4$, 500 μM KH_2PO_4, 10 μM Fe EDTA, 250 μM K_2SO_4, 1 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.1 μM $CoSO_4$, 0.1 μM Na_2MoO_4 (Broughton and Dilworth, 1971) and 5 mM KNO_3. Grain set index was assessed at maturity on five ears from each replication for each line.

The second set of germplasm consisted of 1,108 lines from the 2000/01 international bread wheat (33IBWSN, 18SAWSN), durum wheat (33IDYN) and triticale (33ITSN) nurseries from CIMMYT and a barley nursery (28IBON) from CIMMYT/ICARDA. Entries were sown on soil with 0.1 mg HWS B kg^{-1} in single 5 m rows, with 0.25 m between rows and a row each of the B efficiency checks inserted after every 19 entries. The B efficiency checks were BRB9604 and BRB96-9 (Jamjod and Rerkasem, 1999) for the 28IBON, and Fang 60 and SW41 (Anantawiroon et al., 1997) for the other nurseries. At maturity the GSI was determined from 20 randomly selected ears for each row.

3. Results

3.1 Responses to boron in bread wheat, durum wheat and triticale

Bread wheat, triticale and durum wheat all exhibited responses to B, where number of grains per ear and GSI, that varied significantly among the genotypes (Table 1). Symptoms of B deficiency observable in bread wheat in B0.1 at anthesis included shrivelled anthers, poorly developed pollen that did not stain with iodine, and florets that failed to fertilize and remained open, giving the ears a translucent, "paper lamp" appearance. The paper lamp effect was also observed in triticale and durum in B0.1 and sometimes in B0.2. In these low B levels some triticale lines showed a "rat-tail" symptom of the ear tip, in which terminal spikelets were reduced in size and sometimes completely degenerated, leaving remnants of dead, papery white tissue. These symptoms did not occur in bread or durum wheat lines. There was a significant interaction between the genotype and B effect on the number of spikelets per ear in triticale, but not in bread wheat or durum. The number of ears m^{-1} was not affected by B in all three species .

At B0.1, the B efficient check Fang 60 set grain normally with GSI >85%, while genotypes evaluated ranged in GSI from 0% to 100%. Entries with grain set failure in half or more of their competent florets in B0.1 accounted for three-quarters of the 1996/97 germplasm (Table 2). These included all of the durum lines and all but eight of the triticale lines. Forty percent of the germplasm was most severely affected by B deficiency in B0.1, with grain set in only one in five of their competent florets or fewer. These included most of the durum (82%), half of the triticale, and 14% of the bread wheat lines. Six out of 98 entries of bread wheat, but none of durum and triticale, had GSI in B0.1 exceeding 85%, the same as B efficient Fang 60.

The species differences were also evident in the responses to B in means of GSI and the number of grains per ear. The whole nursery mean GSI in B0.1 was highest in bread wheat, followed by the 28ITYN and 28IDYN, in that order (Table 3). Increasing B level increased GSI in the B inefficient classes in all three species. In the moderately inefficient classes the GSI was approaching maximum when B was increased to B0.2, but in the more inefficient classes, which included all of the durum lines and almost all of the triticale lines, maximum GSI was not reached until B0.3. Responses to B in the number of grains per ear (Table 4) followed closely their GSI responses ($r^2 = 0.74$ for the 4HTWYT and 17ESWYT combined; 0.61 for 28ITYN and 0.62 for 28IDYN, all significant at $p < 0.001$).

In sand culture to which B had not been applied (B0) the 4HTWYT, 28ITYN and 28IDYN lines responded to B deficiency in the same way as in the field at B0.1. In all three species, the GSI means for each nursery and for each B efficiency class in B0 (Table 5) were almost identical to those in B0.1 (Table 4), r^2 for the 4HTWYT measured at 0.90. Some of the B

efficient and inefficient bread wheat lines are identified in Table 6. For these individual lines from the 4HTWYT, their GSI in the sand culture without added B also closely followed those in the low B soil.

In triticale, the number of spikelets per ear in about one third of the entries was depressed by B deficiency, with up to 50% depression in the most severe cases (Table 7). The ear size response to B in the 28ITYN, however, did not correlate with the GSI response ($r^2 = 0.002$, not significant at $p < 0.05$), neither did those in the 4HTWYT, 17ESWYT ($r^2 = 0.006$ and 28IDYN ($r^2 = 0.0004$).

3.2 Boron efficiency in the 2000/2001 international germplasm

Through out the experimental plot, the GSI in the B efficient check wheat, Fang 60, was approaching 100%, and was 84% in BCMU96-9 barley, while it ranged between 70% and 80% in the moderately inefficient wheat SW41 and 60% in BRB9604 (Table 8). Lines with GSI exceeding 90% included 10% of the 33IBSWYN, 8% of the 18SAWSN, and none of the 28IBON, 33IDYN and 33ITSN. Entries with GSI in the low B soil in same range as or lower than SW41, the moderately inefficient wheat check, accounted for 71% of the bread wheat, 96% of the triticale and all of the durum wheat and barley in the 2000/01 nurseries. The rat-tail symptom was observed on many of the barley as well as triticale entries.

4. Discussion

The predominance of B inefficiency in the bread and durum wheat, barley and triticale germplasm evaluated has a potential to cause serious problems in many of the world's

growing areas. Contrary to the often held view of low B requirement and insensitivity to B deficiency of wheat and related small grains (Lamb, 1967; Marten and Westermann, 1991), reports of yield loss due to B deficiency have come from many regions. Boron deficiency has been reported in the field on at least 132 crops in 80 countries in all continents (Shorrocks, 1997). Large areas of low B soils have been identified in the Americas, Europe, Africa and Asia, with the single largest contiguous area of B deficiency is in China (Liu et al., 1981).

Failure of commercial wheat crops due to B deficiency has been reported in China from Heilongjian in the northeast, (Li et al, 1978) to Yunnan in the southwest (Yang, 1992). The most extensive area of B deficient wheat so far identified is in one of the poorest corners of Asia, that extends from the northwest of India into Nepal and Bangladesh, where about one million ha of crop land is estimated to be adversely affected by B deficiency (Kataki et al., 2002). Low B soils and incidences of B deficiency are prevalent in the Indian northwestern states of Bihar, West Bengal, Orissa, Meghalaya and Assam (Sakal and Singh, 1995), where grain set failure and responses to B in wheat have been reported (e.g. Singh et al., 1976; Sarkar and Chakraborty, 1980; Mandal and Das, 1988; Dwivedi et al., 1990). A common local knowledge that the problem extends into neighboring areas of Bangladesh (Reuter, 1987) has been recently confirmed (Kataki et al. 2002). In Nepal B deficiency in wheat and other crops has been commonly reported from the Terai or flat plains, where half of the country's wheat crop is grown (Subedi et al., 1996). Reports of B deficiency-induced sterility have also begun to come from "less likely" regions. Incidences of B deficiency in wheat have been reported from Pakistan (Rashid et al., 2002), which is known for high levels of soil B (Sillanpaa, 1982) or even from areas prone to B toxicity problem. That pockets of B deficiency may occur among soils with toxic level B has been documented in the Anatolia in

Turkey (Gezgin et al. 2002). In one such pocket B application has been reported to increase grain yield of durum wheat (cv. Ç-1252) by 16% (Topal et al., 2002). Bread and durum wheat, barley and triticale germplasm with the same level of B efficiency as the majority of those evaluated in this study should be expected to do poorly in all such growing regions that are prone to B deficiency.

The international germplasm from CIMMYT, and ICARDA in the case of barley, consists of superior genetic materials that have incorporated desirable characteristics such as high yielding capacity and resistance to important diseases. Many developing countries, Nepal and Bangladesh included, depend on the CIMMYT germplasm as the source of their new cultivars. In areas prone to B deficiency, the genetic yield potential of this introduced germplasm cannot be fully utilized because of the B deficiency constraint. Just as we have found with the CIMMYT germplasm in general, very high proportions of the recommended and widely grown cultivars in Nepal (Subedi et al., 1997) and Bangladesh (Ahmed et al., 2002) have been rated as B inefficient. It is not surprising that grain set failure is still common in farmers' wheat fields in Bangladesh, especially on the very low B soils in the northwestern part of the country (Kataki et al, 2002), and Nepal (Pant, 1994; Subedi et al., 1996). One solution would be to apply B fertilizer, as is routinely done in Brazil. However, persistence of the problem in many countries indicates that this seemingly simple and inexpensive option is not always available to growers. Furthermore, new improved varieties selected on research stations where B fertilizer has been applied can be expected to fail commercially, unless advanced breeding lines are assessed for B efficiency before they reach on-farm trials.

Under the low B condition that completely depressed grain set in other genotypes, a few bread wheat lines have been found to set grain normally, along with the B efficient check Fang 60. This B efficiency trait confers a remarkable adaptation to low B soils, so offers a solution to B deficiency in wheat production. Grain set failure and yield losses may be prevented by ensuring that germplasm destined for areas prone to B deficiency is B efficient. A cross between B efficient bread wheat Fang 60 and very inefficient Bonza demonstrated that gene(s) for B efficiency may be easily transferred (Ngorian, 2001). Responses to B in the progenies suggested a few major genes controlling B efficiency. There is real scope for genetic improvement. The source of genes for B efficiency already exists in bread wheat, screening can be done in the field on soil with low B or in a simple sand culture, as we have demonstrated. Many of the B efficient genotypes identified were advanced breeding lines, i.e. ready to be released as cultivars, that were included in CIMMYT international yield nurseries such as the 4HTWYT and 17ESWYT. For triticale, durum wheat and barley, a transfer of relevant genes from B efficient bread wheat such as Fang 60 will probably be essential. As it is present in the more B efficient bread wheat but not in the less B efficient durum wheat, triticale and barley, the D genome is of great interest as a possible source of genes for increasing B efficiency.

The primary effect of B deficiency in bread and durum wheat, barley and triticale was grain set failure. Boron levels affected grain set in bread wheat lines in the same manner as previously reported for wheat (Li et al., 1978; da Silva and de Andrade, 1980) and barley (Ambak and Tadano, 1991). The present study has established that B deficiency has similar effects on grain set in durum wheat and triticale, and that the GSI may be used to assess B efficiency in these species as well as bread wheat (Rerkasem and Loneragan, 1994) and barley (Jamjod and Rerkasem, 1999). The effect of B deficiency on grain set was equally

well described using either the GSI or number of grains per ear. However, as the number of competent florets and the potential number of grains may vary with genotypes and environment, a B sufficiency control is required to measure the effect of B on the number of grains per ear in different genotypes and growing conditions. In contrast, the GSI is very useful to evaluate responses to low B in nurseries with large entry numbers without the need for a B sufficiency control. The extent of B deficiency in the screening environment may be indicated by inclusion of a set of check genotypes covering a range of responses to low B. The GSI is also useful to quantify the problem of grain set failure in wheat, triticale and barley under a wide range of environmental condition on-farm. The presence of common varieties with known susceptibility to B deficiency allows verification of B deficiency as the likely limiting factor.

In some cases, triticale also responded to low B with a depression the number of spikelets per ear, similar to a previous report on barley (Jamjod and Rerkasem, 1999). The poor correlation between the effect of B on the number of spikelets per ear and the GSI for individual genotypes and species suggests that a different process from ear size response may govern the grain set response. The rat-tail symptom provides a convenient measure for the effect of B deficiency on spikelet number in barley and triticale. A slight improvement in the precision of determination of the B effect on triticale may be expected from combining the GSI and ear size response, but this may not be worth the extra cost of a B sufficiency control.

It is as yet unclear what mechanisms are involved in B efficiency. The differential effects of B deficiency on B efficient and inefficient genotypes appear not to be reflected in the concentration of B in their flag leaf or developing ear (Rerkasem and Loneragan, 1994) or the way they partition B among the various organs (Subedi et al., 1999). Triticale has been

reported to have derived genes for Cu efficiency (Graham, 1979) and Zn efficiency (Cakmak et al., 1997) from the rye chromosomes. Since triticale is largely B inefficient, the mechanism for B efficiency is unlikely to be related to those involved in Cu and Zn efficiency. For tolerance to B toxicity, exclusion of B has been suggested as a primary mechanism (Nable et al., 1997). In areas where soils have both deficient and toxic levels B occurring in close proximity, it may be necessary to ensure that selection and breeding for B efficiency does not inadvertently increase susceptibility to B toxicity, especially in an international germplasm such as that generated and distributed by CIMMYT and ICARDA.

5. Conclusion

The predominance of B inefficiency in germplasm such as that generated and distributed by CIMMYT and ICARDA poses a constraint to the realization of its full genetic yield potential. However, the presence of B efficiency in the germplasm suggests a solution to the problem of B deficiency in wheat production. We have demonstrated that the B efficiency trait may confer adaptation to low B soils, and effectively prevent grain set failure and yield losses. We have further shown that screening for B efficiency can be done easily and recommend that B efficiency should be included in the breeding objectives for bread and durum wheat, barley and triticale germplasm destined for growing areas prone to B deficiency.

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References

Ahmed, M., Jaihiruddin, M., Jamjod, S. and Rerkasem, B. 2002. Boron efficiency in wheat germplasm from Bangladesh. In: Goldbach, H. E., Rerkasem, B., Wimmer, M. A., Brown, P. H., Thellier, M., and Bell, R.W. (Eds.), All Aspects of Plant and Animal Boron Nutrition, Kluwer and Plenum Academic Publishers, Dordrecht, The Netherlands, pp. 299-303.

Ambak, K. and T. Tadano. 1991. Effect of micronutrient application on the growth and occurrence of sterility in barley and rice in a Malaysian deep peat soil. Soil Sci. Plant Nutr. 37, 715-724.

Anantawiroon, P., Subedi, K. and Rerkasem, B. 1997. Screening wheat for boron efficiency. In Bell, R.W. and Rerkasem, B. (Eds.), Boron in Soils and Plants. Dordrecht, The Netherlands: Kluwer Academic Publishers, pp. 101-104.

Cakmak, I., Derici, R., Torun, B., Tolay, I., Braun, H.J., andSchelgel, R. 1997. Role of rye chromosomes in improvement of zinc efficiency in wheat and triticale. Plant Soil 196, 249-253.

da Silva, A.R. and de Andrade, J.M.V. 1980. A Cultura do Trigo nas Varzeas de Minas Gerais – possibilidades e dificuldades. Embrapa, Centro de Pesquisa Agropecuária dos Cerrados – CPAC. Circular Técnica no. 2. 69 p. (In Portuguese)

da Silva, A.R. and de Andrade, J.M.V. 1983 Efeito micronutrientes no chochamento do trigo de seqqueiro e nas culturas de soja e arroz, em latossolo vermelho-amarelo. [Influence of micronutrients on the male sterility, on upland wheat and on rice and soybean yields, in red-yellow latosol] Pesq. Agropec. Bras., Brasilia 18, 593-601. (In Portuguese with English Abstract)

Dwivedi, G.K., Dwivedi, M. and Pal, S.S.. 1990. Modes of application of micronutrients in acid soil in soybean-wheat crop sequence. J. Indian Soc. Soil Sci. 38, 458-463.

Dwivedi, G.K., Dwivedi, M. and Pal, S.S.. 1990. Modes of application of micronutrients in acid soil in soybean-wheat crop sequence. J. Indian Soc. Soil Sci. 38, 458-463.

Gezgin, S., Dursun, N., Hamurcu, M., Harmankaya, M., Önder, M., Sadea, B., Topal, A., Soylu, S., Akgün, N., Yorgancilar, M., Ceyhan, E., Çiftçi, N., Acar, B., Gültekin, I., Iş ik, Y., Şeker, C. and Babaoglu, M. 2002. Boron content of cultivated soils in central-southern Anatolia and its relationship with soil properties and irrigation water quality. In: Goldbach, H. E., Rerkasem, B., Wimmer, M. A., Brown, P. H., Thellier, M., and Bell, R.W. (Eds.), All Aspects of Plant and Animal Boron Nutrition. Kluwer and Plenum Academic Publishers, Dordrecht, The Netherlands, pp. 391-400.

Graham, R.D. 1979. The sensitivity of hexaploid and octaploid triticale and their parents to copper deficiency. Aust. J. Agric. Res. 30, 791-799.

Graham, R.D. 1984. Breeding for nutritional characteristics in cereals. Adv. Plant Nutr. 1, 57-102.

Jamjod, S., Mann, C.E. and Rerkasem, B. 1992. Screening for boron deficiency in wheat. In: Mann, C.E. and Rerkasem, B. (Eds.), Boron deficiency in wheat. CIMMYT Wheat Special Report No. 11, International Maize and Wheat Improvement Center, Mexico, D.F., pp. 79-82.

Jamjod, S. and Rerkasem, B. 1999. Genotypic variation in responses of barley to boron deficiency. Plant Soil 215, 65-72.

Kataki, P.K., Srivastava, S.P., Saifuzzaman, M. and Upreti, H.K. 2002. Sterility of wheat and response of field crops to applied boron in the Indo-Gangetic Plains. J. Crop Production 4, 133-165.

Lamb, C.A. 1967. Physiology. In: Quisenberry, K.S. and Reitz, L.P. (Eds.) Wheat and Wheat Improvement. Agronomy Monograph 13. ASA, Madison, WI., pp. 181-223.

Li, B.H., Li, W.H., Kui, M.C., Chao, W.S., Jern, H.P., Li, C.R., Chu, W.J. and Wang, C.L. 1978 Studies on cause of sterility of wheat. J. Northeastern Agric. Coll. 3, 1-19. (In Chinese)

Liu, Z., Zhu, Q. and Tang, L. 1981. Boron deficient soils and their distribution in China. Soil Research Report No. 5. Institute of Soil Science, Academia Sinica, Nanjing, China.

Mandal A B and Das A D 1988. Response of wheat (Triticum aestivum) to boron application. Indian J. Agric. Sci. 58:681-683.

Marten, J.M. and Westermann, D.T. 1991. Fertilizer applications for correcting micronutrient deficiencies. In: Mordtvedt, J.J. et al. (Eds.) Micronutrients in Agriculture. SSSA Book Series no. 4. SSSA, Madison, WI, pp. 549-592.

Misra, R., Munankarmi, R.C., Pandey S.P. and Hobbs P.R. 1992. Sterility work in wheat at Tarahara in the eastern Terai of Nepal. In: Mann, C.E. and Rerkasem, B. (Eds.), Boron deficiency in wheat. CIMMYT Wheat Special Report No. 11, International Maize and Wheat Improvement Center, Mexico, D.F., pp. 67-71.

Nable, R.O., G.S. Banuelos, and J.G. Paull. (1997) Boron toxicity. Plant Soil 193, 181-198.

Ngorian, S. 2001. Response of Segregating Wheat Populations to Boron Deficiency. M.Sc. Thesis (Agronomy). Graduate School, Chiang Mai University.

Pant, J. 1994. Genotypic response of wheat to boron application under irrigated and water stress conditions. M.Sc. Thesis (Agricultural Systems). Graduate School, Chiang Mai University.

Rashid, A., Muhammad, S. and Rafique, E. 2002. Gynotypic variation in boron uptake and utilization in rice and wheat. In: Goldbach, H. E., Rerkasem, B., Wimmer, M. A., Brown, P. H., Thellier, M., and Bell, R.W. (Eds.), All Aspects of Plant and Animal

Boron Nutrition, Kluwer and Plenum Academic Publishers, Dordrecht, The Netherlands, pp. 305-310

Rerkasem, B. 1996. A general survey of the incidence of wheat sterility. In: Rawson, H.M. and Subedi, K.D. (Eds.), Sterility in wheat in subtropical Asia: extent, causes and solution. ACIAR Proceedings No. 72., Australian Centre for International Agricultural Research, Canberra, ACT. pp. 8-12.

Rerkasem, B. and Jamjod. S. 1997. Boron deficiency induced male sterility in wheat (*Triticum aestivum* L.) and implications for plant breeding. Euphytica 96, 257-262.

Rerkasem, B. and Loneragan, J.F. 1994. Boron deficiency in two wheat genotypes in a warm, subtropical region. Agron. J. 86, 887-890.

Reuter, D.J. 1987. An Assessment of Wheat Nutrition Research in Bangladesh. A consultancy report for CIMMYT and CIDA. SAGRIC International. 45 p.

Sakal, R. and Singh, A.P. 1995. Boron research and agricultural production. *In* Tandon, H.L.S. (Ed.), Micronutrient research and agricultural production. Fertilizer Development and Consultation Organization, New Delhi, India, pp. 1-31.

Sarkar, A.K. and Chakraborty, S. 1980. Effect of micronutrient on yield of major field crops grown in the Gangetic delta region. Indian Agric. 24, 259-266.

Shorrocks, V.M. 1997. The occurrence and correction of boron deficiency. Plant Soil 193, 121-148.

Sillanpaa, M. 1982. Micronutrients and nutrient status of soils – a global study. FAO Soil Bulletin 48. FAO, Rome.

Singh, R.P., Sinha ,S.D. and Prasad, R.B.. 1976. Effect of boron on seed setting in wheat under North Bihar conditions. Indian J. Agron. 21, 100-101.

Subedi, K.D., Budhathoki, C.B. and Subedi, M. 1997. Variation in sterility among wheat (*Triticum aestivum*) cultivars in response to boron deficiency in Nepal. Euphytica 95, 21-26.

Subedi K.D., Budhathoki, C.B., Subedi, M. and Yubak, D.G.C.. 1996. Effect of sowing time and boron on sterility in four genotypes of wheat (*Triticum aestivum* L.) in the Western Hills of Nepal. In: Rawson, H.M. and Subedi, K.D. (Eds.), Sterility in wheat in subtropical Asia: extent, causes and solution. ACIAR Proceedings No. 72., Australian Centre for International Agricultural Research, Canberra, ACT., pp. 51-61.

Subedi, K.D., Greogory, P.J. and Gooding, M.J. 1999. Boron accumulation and partitioning in wheat cultivars with constrasting tolerance to boron deficiency. Plant Soil 214,141-152.

Tandon, J.P. and Naqvi, S.M.A. 1992. Wheat varietal screening for boron deficiency in India. In: Mann, C.E. and Rerkasem, B. (Eds.), Boron deficiency in wheat. CIMMYT Wheat Special Report No. 11, International Maize and Wheat Improvement Center, Mexico, D.F., pp. 76-78.

Topal, A., S. Gezgin, N. Akgün, N. Dursun and M. Babaoglu. 2002. Yield and yield attributes of durum wheat (*Triticum durum* Desf.) as affected by boron application. In: Goldbach, H. E., Rerkasem, B., Wimmer, M. A., Brown, P. H., Thellier, M., and Bell, R.W. (Eds.), All Aspects of Plant and Animal Boron Nutrition, Kluwer and Plenum Academic Publishers, Dordrecht, The Netherlands, pp. 401-406.

Yang, Y.H. 1992. Wheat boron deficiency in Yunnan, China. In: Mann, C.E. and Rerkasem, B. (Eds.), Boron deficiency in wheat. CIMMYT Wheat Special Report No. 11, International Maize and Wheat Improvement Center, Mexico, D.F., pp. 72-75.

www.cimmyt.org/Research/wheat Accessed February 1, 2002

Table 1. The effects of soil B levels (B) by genotypes (G) on the grain set index (GSI), the number of grain per ear, spikelets per ear and ears per m in bread wheat (4HTWYT, 17ESWYT), triticale (28ITYN) and durum (28IDYN).

	Bread wheat		Triticale	Durum
	4HTWYT	17ESWYT	28ITYN	28IDYN
df (B)	1	1	2	2
Error 1	1	1	2	2
df (G x B)	49	49	98	98
Error 2	98	98	152	152
GSI (%)				
B	***	*	***	***
G x B	*	*	*	*
Grains ear^{-1}				
B	***	*	***	**
G x B	*	*	*	*
Spikelets ear^{-1}				
B	NS	NS	NS	NS
G x B	NS	NS	*	NS
Ears m^{-1}				
B	NS	NS	NS	NS
G x B	NS	NS	NS	NS

*Significant at $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

NS not significant at $P < 0.05$.

Table 2. Frequency distribution of genotypes of bread wheat, triticale and durum by their grain set index (GSI) in soil in B0.1.†

Class by GSI in B0.1	Bread wheat		Triticale	Durum	Total
	4HTWYT	17ESWYT	28ITYN	28IDYN	
	Frequency (%)				
0-20%	14.3	14.3	51.0	81.6	40.3
21-50%	57.1	30.6	32.7	18.4	34.7
51-70%	12.2	30.6	16.3	0.0	14.8
71-85%	12.2	16.3	0.0	0.0	7.1
>85%	4.1	8.2	0.0	0.0	3.1
Total	49	49	49	49	196
†GSI (%±SE) of B efficient check, Fang 60					
	94.3±4.1	85.5±5.3	91.3±8.8	98.3±1.4	

Table 3. Responses to boron in the field in bread wheat, durum wheat and triticale, by B efficiency class.

Soil boron level	Class of genotypes, by GSI in B0.1					Nursery mean	Fang 60
	0-20%	21-50%	51-70%	71-85%	>85%		
	Grain set index (%)						
Bread wheat (4HTWYT)							
0.1	10.2a	35.8a	59.1a	79.3a	89.2a	42.5a	94.3a
0.2	71.8b	82.4b	84.4b	89.5b	95.4a	82.5b	98.5a
Bread wheat (17ESWYT)							
0.1	13.5a	35.3a	60.9a	77.5a	87.6a	51.6a	85.5a
0.2	71.5b	79.5b	85.7b	91.7b	95.5a	83.7b	99.0b
Triticale (28ITYN)							
0.1	9.9a	29.3a	57.6a	ne[†]	ne	24.7a	91.3a
0.2	62.5b	80.6b	84.5b	ne	ne	72.3b	98.2a
0.3	95.2c	95.1c	97.9c	ne	ne	95.6c	96.3a
Durum wheat (28IDYN)							
0.1	6.5a	31.4a	ne	ne	ne	12.8a	98.3a
0.2	54.7b	72.3b	ne	ne	ne	58.5b	100.0a
0.3	88.9c	95.8c	ne	ne	ne	90.3c	99.5a

Significant effect of B in each class of genotypes is denoted by different letters by means of LSD ($P < 0.05$)

[†]ne = no entry in this class

Table 4. Number of grains per ear in B0.1 relative to B sufficiency (± standard error) in different classes of genotypes of bread wheat, triticale and durum wheat grown in the field.

Class of genotypes, by GSI in B0.1	Bread wheat		Durum	Triticale
	4HTWYT	17ESWYT	28ITYN	28IDYN
	Number of grains ear^{-1} in B0.1 relative to B sufficiency†			
0-20%	0.20±0.11	0.33±0.08	0.10±0.01	0.06±0.08
21-50%	0.39±0.18	0.53±0.20	0.25±0.01	0.14±0.03
51-70%	0.69±0.15	0.57±0.18	0.45±0.07	ne
71-85%	0.80±0.18	0.60±0.11	ne‡	ne
>85%	0.97±0.19	0.75±0.14	ne	ne
Fang 60	0.94±0.26	1.01±0.04	1.05±0.27	1.14±0.05
Mean	0.49±0.29	0.56±0.29	0.22±0.17	0.06±0.10

† B sufficiency was B0.2 for bread wheat and B0.3 for durum wheat and triticale.

‡ne = no entry in this class

Table 5. Mean grain set index (GSI) of bread wheat, durum wheat and triticale lines in different boron efficiency classes in sand culture without added boron (B0), compared with a boron-efficient check, Fang 60.

Class by GSI in B0.1	Bread wheat 4HTWYT	Triticale 28ITYN	Durum wheat 28IDYN
	Grain set index in B0 (%±SD)		
0-20	28.4±20.2	10.4±16.7	8.3±11.5
21-50	58.6±18.6	17.0±18.2	10.3±10.0
51-70	63.5±20.0	39.1±26.4	ne†
71-85	67.3±19.8	ne	ne
>85	92.0±11.4	ne	ne
Nursery mean	57.2±22.7	17.9±23.9	10.6±16.6
Fang 60	95.0±7.1	96.4±3.2	97.2±4.0

†No entry in this class

Table 6. Grain set index (GSI) of various boron efficient and inefficient bread wheat lines in a soil low in B (0.1 mg HWS B kg^{-1}, B0.1) and sand culture without added B (B0). (Each GSI number is mean of two replicates, ±standard deviation)

Boron condition for screening		Soil (B0.1)	Sand (B0)
Nursery, entry no., variety or cross		Grain set index (%±SD)	
4HTWYT			
1	Fang 60	94.3±1.7	95.0±7.1
4	MOCHIS T 88	29.3±8.8	38.8±14.5
5	FASAN	3.6±12.0	8.3±18.0
12	PAT10/ALD//PAT72300/3/PVN/4/BOW	92.7±2.4	89.4±1.9
13	PAT10/ALD//PAT72300/3/PVN/4/BOW	85.8±0.4	91.6±3.4
15	TRAP#1/BOW	83.5±15.6	85.3±2.1
20	PRINIA	0±0.0	15.8±0.0
30	TIA.1	85.0±4.2	82.4±7.1
17ESWYT			
1	Fang 60	85.5±14.1	nd[†]
6	WEAVER	2.8±3.2	nd
7	CHIL/2*STAR	89.0±0.6	nd
9	TURACO/CHIL	17.8±1.8	nd
28	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN	5.3±0.4	nd
31	STAR//KAUZ/PVN	87.0±1.4	nd
43	KAUZ*2//SAP/MON/3/KAUZ	88.3±11.0	nd
47	CPAN 3004	86.0±16.3	nd

[†]nd = no data

Table 7. Effect of B on ear size (number of spikelets per ear) in triticale grown in the field at different levels of B, with ranges in parentheses.

B response class†	Number of entries	B0.1	B0.2	B0.3
		Spikelets per ear		
Responsive	19	14.66 (12.7-15.4)	19.44 (14.8-25.5)	22.81 (19.4-27.9)
Non-responsive	30	18.04 (12.8-24.8)	20.73 (14.1-24.8)	20.29 (13.4-25.6)
Fang 60		12.75	14.10	13.40
LSD ($P < 0.05$)			5.84	

†Separation between responsive and non-responsive classes by means of the difference for each genotype between B0.1 and B0.3 in number of spikelets per ear that is either greater or less than the LSD.

Table 8. Frequency distribution (%) of boron efficiency classes in selected sets of CIMMYT 2000/01 international germplasm grown in soil with 0.1 mg HWS B kg^{-1}.

Grain set index class (%)	Bread wheat 33IBS-WYN	Bread wheat 18SA-WSN	Barley 28IBON†	Durum wheat 33IDYN	Triticale 33ITSN	Whole germ-plasm
	Frequency (%)					
< 20	36.4	23.0	37.0	34.7	42.8	35.4
20-49	25.0	23.0	39.0	51.0	25.5	29.2
50-74	8.0	28.7	24.0	14.3	27.5	19.5
75-90	20.6	17.2	0.0	0.0	4.1	11.0
> 90	10.1	8.0	0.0	0.0	0.0	4.9
Total	388	190	265	49	216	1108
GSI of B efficiency checks (%, with standard deviation in brackets)						
SW41	71.1±7.4	71.6±5.5	60.5±6.6‡	82.8±1.0	70.1±4.3	
Fang 60	97.2±0.8	96.8±1.2	84.3±2.3§	99.3±0.1	96.5±0.8	

† From CIMMYT/ICARDA

‡ BCMU96-9 and § BRB9604

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Genetic control of boron efficiency in wheat (*Triticum aestivum* L.)

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Key words: boron, deficiency, efficiency, wheat

Abstract

The genetic control of boron (B) efficiency in wheat (*Triticum aestivum* L.) was studied for three genotypes representing B inefficient (I, Bonza), moderately B inefficient (MI, SW 41) and B efficient (E, Fang 60) categories. Boron efficiency was expressed as a partially dominant character but the phenotypes of F_1 hybrids, relative to parents, indicated genetic control varied from recessive to additive to completely dominant with different cross combinations and B levels. Major genes were identified from the evaluation of F_2-derived F_3 populations derived from intercrosses between the three parents. Monogenic segregation was found in Bonza x SW 41 and SW 41 x Fang 60 crosses and digenic segregation resulted between Bonza x Fang 60 cross. Among the three wheat genotypes with widely different B efficiency, genetic variation for response to B could be accounted for by two genes, *Bo$_d$1* and *Bo$_d$2*.

Abbreviations: B, boron; E, efficient; GSI, grain set index; HWS, hot water soluble; I, inefficient; MI, moderately inefficient

Introduction

Low boron (B) soils are widespread in many subtropical wheat growing areas (Sillanpaa, 1982; Shorrocks, 1997). These include the northern region of Thailand, where wheat is being promoted (Rerkasem & Jamjod, 1989) and established wheat growing areas of China (Li et al., 1978), Bangladesh (Kataki et al., 2002), northwestern India (Sakal & Singh, 1995) and the Terai and mid-hills of Nepal (Subedi et al., 1996). Boron deficiency causes yield reduction by inducing male sterility, resulting in grain set failure (Rerkasem & Loneragan, 1994). A wide range of genotypic variation for response to low B has been identified and genotypes were classified into distinct B efficiency classes, namely, efficient, moderately efficient, moderately inefficient and inefficient (Rerkasem & Jamjod, 1997). Below a critical B level, inefficient genotypes were completely sterile and set no or just a few grains, while efficient genotypes set grain normally.

Genotypic variation offers a solution to sterility of wheat in low B soils. Boron efficient genotypes have been found to avoid sterility in the field in low B soils in Bangladesh, Nepal and northern Thailand where inefficient genotypes sustained serious yield losses (Rerkasem & Jamjod, 1989; Subedi et al., 1996; Kataki et al., 2002). Avoidance of B deficiency through selection and breeding for B efficient cultivars appears to be a promising approach for wheat. Furthermore, the response to low B in wheat is unique in that at certain levels of B deficiency male fertility, easily quantified as grain set, is the only adverse effect observed (Rerkasem et al., 1997). Wheat therefore offers a model plant on which the effect of B deficiency on male fertility may be studied without the confounding effects from other physiological

processes. However, the lack of understanding of genetic control of B efficiency traits involved in grain set hampers selection and breeding for B efficiency as well as genotypic variation studies. In this study, we evaluated responses to B of F_1 hybrids and F_2-derived F_3 populations in comparison to those of their B efficient, moderately inefficient and inefficient parents, to determine their genetic control.

Materials and Methods

Genetic materials

Fang 60 (B efficient; E, Jamjod et al., 1992), SW 41 (moderately B inefficient; MI, Rerkasem & Loneragan, 1994) and Bonza (B inefficient; I, Rerkasem & Jamjod, 1997) were used as parents. F_1 hybrid plants of three crosses, Bonza x SW 41, SW 41 x Fang 60 and Bonza x Fang 60, including reciprocal crosses were tested in Experiment 1 and sown in B sufficient soil to produce F_2 and F_3 generations. F_3 populations were tested in sand culture in Experiment 2. The first backcross population (BC_1) was made for the Bonza x SW 41 cross using Bonza as a recurrent parent, i.e. Bonza x (Bonza x SW 41), and used in Experiment 3.

Experiment 1: Evaluation of F_1 hybrids to B levels

Parents and F_1 populations were sown in a low B soil (0.1 mg hot water soluble B kg^{-1}) in the field at Chiang Mai University. The experiment was arranged as a split plot design with 3 replications. Three B levels were arranged in main plots, including nil (B0), limed at the rate 2 t ha^{-1} (BL), to accentuate B deficiency (Rerkasem & Jamjod,

1989) and boron at the rate 10 kg borax ha^{-1} (B+) applied to the soil. Three parents (Fang 60, SW 41 and Bonza) and their F_1 hybrids were arranged in sub plots. In each plot, 5 plants of each parent and F_1 were sown in single rows with 0.1 m between plants and 0.25 m between rows. At boot stage, the first two ears of each plant were bagged to prevent outcrossing. At maturity, the bagged ears were harvested and the effect of B deficiency quantified as the grain set index (GSI, percentage of the 20 basal florets from 10 central spikelets with grain; Rerkasem & Loneragan, 1994). Responses to B of parents and F_1 hybrids were compared by Duncan's Multiple Range Test (DMRT).

Experiment 2: Evaluation of F_2-derived F_3 families

Three populations of F_2-derived F_3 families examined included (Bonza x SW 41), (SW 41 x Fang 60) and (Bonza x Fang 60). Families were sown in freely drained earthenware pots (0.3 m diameter and 0.3 m deep) containing washed river quartz sand with no detectable available B. The sand was watered twice daily with an otherwise complete nutrient solution without added B (B0). The nutrient solution was adapted from Broughton & Dilworth (1971) and consisted of (μM): $CaCl_2$, 1000; $MgSO_4$, 250; KH_2PO_4, 500; FeEDTA, 10; K_2SO_4, 250; $MnSO_4$, 2; $ZnSO_4$, 0.5; $CuSO_4$, 0.2; $CoSO_4$, 0.1, Na_2MoO_2, 0.1 and KNO_3, 5000. This sand culture system has been successfully used in screening for B efficiency in wheat (Rerkasem & Jamjod, 1997) and barley (Jamjod & Rerkasem, 1999). In each pot, eleven plants per family or parental line were sown with one plant of the B inefficient genotype, Bonza, at the center, i.e. 12 plants per pot. The number of families for each combination were 85 for (Bonza x SW 41)F_3, 92 for (SW 41 x Fang 60)F_3 and 114 for (Bonza x Fang 60)F_3. Twelve pots of each parental line were included. At boot stage, the first

two ears from each plant were bagged. At maturity, the bagged ears were harvested and assessed for grain set with the GSI.

To classify families into each type of response to B, mean GSI and variance within family were calculated and related to those of the parents. With respect to B, the families were classified as homozygous efficient or homozygous inefficient when their mean and variance were in the range of either efficient or inefficient parents. Families with means outside the range of the parents but having a variance within the range of the parents were classified as homozygous intermediate. Families with a variance higher than those of parents were classified as segregating. Chi-square analysis was used for testing goodness of fit of the observed segregation ratio to the value expected for each of two models. Families for each population were tested for the monogenic ratio of 1 homozygous efficient : 2 segregating : 1 homozygous inefficient and for the digenic ratio of 1 homozygous efficient : 14 (homozygous intermediate + segregating) : 1 homozygous inefficient.

Experiment 3 Evaluation of backcross population

The backcross population (BC_1) of Bonza x (Bonza x SW 41), B inefficient recurrent parent; Bonza and donor parent, SW 41 were tested for response to B in sand culture without added B as described in Experiment 2. One hundred of BC_1F_1 plants and 50 plants of each parent were sown. At boot stage, the first two ears from each plant were bagged. At maturity, plants were harvested and grain set determined based on the GSI of bagged ears. Chi-square tests were conducted to examine the segregation ratio of the backcross population with the expected ratio of 1 Bonza type to1 heterozygote.

Results

Response of F_1 hybrids

There was a significant difference in the response to B among the three wheat genotypes and their F_1 hybrids (Table 1). The GSI in all genotypes exceeded 80% when B was applied (B+). The parents differed in GSI in response to the lower B treatments, BL and B0. Fang 60 was the most efficient and did not show an adverse effect of B deficiency. SW 41 showed an intermediate B efficiency, with GSI of 58.3% in B0 and 44.2% in BL. With GSI of 32.9% in B0 and 0.2% in BL, Bonza was the most inefficient.

The GSI of F_1 hybrids relative to their parents varied with the parental combinations and B treatments. There was no significant difference between reciprocal crosses at each B level.

With the BL and B0 treatments the GSI of the F_1 hybrids from SW 41 x Fang 60 combinations were not significantly different from the efficient parent, Fang 60. Those from Bonza x Fang 60 combinations were intermediate between the two parents but closer to Fang 60 at BL and not different from Fang 60 at B0. In contrast, F_1 hybrids of Bonza x SW 41 were not significantly different from the inefficient parent (Bonza) when grown at BL and were similar to the two parents at B0.

Response of F_2-derived F_3 populations

As in low B soil, the three parental lines and their F_3 populations tested in this study showed a large range of responses to low B in sand culture (Table 2). Mean GSI of Bonza, SW 41 and Fang 60 parental lines were between 0-3, 33-69 and 87-99%, respectively. Mean GSI of F_2 derived families were within the range of their respective parents. Within line variances of the inefficient Bonza and efficient Fang 60 parents were between 0-189 while those of the intermediate, moderately inefficient SW 41 parents were between 657-1328. Within family variances of the F_3 varied from 0 to 2508 (Table 2).

Means and variances of F_3 families for each cross were compared to parents and classified into homozygous efficient, segregating, homozygous intermediate and homozygous inefficient types. Families from Bonza x SW 41 and SW 41 x Fang 60 could not be clearly classified into homozygous SW 41 and segregating classes due to high phenotypic variance within the SW 41 parent (Figure 1a and 1b). Therefore, families were classified into homozygous inefficient (I) and segregating plus homozygous moderately inefficient (Seg + MI) for the Bonza x SW 41 cross and segregating plus homozygous moderately inefficient (Seg + MI) and homozygous efficient (E) for the SW 41 x Fang 60 cross (Figure 1c). Chi-square analysis demonstrated that segregation of F_3 families from the crosses Bonza x SW 41 and SW 41 x Fang 60 was consistent with the single gene model (Table 3). Segregation of F_3 families from the cross Bonza x Fang 60 deviated from the monogenic ratio and was consistent with a two gene model (Table 3).

Response of backcross population

When tested in B0, the inefficient recurrent parent, Bonza set no grain (Figure 2). The GSI of the donor SW 41 parent ranged between 25% and >75%. Nearly 50% of the BC_1 population expressed the same GSI as the recurrent parent. Chi-square analysis was consistent with 1:1 ratio of Bonza type: heterozygote ($\chi 2 = 1.17$, $p = 0.5945$).

Discussion

The segregation patterns of F_2-derived F_3 families and the BC_1 population between parents with contrasting levels of B efficiency were consistent with B efficiency being under the control of major genes. The full response range of the three parents was shown to be controlled by two major independent loci, with the efficient Fang 60 having efficiency alleles at both loci and the moderately inefficient SW 41 having efficiency alleles at one locus. The B inefficient Bonza is expected to have alleles for inefficiency at both loci. If the gene symbol *Bo$_d$* is assigned to the loci controlling B efficiency, the proposed genotypes of these parental lines are as follows: Fang 60 *Bo$_d$1Bo$_d$1 Bo$_d$2Bo$_d$2*, SW 41 *bo$_d$1bo$_d$1 Bo$_d$2Bo$_d$2* and Bonza *bo$_d$1bo$_d$1 bo$_d$2bo$_d$2*. This study presents the first clear evidence of genes controlling B efficiency in wheat, although there have been reports in other crops. For example, Xu et al. (2001) suggested one major gene controlled B efficiency in oil seed rape, but that possibly another three minor genes were also associated with B efficiency. Major gene control of B efficiency has also been reported for celery (Pope & Munger, 1953), tomato (Wall & Andrus, 1962) and red beet (Tehrani et al., 1971).

The results of this study suggested that the responses of F_1 hybrids compared to parents varied with B treatments and parent combinations. Responses of the F_1 from the Bonza x SW 41 were close to the B inefficient Bonza at BL and intermediate to the two parents at B0, suggesting recessive and additive control of B efficiency, respectively. In contrast, for the crosses SW 41 x Fang 60 and Bonza x Fang 60 at BL and B0, the GSI of the F_1 hybrids were close to the B efficient Fang 60, indicating completely dominant control of B efficiency. The changing response of F_1 hybrids according to their parental combinations and treatments was also found in the study of tolerance to high concentrations of B (Paull et al., 1991). These responses were consistent with the hypothesis of Knight (1973) that for a quantitative trait the response of an F_1 hybrid relative to its parents, will vary according to the environmental conditions.

The nature of the expression of B efficiency will influence the level of the treatments selected for screening segregating populations in a breeding programme. For example, B0 in sand culture was able to identify homozygous inefficient genotype but could not differ heterozygote from homozygous moderately inefficient genotypes from an I x MI cross (Figure 2) whereas BL in soil suppressed grain set of both genotypes (Table 1). However, low levels of B could not be used to discriminate heterozygotes and homozygous efficient (E) genotypes when screening an F_2 and other segregating generations due to the dominant gene action (Table 1, Ngorien, 1999). Progeny testing, as shown in the study of F_3 (Figure 1), is suggested for screening segregating populations involving B efficient (E) parents.

As B efficiency in wheat is controlled by major genes, the backcross method is the most efficient way for transferring B efficiency into locally adapted, inefficient varieties. Backcross-derived lines between SW 41 (Bo_d2) as the donor parent and Bonza as the recurrent parent are now being developed at Chiang Mai University and will be evaluated for yield advantage in the next growing season. The Bonza derivatives carrying Bo_d2 will be selected and following further crosses with Fang 60 as a donor parent, lines carrying Bo_d1 and Bo_d2 will be developed for evaluation under low B conditions.

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References

Broughton, W.J. & M.J. Dilworth, 1971. Control of leghaemoglobin synthesis in snake bean. Biochem. J. 125: 1075-1080.

Jamjod, S., & B. Rerkasem, 1999. Genotypic variation in response of barley to boron deficiency. Plant and Soil 215: 65-72.

Jamjod, S., C.E. Mann & B. Rerkasem, 1992. Screening for boron deficiency in wheat. In: C.E. Mann & B. Rerkasem (Eds.), Boron Deficiency in Wheat, pp. 79-82. CIMMYT What Special Report No. 11, CIMMYT, Mexico.

Kataki, P.K., S.P. Srivastava, M. Saifuzzaman & H.K. Upreti, 2002. Sterility of wheat and response of field crops to applied boron in the Indo-Gangetic Plains. J. Crop Prod 4: 133-165.

Knight, R., 1973. The relation between hybrid vigour and genotype-environment interactions. Theo. Appl. Genet. 43: 311-318.

Li, B.H., W.H. Li, M.C. Kui, W.S. Chao, H.P. Jern, C.R. Li, W.J. Chu & C.L.Wang, 1978. Studies on cause of sterility of wheat. J. Northeastern Coll. 3: 1-19 (*in Chinese with English translation*).

Ngorien, S. 1999. Response of segregating wheat populations to boron deficiency. MS. Thesis, Chiang Mai University, Chiang Mai, Thailand. 68 p.

Paull, J.G., A.J. Rathjen & B. Cartwright, 1991. Major genes control of tolerance of bread wheat (*Triticum aestivum* L.) to high concentrations of boron. Euphytica 55: 217-228.

Pope, D.T. & H.M. Munger, 1953. The inheritance of susceptibility to boron deficiency in celery. Proc. Am. Soc. Hortic. Sci. 61: 481-486.

Rerkasem, B. & J. F. Loneragan, 1994. Boron deficiency in two wheat genotypes in a warm subtropical region. Agron. J. 85: 887-890.

Rerkasem, B. & S. Jamjod, 1997. Boron deficiency induced male sterility in wheat (*Triticum aestivum* L.) and implications for plant breeding. Euphytica 96: 257-262.

Rerkasem, B. & S. Jamjod, 1989. Correcting boron deficiency induced ear sterility in wheat and barley. Thai J. Soils and Fertilizers 11: 200-209. (*in Thai with English summary*).

Rerkasem, B. Lordkaew, S. & Dell, B. 1997. Boron requirement for reproductive development in wheat. Soil Sci. Plant Nutr.43: 953-957.

Sakal, R. & A.P. Singh, 1995.Boron research and agricultural production. In:H.L.S. Tandon (ed.), Micronutrient Research and Agricultural Production, pp. 1-31. Fertilizer Development and Consultation Organization, New Delhi, India.

Shorrocks, V.M. 1997. The occurrence and correction of boron deficiency. Plant and Soil 193: 121-148.

Sillanpaa, M. 1982. Micronutrients and nutrient status of soils. FAO Bull. 48, FAO, Rome.

Subedi, K.D., C.B. Budhathoki, M. Subedi & D.G.C. Yubak, 1996. Effect of sowing time and boron on sterility in four genotypes of wheat (*Triticum aestivum* L.) in the Western hills of Nepal. In: H.M. Rawson & K.D. Subedi (eds.), Sterility in Wheat in Subtropical Asia: Extent, Causes and Solution, ACIAR Proceedings No. 72, pp. 51-61. ACIAR, Canberra.

Tehrani, G., H.M. Munger, R.W. Robinson & S. Shannon, 1971. Inheritance and physiology of response to low boron in red beet (*Beta vulgaris* L.). J. Am. Soc. Hortic. Sci. 96: 226-230.

Wall, J.R. & C.F. Andrus, 1962. The inheritance and physiology of boron response in the tomato. Am. J. Bot. 49: 758-762.

Xu, F.S., Y.H. Wang & J. Meng, 2001. Mapping boron efficiency gene(s) in *Brassica napus* using RFLP and AFLP markers. Plant Breeding 120: 319-324.

Table 1. GSI (%) of three parents, F_1 hybrids and reciprocal crosses in response to B levels. BL = limed soil with no added B; B0 = unlimed soil with no added B; B+ = 10 kg Borax ha^{-1} added to unlimed soil.

Parents/F_1 hybrids	B treatments[a]		
	BL	B0	B+
Bonza	0.2 d	32.9 b	86.0 a
SW 41	44.2 c	58.3 b	90.8 a
Fang 60	98.1 a	99.1 a	97.9 a
Bonza x SW 41	3.3 d	46.0 b	80.6 a
SW 41 x Bonza	9.7 d	38.7 b	94.2 a
Bonza x Fang 60	65.8 bc	91.5 a	96.9 a
Fang 60 x Bonza	87.5 ab	97.2 a	97.8 a
SW 41 x Fang 60	93.2 a	97.0 a	93.6 a
Fang 60 x SW 41	93.1 a	98.6 a	94.7 a
F-test	B^{**}, G^{***}, BxG^{***}		

** and *** Significant at 0.01 and 0.001 probability levels, respectively.

[a] Means within a column with the same letter do not differ significantly at 5% level with Duncan's Multiple Range Test.

Table 2. Range of mean GSI (%) and variance within family/line of 3 parents and F_2-derived families, from 3 crosses grown in sand culture without added B (B0). Values are based on 11 plants per F_2-derived family.

Line/Family		Mean GSI (%)			Variance		
	n	Min	Mean	Max	Min	Mean	Max
Parents							
Bonza	12	0	1	3	0	12	51
SW 41	10	33	52	69	657	953	1328
Fang 60	12	87	94	99	4	75	189
F_3 families							
(Bonza x SW 41) F_3	85	0	17	71	0	466	1907
(SW 41 x Fang 60) F_3	92	30	69	98	10	625	1586
(Bonza x Fang 60) F_3	114	0	41	100	0	759	2508

n = number of parental lines or F_3 families

Table 3. Chi-square analysis of the responses to B deficiency of F_2-derived F_3 families from three crosses grown in sand culture without added B.

Cross	Model[a]		Number of families[b]			$\chi 2$	P
			I	Seg+MI			
Bonza x SW 41	1:3	Exp.	21.25	63.75		0.18	0.6611
	1:15	Exp.	5.31	79.69		18.65	<0.001
		Obs.	23	62			
				Seg+MI	E		
SW 41 x Fang 60	3:1	Exp.		69	23	1.06	0.3355
	15:1	Exp.		86.25	5.75	11.65	<0.001
		Obs.		73	19		
			I	Seg+Int.	E		
Bonza x Fang 60	1:2:1	Exp.	28.5	57	28.5	61.34	<0.001
	1:14:1	Exp.	7.125	99.75	7.125	5.26	0.078
		Obs.	12	92	10		

[a] Exp. – expected ratio; Obs. – observed ratio.

[b] E = homozygous efficient, MI = homozygous moderately inefficient, I = homozygous inefficient, Seg = segregating, Int. = homozygous intermediate.

Figure 1. Mean GSI (%) and variance within families of F_2-derived F_3 families and parents grown in sand culture without added B. Note: Mean GSI and variance of Bonza parent were both 0 and obscured by F_3 families' data.

a) Bonza x SW 41

b) SW 41 x Fang 60

c) Bonza x Fang 60

Figure 2. Response of GSI (%) of backcross population (BC_1), recurrent (Bonza) and donor (SW 41) parents grown in sand culture without added B.

Figure 1.

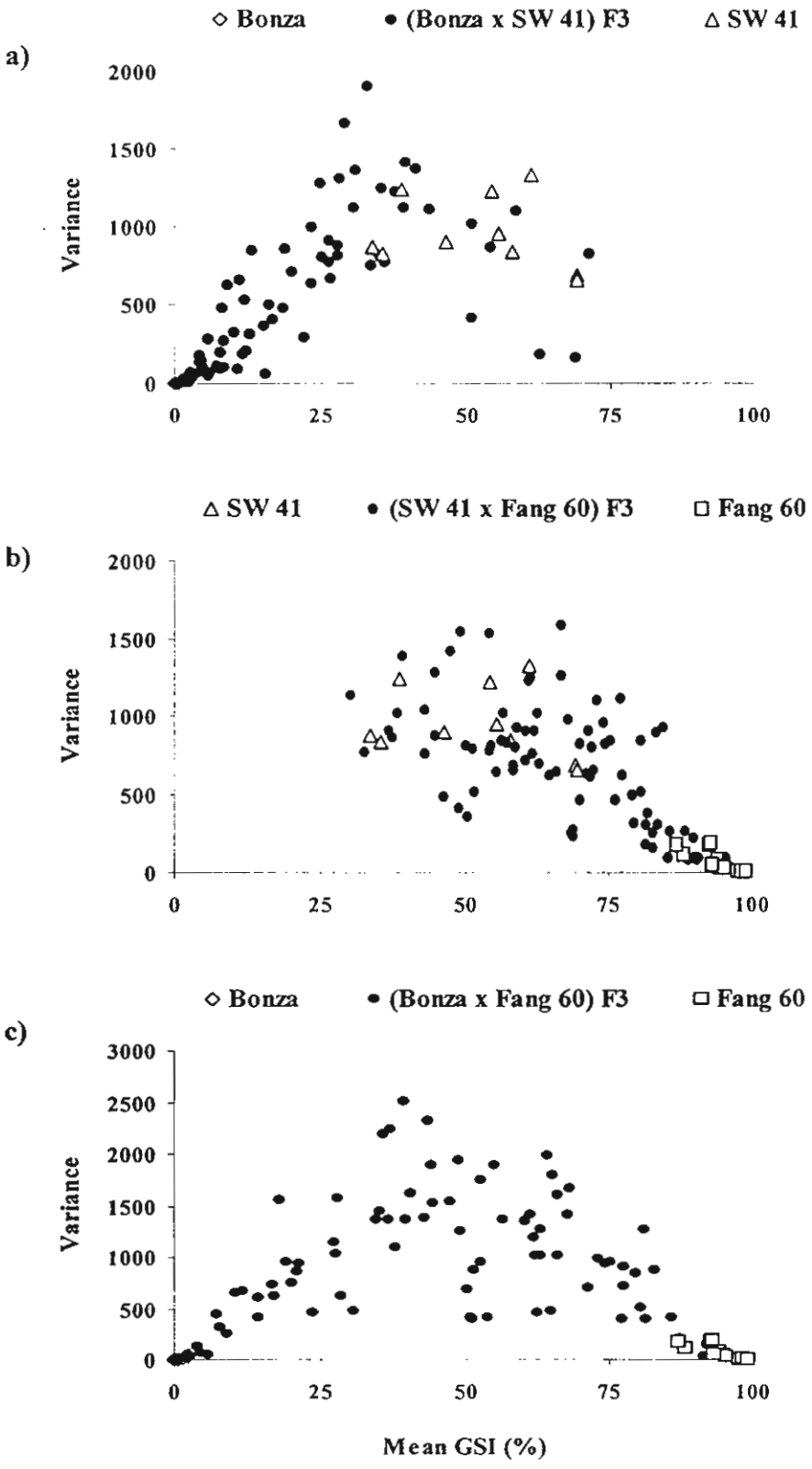
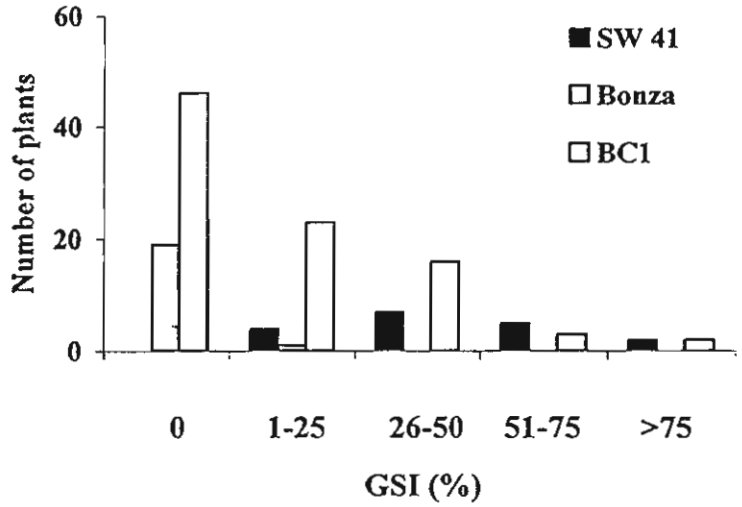

◇ Bonza
• (Bonza x SW 41) F3
△ SW 41
a)
Variance
2000
1500
1000
500
0
0
25
50
75
100
△ SW 41
• (SW 41 x Fang 60) F3
□ Fang 60
b)
Variance
2000
1500
1000
500
0
0
25
50
75
100
◇ Bonza
• (Bonza x Fang 60) F3
□ Fang 60
c)
Variance
3000
2500
2000
1500
1000
500
0
0
25
50
75
100
Mean GSI (%)

Figure 2.


60
40
20
0
Number of plants
SW 41
Bonza
BC1
0
1-25
26-50
51-75
>75
GSI (%)

Contrasting responses to boron deficiency in barley and wheat

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Keywords: Barley, Boron deficiency, Boron efficiency, Genotypic variation, Wheat

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Contrasting responses to boron deficiency in barley and wheat

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Abstract

To determine if boron (B) deficiency, commonly reported to depress grain set in wheat, has the same effect in barley, a set of experiments compared five wheat and seven barley genotypes at various B levels in sand culture and in the field. In sand culture, plants were grown with levels of added B, from 0 to 10 μM. In the field, they were sown in a low B soil [0.15 mg hot water soluble (HWS) B kg^{-1}] with three B treatments (nil, 2 t lime ha^{-1}, 1 kg B ha^{-1}). In sand culture without added B, the genotypes ranged in grain set index (GSI) from 0 to 93 % for wheat and 0 to 67 % for barley. Boron concentration of the spike and flag leaf at booting in wheat and barley correlated ($r = 0.8 - 0.9$, $p < 0.01$) with the effect of B on GSI. Grain set was the only response, measurable in decreased number of grain $spike^{-1}$ and grains $spikelet^{-1}$, to low B in wheat. In barley, low B also depressed the number of spikelet $spike^{-1}$ by 23 to 75 % and induced a "rat-tail" symptom of terminal spikelet degeneration. There was a weak correlation between spike and flag leaf B and the effect of B on spike size in barley ($r = 0.47$ and 0.37, respectively, $p < 0.1$). In some barley genotypes, the low B level that depressed grain set sometimes also delayed spike emergence and depressed the number of spikes $plant^{-1}$ but sometimes increased tillering and dry weight of straw. These results demonstrate that the phenotype of plant response to low B is more complex in barley than wheat and may require different strategies for managing B nutrition of barley including different approaches for selecting B efficient genotypes.

Introduction

Although it is often reported that cereals have low sensitivity to B deficiency (Marten and Westerman, 1991; Shorrocks, 1997), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) can be adversely affected by B deficiency in the field. In both species, B deficiency depresses male fertility, resulting in grain set failure (e.g. see Li et al., 1978 and da Silva and de Andrade, 1983 for wheat and Ambak and Tadano, 1991 for barley). Genotypic variation in the response to low B supply has been reported in wheat (Rerkasem and Jamjod, 1997) and barley (Jamjod and Rerkasem, 1999). Those genotypes that are able to grow and yield well in soils in which other genotypes are adversely affected by B deficiency have been called B-efficient genotypes (Rerkasem and Jamjod, 1997). In wheat, genotypic variation to B has only been expressed in reproductive growth (Rerkasem and Loneragan, 1994), whereas in barley, it has been reported in both reproductive and vegetative growth. In barley, however, the B response is somewhat confusing as B deficiency that depresses male fertility and grain set has been reported to either enhance (Ambak and Tadano, 1991) or depress (Jamjod and Rerkasem, 1999) vegetative growth. This study aims to determine how differently barley and wheat phenotypes respond to B deficiency by comparing a range of wheat and barley genotypes under defined conditions of B supply. This information has relevance for managing B nutrition in the field and also for selecting B-efficient genotypes in the future.

Materials and Methods

Barley and wheat genotypes covering a wide range of B efficiency (Jamjod and Rerkasem, 1999, Anantawiroon et al., 1997) were compared at various levels of B supply in two sand culture experiments, and one experiment in the field and sand culture in Chiang Mai, Thailand. These genotypes have been identified from extensive screening of germplasm from the International Maize and Wheat Improvement Center (CIMMYT). In experiment 1, three genotypes of two-row barley (BRB 9, BCMU 96-9 and CMBL 92029) and wheat (Fang 60, SW 41 and Tatiara) were grown in sand culture with three levels of added B, 0, 0.1, 0.3 and 5 μM B. Experiment 2, also in sand culture, compared one genotype of barley (BRB 9) with one wheat (SW 41) at 0 and 10 μM B. Experiment 3 evaluated seven

genotypes of two row (BRB 9604, BRB 9, BCMU 96-9, and Stirling) and six row (BRB 2, FNBL 8309 and LARTC 9408) barley and five genotypes of wheat (Fang 60, Flycatcher, SW 41, Bonza and Tatiara) in sand culture with two levels of added B, 0 and 10 μM. All of the sand culture experiments were arranged in three replicates. The same genotypes were also grown in the field as subplots in main plots of three B levels: BL (soil limed at 2 t ha^{-1} to accentuate B deficiency), B0 (nil) and B10 (10 kg borax ha^{-1}), arranged in four replicated blocks.

In the sand culture, plants were grown in earthenware pots (30 cm in diameter, 30 cm deep) containing washed river quartz sand. Each pot was watered twice daily with 1 liter of nutrient solution containing varying levels of added B as described above, and other nutrients including 1000 μM $CaCl_2$, 500 μM KH_2PO_4, 250 μM $MgSO_4$, 250 μM K_2SO_4, 10 μM Fe EDTA, 1μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.1 μM Na_2MoO_4 (Broughton and Dilworth, 1971) and 5 mM KNO_3. This nutrient solution has been successfully used to screen barley (Jamjod and Rerkasem, 1999) and wheat germplasm (Rerkasem and Jamjod, 1997). The field experiment was on a Tropaqualf sandy loam soil of San Sai series with 0.15 mg hot water soluble (HWS) B kg^{-1}. The subplot containing each genotype consisted of 4 x 1 m rows, with 20 cm between rows. For experiments 1 and 2, there was one complete set of pots for each of two harvests. The first harvest, at boot stage (GS 45, Zadok et al., 1974), was for determination of spike and flag leaf B. Plant samples were dried, dry ashed and B concentration determined by the azomethine-H method (Lohse, 1982). Grain set and the number of spikelets $spike^{-1}$ were determined, at maturity in all experiments, on all plants and tillers in each pot in sand culture, from 20 randomly selected spikes from the 0.6 m middle section of two central rows in the field experiment. Grain set in wheat was assessed by the grain set index (GSI), percentage grain set, in the first two florets of 10 central spikelets (Rerkasem and Loneragan, 1994). The GSI in barley was assessed in the two side florets of the central spikelets for six-row barley, and the single fertile median florets in two-row barley (Jamjod and Rerkasem, 1999). Other effects of B that were recorded included number of tillers $plant^{-1}$, spikes $plant^{-1}$, spikelets $spike^{-1}$, days to floral initiation and spike emergence, shoot dry weight at boot stage and grain yield.

Results and discussion

There were both similarities and major differences in the response to low B of barley and wheat. Although B deficiency was moderate in the field and extreme in the pot trials, for the most part, trends were similar across experiments. In the sand culture without added B, grain set measured with GSI ranged from 0 to >90% in wheat and from 0 to 67% in barley genotypes (Tables 1, 2). The two most tolerant genotypes were wheat, Fang 60 and Flycatcher (Table 2). They already had GSI that exceeded 90% even without added B, while increasing B increased grain set in the other wheat and all barley genotypes. Most sensitive to B deficiency were Bonza, Tatiara (wheat) and Stirling (barley), which consistently had the lowest grain set in the lower B levels in pots and in the field (Tables 1 and 2). None of the barley genotypes was as tolerant as Fang 60, the most tolerant wheat genotype. In addition to its adverse effect on grain set, B deficiency depressed the number of spikelets spike^{-1} in barley by 21 to 75 % but not in wheat (Tables 3, 4, 5). Barley spikes that were B deficient exhibited the "rat-tail" symptom (Figure 1e) in which terminal spikelets had degenerated into wisps of white papery tissues similar to copper deficiency (Snowball and Robson, 1983). The lack of response to low B of the spikelet number in wheat is consistent with previous reports where a large number of genotypes have been evaluated under a wide range of conditions (e.g. Subedi et al. 1997; Anantawiroon et al. 1997).

In many of the barley genotypes and one of the wheat (Tatiara), tillering was increased at the level of B that depressed grain set (Tables 4, 5). This stimulating effect of low B was observed even when grain set was completely suppressed. That reproductive growth can be limited by B deficiency, at a level that is not limiting to vegetative growth, has been previously reported for both barley (Ambak and Tadano, 1991; Jamjod and Rerkasem, 1999) and wheat (Rerkasem et al., 1997). The increased tillering in low B was reflected in a significant increase in plant dry weight. However, these extra tillers were largely barren. The number of spikes plant^{-1} was actually depressed by B deficiency in some of the barley genotypes, by up to 90%. The extra tillers developed into extra spikes only occasionally, in BRB 9 barley in experiment 2 and Tatiara wheat in experiment 3. Boron deficiency delayed spike emergence in some barley but in none of the wheat genotypes (Tables 4, 5). Boron deficiency delaying spike emergence in barley has been previously reported (Phasook, 2000). The author also showed that there was no effect of B deficiency on the rate of barley development to the double ridge stage of floral initiation. Apparently unrelated to the

induction of the reproductive primordia, the delay in spike emergence in barley is likely to be associated with arrested subsequent ontogeny of the primordia, leading to abnormal morphology (Figure 1b-d). The relationship between various primary and secondary reproductive and vegetative responses to low B in barley may not be direct and straightforward. Responses to B in terms of grain set did not correlate with relative responses to B in the number of tillers plant^{-1}, spikes plant^{-1} and spikelets plant^{-1} ($p < 0.05$)

Boron concentration in the flag leaf and spike of wheat and barley at boot stage (Tables 6, 7 and 8) correlated well with the effect of B on their GSI ($r = 0.8 - 0.9$, $p < 0.01$). However, the grain set response to B could be predicted by tissue B only in some genotypes. The more severe effect of low B on grain set in Tatiara was clearly associated with lower B concentrations in its flag leaf and spike. The difference was less clear-cut between Fang 60 and SW 41, and among the barley genotypes covering a relatively narrow range of sensitivity to B deficiency. Previous reports also found SW 41 to be indistinguishable from Sonora 64, another B efficient wheat genotype (Rerkasem and Loneragan, 1994) as well as from Fang 60 (Subedi et al., 1999) by their tissue B concentration. Boron deficiency depresses grain set in wheat by adversely affecting pollen development and stigma function (Rerkasem et al., 1993), and the same possibly applies in barley. The grain set response to B in wheat correlated more closely with B concentration in the anther and carpel than in the secondary reproductive parts of the spike such as lemmas and palea (Rerkasem et al., 1997). Thus anthers and carpel B may better predict grain set response in different genotypes than in leaf and ear.

Wheat and barley had the same inflorescence type and are similar in the enclosure of developing spikes within leaf sheaths. The differential effects of B on spikelet number in wheat and barley were not reflected in their tissue B concentration. The species had about the same range of B concentrations in the spike and flag leaf (Tables 6, 7, 8) at the booting stage, while B deficiency depressed spikelet and spike number only in barley and not in wheat. Among the barley genotypes, the effect of B on spikelet number in barley correlated weakly ($p < 0.1$) with spike ($r = 0.47$) and flag leaf B ($r = 0.37$). The response to low B in spikelet number may be related to differences in B requirement for spikelet development or the ability to supply B to the growing spike inside the leaf sheath. Wheat could be more effective in transporting B into developing spikes than barley. However, for wheat genotypes most sensitive to low B in term of grain set such as Bonza and Tatiara this would have to happen in spite of a very poor ability to supply B for development and function of

the primary reproductive organs, anthers and carpel. There is a possibility that differential demand or supply for B may be related to specific parts of the developing spike and spikelets, at critical stages of their development.

While mechanisms behind the difference between wheat and barley remain to be explained, agronomic implications of the findings are clear. In both wheat and barley, the role of B is critical for grain yield and consideration needs to be given to the time taken for fertilizer B to enter critical sites in the spike at critical times. Wheat grain yield is depressed by B deficiency primarily through grain set. The effect may be simply predicted by the GSI, which may be used to evaluate large numbers of wheat lines for B efficiency (Anantawiroon et al., 1997; Rerkasem and Jamjod, 2001). In barley, in contrast, the effect of B on grain set may be attenuated by other responses, depending on the genotype. The combined effect of low B on grain set and spikelet number on the number of grain spike^{-1} and grain yield in BRB 9 barley (Table 5) is likely to be found in genotypes that behave like BRB 9604 and BRB 2 (Table 4). For other genotypes the grain yield may be depressed even further through the adverse effect of low B on the number of spike plant^{-1}, as in BCMU 96-9, FNBL 8309 and LARTC 9408. However, a particularly severe effect of B deficiency (e.g. grain set in BRB 2 and Stirling and the number of spike plant^{-1} in BCMU 96-9), may override all other responses. The GSI is used to evaluate for B efficiency without the need for a B sufficiency control, the degree of severity in B deficiency is indicated by a set of B efficiency (Anantawiroon et al., 1997). The index could also be used in a first round identification of those barley genotypes that are most sensitive to B deficiency in grain set. However, genotypes in which grain set is tolerant to low B will need to be assessed for other responses such as spikelet number and spike number, which can be done only in comparison with a B sufficiency control. Considerable savings may be achieved by such two-step screening especially with germplasms with a high proportion of genotypes in which grain set is sensitive to B deficiency. The rat-tail symptom provides a useful visual marker for the more severe effect of B deficiency on spikelet number.

This paper has shown that the phenotypic response to B deficiency of barley is more complex than that in wheat. Different strategies for managing B nutrition are required, including different approaches to select for B efficient genotypes.

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References

Ambak K and Tadano T 1991 Effect of micronutrient application on the growth and occurrence of sterility in barley and rice in a Malaysian deep peat soil. Soil Sci. Plant Nutr. 37, 715-724.

Anantawiroon P, Subedi K D and Rerkasem B 1997 Screening wheat for boron efficiency. *In* Boron in Soils and Plants. Dds R W Bell and B Rerkasem. Kluwer Academic Publishers, The Netherlands. pp 101-104.

Broughton W J and Dilworth M J 1971 Control of leghaemoglobin synthesis is snake beans. Biochem. J. 125, 1075-1080.

da Silva A R and de Andrade J M V 1983 Influence of micronutrients on the male steriltiy, on upland wheat and on rice and soybean yields, in red-yellow latosol. Pesq. agropec. bras., Brasilia 18, 593-601. (In Portuguese with English Abstract)

Gupta U C 1993 Introduction in boron and its role in crop production. Ed U C Gupta. P1. CRC Press, Boca Raton, FfL, USA.

Jamjod S and Rerkasem B 1999 Genotypic variation in response of barley to boron deficiency. Plant Soil. 215, 65-72.

Li B H, Li W H, Kui M C, Chao W S, Jern H P, Li C R, Chu W J and Wang C L 1978 Studites on cause of sterility of wheat. Journal of Northeastern Agricultural College. 3, 1-19.

Lohse G 1982 Microanalytical azomethine-H method for boron determination in plant tissues. Commun. Soil Sci. Plant Anal. 13,127134.

Marten J M and Westermann D T 1991 Fertilizer applications for correcting micronutrient deficiencies. *In* Micronutrients in Agriculture. 2nd ed. Eds. J J Mordtvedt, F R Cox, L M Shuman and R M Welch. pp. 549-592. SSSA Book Series no. 4. SSSA, Madison, WI.

Phasook T 2000 Boron efficiency in two barley genotypes. M.Sc. (Agronomy) Thesis. Graduate School, Chiang Mai University, Thailand.

Rerkasem B and Jamjod S 1989 Correcting boron deficiency induced spike sterility I wheat and barley. Thai J. Soils and Fertilizers 11, 200-209 (in Thai with English summary)

Rerkasem B and Jamjod S 1997 Boron deficiency induced male sterility in wheat (*Triticum aestivum* L.) and implications for plant breeding. Euphytica 96, 257-262.

Rerkasem B and Jamjod S 2001 Overcoming wheat sterility problem with boron efficiency.

In Plant nutrition-Food security and sustainability of agro-ecosystems. Eds WJ Horst, M K Schenk, A Bürkert, N Claassen, H Flessa, W B Frommer, H Goldbach, H-W Olfs, V Römheld, B Sattelmacher, U Schmidhalter, Shubert, N v Wirén and L wittenmayer. Kluwer Academic Publishers, The Netherlands. pp 82-83.

Rerkasem B and Loneragan J F 1994 Boron deficiency in two wheat genotypes in a warm, subtropical region. Agron. J. 86, 887-890.

Rerkasem B, Netsangtip R, Lordkaew S and Cheng CH 1993 Grain set failure in boron deficiency wheat. Plant Soil. 155/156, 309-312.

Rerkasem B, Lordkaew S and Dell B 1997 Boron requirement for reproductive in wheat. Soil Sci. Plant Nutr. 43, 953-957.

Shorrocks V M 1997 The occurrence and correction of boron deficiency. Plant Soil. 193, 121-148.

Snowball K and Robson A D 1983 Symptoms of Nutrient Deficiencies. Department of Soil Science and Plant Nutrition. Institute of Agriculture, University of Western Australia.

Subedi K D, C B Budhathoki and M Subedi 1997 Variation in sterility among wheat (*Triticum aestivum* L.) genotypes in response to boron deficiency in Nepal. Euphytica. 95, 21-26.

Subedi K D, P J Gregory and M J Gooding 1999 Boron accumulation and partitioning in wheat cultivars with contrasting tolerance to boron deficiency. Plant Soil. 214 (1-2), 141-152.

Zadok J C, Cheng T T and Konzak C F 1974 A decimal code for the growth stages of cereals. Weed Res. 14, 415-420.

Table 1. Response in Grain Set Index (%) in two-row barley and wheat to boron supply in sand culture (Experiment 1).

Species/genotype	Added B (μM)			
	0	0.1	0.3	5.0
Barley				
BRB 9	32.2 aD	57.5 bD	67.6 cC	76.7 dC
BCMU 96-9	23.4 aC	24.8 aC	28.4 aB	54.6 bB
CMBL 92029	12.5 aB	13.5 aB	22.0 bB	41.6 cA
Wheat				
Fang 60	94.8 aF	94.4 aF	98.7 aE	98.5 aD
SW 41	67.3 aE	74.6 bE	77.4 bcD	81.9 cC
Tatiara	0.2 aA	1.9 aA	13.0 bA	61.2 cB
Effects	Boron (B)	Genotype (G)	B x G	
F-test	**	**	**	

Differences (by LSD $p < 0.05$) in the same row are indicated by different lowercase letters and in the same column by different uppercase letters. ** significant at $p < 0.01$

Table 2. Response in Grain Set Index (%) in barley and wheat grown in sand culture with and without added boron; and in the field with limed soil (BL), no added boron (B0) and boron treated soil (B+) (Experiment 3).

Species/	Sand culture (μM B)		Field soil treatment		
genotype	0	10	BL	B0	B+
Two row barley					
BRB 9604	66.9 aC	94.5 bC	86.7 aDE	93.4 aF	93.6 aBC
BRB 9	57.3 aC	94.4 bC	61.7 aC	69.1 aDE	83.0 bAB
BCMU 96-9	25.9 aB	76.1 bA	74.5 aCD	92.4 bF	87.5 abBC
Stirling	0 aA	92.2 bBC	37.7 aB	52.2 bC	89.6 cBC
Six row barley					
FNBL 8309	56.9 aC	79.0 bAB	80.7 aDE	83.4 aF	90.5 aBC
LARTC 9408	29.8 aB	86.7 bABC	69.0 aCD	79.4 abEF	91.1 bBC
BRB 2	8.7 aA	83.5 bBC	63.9 aCD	65.4 aCD	73.6 aA
Wheat					
Fang 60	93.3 aD	100 aC	98.2 aE	97.8 aF	98.6 aC
Flycatcher	92.7 aD	95.0 aC	72.0 aCD	92.5 bF	95.2 abBC
SW 41	60.8 aC	91.7 bBC	72.6 aCD	93.4 bF	95.4 bBC
Bonza	1.3 aA	97.4 bC	26.0 aB	31.3 aB	87.8 bBC
Tatiara	0 aA	75.3 bA	1.8 aA	13.6 aA	71.5 bA
	Effects	Boron (B)	Genotype (G)	B x G	
	F-test	**	**	**	

Differences (by LSD $p < 0.05$) in the same row are indicated by different lowercase letters and in the same column by different uppercase letters. ** significant at $p < 0.01$

Table 3. Response in the number of spikelets spike^{-1} in two-row barley and wheat to boron supply in sand culture (Experiment 1).

Species/genotype	Added B (μM)			
	0	0.1	0.3	5.0
Barley				
BRB 9	8.9 aA	9.9 aA	10.0 aA	9.2 aA
BCMU 96-9	20.2 aE	25.6 cE	24.0 bE	24.2 bE
CMBL 92029	13.0 aB	15.0 bCD	15.9 bD	17.6 cD
Wheat				
Fang 60	14.3 aC	14.2 aBC	14.6 aBC	14.4 aBC
SW 41	13.0 aB	13.7 aB	13.9 aB	14.0 aB
Tatiara	15.7 aD	15.8 aD	15.4 aCD	15.6 aC
Effects	Boron (B)	Genotype (G)	G x B	
F-test	**	**	**	

Differences (by LSD $p < 0.05$) in the same row are indicated by different lowercase letters and in the same column by different uppercase letters. ** significant at $p < 0.01$

Table 4. Relative boron responses (B0 as % of B10) for tillers, spikes, spikelets, Grain Set Index (GSI) and time of spike emergence in barley and wheat grown in sand culture (Experiment 3).

Species/ genotype	Tillers plant^{-1}	Spikes plant^{-1}	Spikelets spike^{-1}	GSI (%)	Days to spike emergence
Two-row barley					
BRB 9604	103.0 ab	84.7 b	45.1 b	70.8 c	112.0 b
BRB 9	204.0 cd	94.5 b	27.9 a	60.7 c	131.9 d
CMU 96-9	260.7 d	10.8 a	24.9 a	34.0 b	125.4 cd
Stirling	110.9 ab	11.6 a	35.2 ab	0.0 a	118.6 bc
Six-row barley					
FNBL 8309	119.7 ab	55.6 ab	76.9 c	72.0 c	125.4 cd
LARTC 9408	237.7 d	55.6 ab	45.6 b	34.4 b	95.0 a
BRB 2	186.7 cd	74.2 b	36.8 ab	10.4 a	118.8 bc
Wheat					
Fang 60	103.0 ab	98.0 b	95.0 d	93.3 d	100.6 a
Flycatcher	82.4 a	67.6 b	87.8 cd	97.6 d	100.0 a
SW 41	109.4 ab	98.0 b	88.1 cd	66.3 c	101.7 a
Bonza	95.8 ab	79.7 b	90.6 cd	1.3 a	99.0 a
Tatiara	151.9 bc	154.9 c	97.8 d	0.0 a	102.3 a
Analysis of variance					
Effects	F-test				
Boron (B)	**	**	**	**	**
Genotype (G)	**	**	**	**	**
B x G	**	**	**	**	**

Differences (by LSD $p < 0.05$) in the same column are indicated by different letters.

** Significant at $p < 0.01$

Table 5. Response of vegetative and reproductive growth in wheat (SW 41) and barley (BRB 9) to boron supply in sand culture (Experiment 2).

Plant response	Added B (μM B)				F-test
	0	10	0	10	Genotype x Boron
	Barley (BRB 9)		**Wheat (SW 41)**		
Shoot dry weight (g pot^{-1})	40.4 b	29.6 a	65.4 c	67.2 c	*
Tillers plant^{-1}	32.4 b	15.5 a	12.0 a	9.6 a	**
Day of spike emergence	49.1 b	45.8 a	56.9 c	57.3 c	*
Spikes plant^{-1}	18.8 c	13.1 b	7.2 a	6.4 a	*
Spikelets spike^{-1}	8.9 a	16.9 b	17.6 bc	19.0 c	*
Grains spike^{-1}	2.7 b	16.7 c	0.1 a	44.5 d	*
Grain yield (g pot^{-1})	5.2 a	67.2 b	1.3 a	82.6 c	*
Grain Set Index (%)	24.6 b	98.0 c	0.3 a	97.7 c	*

F-test of genotype by boron interaction, significant level: * $p < 0.05$, ** $p < 0.01$. Differences between B levels in each species are indicated by different lowercase letters (by LSD $p < 0.05$).

Table 6. Boron concentration (mg B kg^{-1}) in the flag leaf at booting of barley and wheat grown at four levels of boron in sand culture (Experiment 1).

Species/genotype	Added B (μM)			
	0	0.1	0.3	5.0
Barley				
BRB 9	13.2 aD	13.4 abD	14.6 bcE	15.6 cBC
BCMU 96-9	4.6 aA	5.4 abA	6.5 bB	8.7 cA
CMBL 92029	6.9 aB	7.0 aB	7.7 aB	7.7 aA
Wheat				
Fang 60	9.3 aC	10.9 bC	12.1 cD	16.2 dC
SW 41	7.5 aB	7.7 aB	9.3 bC	14.3 cB
Tatiara	3.8 aA	4.1 aA	4.5 aA	8.1 bA
Effects	Boron (B)	Genotype (G)	B x G	
F-test	**	**	**	

Differences (by LSD $p < 0.05$) in the same row are indicated by different lowercase letters and in the same column by different uppercase letters. ** significant at $p < 0.01$

Table 7. Boron concentration (mg B kg^{-1}) in the spike at booting of barley and wheat genotypes grown in sand culture at four levels of boron (Experiment 1).

Species/genotype	Added B (μM)			
	0	0.1	0.3	5.0
Barley				
BRB 9	6.6 aB	9.5 bC	12.3 cD	12.8 cE
BCMU 96-9	9.3 bD	8.5 abBC	8.2 aB	9.3 bC
CMBL 92029	4.1 aA	5.0 abA	5.7 bcA	6.1 cA
Wheat				
Fang 60	8.5 aCD	8.8 aBC	9.9 bC	10.4 bD
SW 41	7.8 aC	8.2 aB	10.9 bC	10.9 bD
Tatiara	4.9 aA	5.4 aA	6.4 bcA	7.3 bB
Effects	Boron (B)	Genotype (G)	B x G	
F-test	**	NS	**	

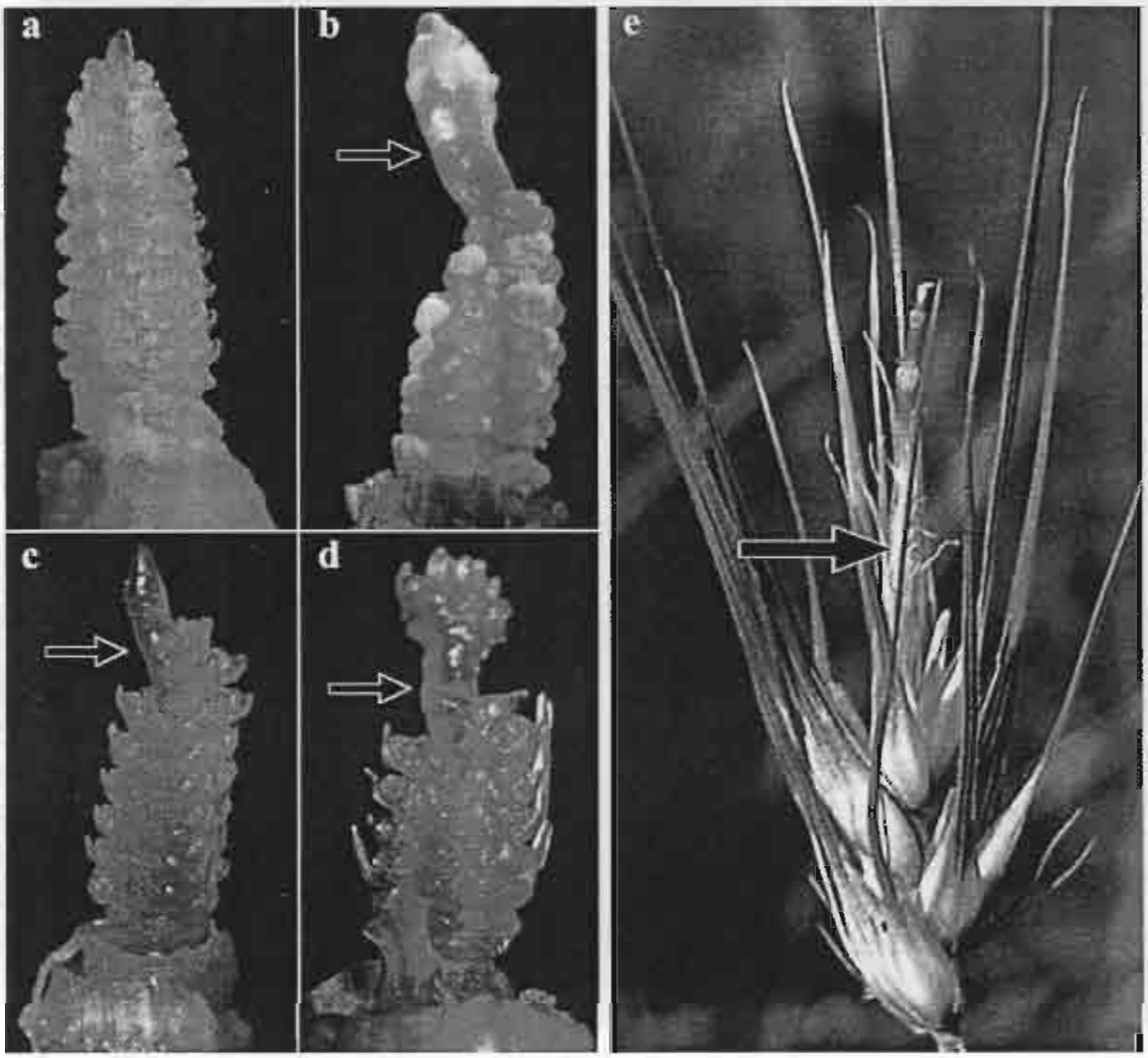
Differences (by LSD $p < 0.05$) in the same row are indicated by different lowercase letters and in the same column by different uppercase letters. ** significant at $p < 0.01$

Table 8. Boron concentration in the whole spike and flag leaf of the main stem at booting in wheat and barley grown with (B10) or without (B0) boron in sand culture (Experiment 2).

Species/genotype	Spike B (mg B kg^{-1})		Flag leaf B (mg B kg^{-1})	
	B0	B10	B0	B10
Barley, BRB 9	3.1	4.2	4.3	11.2
Wheat, SW 41	5.2	5.8	3.4	11.5
Effects	F-test			
Boron (B)	**		**	
Genotype (G)	**		NS	
B x G	NS		NS	

** significant at $p < 0.01$, NS = not significant, $p < 0.05$

Figure 1. The primordia of barley (cv. BRB 9) at lemma - awn primordium stage (Zadok et al., 1974) showing complete spikelet terminal in sufficient B supply (a) compared with abnormal primordia (b-d) in B deficiency (arrows showing arrested development of terminal spikelets). B oron deficient barley spike exhibiting the rat-tail symptom, in which terminal spikelets had degenerated in to wisps of white papery tissue (e).


a
b
c
d
e

Genotypic Variation in Boron Long Distance Transport into the Reproductive Organ of Wheat

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ABSTRACT

Genotypic variation in Boron (B) efficiency in wheat (*Triticum aestivum* L.) is expressed as large differences in grain set and pollen sterility under low soil B, but the mechanisms responsible for such differences are unknown. This paper aims to determine the mechanism for cultivar difference in B efficiency by comparing B-efficient (Fang 60) and B-inefficient (SW41) cultivars. Plants were grown with adequate ^{11}B (10 μM), until the premeiotic interphase stage in anther development, then transferred into ^{10}B at 0.1 or 10 μM. After five days, ending at the young microspore stage, all plants were transferred into adequate ^{11}B. Plants were sequentially harvested at 0, 1 and 5 days after transferring into ^{10}B, and at anthesis, anthers were collected and fresh pollen examined for viability. After 5 days in 0.1 μM B, during the critical stage of pollen development, pollen viability in SW41 was depressed by 47 %, but Fang 60 was not affected. Fang 60 maintained total B concentration in the ear at 6.8 mg kg^{-1} DW, in contrast to 3.8 mg kg^{-1} DW in SW 41. Insensitivity to B deficiency of Fang 60 was associated with its greater capacity to supply adequate ^{10}B to the ear directly from the root, but not from the previously taken up ^{11}B when external supply was restricted.

INTRODUCTION

There are numerous reports of genetic variation in B efficiency among wheat genotypes (Li et al, 1978; Tandon and Naqvi, 1992; Subedi et al, 1993; Rerkasem et al, 1993; Rerkasem and Jamjod, 1997) and other cereals such as barley (Rerkasem and Jamjod, 1989). Differences in B efficiency in wheat are generally associated with differences in the degree of pollen sterility or the ability to set grain in low B soil (Subedi et al, 1993; Rerkasem and Loneragan, 1994; Rerkasem and Jamjod, 1997). Pollen development in susceptible cultivars is impaired by B limitation resulting in pollen grains that are small and misshapen and do not accumulate starch (Rerkasem et al, 1989; Subedi et al, 1997; Anantawiroon et al, 1997). It has been suggested that the critical phase of anther development is surrounding pollen meiosis (Rawson, 1996), especially the period from premeiotic interphase through meiosis to late tetrad development (Huang et al, 2000; Dell and Huang, 2002). However, the mechanisms underlying cultivar differences in B efficiency in wheat remain unknown, but clearly they are associated with B supply to the ear during critical stages of microsporogenesis.

In broccoli (*Brassica oleracea* var. *italica* Plenck ~~cv. Commander~~), greater remobilization of B into the inflorescence was responsible for the avoidance of anatomical disorders in the inflorescence due to B deficiency in some cultivars (Shelp et al, 1992). Similarly, Stangoulis (2001) recently reported that greater B efficiency in ~~the~~ oilseed rape (*Brassica napus* L.) cv.~~ultivar~~ Huashuang) was associated with retranslocation of ^{10}B from old leaves whereas B-inefficient cultivars lacked this capacity. However, both of these studies were carried out with *Brassica* species which exhibit a degree of phloem mobility of B in contrast to many other species

which do not (Brown and Shelp, 1997). Huang et al (2001) showed that some ^{10}B, incorporated into vegetative plant parts following absorption from the external solution, was capable of retranslocation into the ear of wheat. However, the authors concluded that the amount of ^{10}B retranslocated was insignificant relative to the B demand of the ear. Since only a B-inefficient cultivar, Wilgoyne, was used in this study (Rerkasem unpublished), the possibility remains that the capacity to retranslocate B into the ear may differ across cultivars, and that this difference may allow the~~the~~ mechanism of B efficiency in wheat to be identified. The avoidance of male infertility in B-efficient cultivars may involve the ability to supply B adequately into the nontranspiring anthers or the developing ear. This experiment set out to examine B responses of efficient and inefficient wheat genotypes during the most critical stage in pollen formation and to compare the routes of B mobilization into the ear.

RESULTS AND DISCUSSION

Huang et al. (2000) suggested that the period of pollen development from the premeiotic interphase to the late tetrad was the main phase sensitive to B deficiency. They also postulated that B deficiency during meiosis might impair the formation of pollen cell walls and cell expansion, leading to a reduction in pollen viability in wheat. Pollen viability in B-inefficient SW 41 was nearly halved when the external B supply was interrupted during this critical stage (Figure 1). The same treatment had no effect on pollen viability of Fang 60. This is consistent with known differences in the sensitivity to B deficiency of the genotypes as represented by their male fertility and grain set in low B (Anantawiroon et al, 1997; Subedi et al, 1999).

The differential response to short-term B deficiency in SW 41 and Fang 60 was associated with significantly lower concentration of B in the ear of SW 41 (Table 2).

Thus, the B-efficient and inefficient genotypes in this study are distinguishable by the B concentration in their ears. This difference was not detected by others who have reported genotypic variation in responses to low B for male fertility and grain set (Rerkasem and Lordkaew, 1996) including a comparison between SW 41 and Fang 60 (Subedi et al., 1999). In these previous studies, determination of B in the ear was not done until booting. By this time, the B deficient wheat ear may have continued to accumulate B in the palea, lemma and other secondary sexual parts of the ear although damage to the pollen had already occurred. The present results suggest that it may be feasible to distinguish between the B-efficient and inefficient genotypes by early B analysis of the ear. However, timing of the analysis will be critical. Sampling for the ear has to be done immediately upon the completion of the critical meiotic stage of pollen development.

Boron concentrations in upper canopy leaves of Fang 60 were also greater than those in SW 41 after 5 days of low B supply (Table 2). This suggests that cultivar difference in sensitivity to B deficiency may be associated with the pattern of B distribution within the plant when B was limited. The greater ability of Fang 60 to distribute B into the developing ear may contribute to its tolerance to low external B. The reproductive ~~plant~~ parts of wheat, the anthers and carpels, were found to require B at greater concentration for their normal development than leaves (Rerkasem et al, 1997; Huang et al, 2000). The greater amount of B distributed into the apical regions of the plant may increase the opportunity for reproductive success.

A suggestion has been made by some that previously accumulated B might be effectively remobilized to supply the ear of B-efficient genotypes, such Fang 60, when external supply becomes limited (Rawson, 1996; Subedi et al, 1999). We found this to be unsupportable by three sets of results, which instead suggested that the

primary mechanism for B efficiency in Fang 60 to be associated with its long distance transport directly from the root. Firstly, accumulation of ^{11}B in the ear of both SW 41 and Fang 60 seemed to have stopped after ^{11}B in the nutrient solution was replaced by ^{10}B. The content of ^{11}B in the ear of either SW 41 or Fang 60 did not increase after the ^{10}B treatments were imposed (Table 3). Data on the content of ^{10}B in the ear provided the second piece of supporting evidence that the mechanism for B efficiency is related to direct supply from the root and not recycled B from some plant parts. In the low B treatment, the ^{10}B content in the ear of B-efficient Fang 60 was three times that of the B-inefficient SW 41 (Table 4). Examination of the ^{10}B:^{11}B ratio provides the third piece of evidence. The ratio of ^{10}B:^{11}B increased significantly with time during the 5 days that the B^{11} supply was replaced by ^{10}B (Table 5) but at different rates in the two genotypes. The increase in the ratio of B^{10}:B^{11} with time after the transfer from ^{11}B to ^{10}B was much stronger in Fang 60 than SW 41, especially with lower external B. During the 5 days in which external B was lowered to 0.1 μM, the ^{10}B:^{11}B ratio in Fang 60 was increased from 0.17 to 1.15 and less so in SW 41, from 0.11 to 0.38.

In conclusion, pollen viability of the B-efficient Fang 60 was not affected by withholding B during the critical stage of microsporogenesis while pollen viability in inefficient SW 41 was nearly halved. The genotypic difference in B efficiency is related to the greater ability of Fang 60 to accumulate and distribute B into the developing ear than SW41. By using ^{11}B and ^{10}B, we were able to demonstrate that net B movement into the ear, when external supply was restricted, did not come from the ^{11}B previously taken up by the plant. The greater amount of ^{10}B accumulated by Fang 60 in low B further confirmed that the primary mechanism for B efficiency in

Fang 60 is its greater capacity to supply adequate B to the ear directly from the root, enabling Fang 60 to avoid pollen sterility caused by B deficiency.

MATERIALS AND METHODS

Plant material and culture

This experiment selected two spring wheat cultivars from the efficient (cv. Fang 60) and inefficient (cv. SW 41) classes of B efficiency determined by Rerkasem and Jamjod (1997). Seeds were imbibed in aerated 2 mM $CaSO_4$ solution for 24 hours and germinated on paper towels moistened with 2 mM $CaSO_4$ for 48 hours in the dark at 25 °C. Seeds of SW41 were germinated 1 day before seeds of Fang 60 in order to synchronize the stage of ear development at microsporogenesis. Seedlings were transferred into trays containing 8 L of 1/3 strength nutrient solution (Huang et al, 1996) with a concentration of 0.1 μM ^{11}B and 5 mM 2-[N-Morpholino]ethanesulfonic acid. The pH was adjusted daily to 6.0 ± 0.2 with 1 M KOH or 10 % H_2SO_4. Four days after germination, the plants were grown in a 5 L full-strength basal nutrient solution that contained 10 μM ^{11}B and aliquots of all nutrients were added to each pot during the experiment by the programmed nutrient addition as described in Huang et al. (1996). Tillers were restricted to a maximum of 4 tillers plant^{-1}, by removing extra tillers as they emerged from the 5th onward. The stage of growth was determined by dissection of spare plants at the 6, 7 and 8 leaf stages and the stage of pollen development was determined on extra B–adequate plants ~~with~~ using DAPI (4'-6-Diamidino-2-phenylindole 2HCl, Sigma Lot 104F-0542) ~~test~~ fluorescence (Vergne, Delvallee and Dumas,is this style of 3 authors OK? 1987). Ten μM ^{11}B was supplied continuously up to ~~the~~ late premeiotic interphase /early meiosis in the main stem (Bennett et al, 1973). The plants were then treated with either 0.1 or 10 μM of 99.43 % ^{10}B-enriched boric acid continuously up to the

late tetrad, after which the plants were returned to solutions with 10 μM ^{11}B up to anthesis (Table 1). ^{10}B was used as a tracer for B distribution to the ear during critical stage of microsporogenesis, while ^{11}B was used as a tracer for B remobilization to the ear.

The pH was adjusted to 6.0 -± 0.2 with 1 M KOH or 10 % H_2SO_4. The pots were randomly distributed in temperature-controlled water baths (18-22 °C) and repositioned daily within the baths and shifted between baths every 3 days. The growth conditions in the glasshouse were: mean air temperature 27.5 °C (range:20-35); mean PAR (photosynthetic active radiation) 1165 μmol m^{-2} s^{-1} (range: 960-1900) . Nutrient solutions wereas aerated with filtered air and the dry weight increments of extra plants were used to calculate the amount of nutrients required to maintain nutrient supply by programmed nutrient addition (Asher and Blamey, 1987). Analytical grade chemicals were used to make up the nutrient solutions and water, purified by passing through a column packed with B-specific resin (IRA-743, Sigma Chemical Co.), was used for making up all nutrient solutions. Between the treatments, the roots were washed with DI water, then rinsed three times in 5 mM $CaSO_4$ solution, in order to remove unbound B from root free space.

Sampling and plant analysis

The first harvest was taken before the ^{10}B treatment at the beginning of the critical stage of pollen development in the main stem (Huang et al, 2000) (Table 1). The second and third harvests were taken 1 day and 5 days (late tetrad stage) after the ^{10}B treatment began. At each harvest, three pots (replicates) of plants (two plants each) were taken from each cultivar. The plants were subdivided into flag leaf, ear, penultimate leaf, stem between flag leaf and penultimate leaf of the main stem. All samples were analysed for ^{11}B and ^{10}B- content. The fourth harvest was taken at

anthesis. From three replicate pots (~~replicates~~two plants per pots) ~~of plant (two plants each) from~~ of each cultivar, pollen was taken from the central 4 spikelets of the main shoot ear. Pollen at anthesis was tested for viability with the fluorochromatic (FCR) method (Heslop-Harrison and Shivanna, 1984). The plant samples were oven-dried (70 °C). Dry samples were ground in a stainless steel mill and dry-ashed in 1 % nitric acid as described in Huang et al, (2001) and B concentration was determined using~~by~~ an inductively coupled plasma automatic emission spectrometer (ICP-AES) (Zarcinas et al, 1987).

Statistical analysis

Data were analyzed statistically by analysis of variance. Significantly different means were separated at the 0.05 probability level.

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LITERATURE CITED

Anantawiroon P, Subedi KD and Rerkasem B (1997) Screening wheat for boron deficiency . In Boron in Soil and Plant. Eds RW Bell and B Rerkasem, Kluwer Academic Publishers Dordrecht, New York, 101-104; ?

Asher CJ and Blamey FPC (1987) Experimental control of plant nutrient status using programmed nutrient addition. J. Plant Nutr 10: 1371-1380? Check others

Bennet MD, Rao MK, Smith JB and Bayliss MW (1973) Cell development in the anther, the ovule and the young seed of *Triticum aestivum* L. var. Chinese Spring. Phil. Trans. Royal Soc. London Ser. B 266: 39-81.

Brown PH,?and Shelp BJ (1997) Boron mobility in plants. Plant and Soil 193: 85-101

Cheng C and Rerkasem B (1993) Effect of boron in the pollen viability of wheat. Plant Soil 155/156: 313-315

Dell B, Huang L and Bell RW (2002) Boron in Plant Reproduction. In HE Goldbach, B Rerkasem, MA Wimmer, PH Brown, M Thellier and RW Bell eds, Boron in Plant and Animal Nutrition, Kluwer Academic/Plenum, New York, 103-117

Heslop-Harrison J, Heslop-Harrison Y, Shivahna KR (1984) The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. Theor Appl Genet 67: 367-375.

Huang L, Plant J, Bell RW, Dell B and Deane K (1996) Effects of boron deficiency and low temperature on wheat sterility. In: Rawson HM and Subedi KD eds. Sterility in Wheat I Sub-Tropical Asia: Extent, Causes and Solutions. ACIAR Proceedings No. 72, ACIR, Camberra, 90-101

Huang L, Plant J, Dell B, and Bell WR (2000) Effects of boron deficiency on anther development and floret fertility in wheat (Triticum aestivum L. Wilgoyne). Ann. Bot. 85: 493-500

Huang L, Plant J, Dell B, and Bell WR (2001) Boron supply into wheat (*Triticum aestivum* L. cv. Wilgoyne) ear whilst still enclosed within leaf sheaths. J. Exp. Botany 52: 1731-1738

Li BH, Li WH, Kui MC, Chaows, Jern HP, Li CR, Chu WJ and Wang CL (1978) Studies on cause of sterility of wheat. J. Northeast Agric. College 3: 1-19 (In Chinese, with English translation)

Rawson HM and Hofstra G (1969) Translocation of remobilization of 14C assimilated at different stages by each leaf of the wheat plant. Aust. J. Biol. Sci 22: 321-331

Rawson HM.? (1996) The developmental stage during which boron limitation causes sterility in wheat genotypes and the recovery of ferility. Aust. J. Plant Physiole 23: 709-717

Rerkasem, B and Jamjod, S (1989) Correcting boron deficiency induced ear sterility in wheat and barley. Thai J. Soils Fert 11: 200-209 (in Thai with English summary)

Rerkasem B and Loneragan J F (1994) Boron deficiency in two wheat genotypes in a warm, subtropical region. Agron. J. 86: 887-890

Rerkasem B and Lordkaew S (1996) Tissue Boron. In Sterility in Wheat in Sub-Tropical Asia Extent, Causes and Solutions. Eds HM Rawson and KD Subedi, ACIAR Proc. No. 72, 36-38.

Rerkasem B, Lordkaew S, Dell B (1997) Boron requirement for reproductive development in wheat. In: T Ando, K Fujita, T Mae, H Matsumoto, S Mori, J Sekiya, eds. Plant nutrition for sustainable food production and environment. Dordrencht Kluwer Academic Publishers, New York, 69-73

Rerkasem B, Netsangtip R, Lordkaew S and Cheng C (1993) Grain set failure in boron deficient wheat. Plant Soil 155/156: 309-312

Rerkasem B and Jamjod S (1997) Genetypic variation in plant response to low boron and implications for plant breeding. Plant Soil. 193: 169-180

Bens refs are not in the correct order

Rerkasem B, Saunders DA and Dell B (1989) Grain set failure and boron deficiency in wheat in Thailand. J. Agric. (CMU) 5:1-10 Font?

Shelp BJ, Penner R and Zhu Z (1992) Broccoli (*Brassica oleracea* var. *italica*) cultivar response to boron deficiency. Can. J. Plant Sci. 72: 883-888

Stangoulis JCR, Brown PH, Bellaloui N, Reid RJ and Graham RD (2001) The

efficiency of boron utilisation in canola. Aust. J. Plant Physiol. 28:1109-1114

Subedi KD, Budhathoki CB, Subedi M and Tuladhar JK (1993) Survey and research report on wheat sterility problem (1992/93). LARC working Paper No. 93/49. Nepal, Lumle Agricultural Research Centre.

Subedi KD, Budhathoki CB, Subedi M and GC Yubak D (1997) Response of wheat genotypes to sowing date and boron fertilization aimed at controlling sterility in a rice-wheat rotation in Nepal. Plant Soil 188: 249-256

Subedi KD, Gregory PJ and Gooding MJ (1999) Boron accumulation and partitioning in wheat cultivars with contrasting tolerance to boron deficiency. Plant Soil 214: 141-152

Tandon JP and Naqvi SMA (1992) Wheat varietal screening for boron deficiency in india. In: CE Mann and B Rerkasem, eds. Boron Deficiency in wheat, Wheat Special Report No.11. Mexico DF, CIMMYT, 76-78

Vergne P, Delvallee I, Dmas C (1987) Rapid assessment of microspore and pollen development stage in wheat and maize using DAPI and membrane permeabilization. Stain Technology 62: 299-304

Zarcinas BA, Cartwright B, Spouncer LR (1987) Nitric acid digestion and multielement analysis of plant material by inductively coupled plasma spectrometry. Comm. Soil Sci. Plant Anal. 18: 131-146

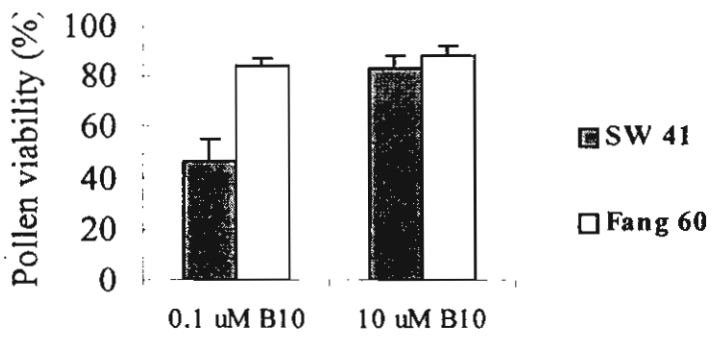

Figure 1. Effect of short term B deficiency during microsporogenesis (days 0-5: see Table 1) on pollen viability in two

Table 1. *Boron treatments, harvest times and stage of pollen development at each harvest of the main stem.*

Days*	Harvest	B treatment	Pollen development stage during treatment
		10 μM ^{11}B	Seedling to white anther (premeiotic stage)
0	1	10 μM ^{11}B	Late premeiotic stage/ early meiosis
1	2	0.1, 10 μM ^{10}B	After late premeiotic stage by 24 hr. still unclear
5	3	0.1, 10 μM ^{10}B	Late tetrad to young microspore stage
	4	10 μM ^{11}B	Anthesis

*= duration of low and adequate ^{10}B treatment.

Table II. *Total B concentration in plant parts (mg kg^{-1} DW) after 5 days of varied B supply treatment. Values are means of three replicates ± SE.*

Plant part	B treatment	Genotype	
	(μM ^{10}B)	Fang 60	SW 41
Ear	0.1	6.8 ± 0.7	3.8 ± 0.3
	10	12 ± 1.4	7.8 ± 0.5
Flag leaf	0.1	13 ± 2.3	9.3 ± 0.6
	10	21 ± 1.4	20 ± 0.5

Penultimate leaf	0.1	17 ± 0.4	13 ± 0.7
	10	19 ± 0.5	20 ± 0.8
Stem segment between flag	0.1	4.0 ± 0.4	4.7 ± 0.3
~~leaf~~ and penultimate	10	6.2 ± 0.9	5.9 ± 0.6
leaves~~f~~			

Table III. *The content of ^{11}B in the ear ($\mu g\ ear^{-1}$) at day 0 (D0), day 1 (D1) and day 5 (D5) of B treatment.*

B^{10} treatment	Fang 60			SW 41		
($\mu M\ B^{10}$)	D0	D1	D5	D0	D1	D5
0.1	0.117	0.098	0.108	0.130	0.100	0.104
10	0.117	0.079	0.070	0.130	0.086	0.140

$LSD_{(0.05)} = 0.038$

Table IV. *The content of ^{10}B in the ear ($\mu g\ ear^{-1}$) at day 1 (D1) and day 5 (D5) of B treatment.*

B treatment	Fang 60		SW 41	
($\mu M\ ^{10}B$)	D1	D5	D1	D5
0.1	0.016	0.122	0.011	0.039
10	0.039	0.170	0.043	0.193

$LSD_{(0.05)} = 0.028$

Table V. *The ratio of ^{10}B : ^{11}B in ear at day 1 (D1) and day 5 (D5) of B treatment. Values are means of three replicates.*

B treatment	Fang 60		SW 41	
(μM B^{10})	D1	D5	D1	D5
0.1	0.17	1.15	0.11	0.38
10	0.49	2.62	0.50	1.38

$LSD_{(0.05)} = 0.021$

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Fallow enrichment with pada (*Macaranga denticulata* (Bl.) Muell. Arg.) trees in rotational shifting cultivation in northern Thailand

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Key words: Improved fallow, *Macaranga denticulata* (Bl.) Muell. Arg.), Slash and burn, Upland rice,

Abstract

Shifting cultivators in Thailand widely attribute the maintainance of crop productivity to pada (*Macaranga denticulata* (Bl.) Muell. Arg.), rotation cycles having become much shorter than the customary 10–20 years. This paper examines the use of pada in a 7-year rotation on an acid soil with low available soil P (2–4 mg kg^{-1} by Bray II). Dense pada patches in 7-year-old fallow averaged 43 tons ha^{-1} of above ground biomass, 20% more than sparse patches. The biomass in dense pada contained disproportionately more P, K, Ca and Mg (34%, 92%, 80% and 107% more, respectively) than in sparse pada patches. Slashing and burning 7-year-old fallow with dense pada produced a subsequent rice yield that was three time that with sparse pada. Rice grown after dense pada had been slashed and burned after three years yielded less than one third of that after a full 7-year rotation. It is, as yet, unclear how rice yield in dense pada patches is enhanced in the full 7-year rotation. Nutrient concentrations in the mature rice were generally either the same or higher in the sparse than dense pada patches. In dense pada patches rice accumulated twice to four times as much nutrients as in sparse pada patches, and a much larger fraction of the nutrients was stored in the fallow. Uptake of nutrients in the sparse pada patches may have been limited by some factor that either governs availability of the nutrients released by burning or depressing rice growth and so its nutrient demand.

Introduction

Rotational shifting cultivation has been shown to be a productive and sustainable form of land use in mountainous areas where land is sufficiently plentiful to allow a fallow period of 10–20 years (Kunstadter 1978). Population pressure combined with an increasing demand for conservation (from watersheds, erosion control, carbon sequestration to biodiversity) by society at large, however, have made certain that this luxury of very long fallows is now no longer an option for most shifting cultivators in Southeast Asia (Rerkasem and Rerkasem 1995) and elsewhere. Considerable interest, therefore, has arisen in approaches that might maintain crop productivity with shorter fallow. Numerous efforts, especially in Africa, have gone into identification of trees and other plants as fallow–enriching species (e.g., Tarawali (1991) and Kwesiga and Coe (1994), Mafongoya and Nair (1997), Kaya and Nair (2001)). This paper reports on the use of a local tree called *pada* for fallow improvement that has helped to maintain upland rice yield at reasonable levels of 2 to 4 Mg ha^{-1} on a seven years rotation.

Pada (*Macaranga denticulata* (Bl.) Muell. Arg.) is well known for its fallow-enriching property amongst the various ethnic groups who make a living on rotational shifting cultivation in northern Thailand. It is known as *Teen Tao* amongst the *Khamu* and *H'tin* who populate the northeastern mountains, on the border with Laos. *Pada* is the name in *Skaw Karen* (Thailand's largest minority group, now concentrated along

the western border with Myanmar), while the *Pwo Karen* call it *Letha*. The *Lua* (who are believed to have been the dominant group in the region until about a thousand years ago) call it *Tong Coab*. The *Akha* (a group not known to practice rotational shifting cultivation in Thailand) call the tree *Loom Piah*. Amongst lowland *Thai* it is variously called *Tong Taeb, Tong Tao, Tao Maew, Por Khee Haed* or *Bai Hoo Chang*. Pada is a small evergreen tree of the Euphorbiaceae family, that can reach 19 m in height and up to 40 cm in diameter at breast height. *Macaranga* is a relatively large genus of pioneer species (Whitmore 1982). Some 80 species have been identified in Africa and 200 in the Eastern Tropics, although not all are pioneer species. *M. gigantea* and *M. kurzii* also occur in the study village of Huai Tee Cha, but according to farmers pada is the only species with a fallow-improving property. The presence of pada in the fallow is believed to be responsible for maintaining productivity of upland rice in the shorter rotation. This study set out to measure the effect of pada on nutrient accumulation and upland rice yield in farmers' fields in a village where rotational shifting cultivation is still the dominant cropping system.

Materials and methods

The study was conducted in the rotational shifting cultivation fields of the village of Huai Tee Cha (19°78′ N, 93°84′ E), Sob Moei District, Mae Hong Son Province, about 250 km southwest of Chiang Mai. The soil is reddish clay loam. Before commencing plant and soil sampling, we took 30 days to become acquainted with the system by interviewing farmers and extensive field walks. The communally managed shifting cultivation area was divided into fields that are cropped in different years, designated by the year in which rice was last grown (Figure 1). According to the villagers this rotation has taken place for about 200 years, ever since Tee Cha was settled from a neighbouring village. A preliminary study of vegetation composition of the fallow forests was carried out in February and March (the village's normal slashing and burning season) of 2000. Trees were recognized as pada or 'others'. From areas with high and low density pada (defined in collaboration with farmers and designated dense and sparse pada), density of pada and other species was determined in three replicates of 10 × 10 m quadrats. Dominant 'other' species were *Microcos peniculata, Lithocarpus sp., Phoebe lanceolata* and *Glochidion sphaerogynum* (Further information about vegetation composition as well as tree density, biomass and nutrient contents of fallow areas with different ages will be reported elsewhere).

Mature fallow before slashing and burning

As field 1994/2000 was to be slashed and burned it was sampled for biomass and nutrient contents. Dry weight (sub-samples dried to constant weight at 80 °C) of the above ground live biomass, divided into pada and others, and litter were determined. The samples, whole plants in case of pada and others, were analyzed for N, P, K, Ca and Mg. Before burning soil samples were taken from the same area at 0–30 and 30–60 cm for determination of pH (water, 1:1), organic matter content (Walkley-Black), available P (Bray II, Wanatabe and Olsen (1962)), K, Ca and Mg (1 N NH_4OAc pH7).

The upland rice crop

A detailed study of the upland rice was carried out on the crop belonging to one farmer, Nopporn. At 30 days from sowing, samples (whole tops) of ten rice plants each were taken from the sparse and dense pada area for determination of N, P, K, Ca and Mg. At the same time soil samples were taken for determination of fertility characteristics at 0–30 and 30–60 cm depth. At maturity, samples of the rice crop were taken in 2 × 5 m quadrats from the sparse and dense pada areas. In addition, 1 × 1 m samples of rice at maturity were also taken from sparse and dense pada areas from five other farmers in the same village. The rice samples were threshed in the field and separated into grain and straw. Grain yield was determined after 3 days of sun drying (to water content of about 12%), straw yield was measured after drying for 48 hours at 80 °C. Sub-samples from Nopporn's field were also evaluated for the yield components, i.e. number of hills m^{-2}, plants $hill^{-1}$, tillers $hill^{-1}$, panicles $plant^{-1}$ and 1,000 seed weight, and analyzed for N, P, K, Ca and Mg in the grain and straw.

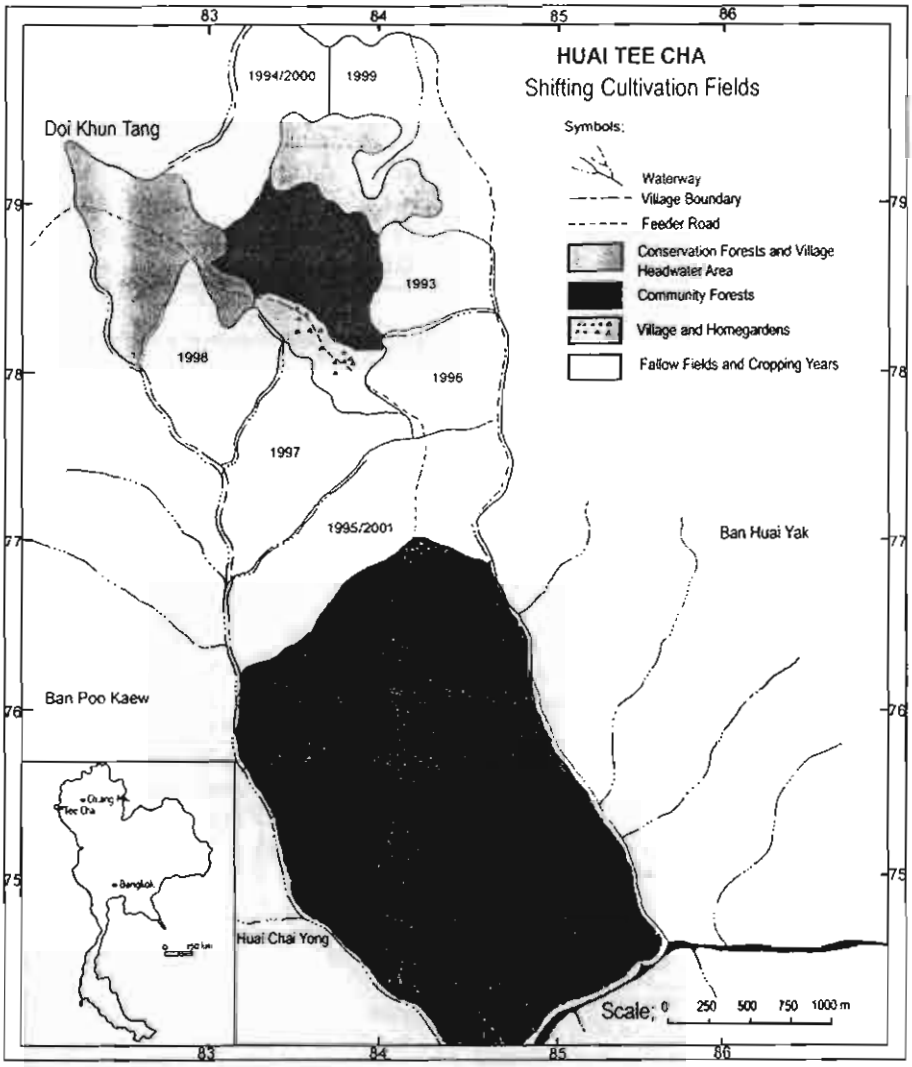

Figure 1. Rotational shifting cultivation fields of Huai Tee Cha village in northern Thailand indicating year of cropping.

Results and discussion

Fallow biomass and nutrient content

After seven years of regrowth (the rice cropping year is considered year 1 of regrowth, as fallow regeneration begins with upland rice emergence) above ground biomass averaged 43 Mg ha^{-1} in dense pada patches, about 20% more than that in sparse patches (Table 1). Pada contributed more than half of the biomass in the dense patches, and only 39% in sparse patches. There was also considerably more litter in dense pada patches. The above ground biomass in dense pada patches contained 536 kg N, 38 kg P, 253 kg K, 132 kg Ca and 46 kg Mg ha^{-1}. This was 10% more N, 34% more P, 92% more K, 80% more Ca and 107% more Mg than that in the sparse pada patches (Table 2). In the mature fallow of 8 years rotation in the village of Pa Pae (18°15′ N 98°3′ E) in the same vicinity where no pada was found (Sabhasri 1978) 143 kg N, 16 kg P and 176 kg K ha^{-1} were reported to have accumulated in the 63 t of above ground biomass (Zinke et al. 1978). The slight difference in total biomass N between sparse and dense pada patches in this study is accounted for by other species, which in sparse pada contributed as much N as pada in the dense pada patches. Pada was clearly crucial to the accumulation of extra P, K, Ca and Mg, though not so much for N. The higher amount of nutrients accumulated in dense pada came largely from

Table 1. Total aboveground biomass in the fallow vegetation after seven years of regeneration of a rotational shifting cultivation system in northern Thailand.

	Sparse pada	Dense pada	Significant difference by t-test
	Mg ha^{-1}		
Pada	9.4	22.2	$p < 0.01$
Other species	21.6	14.2	$p < 0.01$
Litter	4.7	6.4	$p < 0.01$
Total	35.7	42.8	$p < 0.05$

Table 2. Above ground nutrient contents in the fallow vegetation after seven years of regeneration of a rotational shifting cultivation system in northern Thailand.

Vegetation type	Nutrient element	Dense pada	Sparse pada	Significant difference
		Nutrient content (kg ha^{-1})		
Pada	N	289	131	$p < 0.01$
	P	21	6	$p < 0.01$
	K	97	32	$p < 0.01$
	Ca	72	21	$p < 0.01$
	Mg	10	6	$p < 0.05$
Other	N	191	297	$p < 0.01$
	P	12	18	$p < 0.05$
	K	118	85	$p < 0.01$
	Ca	28	36	NS
	Mg	23	6	$p < 0.01$
Litter	N	56	59	NS
	P	5	4	NS
	K	38	14	$p < 0.01$
	Ca	32	16	$p < 0.05$
	Mg	13	10	NS
Total	N	536	488	$p < 0.01$
	P	39	29	$p < 0.05$
	K	253	132	$p < 0.01$
	Ca	132	73	$p < 0.01$
	Mg	46	22	$p < 0.01$

pada in the case of P, pada and litter in the case of Ca, and from other species as well as pada and litter in the case of K and Mg. Pada roots were found to be associated with a diversity (genera and species) and abundance of arbuscular mycorrhizal fungi, both in the number of spores found in the rhizosphere and in root colonization (Youpensook et al. (in press)).

Soil fertility characteristics before slashing and burning and under rice

The study site was on a very acid soil, with pH 4.3 (1:1 water) before burning (Table 3). As others have previously shown (e.g., Nye and Greenland (1964) and Sanchez (1976)), burning clearly had a strong liming effect. Thirty days after rice sowing, pH of the surface soil measured 4.8, and slightly less deeper in the soil profile. Soil organic matter content declined slightly from around 4% before burning to 3.6% by the time rice was 30 days old. The first rainfalls of the season that germinated the rice may have contributed to this decline by stimulating microbial activity. Available soil P was generally very low at 2–4 mg kg^{-1}. There were significant but very small effects of pada density and burning on available P. The highest P value (Bray II) was 4.3 mg kg^{-1}, in dense pada patches after burning, which is very low indeed compared with about 12 mg kg^{-1} considered to be suffi-

Table 3. Fertility characteristics of the soil of the study area, in a rotational shifting cultivation system in northern Thailand, before burning and 30 days after rice sowing.

Pada density	Time	pH (1:1 water)	Organic matter (%)	P	K	Ca	Mg
				(mg kg^{-1})		(meq 100 g^{-1})	
Depth, 0–30 cm							
Sparse	Before[a]	4.30	3.96	2.23	133	0.53	0.27
	After[b]	4.79	3.60	3.34	181	0.64	0.48
Dense	Before	4.26	4.19	2.62	148	0.78	0.65
	After	4.83	3.84	3.70	198	0.75	0.67
Depth, 30–60 cm							
Sparse	Before	4.29	3.87	1.53	107	0.38	0.13
	After	4.41	3.43	3.00	156	0.47	0.49
Dense	Before	4.24	3.97	2.02	110	0.56	0.37
	After	4.58	3.73	4.29	178	0.59	0.50
Significant effects by analysis of variance							
Effects	Pada	NS	NS	*	NS	*	*
	Time	*	*	*	*	NS	NS
	Depth	NS	NS	NS	*	*	*
PxT, TxD, PxT, PxTxD		NS	NS	NS	NS	NS	NS

a) Before burning; b) After burning and 30 days from rice sowing

cient (Sanchez 1976). There was no significant difference in soil pH, organic matter and extractable K content between dense and sparse pada patches (Table 3). On the other hand, dense pada was associated with 47% more extractable Ca and twice to three times as much extractable Mg than in the sparse pada patches.

Pada establishment

As upland rice germinated after the first rains, pada emerged in thick pink carpets among the rice. Farmers do not treat pada as weeds, they are not routinely removed when the rice is weeded by hand. Thinning may be done where the density is considered too high and some attempts are sometimes made to transplant seedlings to low density areas. At rice maturity, about six months after sowing, and when pada had reached almost 1.5 m height, the number of pada averaged about 7 plants m^{-2} in dense patches, twice that in sparse patches (Table 4). In the fields cropped in different years, there was a trend of pada density decrease with fallow age (data not shown). In the 1995 field (slashed and burned in 2001), dense pada patches averaged 0.4 pada-tree m^{-2}, and 0.1 pada-tree m^{-2} in sparse patches. Since these are a fraction of the numbers of pada seedlings in the first year, the problem of low pada density must have been associated with survival of the seedlings rather than recruitment.

Table 4. Density of trees, pada and other species, after 6 months and 7 years in a rotational shifting cultivation system in northern Thailand.

Species	Pada density	Trees/m^2	
		6 months[a]	7 years[b]
Pada	Sparse	3.27 ± 0.32	0.10 ± 0.01
	Dense	6.60 ± 0.12	0.42 ± 0.02
Others†	Sparse	ND	0.12 ± 0.01
	Dense	ND	0.21 ± 0.01

a) At rice harvest ND = not determined, very few other plants at this time; b) Before slashing and burning for the next crop, the rice year is year 1 of regrowth; † Major species included *Microcos peniculata, Lithocarpus sp., Phoebe lanceolata* and *Glochidion sphaerogynum*. Values are mean ± standard errors of three replicates.

Upland rice nutrition and yield

Rice plants at one month in dense pada contained N and K at significantly higher concentrations than those in sparse pada patches (Table 5). No significant difference was found in the concentration of P, Ca and Mg. Based on published data (Reuter et al. 1997), the rice crop at 30 days was deficient in N and K in sparse pada area, and deficient in P in both sparse and dense pada area.

Table 5. Nutrient concentration in the upland rice (whole tops) at 30 days from sowing, in areas following pada at low and high densities in a rotational shifting cultivation system in northern Thailand.

Concentration (%)	Sparse pada	Dense pada
N	2.76[a]	4.06[b]
P	0.29	0.32
K	3.60[a]	4.99[b]
Ca	0.19	0.26
Mg	0.22	0.24

a) For same nutrient element, different letters designate significant difference by LSD ($p < 0.05$).

In 7-year rotation, rice grain yield in the dense pada area determined from detailed measurements in a field belonging to one farmer, averaged 2.57 Mg ha^{-1}, three times that in the sparse pada area (Table 6). Similar effects of pada density on the rice grain and straw yield were observed in 7-year rotation fields belonging to five other farmers (Table 7). The higher yield in dense pada patches was associated largely with a higher number of panicles and percentage of fertile tillers, and to a less extent higher plant density and number of tillers $hill^{-1}$ (Table 8). The effect was clearly cumulative, requiring more than three years of fallow.

How did high pada density increase the rice yield? Rice grown in dense pada patches that were slashed and burned after three years of fallow yielded only 0.74 Mg ha^{-1} of grain (Table 6). Clearly, the higher rice yield was not due to some pre-existing condition in the dense pada patches. As discussed above, the growth of rice at 30 days in sparse pada patches may have been limited by N and K deficiency, and more severely by P deficiency. By maturity, the concentration of N and Mg in the rice grain and P in both grain and straw were higher in the sparse than in the dense pada area (Table 9). The concentration of straw N and Mg and grain K were not different between rice in sparse and dense pada area. Straw K was the only case of nutrient concentration in rice in dense pada exceeding that in the sparse area. Critical K deficiency concentrations have been reported at 0.4% in the grain and 1% in the straw (Reuter et al. 1997). Compared with these, K deficiency is not indicated by the the grain K concentration at 0.45% and straw K at 1.91% in the rice in the sparse pada patches.

At maturity, the rice crop accumulated twice as much N, P, Ca and Mg and four times K in the dense as in the sparse pada patches (Table 9). However, the amount of N, P and K taken up by the rice crop accounted for a much smaller fraction of the nutrients accumulated above ground in 7-year old-fallow in the sparse (4%, 15% and 16%, for N, P and K, respectively) than in the dense pada patches (9%, 25% and 30%, respectively). The rice Ca and Mg uptake relative to the nutrient in fallow biomass were somewhat closer between sparse (13% for Ca; 5% for Mg) and dense (15% and 6%, respectively) pada patches. Although much of the above ground N in the fallow would have been lost with burning, most of the P, K, Ca and Mg could be assumed to remain in the ash. Much more nutrients would have been present in the root zone of rice than was taken up by the rice crop. The much smaller fraction of fallow accumulated P and K taken up by rice in sparse compared with dense pada patches suggested that the uptake of these nutrients was limited by some factor(s). Such a factor may limit uptake through demand by depressing growth and yield of the rice or through availability of the nutrients released by burning. Further explanation is not yet possible from data obtained so far.

This paper has shown that upland rice in a 7-year rotation yielded three times as much grain with pada at 0.42 trees m^{-2} as with 0.10 trees m^{-2} in the fallow, and that the effect was not due to some pre-existing condition in the high pada patches. The factor most immediately relevant to the farmers of Huai Tee Cha, and others who similarly depend on rotational shifting cultivation for their living in the mountainous region of mainland Southeast Asia, is if and how this effect of dense pada may be transferred to areas with sparse or no pada. The answer may be dependent on identification of (a) factors that are limiting to the survival of pada seedlings, and (b) how pada affects rice yield. Both of these are currently under investigation.

Table 6. The yield of upland rice in seven and four years rotation, in areas following pada at low and high densities during the fallow period, in a rotational shifting cultivation system in northern Thailand.

Pada density	Dense	Sparse	Dense	Rotation effect
Rotation	7 years	7 years	4 years	
	Yield (Mg ha^{-1})			
Grain	2.57[a]	0.83[b]	0.74[b]	$p < 0.01$
Straw	2.35[a]	0.97[b]	0.72[c]	$p < 0.01$
Total	4.92[a]	1.79[b]	1.47[c]	$p < 0.01$
Harvest Index	52.3	46.1	50.7	

a) Numbers in same row followed by different letters are significantly different by LSD ($p < 0.05$).

Table 7. Range and variation of rice yield in seven year rotation, in areas following pada at low and high densities during the fallow period, in a system of rotational shifting cultivation in northern Thailand.

Yield	Grain		Straw	
Pada density	Dense	Sparse	Dense	Sparse
	Mg ha^{-1}			
Maximum	4.53	1.56	3.80	1.75
Minimum	2.48	0.71	2.21	0.86
Mean (of 8 fields, 6 farms)	3.04	1.15	2.74	1.19
Standard deviation	0.71	0.33	0.59	0.30

Table 8. Yield components of upland rice in a rotational shifting cultivation system in northern Thailand with dense and sparse pada densities during the fallow period.

Yield component	Pada density		Significant Difference[a]
	Dense	Sparse	
Hills m^{-2}	6.2	5.5	**
Tillers hill^{-1}	13.9	10.3	*
Panicles hill^{-1}	12.3	7.1	**
Panicles m^{-2}	76.7	39.0	**
Fertile panicles (%)	90.1	66.6	**
1000 seed weight (g)	29.1	29.1	NS

a) NS = not significant ($p < 0.05$); ** = significant ($p < 0.01$)

Table 9. Nutrient concentration and contents at maturity of the rice after fallow with dense and sparse pada in a rotational shifting cultivation system in northern Thailand.

	Grain[a]		Straw[a]	
Pada density	Dense	Sparse	Dense	Sparse
(a) Nutrient concentration (%)				
N	1.13[a]	1.42[b]	0.48	0.63
P	0.23[a]	0.31[b]	0.04[a]	0.10[b]
K	0.42	0.45	2.47[b]	1.91[a]
Ca	0.11	0.10	0.33	0.36
Mg	0.11[a]	0.13[b]	0.13	0.13
(b) Nutrient content (kg/ha)				
N	34.1[b]	13.9[a]	13.6[b]	7.3[a]
P	7.6[b]	3.3[a]	2.1[b]	1.0[a]
K	13.1[b]	5.4[a]	62.9[b]	16.1[a]
Ca	3.1[b]	1.1[a]	8.1[b]	3.5[a]
Mg	3.4[b]	1.4[a]	3.3[b]	1.5[a]

a) Different letters designate significant difference (by LSD, $p < 0.05$) between dense and sparse pada patches for grain or straw yield.

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References

Kaya B. and Nair P.K.R. 2001. Soil fertility and crop yields under improved-fallow systems in southern Mali. Agrof Syst 52: 1–11.

Kwesiga F. and Coe R. 1994. The effect of short rotation *Sesbania sesban* planted fallows on maize yield. For Ecol Mgt 64: 199–208.

Kunstadter P. 1978. Subsistence agricultural economics of Lua' and Karen Hill Farmers, Mae Sariang District, Northwestern Thailand. In: Kunstadter P., Chapman E.C. and Sabhasri S. (eds), Farmers in the Forest. The University Press of Hawaii, Honolulu, pp. 71–133.

Mafongoya P.L. and Nair P.K.R. 1997. Multipurpose tree prunings as a source of nitrogen to maize under semiarid conditions in Zimbabwe. Part 1. Nitrogen-recovery rates as influenced by pruning quality and methods of application. Agrof Syst 35: 31–46.

Nye P.H. and Greenland D.J. 1964. Changes in the soil after clearing a tropical forest. Plant Soil 21: 101–112.

Rerkasem K. and Rerkasem B. 1995. Montane mainland South-east Asia: agroecosystems in transition. Global Environmental Change 5: 313–322.

Reuter D.J., Edwards D.G. and Wilhelm N.S. 1997. Temperate and tropical crops. Plant Analysis, an Interpretation Mannual. : 81–285.

Sanchez P.A. 1976. Properties and Management of Soils in the Tropics. John Wiley & Sons New York, New York, 618 pp.

Sabhasri S. 1978. Effects of forest fallow cultivation on forest production and soil. In: Kunstadter P., Chapman E.C. and Sabhasri S. (eds), Farmers in the Forest. The University Press of Hawaii, Honolulu, pp. 160–184.

Tarawali G. 1991. Effect of Stylosanthes fodder banks on grain yield of maize. Trop Grass 25: 26–31.

Wanatabe F.S. and Olsen S.R. 1962. Colorimetric determination of phosphorus in water extracts of soil. Soil Sci 93: 183–188.

Whitmore T.C. 1982. On pattern and process in forests. In: Newman E.I. (ed.), The Plant Community as Working Mechanism: Special Publication of the British Ecological Society Produced as a Tribute to A.S. Watt. Blackwell Scientific Publication, Oxford, pp. 45–59.

Youpensook S., Lumyong S., Yimyam N. and Dell B. Biodiversity of Arbuscular mycorrhizal fungi from rhizosphere of *Macaranga denticulata* (Euphorbiaceae) on the highland rotational shifting cultivation area in Thailand. Aust. J. Botany (in press).

Zinke P., Sabhasri S. and Kunstadter P. 1978. Soil fertility aspects of the Lua forest fallow system of shifting cultivation. In: Kunstadter P., Chapman E.C. and Sabhasri S. (eds), Farmers in the Forest. University Press of Hawaii, Honolulu, pp. 134–159.

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Contents

MINI REVIEW

Cover photo: Gene Hettel

Determinants of a premium-priced, special-quality rice

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Thai Jasmine or Thai Hom Mali is Thailand's special-quality rice for which local consumers and export markets are willing to pay a premium price. Thai Jasmine rice does not always receive a premium price from buyers. The price varies, depending on where the crop is grown. For example, milled rice (special grade, 100% head rice) from Prao is sold at a retail price of 22 baht kg^{-1} (US$1 = 43 baht), that from Sanpatong costs 20 baht kg^{-1}, whereas Mae Chan rice fetches 16 baht kg^{-1}. Milled rice from Prao with 5% broken grain is priced at 17 baht kg^{-1}. In this paper, we examined the relationship between quality parameters used by commercial rice buyers and the price they pay for KDML 105.

Twenty-seven 500-g samples of unhusked KDML 105 were collected from farmers' fields in Chiang Mai in the upper part of northern Thailand, as well as 20 samples from Nakornsawan in the lower north. The samples were priced and rated for quality characteristics by a commercial rice buyer (Chiangmai Chaiwiwat Ricemill Co., Ltd.). The quality characteristics evaluated were grain moisture, percentage head rice yield, aroma, vitreousness, and translucency. Grain moisture was measured by a Riceter series L grain moisture tester (Kett Electric Laboratory). A small sample (20–30 g) of rice was unhusked on a board with a roller. An experienced rice buyer rated percentage head recovery, vitreousness, translucency, and aroma, using a 0–3 scale.

All 27 samples from Chiang Mai were priced above 7,000 baht t^{-1} (US$162). In contrast, samples from Nakornsawan were more varied and received prices ranging from less than 5,500 to more than 7,000 baht t^{-1}. Only 10% of the samples from Nakornsawan were judged to be of premium quality and priced at more than 7,000 baht t^{-1}; 40% were priced at less than 5,500 baht t^{-1}. Grain moisture did not exceed the standard 14% in any of the samples—those from Chiang Mai ranged from 13.3% to 14.0%, while the Nakornsawan samples had 11.2–12.0%. The prices of Nakornsawan samples were determined primarily by their aroma and vitreousness scores, with percentage head rice and translucency having relatively minor effects (equation 1).

$$P = 3{,}259.7 + 28.8X_1^{*} + 403.5X_2^{*} + 357.1X_3^{*} + 71.9X_4^{ns} \quad r^2 = 0.82^{***} \quad (1)$$

where P = sample price, X_1 = percentage head rice, X_2 = aroma score, X_3 = vitreousness, score, and X_4 = translucency score.

All 27 samples from Chiang Mai received the full score of 3 for vitreousness, translucency, and aroma. Price was determined by only one quality characteristic, percentage head rice (equation 2).

$$P = 6{,}969.4 + 11.3X_1^{***} \quad (2)$$
$$r^2 = 0.92^{***}$$

These results illustrate the effect of geographic differences on special-quality rice. The Chiang Mai samples represent areas that produce rice with high-quality characteristics in terms of aroma, grain translucency, and vitreousness. In such a situation, attention to factors that influence percentage head rice would ensure that farmers are paid premium prices for their harvest. Grain moisture at harvest and during application of N fertilizer has been shown to strongly affect head rice percentage (Nangju and De Datta 1970, Jongkaewattana et al 1993). The problem is much more complicated for farmers in areas where important quality parameters, such as aroma and vitreousness, are highly variable as we found in Nakornsawan. In such a situation, production of Thai Jasmine rice that will fetch premium prices will not be possible until environmental factors governing these quality parameters are identified and controlled.

References

Jongkaewattana S, Geng S, Brandon DM, Hill JE. 1993. Effect of nitrogen and harvest grain moisture on head rice yield. Agron. J. 85:1143-1146.

Nangju D, De Datta SK. 1970. Effect of time of harvest and nitrogen level on yield and grain breakage in transplanted rice. Agron. J. 62:468-474.

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The effect of nitrogen on rice grain iron

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Previous studies have shown that iron (Fe) content of rice grain may vary widely among rice genotypes (Senadhira et al., 1998; Prom-u-thai and Rerkasem, 2001). In addition, grain Fe may also be affected by environmental and management conditions. This experiment measured grain Fe concentration in five rice genotypes (KDML105, IR68144, UBON2, BASMATI370 and RD6) grown under three levels of N (0, 60, 120 kg N ha^{-1}). The field experiment was in a split plot design with three replications. Basal fertilizer consisted of 15 kg P_2O_5 and 15 kg K_2O ha^{-1}. The basal fertilizer and half of the N were applied at transplanting, and the other half of N was applied after four weeks. Fe concentration was determined in mature grain, as unhusked (whole grain with palea and lemma intact) and brown rice (palea and lemma removed), husk (palea and lemma) and polished grain (30 second), by dry-ashing and atomic absorption spectrometry (Emmanuel *et al.*, 1984).

In all five genotypes, grain yield was increased slightly by the application of 60 kg N ha^{-1}, but there was no further effect of increasing N to 120 kg N ha^{-1} ($p < 0.05$) (Table 1). Nitrogen fertilizer generally increased N content of the rice grain (Table 2). On the other hand, grain Fe concentrations in the five genotypes were affected by N rate differently (Table 3). The Fe concentration in unhusked rice of KDML105, IR68144, Basmati370 and RD6 increased with N to the rate 120 kg N ha^{-1}. Nitrogen had no effect on Fe in unhusked grain of UBON2. Much higher concentrations of Fe were found in the rice husk compared with the rest of the grain. Nitrogen levels had no effect on Fe concentration in brown rice and husk. Basmati370 was an exception, the Fe in brow rice

of this genotype was increased with N to the rate of 120 kg N ha^{-1}. To produce white rice, the mill normally polishes brown rice for about 30 seconds. In this study, we found that grain Fe concentration generally declined after polishing, indicating that a high proportion of Fe is contained in the bran or polishing. In UBON2 and RD2, grain Fe in white rice in N60 and N120 was about twice as much that in N0. There was similar, although slightly less, effect of N in Basmati370. In IR68144 the grain Fe in white rice at N120 was slightly less than these at N0 and N60, but in KDML105, the grain Fe in white rice showed no response to nitrogen. However, the grain Fe in unhusked, brown and white rice were not correlated with grain N in rice grain and grain yield at three levels of N application.

Different parts of the rice grain: the husk, the bran and the endosperm, appeared to contain Fe at different concentrations and their Fe contents responded differently to nitrogen fertilizer. The effect of nitrogen fertilizer on grain Fe appeared to be mostly in the husk. The Fe concentration in unhusked rice was only weakly correlated with that in brown rice ($r = 0.66$) and white rice ($r = 0.42$).

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Refferences

Emmanuel D, Dell B, Kirk G, Loneragan J, Nable R, Plaskett D, Webb M. 1984. Manual of Research Procedures. First edition. Plant nutrition research group school of environmental and life science. Murdoch University, Australia.

Prom-u-thai C, Rerkasem B. 2001. Grain iron concentration in Thai rice germplasm. *Dev. Plant Soil Sci.* 92, 350-351.

Senahira D, Gregorio G, Graham RD. 1998. Breeding iron and zinc – dense rice. Paper presented at the International Workshop on Micronutrient Enhancement of Rice for Developing Countries, September 3, 1998. At Rice Research and Extension Center, Stuttgard, Arkansas.

Table 1 Grain yield (t ha^{-1}) of five genotypes grown at three levels of nitrogen (0, 60, 120 kg N ha^{-1})

Genotypes	Grain yield (t ha^{-1})		
	N0	N60	N120
KDML105	3.6	4.0	3.8
IR68144	2.7	4.7	3.9
UBON2	4.1	4.0	3.7
RD6	3.1	3.4	3.3
BASMATI370	3.7	3.3	3.4
	3.2 a	3.8 b	3.6 b
Analysis of variance			
p(Genotypes)	ns		
p(Nitrogen)	< 0.05		
p(G X N)	ns		
LSD (nitrogen, 0.05)	0.4		

Table 2. The N concentration (%N) in unhusked rice of five genotypes grown under three levels of N (0, 60, 120 kg N ha^{-1})

Genotypes	Grain N concentration (%N)		
	N0	N60	N120
KDML105	1.3 aA	1.6 aB	1.8 bB
IR68144	1.5 aA	1.6 aA	1.9 bcB
UBON2	1.5 aA	1.6 aA	2.0 cB
RD6	1.3 aA	1.6 aB	1.8 bB
BASMATI370	1.3 aA	1.5 aA	1.5 aA
Analysis of variance			
p(Genotypes)	< 0.01		
p(Nitrogen)	< 0.01		
p(G X N)	< 0.01		
LSD (nitrogen, 0.05)	0.30		
LSD (genotypes, 0.05)	0.20		

Lower case for comparison of the genotype effect

Upper case for comparison of the N effect

Table 3. The Fe concentration in unhusked, brown rice husk and polished grain (white grain) at 30 and 60 seconds of five genotypes grown under three levels of N (0, 60, 120 kg N ha^{-1}

Genotypes	N	Fe concentration (mg Fe kg^{-1})			
		Unhusked	Brown rice	White (30s)[§]	Husk
KDML105	N0	13.0 a	7.8 a	7.9 a	36.0 a
	N60	14.6 ab	8.2 a	7.2 a	32.6 a
	N120	16.1 b	8.8 a	7.3 a	37.4 a
IR68144	N0	15.8 a	13.5 a	12.3 b	37.9 a
	N60	17.2 a	13.0 a	13.6 b	38.3 a
	N120	21.1 b	13.1 a	10.0 a	48.6 a
UBON2	N0	13.8 a	9.3 a	4.6 a	42.7 a
	N60	13.6 a	8.1 a	8.8 b	44.8 a
	N120	13.5 a	8.3 a	6.5 ab	48.5 a
RD6	N0	15.2 a	8.2 a	5.1 a	39.8 a
	N60	15.8 b	8.3 a	10.3 b	51.3 a
	N120	18.3 b	9.0 a	8.2 b	44.2 a
BASMATI	N0	15.6 a	10.8 a	7.6 a	35.8 a
	N60	16.3 a	12.1 ab	6.5 a	37.5 a
	N120	18.3 b	12.4 b	10.2 b	47.3 b
Analysis of variance					
p(Genotypes)		< 0.01	< 0.01	< 0.01	< 0.01
p(Nitrogen)		< 0.01	ns	< 0.05	< 0.05
p(G X N)		< 0.05	< 0.05	< 0.01	< 0.05
LSD (0.05)		2.6	1.5	2.3	11.5

*LSD used to comparison the different at the same genotypes at different treatment
[§] Polished for 30 seconds

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Agrodiversity Lessons in Mountain Land Management

Intensification of crop production in the mountains has long been perceived as unsustainable. However, since the late 1980s it has become increasingly evident that decline and collapse are not always inevitable. The present article provides examples from the highlands of northern Thailand to show that local food security can be improved while impacts on the resource base and the environment are minimized. This was achieved with the help of cropping systems developed and adapted by farmers themselves (Figure 1). Studying farmers' management techniques will allow this success to be repeated elsewhere, but only if it is based on the idea of dynamic variation in cropping system management that occurs within and between mountain agroecosystems, defined as agrodiversity.

FIGURE 1 A mosaic of mountain land uses: vicinity of Mae Cham village, Chiang Mai, Thailand. (Photo by Kanok Rerkasem)

A mountain agroecosystem on the verge of collapse

The following account of the Hmong village of Pah Poo Chom (see following description) found in the files of the Tribal Research Center in Chiang Mai aptly portrays the dire situation of the highlands of northern Thailand in the 1970s and 1980s:

> After seven years most of the surrounding forests had been cleared and cropped with rice, opium and maize. There was increasing competition for land from the neighboring villages of Thai, Lahu and Lisu. In 1970 the village grew 35 *rai* (5.6 ha) of opium, but the yield was very low. The village was extremely poor, and more than 55% of the total adult population was addicted to opium. Many had to make a living from employment outside the village.

The highlands of northern Thailand are populated by several ethnic groups. The people originally made a living from slash-and-burn systems of land use broadly classified as rotational and pioneer types of shifting cultivation. Rotational shifting cultivators (Karen, H'tin, Lua, and Khamu) typically settled in one place to grow rice and associated crops in a system of rotation involving 1 year of cropping and 9–14 years of fallow. Pioneer shifting cultivators (Hmong, Lahu, Lisu, Yao, Akha, and Haw) were migratory.

It was a harsh way of life with a constant risk of crop failure. The system was

Dear Readers,

This issue of Mountain Research and Development *is the first in the International Year of Mountains (IYM2002). The basic purpose of IYM2002, as declared by the UN General Assembly, is "to promote the conservation and sustainable development of mountain regions, thereby ensuring the well-being of mountain and lowland communities."*

In order to achieve this purpose, natural resources in mountain regions need to be used in a sustainable way that avoids overuse and degradation. The Global Assessment of Soil Degradation (GLASOD) published by the United Nations Environment Programme (UNEP) and the International Soil Reference and Information Centre (ISRIC) in 1990 showed that one-fourth of the world's agricultural land—corresponding to one-tenth of the Earth's land area—has been damaged by long-term soil degradation owing primarily to water and wind erosion. Although there has been no update of this global assessment, the issue of land and soil degradation has been included in several global agendas and formal frameworks.

Mountains are particularly susceptible to soil erosion caused by surface runoff due to high rainfall, steep slopes with erodible soils, growing pressure to use marginal lands for agriculture in some areas, abandonment of agropastoral land in other areas, and the construction of infrastructure for economic activities. Because mountains also serve as water towers, providing water not only in mountain areas but for the surrounding lowlands as well, land degradation in mountains has serious impacts on the global supply of fresh water and on growing water-related conflicts.

The present issue focuses on efforts to avoid and combat land degradation; experiences need to be documented and exchanged so that existing knowledge can be used for better land management. Hence the development section presents examples of successful soil and water management, focusing on revival of a degraded region in Serbia, agrodiversity in Thailand, contour bunds and hedgerows in the Philippines, live fences to protect sloping lands in Ecuador, and spring sanctuaries in the Indian Himalaya. An article on the World Overview of Conservation Approaches and Technologies (WOCAT), a knowledge management system in which over 35 institutions worldwide collaborate, illustrates tools that can be used to document and exchange local experiences at the global level. Articles in the research section demonstrate the wealth of knowledge about land use, land cover change, resource management in mountain areas, and the potential for improved use of resources.

Additional efforts are needed at the local, national and global levels to raise awareness of the possibilities of reducing land degradation, showing the potential for improved land management in mountains regions as well as the impact of successful management techniques on the livelihoods of people in the mountains and the surrounding lowlands. This will be a significant contribution to the overall purpose of IYM2002.

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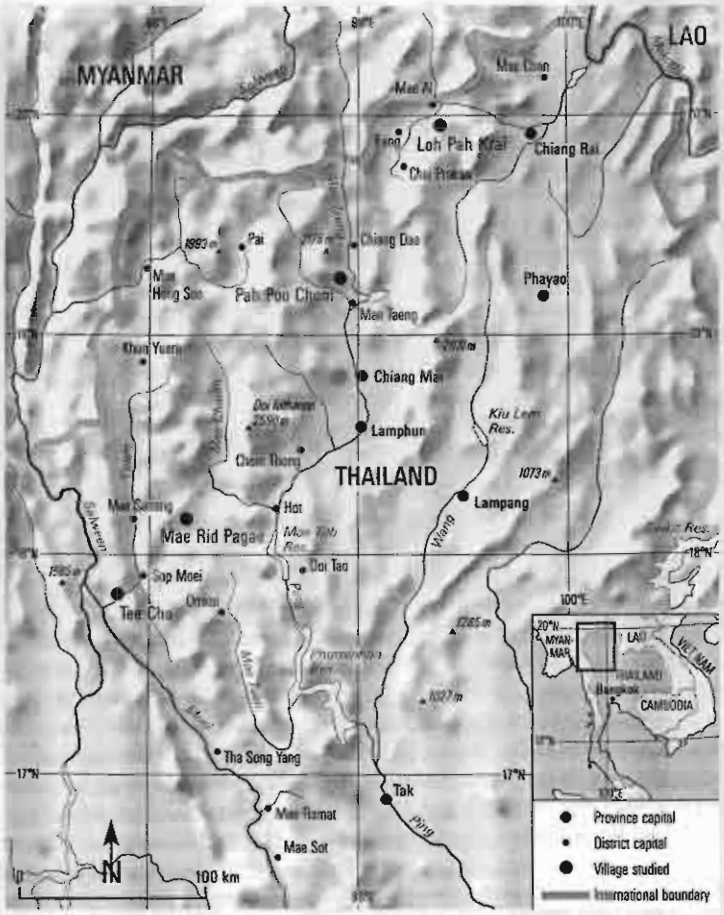
nevertheless sufficiently productive and sustainable as long as land and mature forests were plentiful. Since the 1950s, however, internal and external pressures have been mounting. These include rapid population growth and growing national concerns about loss of forest cover, soil erosion, and other impacts on the environment and natural resources, especially water. Agricultural land has been drastically reduced by demarcation of large areas for watersheds, national parks, and wildlife sanctuaries.

Coping with change

In addition to increased pressure on the land, there were other changes in the 1980s. Law enforcement against opium became more rigorous. New opportunities emerged as the result of increased integration into the national economy, better communications, and access by road. New cropping systems and modification of old ones appear to have raised productivity. Some form of forest conservation is practiced in almost every village. Four sample villages (Figure 2) illustrate that success was not restricted to any particular ethnic group and that it did not come about because of the transfer of any single magic external technology. It was the product of many different innovations and practices, some originating locally, others imported into the mountains, but all adapted to individual fields, farms, and villages.

Pah Poo Chom is a Blue Hmong village situated in Chiang Dao, 100 km north of Chiang Mai. The community first settled on a mountain ridge in 1963. Most of the surrounding forests had been cleared by 1970 and cropped with rice, maize, and opium. A major investment during the 1970s to develop wetland rice cultivation was unsuccessful. Productivity was too low to feed a population of 116; the village was on the brink of collapse. But Pah Poo Chom has become a very different village since the early 1990s. With a population of about 250, it now uses only about 30% of its land. The steeper slopes are covered with forests. Obvious signs of prosperity can be seen in the number of pick-up trucks and other consumer goods. Cash cropping, with sprinkler-irrigated lichee and vegetables, has been adopted on a major scale, but successful management of local plant genetic resources has also contributed to food security and income generation.

FIGURE 2 Map of 4 sample villages: Pah Poo Chom, Mae Rid Pagae, Loh Pah Krai, and Tee Cha. (Map by Andreas Brodbeck)



Loh Pah Krai was a typical opium-growing Lahu village. With their pioneering form of shifting cultivation, the villagers moved through the highlands growing opium, maize, and upland rice. After "eating up forests in three provinces," they settled at Mae Ai, north of Chiang Mai, in the mid-1960s. A sizeable tract of irrigated rice land was bought from lowland Thais. By the early 1990s the village was making a living from rice–soybean cropping systems in the irrigated fields and maize–legume systems on the slopes. Several farmers now have elaborate home gardens with 30–40 species, including annual crops, fruit and other trees, local and introduced vegetables, herbs and spices, and various semidomesticated species.

FIGURE 3 Upland rice field after harvest, with *pada*. (Photo by Kanok Rerkasem)

Mae Rid Pagae is a Skaw Karen village in Mae Hong Son, near Myanmar. In the past, the village subsisted on rotational shifting cultivation supplemented by limited irrigated wetland rice and off-farm employment in the nearby town of Mae Sariang in bad years. The introduction of cabbage in the early 1980s raised productivity significantly. The success of Mae Rid Pagae, however, is not a matter of just growing cabbage; new cropping systems have evolved, incorporating cabbage production and components of traditional cropping systems. Cabbage is grown in paddies with irrigation in the dry season, after wet season rice. On the slopes, upland rice and cabbage are grown in rotation. Rice yield has doubled or tripled, possibly because of the residual effects of fertilizers and clean weeding of cabbage.

Tee Cha is a Pwo Karen village, established more than 200 years ago, in the Salween watershed on the Myanmar border in Mae Hong Son. It is one of the few villages where rotational shifting cultivation is still productive. Good forest cover dominates the village landscape. The cropping system in Tee Cha is predominantly subsistence and is managed partly on a communal basis. Although situated in a less densely populated area than most highland villages, it is also experiencing external and internal pressures on the land. In a much-shortened rotation, maintenance of productivity is attributed to an innovative use of local fallow-enriching species, especially *pada* (*Macaranga denticulata* Muell. Arg.) (see Box 1 and Figure 3). In addition, local domesticated, semidomesticated, and wild species contribute significantly to food security.

BOX 1

A local fallow-enriching species, *Macaranga denticulata* Muell. Arg. (*pada*)

Pada is a small tree of the family Euphorbiaceae. Its primary use is in fallow enrichment; the wood may be used for fuel and the leaves for wrapping. During the cropping year, *pada* seedlings emerge in thick carpets among the rice. Farmers manage *pada* in a number of ways; the seedlings are not considered weeds and are thus not eradicated during hand weeding, but dense stands may be thinned. Seedlings may also be transplanted to areas with poor establishment. A good stand of *pada* reaches almost over the farmers' head by the time of rice harvest (Figure 3). Dense stands of *pada* (>4000 trees/ha) are associated with an upland rice yield that is about twice that with 1000 trees/ha or fewer. However, after only 3 years of dense *pada* the rice yield is only 1 quarter of that after 6 years. Attempts to transfer *pada* to neighboring villages have so far been unsuccessful.

The agrodiversity approach

"Agrodiversity" is used here as a means of analyzing and understanding innovation and management of cropping systems in the mountains. Agrodiversity has been defined as the dynamic variation in cropping systems, output, and management practice that occurs within and between agroecosystems. It is characterized by biophysical differences and the many changing ways in which farmers manage diverse genetic resources and natural variability and their practices in dynamic social and economic contexts (Brookfield 2001).

Diversity in the biophysical environment

The highlands are places of great diversity in the biophysical environment. Successful cropping systems are often those that can take advantage of special sets of conditions. Utilizing 16 ha of former paddy for irrigated orchards and vegetable fields, Pah Poo Chom has no major soil erosion problems. Pah Poo Chom is but 1 of numerous highland villages that have taken advantage of gravitation to supply

piped water for domestic use and irrigation. Tee Cha's "luck" in the presence of *pada* and other natural fallow-enriching species is shared by few other shifting-cultivation villages in the neighborhood (see Box 1).

The primary constraint on technology transfer from the outside is poor characterization and documentation of the biophysical environment of the highlands. On the other hand, the farmers' understanding of the local biophysical environment is well known among those familiar with the mountains of northern Thailand (see Box 2). Farmers readily speak about variations in soil, temperature, water, position on the slope, the special characteristics of local plants, and various seasonal conditions. Much can therefore be learned from them, but this requires more than a few hours of visits by national and international "experts."

Diversity in management and innovation

Saophang Saetao (Figure 4) is one of the few farmers in Pah Poo Chom who manage agroforest edges to produce medicinal plants and a special kind of bamboo for making a traditional Hmong musical instrument known as *can*, as well as wild vegetables, timber, and herbs, like other farmers. Saophang's knowledge of forestry and traditional medicine and his skill in crafting the highly priced *can* are especially important to agroforest edge management. Other farmers, mainly women, manage the hedges for traditional food plants. Wild vegetables from Pah Poo Chom's agroforest edges sell well in Chiang Mai City. The managers of agroforest edges in Pah Poo Chom are all old farmers who have retained knowledge and skills from the opium-based cropping systems. Younger farmers tend to focus only on cabbage and lichee.

Saophang and others like him can be called "expert farmers," whose qualifying characteristic is their crop management ability. Those who specialize in food crops other than rice are usually women, whose special gender-related skills and knowledge are exemplified by the management of numerous domesticated and semidomesticated food plants, including 20–30 different kinds sown mixed with rice (Figure 5). It is not always easy to identify expert farmers, however; those who are most capable are not necessarily the most outspoken or articulate.

BOX 2

Knowledge and skill in crop management of mountain farmers

Headman, Hmong village of Khun Sa Nai, Chiang Mai, on wetland rice cultivation in narrow highland valleys:

The soil is warm in the bottom fields. We need one kind of rice. At the top we need another kind because the soil is cold and hard.

H'tin farmer in Namsod, Nan, on effects of trees in upland rice:

Du (Pterocarpus sp) *is good for rice, which you can see growing right up to the trees. We can leave many of these in the field; they are good timber too. But see how no rice grows under this* mamuen (Irvingia malayana); *you don't want them in the rice field. We keep just this one tree for the seed that children love.*

Headman, Hmong village of Khun Sa Nai, Chiang Mai, on rice for poor soils:

Akha rice is good for poor soils. For upland fields with poor soils, we get our rice seeds from the Akha.

Karen farmer, Pang Gorm, Nan, on legumes and upland rice:

You cannot grow these legumes just anywhere among the rice; they are very aggressive and will climb everywhere and smother the rice. We grow a few hills of these on the edge of the field and provide some dead twigs and branches for them to climb. But this tua lawd *or* tua sord *(a cowpea) is one we can grow among the rice. Its branches will grow several meters, but always stay hugging the ground and never climb or do the rice any harm.*

Karen farmer, Mae Rid Pagae village, Mae Hong Son, on the seasonality of insect pests in vegetables:

We have to spray a lot in the dry season, but hardly ever in the wet season. See how everything is growing in the forest when it rains? The insects have plenty to eat and don't need to come and eat our vegetables. But in the dry season there is nothing for them to eat except our crops.

Karen farmer, Mae Sariang, Mae Hong Son, on soil erosion and land formation:

Soil loss from those fields at the top is good. We need a few more years before this land (at the bottom of the slope) can be leveled and bunded for wetland rice. But soil loss from that field up there is no good because it will ruin the wetland rice field just below it. Luckily, the field is under my control, so I can take care that the forest cover is never cleared and the soil not disturbed.

Diversity in local genetic resources

Plant genetic resources available for local use are the key to the successful new cropping systems discussed previously. Production of cabbage, pepper, carrot, lettuce, tomato, potato, etc relies on the most modern varieties of imported seed. These, however, have not displaced traditional local germ plasm, even in villages that have gone into commercial production on

a major scale. Local plant species and varieties continue to contribute significantly to food security, especially where there are problems with the market, for example, when vegetable prices collapse or the lichee trees do not set fruit. Local plant genetic resources are even more valuable to those with limited access to the market, including villages with poor transportation such as Tee Cha and people who have not been highly successful with cash cropping, which includes the village poor everywhere.

Wild and semidomesticated species, as well as rice and the various crop species associated with it, all contribute to food security. Saophang's agroforest hedge in Pah Poo Chom contains 92 species of plants. In addition to *pada*, other soil-improving species are only beginning to be identified and characterized by researchers. Rice is still the primary subsistence crop in most highland villages, although its relative importance has declined with the spread of new cash crops. Virtually all of the rice varieties grown in the mountains are from local germ plasm. At least 15 local varieties have been identified in Tee Cha; each farmer grows 2–5 varieties. Taste preference for traditional varieties is one reason for lack of success with "improved" rice varieties in the highlands. The other reason is that new varieties bred for wide adaptation have failed to meet the diversity of biophysical conditions in the highlands. Through their maintenance of the local germ plasm of native strains of rice, other food crops, and semidomesticated species, these farmers provide the world at large with a valuable service of in situ conservation of plant genetic resources.

FIGURE 4 Saophang Saetao and a *can* made with a special bamboo from his agroforest edge. (Photo by Kanok Rerkasem)

Diversity in institutional arrangements

The ability of local institutions to manage the common resources of land, water, and forests can be the 1 condition on which the success or failure of new cropping systems depends. When former opium-growing villages bought their irrigated rice land in order to settle down, in addition to transfer of technology for growing wetland rice they had to adapt rather complex communal institutions to the management of the irrigation system. The Lahu of Loh Pah Krai have had to learn to manage sharing of irrigation water within the village and with neighboring villages as well as to organize maintenance of the physical structures of the irrigation system. In contrast, conflicts have erupted between highland and lowland villages in many areas where institutions for water sharing were poorly developed. The presence of roaming livestock is often the primary constraint on vegetable production. Common sense might suggest fences, but in Mae Rid Pagae the enforcement of a communal law is an additional factor. Owners of the vegetable fields have a responsibility to make their fences cattle-proof during the day. Livestock owners are fined for damages that occur at night,

FIGURE 5 A seed mixture of rice and 24 other kinds of crops sown by Mrs Inar in Tee Cha. (Photo by Kanok Rerkasem)

when the animals need to be tied or locked up in stalls.

Rotational shifting cultivators such as the Karen of Tee Cha owe the productivity and sustainability of their land use system primarily to traditional land management institutions. Communal management ensures that fallow forests (ie, forests that cover the land during fallow) are allowed to regenerate quickly after 1 crop season and grow undisturbed before slash-and-burn takes place again. Rotational shifting cultivation is less successful where communal land management has broken down or was never practiced in the first place. In remote places, enforcement of local rules and regulations is often the only effective way to protect forests against encroachment, over-harvesting of timber and other forest products, and forest fires.

Conclusion

Land use in the highlands of northern Thailand has undergone dramatic changes under external and internal pressures. Some farmers have been able to cope by adopting cropping systems that are more productive and have minimal impact on the environment. This has been done with local innovations in crop management that have replaced or improved traditional shifting cultivation. The 4 sample villages are not unique; many others can be found throughout the region. The key to these successes is local innovations that have come about through (1) farmers' knowledge of spatial and temporal diversity of their biophysical environment, (2) local availability of plant genetic resources and farmers' knowledge of their special adaptation and other characteristics, (3) the crop management skills of individual farmers, and (4) effective institutional arrangements for dealing with the management of common resources. The ability to manage this "agrodiversity" to the advantage of their specific cropping or land use system is the unique qualification of successful, expert farmers. Analysis of the 4 elements of agrodiversity is useful in any attempt to improve the performance of crop production in mountain agroecosystems by outside experts.

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FURTHER READING

Brookfield H. 2001. *Exploring Agrodiversity*. New York: Columbia University Press.
Brookfield H, Padoch C. 1994. Appreciating agrodiversity: a look at the dynamism and diversity of indigenous farming practices. *Environment* 36:6–11, 37–45.
Suthi C. 1982. Highland agriculture: from better to worse. *In:* McKinnon J, Vienne B, editors. *Hill Tribes Today*. Bangkok and Paris: White Lotus, pp 107–142.

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Adoption and Maintenance of Contour Bunds and Hedgerows in a Dynamic Environment

Experience in the Philippine Uplands

The widespread adoption of soil conservation technologies by farmers (notably contour hedgerows) observed in Guba, Cebu City, Philippines, is not often observed elsewhere in the country. Adoption of these technologies was because of the interaction of such phenomena as site-specific factors, appropriate extension systems, and technologies. However, lack of hedgerow maintenance, decreasing hedgerow quality, and disappearance of hedgerows raised concerns about sustainability. The dynamic nature of upland farming systems suggests the need for a location-specific farming system development framework, which provides farmers with ongoing extension for continual promotion of appropriate conservation practices.

FIGURE 1 An overview of Adlaon Village, a neighbor of Guba, showing cultivation on steep slopes and some conservation farming in the foreground. (Photo by R. V. Gerrits)

A high rate of adoption

The rate of adoption of soil conservation technologies by upland farmers in the Philippines has remained low, with few successful projects. One such project is located in the hinterlands of Cebu City, implemented by the Mag-uugmad Foundation, Inc (*MFI*), a nonprofit, nonstock, farmer-based NGO concerned with the declining status of upland farmers, degradation of upland resources, and the downstream effects of resource degradation. Surveys conducted from 1993 to 1997 in 8 upland project sites throughout the Philippines revealed that the rate at which soil conservation technologies are adopted at the MFI Guba site represents a phenomenon rarely seen elsewhere in the Philippines. A 1-week informal survey was conducted to determine the reasons for this widespread adoption and to analyze sustainability. Interviews with key persons and households, transect walks, and farm visits were conducted in various localities.

Guba is located approximately 25 km northwest of Cebu City center and is relatively accessible by means of 3 direct, unsealed gravel roads. The site's climate is characterized by lack of a pronounced maximum rainy period. Soils are heterogeneous but primarily acidic, heavy clay loams, with slight to severe erodibility and susceptibility to waterlogging. A conservation project begun in 1981 in Barangay Guba, Cebu City, spread to the 9 neighboring *barangays* (villages) of the city, covering a total area of around 78 km^2 (Figure 1).

Livelihood based on farming

Farming is the primary source of livelihood for most households. Local employment opportunities include contract hedging, spraying, harvesting and hauling mangoes, contract firewood and charcoal production, and the purchase and sale of bamboo. Off-farm employment by one or more of the younger farm-household members also provides some cash income.

The average farm size is 1 ha of Alienable and Disposable or Public Forest Lands. Key persons interviewed suggested that 30% of the farmers are tenants who either rent some land in addition to their existing (but small) landholdings or are wholly dependent on rented land. Most tenants have long-term tenancy agreements (in some cases, intergenerational), creating a feeling of security of tenure. The growing accessibility of the site has brought about increased commercialization of farming. This leads farmers to restrict maize cultivation to the first of two annual cropping periods and utilize the second for the cultivation of vegetables. Flowers, fruit

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Easy and rapid detection of iron in rice grain

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Abstract:

One way to improve iron (Fe) intake in those who suffer from Fe deficiency anemia is to increase Fe content in the grain people consume. In this study, we show how a preliminary determination of grain Fe in rice may be made with reaction to Perls' Prussian blue, a stain for Fe (III). Differential localization of Fe in parts of the grain is indicated by intensity of reaction of tissue Fe to the dye. The blue colour reaction was most intense in the embryo, weak in the aleurone layer of the pericarp and invisible in the endosperm. The staining intensity varied with region of the embryo, generally being strongest in the scutellum, intermediate in the coleorhiza and weakest in the coleoptile. Variation in the reaction to Perls' Prussian blue was observed among eleven rice cultivars with varying grain Fe contents. The intensity of the blue colour reaction in the embryo of different rice cultivars was indicative of their grain Fe contents for both brown and white (polished) rice. Those with high grain Fe, >14 mg Fe kg^{-1}, were clearly distinguishable from those with <10 mg Fe kg^{-1} with Perls' Prussian blue. We suggest that this simple staining procedure may be used to quickly screen for high Fe contents in large germplasms containing hundreds of rice entries, using reactions in genotypes with known grain contents as standards.

Keywords: *Oryza sativa*, Rice, Seed, Iron localization, Perl's Prussian blue, Detection

Introduction

Increasing Fe content in the grain people consume is considered one way to increase Fe intake in those suffering from Fe-deficiency anemia.[7] Previous studies have shown that grain Fe content can vary widely among rice genotypes. Most of the commonly eaten rice varieties in Asia contain only about 10 mg Fe kg^{-1} in brown rice, but genotypes with 15 mg Fe kg^{-1} or more have been found.[6,7] Selection and breeding for rice of higher grain Fe content is possible. However, in the past, Fe content of rice grain can only be measured by chemical analysis. This poses a problem when dealing with large numbers and limited amount of samples as in screening of germplasms and evaluation for Fe contents in progenies of crosses. Furthermore, even though the method for chemical analysis for Fe is well established, contamination is still a problem in many labs which has resulted in unusually high grain Fe contents being reported. The application of the Prussian blue stain[5] has made possible rapid estimation of the amount of non-hemoglobin Fe in the marrow and in the blood of humans[2] and other vertebrates.[3] Biologically active Fe is normally very tightly complexed to protein, as in hemoglobin or myoglobin. Fortunately, there are various mechanisms within the tissue which allow the Fe to be detected, in the form of Fe (III) surrounded by hemosiderin. This Fe is reported to be easily stained with Perls' Prussian blue reaction.[8] We set out to see if Perls' Prussian blue may be used to detect the localization of grain Fe in rice, and whether seed with different grain Fe contents may be distinguished by the intensity of staining.

Materials and Methods

Seed of 11 genotypes of rice (*Oryza sativa*) were obtained from plants grown on San Sai soil under wetland condition in the Department of Agronomy field plot at Chiang Mai University, Thailand. The genotypes included IR68144 (a high yielding variety from IRRI), Basmati 370 (a traditional cultivar from Pakistan), KDML 105, RD 6, Hom Klong Luang1 (3 popular aromatic rice cultivars from Thailand), Neaw Ubon 2, Hom Pu Pan, Hom Nang Fa (3 newly released Thai

varieties), and CMU 122, CMU 123, CMU 124 (3 upland Thai varieties). Seed Fe concentration was determined by wet-ashing and Inductive Couple Plasma Spectrophotometry; ICP[9] in mature grain as whole grain brown rice (palea and lemma removed, complete with embryo) or white rice (milled and polished, embryo largely removed). The Perls' Prussian blue reaction was determined on individual grains of brown rice, with three replications for each cultivar. After the husk was removed, the seeds were imbibed in distilled water for 4-5 hours, and were cut in half lengthwise through the embryo with a teflon knife (Advanced Personna Brand) in a Petri dish. The specimens were submerged in freshly prepared Perls' Prussian blue[5] solution (2% hydrocloric acid mixed with 2% potassium ferrocyanide) for 10 minutes. The seed were then gently washed continuously in distilled water for 2 minutes. The ferric Fe is released from any attachments to protein by treatment with dilute hydrochloric acid and then reacts with a dilute solution of potassium ferrocyanide to produce an insoluble compound, ferric ferro cyanide (Prussian blue).[3] The intensity of staining was rated from 0 (no staining), + (weak staining) to +++ (most intense) under a stereo microscope.

Results and Discussion

Perls' Prussian blue staining has been recommended as a method for locating Fe (III) in animal tissue because it is fast, reproducible and the reagent penetrates bulky tissue to give a distinctive blue reaction.[1] The technique has recently been used to report the presence of iron in the aleurone layer of rice grain.[4] In this paper, we show for the first time a pattern of staining with Perls' Prussian blue within the rice grain that clearly indicates differential localization of Fe (Figure 1). The staining was most intense in the embryo, weak in the aleurone layer of the pericarp, and not detectable in the endosperm (Table 1). Differences were also seen in different parts of the embryo (Figure 2, Table 2). The staining was weakest in the coleoptile, intermediate in the coleorhiza and strongest in the scutellum, which was similar to the embryo as a whole. The most intense blue colour (+++) in the embryo (and the scutellum) was associated with the grain Fe content of >13 mg Fe kg^{-1} for brown rice and >10 mg Fe kg^{-1} for polished rice (Table 2). The

intensity of staining was less well correlated with grain Fe at lower concentrations as cultivars with embryo staining rated as + and ++ were not distinguishable by their grain Fe contents. No reaction with Perls' Prussian blue was observed in the endosperm, even at 15.6 mg Fe kg^{-1} as found in white, polished IR68144. The Fe present in the endosperm may not be in the form that reacts with the dye, or possibly the concentration of Fe in the endosperm may be below the limit of visible staining by Perls' Prussian blue. By contrast, staining of the embryo of Neaw Ubon 2 (19.9 ± 1.8 mg Fe kg^{-1}), although faint (+) was still clearly visible. The reaction to Perls' Prussian blue in the aleurone layer of the pericarp also followed roughly the Fe contents of whole grain in individual rice cultivars, but the distinction is less clear between cultivars with high and low grain Fe.

Table 1 Differential staining with Perls' Prussian blue in parts of the grain of 11 rice cultivars.

Variety	Intensity of staining, part of grain *		
	Embryo	Aleurone layer	Endosperm
IR68144	+ + + §	+	0
KDML 105	+ +	+	0
BASMATI 370	+ + +	+	0
Hom Klong Luang 1	+ +	+	0
Hom Nang Fa	+ +	0	0
Hom Pu Pan	+ +	0	0
Neaw Ubon 2	+	0	0
RD 6	+	+	0
CMU 122	+ + +	+ +	0
CMU 123	+ + +	0	0
CMU 124	+ + +	+ +	0

* Whole grain with embryo, without palea and lemma.

§ 0 = no stain; + weak staining; + + moderate staining; + + + intense staining.

Three seeds for each variety gave the same rating.

Table 2 Differential staining with Perls' Prussian blue in different parts of the embryo and grain Fe concentration in 11 rice cultivars.

Variety	Whole	Part of embryo			mg Fe kg^{-1} †	
		Scutellum	Coleoptile	Coleorhiza	Brown rice	White rice
	Intensity of staining					
IR68144	+++‡	+++	+++	+++	19.7 ±	15.6 ±
KDML105	++	++	+	++	7.8 ±	7.2 ±
BASMATI 370	+++	+++	+	+++	14.3 ±	8.0 ±
Hom Klong Luang 1	++	++	++	+	7.9 ±	6.5 ±
Hom Nang Fa	++	++	0	+	8.0 ±	*
Hom Pu Pan	++	++	0	+	8.4 ±	*
Neaw Ubon 2	+	+	0	+	8.1 ±	7.8 ±
RD 6	+	+	+	+	8.6 ±	7.5 ±
CMU 122	+++	+++	++	+++	16.7 ±	13.8 ±
CMU 123	+++	+++	+++	+++	15.8 ±	11.4 ±
CMU 124	+++	+++	++	+++	13.2 ±	10.1 ±

‡ 0 = no colour; + weak staining; ++ moderate staining; +++ intense staining

† Brown rice was whole grain with embryo, without palea and lemma; white rice had palea and lemma removed by milling, and pericarp removed by polishing, embryo largely abscent. Each value was mean of three analyses.

* not determined

Three seeds for each variety gave the same rating.

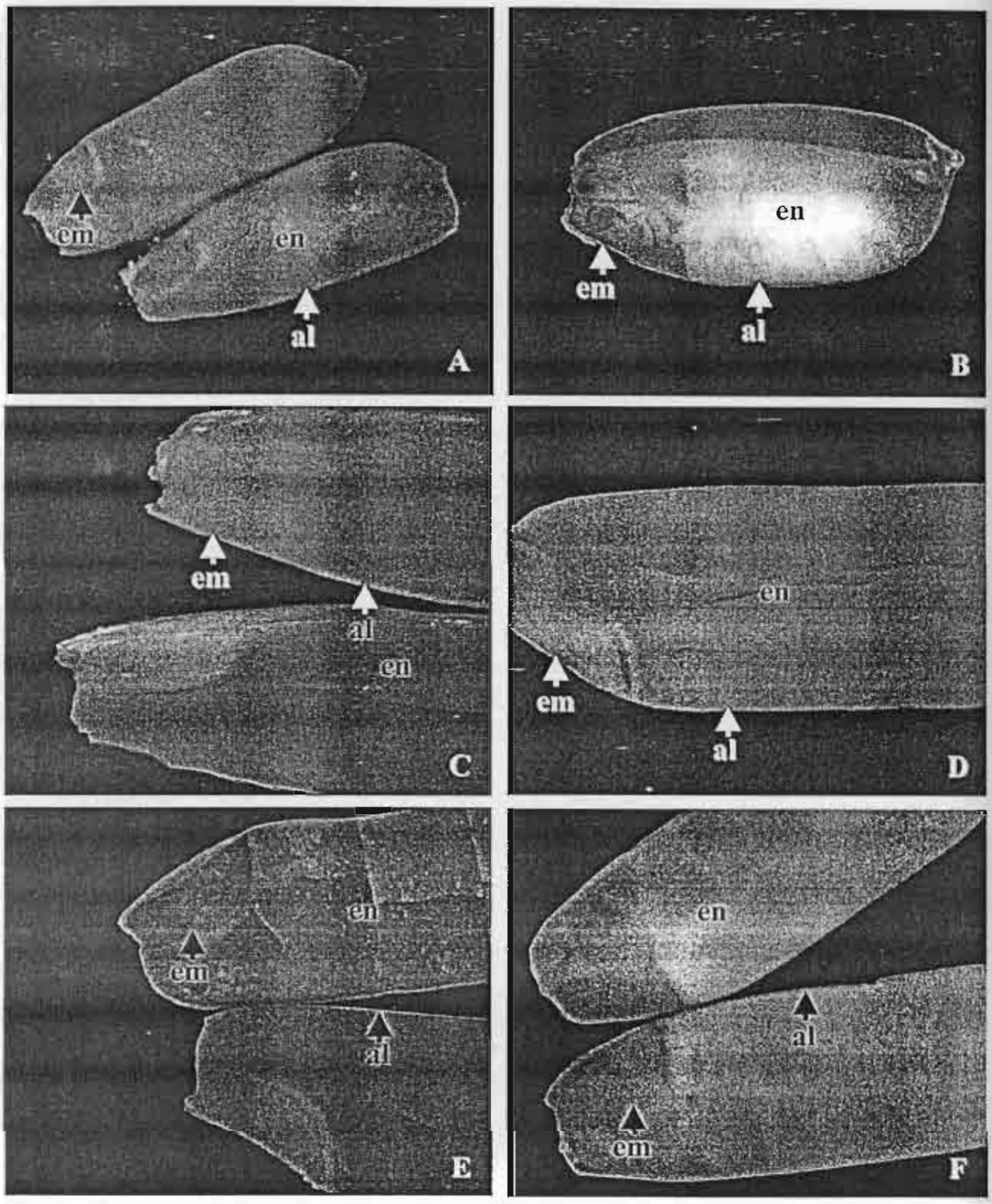

Figure 1 Stereo-micrographs of brown rice grains cut longitudinally in half, showing distribution and intensity of staining with Perls'Prussian blue. Most of the Fe (III) reaction is located in embryo part of the grain. Em, embryo; en, endosperm; al, aleurone layer. Cultivars: A, IR68144; B, CMU122; C, Neaw Ubon 2; D, KDML 105; E, Basmati 370; F, Hom Pu Pan

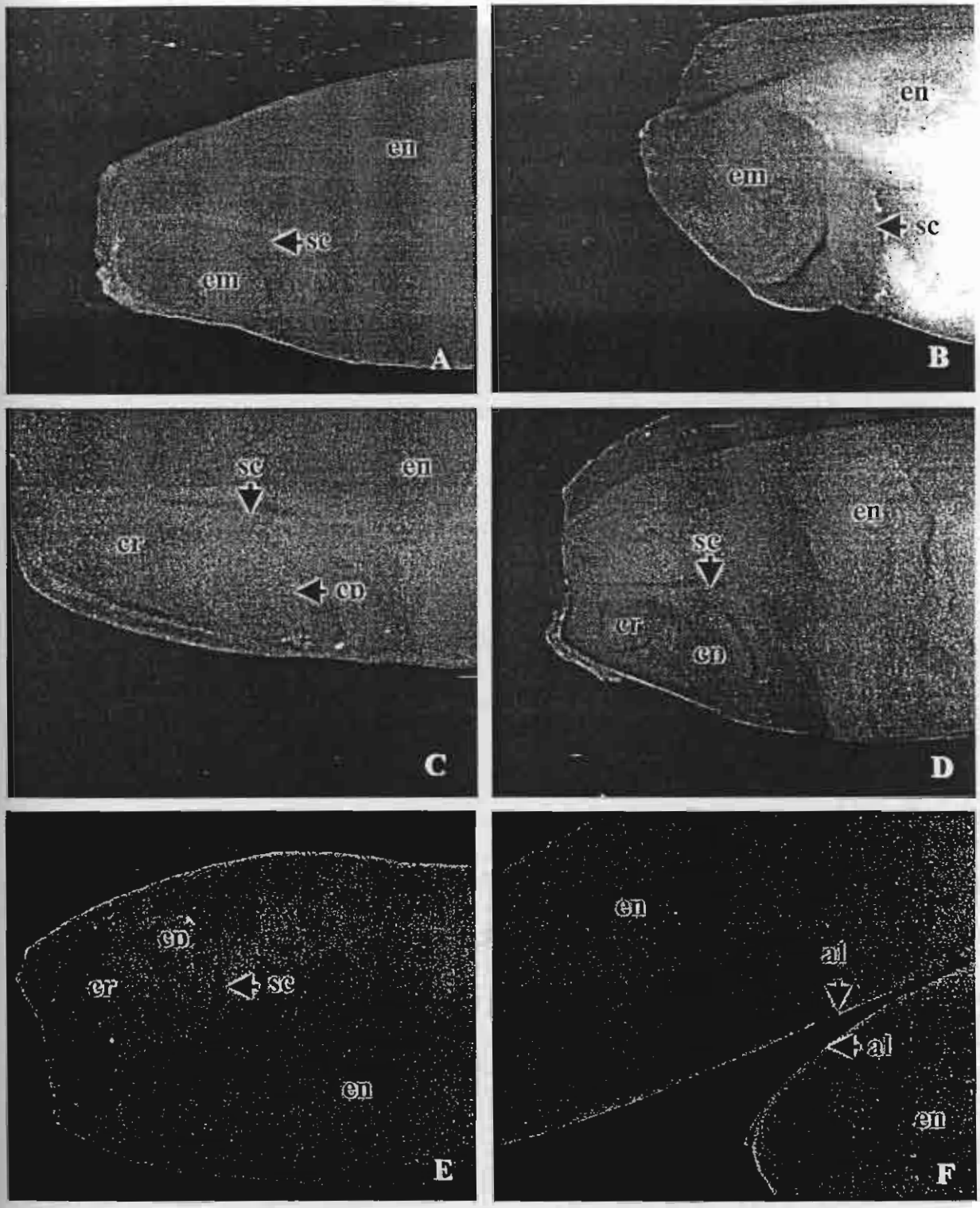

Figure 2 Stereo micrographs of brown rice grain cut longitudinally in half, showing the distribution and density of staining with Perls' Prussian blue. A-E showing the different reaction in different part of embryo: em, en: endosperm, sc: scutellum, cr: coleorhiza, cp: coleoptile. F showing the reaction of Perls' Prussian blue in aleurone layer of KDML 105. Cultivars; IR68144; A, Hom Klong Luang1; B, CMU 124; C, CMU 122, D, Basmati 370; E, KDML 105; F

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References

1. Baker JR (1958) Principles of biological microtechnique. A study of fixation and dyeing. London. pp 304.
2. Dorothy R and Harrette B (1955) The application of the Prussian blue stain to previously stained films of blood and bone morrow. *Blood J.* **10**, 160-166.
3. Doucet MY and Viel (2002) Alveolar macrophage graded hemosiderin score from bronchoalveolar lavage in horses with exercise-induced pulmonary hemorrhage and controls. *J Vet Int Med.* **16**, 281-286.
4. Krishnan S, Ebenezer GAI and Dayanandan P (2001) Histochemical localization of storage components in caryopsis of rice (*Oryza sativa* L.). *Current Science.* **80**, 567-571.
5. Perls M (1867) Nachweis von Eisenoxyd in gewissen Pigmentation. Virchows Archive für pathologische Anatomie and Physiologie and für Klinisch Medizine. **39**, 42-47.
6. Prom-u-thai C and Rerkasem B (2001). Grain iron concentration in Thai rice germplasm. *Dev Plant Soil Sci.* **92**, 350-351.
7. Senahira D, Gregorio G and Graham RD (1998). Breeding iron and zinc – dense rice. Paper presented at the International Workshop on Micronutrient Enhancement of Rice for Developing Countries, September 3, 1998. At Rice Research and Extension Center, . Stuttgard, Arkansas.
8. Stevens A (1977) Pigments and minerals. In: Theory and practice of histological techniques, Bancrofft JD and Stevens A. (eds), pp 186-208, Churchill Livingstone, Edinburg.

9. Zarcinas BA, Cartwright B and Spouncer LR (1987). Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Commun Soil Sci Plant Anal.* **18**, 131-146.

Agrodiversity for *in situ* Conservation of Thailand's Native Rice Germplasm

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Agrodiversity for *in situ* Conservation of Thailand's Native Rice Germplasm

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ABSTRACT

The spread of modern crop varieties has led to a concern about genetic erosion and decline in local crop genetic diversity. To preserve genetic resources it is now generally accepted that in situ conservation is required along side with ex situ conservation. Conservation of natural species of plants and animals may be achieved by conserving their natural habitats. Agricultural environment, however, is influenced by rapidly evolving social and economic forces and continuously emerging technological innovations. The same principles for conserving natural species cannot be applied to in situ conservation of crop diversity. Genetic systems of crop species are also highly dynamic, subject to selection pressure driven by increasingly precise tools for genetic management, including modern biotechnology, changing human needs and preferences. It is unrealistic and unjust to expect farmers to keep their traditional crop varieties in a state of suspended animation. Sustainable and equitable conservation of crop genetic diversity on farm requires two basic sets of understanding. The first is related to the structure and dynamics of the genetic system. This will help to determine (i) what may not be worth the cost of saving and what may worth conserving almost at any cost, and (ii) in what direction future changes may be expected in the germplasm so that management strategies may be adjusted accordingly. The second is related to how farmers manage and make use of local crop varieties. Biophysical differences and the many changing ways in which farmers manage diverse genetic resources and natural variability and their practices in dynamic social and economic context characterize the agricultural environment, or niche, in which crop diversity is to be conserved. Variation in both the genetic system and the niche need to be considered at various organizational levels, from the broadest global level to regional, national, down to local village, farm, field and individual plants. This paper presents the idea of "agrodiversity", as a means to analyze and understand Thai rice farmers' innovation and management of their cropping systems and crop genetic resources. Through agrodiversity analysis, which focuses on the dynamic variation in cropping systems, output, and management practice that occurs within and between agroecosystems, niches for diversity in the local rice genetic resources may be identified and enhanced on farm.

IN SITU CONSERVATION OF LOCAL CROP GENETIC RESOURCES

Widespread adoption of modern high yielding crop varieties has led to a concern about erosion in local crop genetic resources and loss of diversity. Replacement of older varieties by modern improved varieties has accelerated in the past 50 years in what is now commonly

known as the Green Revolution. High Yielding Varieties (HYVs) of rice has almost completely replaced traditional varieties in most rice growing countries in Asia (Kaosa-ard and Rerkasem, 2000). Erosion of local crop germplasm has also resulted from complete change in land use systems. Upland rice, usually grown in some form of shifting cultivation in the mountainous region of mainland Southeast Asia, is rich in genetic diversity (e.g. see Fu and Chen, 1999; Gong et al., 2001; Rerkasem et al., 2002). Land use changes, to wetland rice, extensive plantations of cash and export-oriented tree and industrial crops and large-scale vegetable production, have all resulted in losses of local varieties from farmers' fields.

Responding to this concern, conservation efforts were at first directed at *ex situ* conservation. Seeds of the world's major food crops were collected from throughout the world and preserved at international centers belonging to the CG system (the Consultative Group for International Agricultural Research, also known as the CGIAR) and various other national and international facilities. From the late 1980's weaknesses in the *ex situ* conservation began to be identified. Some pointed out that the evolutionary process that gave rise to genetic diversity is stopped in the cold storage of *ex situ* conservation (Harris, 1989). Many were also worried about the concentration, and thus control, of agricultural genetic resources in developed countries and international centers, and the lack of recognition of the contributions of developing countries and farmers (Fowler and Mooney, 1990). *In situ* conservation is seen by many to be the answer to these problems, and has since received much attention and efforts (e.g. see Smale, 1998; Brush, 1999; Almekinders and De Boef, 2000).

According to the United Nations Convention on Biological Diversity (UN CBD), *in situ* conservation of germplasm involves "the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties" (Reid et al., 1993). For natural populations of wild species, which have reached a steady state or "climax" in a given environment, preserving the environment would then maintain the habitat and so conserving the populations. Throughout the 1990's much efforts and resources have been expended towards *in situ* conservation of crop germplasm as if they were wild species, but it is becoming increasingly clear that this has not worked (Louette, 1999; Almekinders and De Boef, 2000; Julian Berthaud, in CIMMYT, 2001).

To preserve "domesticated or cultivated species, in the surroundings where they have developed their distinctive properties" is to ask farmers to keep their cropping systems in the state of suspended animation. This is unrealistic as well as unjust. It may be possible to provide redress to the economic equity problem by paying farmers to keep their old cropping systems and their traditional varieties. However, such a system of *in situ* conservation will serve no different purpose from the *ex situ* conservation system, but with an added burden of much more complicated management logistics. Furthermore, it has been argued that past evolution of diversity may not be reproduced in such "museum farms", and any genetic changes that may take place in them may be totally irrelevant to future needs (Holden et al., 1993).

Agricultural germplasms are shaped by social and economic as well as biophysical factors. Agricultural habitats and selection pressures are very different from natural ones in

that they are continually modified by management decisions of individual farmers responding to various socio-economic and physical factors and technological breakthroughs. Agricultural habitats are also changeable in a time frame that is much shorter than in natural ecosystems. They can also be highly fragmented into many different niches, often even on single farms, where different genotypes may exist side by side for managerial as well as ecological reasons. Crop genetic systems are subject to the process of human selection and manipulation, which have become most precise and drastic with the advent of molecular biology and genetic engineering. The comparative advantage of *in situ* conservation lies in the capacity of *in situ* populations to store large number of alleles and genotypes (Brown, 1999).

High Yielding Varieties (HVYs), the hallmark of the Green Revolution, are now grown in almost all of the rice cultivating countries of Asia, from China, India, Indonesia, Malaysia, Philippines to Vietnam. Rice yield in individual countries has doubled or tripled, but at the cost of local rice varieties being almost completely replaced by modern HYVs. Thailand is an exception, the new HYVs make up only 20% of its main rice crop, local rice varieties are still grown in farmers' field in many areas of the country. Local rice varieties remain a key component of many rice-based agroecosystems in the country, especially in the North. Northern Thailand lies in the heart of the primary centre of diversity for rice, which extends over remote mountainous neighbouring areas of India, Myanmar, China, Laos and Vietnam. The region is of strategic importance for sustainable management of the world rice genetic system. As Thailand moves along its path in development, even with occasional stumbling like the economic crisis of 1997, the key question for *in situ* conservation is whether there is room for it in the country's rice fields of the future. To answer this we suggest looking at (a) niches for different rice varieties in Thailand's rice-based cropping systems, and (b) structure and dynamics in the local rice genetic systems.

THE NICHE FOR LOCAL RICE GENETIC RESOURCES IN THAILAND'S RICE-BASED CROPPING SYSTEMS

A survey by the Office of Agricultural Economics found over 10 million rai (1.6 million ha) of traditional rice varieties still grown throughout the country in 1996 (Table 1). For efficiency in the production system as well as long term prospect for *in situ* conservation it will be useful to identify how much of the current traditional rice area has resulted from inertia in the extension process and how much has resulted from real biophysical, economic and social constraints. The rice area under traditional varieties is spread through all the four regions (Table 2). Before lack of availability and access to improved varieties is considered as the reason for persistence of traditional varieties, it should be pointed out that research stations or centres of the Thai Rice Research Institute have been located in many of these provinces for half a century or longer, long before the arrival of the Green Revolution in the early 1970's. Some, e.g. Chinat, Surin, Hantra in Ayuthya, and Koksamrong in Lopburi, are among the country's oldest and most famous rice research stations where several "improved" rice varieties have been developed. In other words, lack of access to "improved" varieties is not likely to be the main reason for continued use for many of the local varieties.

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Table 1. Distributions of rice varieties grown in different regions of Thailand, wet season 1996.

Type of varieties	Planted area, by region *(rai)*				
	North	Northeast	Central	South	Country
Traditional	3,014,894	3,094,039	2,607,659	2,082,006	10,798,598
RD6†	2,328,716	13,630,741	3,607	23,962	15,987,026
RD15†	223,709	1,477,434	60,019	19,759	1,780,921
KDML105†	904,483	11,048,752	1,071,809	89,398	13,114,442
Selected traditional‡	2,110,234	1,235,470	1,805,123	76,423	5,227,250
HYVs	4,281,649	1,202,151	4,308,705	590,341	10,382,846
Total	12,863,685	31,688,587	9,856,922	2,881,889	57,291,083

† RD6 and RD15 are derived from KDML105 by mutation with radiation

‡ Local traditional varieties that have be selected by pure line method and released by the Rice Research Institute of Thailand.

Source: Adapted from OAE, 1998.

Table 2. Areas of traditional rice varieties in Thailand

Region: provinces	Area *(rai)*	% of rice land in region
North: Kamphaeng Phet, Sukhothai, Phitsanulok, Pichit, Nakhon Sawan, Uthai Thani, Phetchabun	2,609,751	20.3
Northeast: Loei, Udon Thani, Sakonakorn, Ubon Ratchathani, Srisaket, Surin, Burirum, Khon Kaen, Chaiphum, Nakorn Ratchasima	2,726,469	8.6
Central: Lopburi, Chainat, Ayuthaya, Nakhon Nayok, Prachinburi, Chachoengsao, Sakaew, Kanchanaburi	1,937,813	19.7
South: Chumporn, Surathani, Nakon Si Thammarat, Pattalung, Songkhla, Pattani, Narathiwat	1,743,562	60.5
Total	9,017,595	83.5†
Country‡	10,798,598	18.8

† % of traditional rice area in whole country

‡ Country total includes small areas in other provinces (< 100,000 *rai* each) not included in above total for each region, % of country rice area of 57.3 million *rai* planted to traditional varieties in the whole country

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For many important food crops, numerous traditional varieties or landraces continue to be grown, often along side the HYV's (e.g. potatoes in the Andes, maize in Mexico and wheat in Turkey, cassava in Peru and sorghum in Ethiopia (see review by Brush, 1995; and more extensive reviews for rice, wheat and maize in Smale, 1998; Pingali and Smale, 2002). The last two authors argued that intensification of crop production and productivity gains do not always have to be associated with losses of genetic diversity. Brush (1995) suggested that land fragmentation; marginal agronomic condition, economic isolation and cultural preference and identity are the major reasons for the continuing use and conservation of the landraces. Some of these factors appear to be operating in the case of rice in northern Thailand (Gypmantasiri et al., 1980; Rerkasem and Rerkasem, 1984; Rerkasem et al., 1994), but there may also be other factors. The probability that a Turkish farmer will choose to grow a traditional variety of wheat or not was shown to depend on a complexity of factors including grain quality, yield risk, market opportunity, climatic constraints and agronomic consideration (Meng, et al., 1998). That the continued use and preservation of traditional crop varieties by farmers is determined by a complexity of biophysical, economic and social reasons is increasingly being accepted in the literature (Pingali and Smale, 2000).

A picture of distribution of the various rice varieties, by province, in the whole country has been provided by a survey by the Office of Agricultural Economics in 1996/97 (OAE, 1998). The distribution of major varieties and types by region is shown in Table 1, and by selected provinces in Table 3. Notable are the predominance of HYVs in some provinces, traditional varieties in others and the KDML varieties (KDML105 plus RD6 and RD15, which were derived from KDML105 by mutation) in many others. Together KDML105, RD6 and RD15 accounted for more than half of all the rice planted in the country's main growing season, and almost the entire planted area in some provinces. Wide adaptation to variation in the biophysical environment of the varieties is clearly indicated. This is further enhanced by the shorter growing season of RD15, by about 2 weeks, thus extending the niche into areas with earlier ending of the wet season. The conversion of non-glutinous KDML105 into glutinous RD6 has enabled it to fit neatly into the niche in the upper part of Northern and Northeastern Thailand where glutinous rice is the staple. Being non-responsive to fertilizer, they are usually planted with minimum inputs. All three produce quality rice which find ready markets for local use as well as for export, especially for KDML105 and RD15 which are exported at premium prices as Thai Jasmine or Hom Mali. The HYVs, on the other hand, tend to be grown in irrigated area and are given much higher inputs of fertilizers and pesticides. The average yield for HYVs is about 50% higher than traditional varieties, including the KDML types (OAE, 1998). The price for HYV rice is, however, only about half that of Jasmine rice. What conditions then describe the niche for traditional varieties that are still grown, including in irrigated areas in some provinces?

The means to analyze and understand farmer's innovation and management of their cropping systems and crop genetic resources is here termed "agrodiversity" (Brookfield, 2001). Agrodiversity focuses on the dynamic variation in cropping systems, out put, and management practice that occurs within and between agroecosystems. These may be defined as four different aspects of variations in rice-based cropping systems, namely, diversity in the biophysical environment, diversity in farmers' management innovation and diversity in institutional arrangements and diversity in the local rice genetic resources themselves.

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Table 3. Distribution of major rice varieties in selected provinces, growing season 1996.

	Traditional	KDML type†	HYVs	Selected traditonal†	Total
			Area *(rai)*		
North					
Chiang Rai	83,753	860,210	47,678		991,641
Chiang Mai	31,494	392,722	8,078	84,146	516,440
Nakhon Sawan	1,039,489	185,852	970,260	95,228	2,290,829
Kamphaeng Phet	124,651	76,767	1,048,619	10,365	1,260,402
Phitsanulok	130,357	145,572	792,285	284,760	1,352,974
Northeast					
Udon Thani	131,579	1,492,798	63,065	352,501	2,039,943
Nongkai	48,104	1,048,475	40,369	29,048	1,165,996
Nakon Ratchasima	388,482	2,036,127	588,275	127,782	3,140,666
Ubon Ratchathani	162,959	2,490,683	111,376	126,789	2,891,807
Surin	473,682	2,351,466	0	0	2,825,148
Yasothorn	21,419	970,876	0	3,725	996,020
Central					
Ayuthya	354,515	0	319,345	201,250	875,110
Prachin Buri	407,390	121,808	16,054	188,928	734,180
Nakon Nayok	261,141	18,775	161,095	7,321	448,332
Kanchanaburi	163,947	10,269	197,600	3,223	375,039
South					
Surat Thani	193,677	25,117	67,179	29,670	315,643
Nakon Si Thammarat	603,207	32,116	239,749	946	876,018

† KDML105 plus RD6 and RD15 which have been developed from KDML105 by mutation through radiation
‡ Traditional varieties that have been selected by pure line selction and released by the Thai Rice Research Institute
Source: OAE, 1998.

Diversity in the biophysical environment

Rice in Thailand is grown in six basically different environment related primarily to water and sometimes temperature regimes. Upland rice is grown on dry soil. It is found from about 1,000 m in elevation in the northern part (up to 20o N) of the country down to just a few hundred meters further south (to about 14o N). Mountain wetland rice is grown in flooded soil, with water depth of 20-30 cm, in highland valleys and terraced fields at 600 › 1,000 m in elevation. Irrigated rice, for which the water depth can be controlled at 20 › 30 cm, accounts for some 25% of the country's lowland rice land. Lowland rain-fed rice is grown on relatively flat land, 400 m in elevation or lower, without water control. Drought is the primary constraint. Deep water and floating rice is grown in low-lying areas where water depth may reach several meters. These first five environments are in the wet season, with planting from May to August, harvesting from October to December. The sixth is dry season rice, grown where there is water for irrigation, from about January to June. Archeological evidence indicated that rice has been grown in the North (Gorman, 1969) and Northeast (Solheim, 1972) of the area now called Thailand for at least 6,000 years. Some of these agricultural habitats would have been

in existence for at least a few thousand years. The myriad local populations of rice in each habitat would have gone through as many episodes of meiosis and recombination, and as many seasons of the evolutionary process and selection by farmers. Local populations in these major habitats may be expected to be significantly different from one another, and this would be ground to make sure that each is conserved.

While it is generally known that the high yielding potential of rice HYVs is best expressed where irrigation is available, however, the percentage of rice area planted to HYVs correlated only slightly ($R^2 = 0.36$) with the percentage of irrigated rice land in each province (Figure 1). The relationship between the proportion of rice land that was planted to traditional varieties and irrigation is even weaker ($R^2 = 0.04$). Irrigation is still probably the single most effective factor that removes variation in the biophysical environment for rice. Potential for further increase in irrigation area for the whole of Thailand beyond the current 25% is constrained by numerous economic, ecological, social and political reasons. The picture is slightly different in the highlands, where cultivation of wetland rice is seen as one of the major ways to increase productivity and sustainability of production. Support for investment for the development of highland paddy began with foreign assistance programs, and continues today.

According to the Department of Land Development, only two fifths of the country's rice land is judged suitable for rice growing, about half are only moderately suitable, the rest is affected by some serious constraints (Siamwalla and Na Ranong, 1990). For example, some 3 million *rai* of the Central Plain (Supanburi, Ayuthya, Pathumthani and Nakorn Nayok) are affected by acidity with pH up to 4.5, with another 300,000 rai of acid sulphate soils, affected by extreme acidity of pH < 4.5. In addition, variations in water depth and the timing of inundation have created an enormous diversity in water regimes in the country's river valleys such as the Central Plain (Takaya, 1987). About 5.6 million rai of land in the Northeast are affected by salt with another 16 million *rai* of rice land identified as susceptible to salinization.

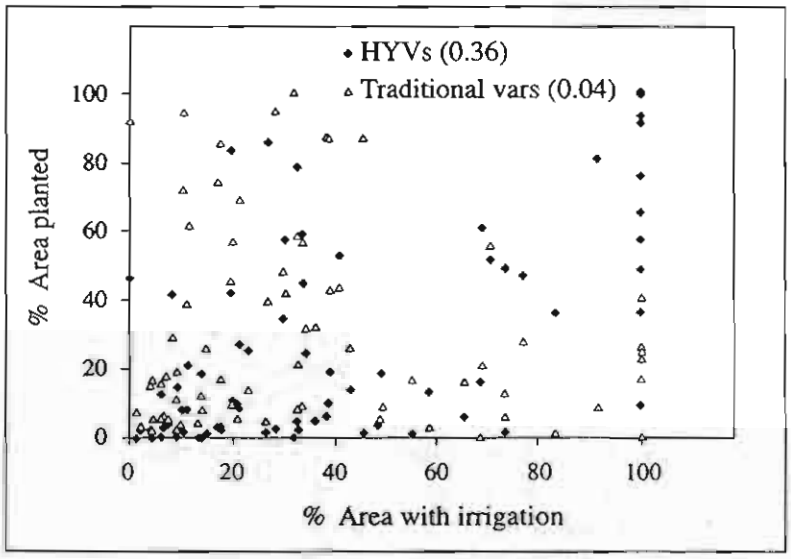

Figure 1. Relationships between percentages of rice land with irrigation and area planted to high yielding (HYVs) and traditional (Traditional vars) rice varieties in Thailand, by province (R2 in brackets).
Source: data from OAE, 1998.

In addition to these broad scale variations, farmer's choice of rice variety may be influenced by local or micro level of variations in the biophysical environment that can occur within single farms. Different varieties may be required for even small differences in water depth of 5-15 cm or delays of 2-3 weeks in field drainage at the end of the season (Rerkasem and Rerkasem, 1984). Places of great micro level variations in the biophysical environment may be found in the highlands, where differences in elevation, slope aspects and gradients and soils may occur over short distances, often within single farms. While there is yet no systematic inventory, there are many anecdotal reports of genetic richness of rice in the highlands of Northern Thailand (e.g. see Dennis, 1987; Pankao, 1996; Chantaraprayoon, 1997; Rerkasem et al., 2002). Differential adaptation in different rice varieties to these different biophysical niches is recognized by farmers. For example: *Bue Chomee* (wild fowl rice) is said to be better adapted to lower temperatures of highland paddies some varieties are more responsive to the improved condition of residual fertility and weed control in rotation with cabbage. Akha rice is believed to be good for poor soils. And so on (Rerkasem et al., 2002). Although less than 1% of the country's rice crop is grown in the mountainous highlands, highland rice is therefore of special importance to *in situ* conservation of Thailand's native rice genetic resources.

At the broad-scale or macro level are variations in soil, temperature and rainfall in the whole of Thailand that have already been characterized and mapped, agricultural zones have been demarcated. Detail maps of the country's soils and agricultural zones are accessible from the website of the Department of Land Development (www.ldd.go.th). Variation at the fields and farms is poorly defined. Although such micro level variations could be characterized with a new technology of "precision agriculture", now being promoted as a fertilizer management tool on individual farms in developed countries. Logistic, economic and technical constraints together make it impossible to imagine such precision being applied in resource poor farms of developing countries. Farmers are the only source of this crucial information. Obviously it is not possible to ask every farmer and neither are all farmers equally knowledgeable. Patterns of local variation may be derived from information provided by those farmers who are well informed on local variation in the biophysical environment (Suthi, 1985; Rerkasem et al., 2002), supported by strategic measurements and instrumentation.

Because different crop genotypes may be adapted to different habitats, diversity in the biophysical environment is the primary basis for diversity in local crop genetic resources. However, field fragmentation is generally considered inefficient in the management of large commercial farms. On small farms it would be tolerated and so local crop genetic diversity preserved only if it does not interfere with farmers' management objectives. Local varieties will be maintained only if they are part of sufficiently productive cropping systems that can meet the need of the farm household better than other alternatives. Such diversity of rice-based cropping systems were commonly found in the Chiang Mai Valley in the early 1980's, and each of the condition for a rice variety was termed "agroecological niche" (Rerkasem and Rerkasem, 1984). They have also been found with other crops in other parts of the world, the term "mutiniche" has been suggested for habitat fragmentation which is economically and socially viable (Bellon, 1996).

Diversity in farmers' management and innovation

Farmers' management and innovation affect local genetic resources in two different

ways. A farmer may influence local crop genetic system by his/her direct choices over the genetic stock, or through various agronomic practices and care given to the crop. The fate of a local rice variety is determined by the collective choice of individual farmers who may choose or not to choose to grow and perpetuate it. Unlike the pure line varieties from plant breeders, local crop varieties are commonly genetically heterogeneous. Genetic make up of a local variety may be affected by how its seed is propagated from season to season, and whether selection pressure is applied by the farmer when he/she pick the seed for growing in the next season. Different varieties are sometimes deliberately mixed together for various agronomic and other reasons (Dennis, 1987). Genetic shifts may also occur with changes in cultivation practices from land preparation, planting, fertilization, pest management to harvest and seed storage. The diversity of habitats and different ways in which the crop genetic system is manipulated are the condition on which local genetic diversity of a particular crop may be derived.

Rice farmers in Thailand have been practicing selection of their seed stock long before anyone even thought about "participatory plant breeding". Evidence of this may be seen in the 20,000 entries of local rice varieties in the national genebank (Vutiyano, 2000). Some of these would have been results of farmer's selection from existing diverse populations. Others could most probably have arisen from progenies of wild X cultivated rice hybridization that commonly emerged in rice fields throughout the country (Oka, 1988; Chitrakorn, 1995). In the field during harvest time in 2001 we saw again the practice common in the Chiang Mai Valley in the 1980s (Rerkasem and Rerkasem, 1984) in which some farmers selected seed for next season planting from panicles with specific appearances. Many who grow glutinous rice also believe that unless they practice seed selection the eating quality will deteriorate and cooked rice becomes hard. Others believe that changing their seed stock by reintroduction of the same varieties from other areas will "re-invigorate" the variety. It would be useful to know what impact these practices have on diversity in the local rice genetic resources, and what will happen when their function is no longer valued on farm. The use of "certified seed", in which genetic homogeneity is ensured, will certainly limit variation within populations and opportunity for selection by farmers.

In addition to the conscious selection of seed stock, which directly affects genetic make up of varieties, the rice genetic system may also be influenced by cultivation practices. Some of these are in the form of simple variety replacement. Thus irrigation would have replaced deep water and floating rice varieties with those that require better water control. When shifting cultivation is replaced by wetland rice in the highlands, whole sets of upland rice germplasm disappeared. Other changes are less obvious. Improved growing condition for upland rice in rotation with cabbage in the highlands means that preference would be given to those varieties that are able to respond to the better condition with higher yield. Another set of information that would be useful to *in situ* conservation of Thailand's native rice germplasm would include answers to the question how local rice genetic system is affected by "modern" practices in the production system. These included planting method, double cropping, chemical weed control, use of chemical fertilizer, combined harvesting, the use of new rice varieties, from those which are more genetically compatible with local wild rice to transgenic rice. In Vietnam, weedy rice has been reported to have become invasive in the south where rice is direct seeded and not in the north where it is transplanted, and more serious in the summer › autumn crop than in other seasons (Chin et al., 2000). Farmers in Kanchanburi and Nakorn

Nayok reported that wild rice that had existed for a long time in the village swamps had become invasive in the rice field since the arrival of combined harvester and chemical weed control.

Diversity in institutional arrangement

As part of the Green Revolution, habitats for wetland rice in Asia were made uniform by large publicly funded irrigation development projects. These provided support for land leveling, irrigation water and support programs that guaranteed cheap and sometimes free inputs of fertilizers and pesticides as well as guaranteed market and prices. Farmers in the Philippines were persuaded to grow "improved" instead of their own varieties by government supported programs that excluded traditional varieties from various services provided (Basilio and Razon, 2000). The adoption of the Green Revolution rice in Asia, from India to Indonesia, was strongly persuaded with various supports and incentives by the government that sometimes enforced at the point of the gun (Pretty, 1995).

Clearly, government actions do not necessarily always have to lead to losses of niches and diversity. The conventional procedure for centralized rice breeding programs in Thailand is the "official adoption" of local "elite" varieties. Genetically heterogeneous local populations have been genetically homogenized through the "pure line selection" method, i.e. the whole population of a particular variety becomes genetically homogenous as every plant is descended from one single homozygous parent. Thus a local elite from Bangkhla near Bangkok named Khao Dawk Mali, became KDML105, Pingaew became Pingaew 56, Nahng Mon became Nahng Mon 4, Muey Nawng became Muey Nawng 48E and Muey Nawng 62M, and so on. It would be useful for national breeding programmes to re-examine a suggestion made many years ago (Allard and Bradshaw, 1964) that gene diversity in populations may bring about populational buffering to stabilize yield. A recent study in China showed experimentally that genetic heterogeneity in the field can help to overcome the vulnerability of crops to diseases (Zhu et al., 2000).

Local traditions and institutions that may affect usage and conservation of crop genetic resources may vary from ceremonial and ritualistic roles of some crop varieties to customary rules governing usage of common resource including sharing and exchanges of germplasm, to market and trade arrangements. In some areas the management of hired or exchanged labor requires that certain crop management practices such as transplanting and harvesting of different fields is staggered over a length of time. In such areas different varieties that require to be planted and harvested at different times will always be needed (Rerkasem and Rerkasem, 1984). Such needs can vary among ethnic groups and from place to place. The effect of cultural difference is most clearly illustrated by the change of dominance between non-glutinous KDML105 and glutinous but otherwise closely related RD6 rice in the North and Northeastern provinces. KDML105 dominates in all those provinces where non-glutinous rice is staple and where glutinous rice is staple RD6 becomes dominant (Table 4). Where crop genetic resources are treated as common property, and are readily exchanged and shared, many will contribute to its conservation and selection, and so genetic variation. Those heirloom varieties that are jealously guarded within clans and families are likely to be unique. The application of intellectual property laws to new crop varieties is generally expected to provide private incentives for crop genetic improvement. With the intention to encourage conservation, Thailand's New Plant Variety Protection Act 2542 also provided protection to community's

right to traditional varieties. It remains to be seen if these legislation have the intended effects. Various other forms of institutional arrangements apart from the national government and its formal laws, regulations and development policies, may also influence local crop genetic resources.

Table 4. Choice between glutinous (RD6) and non-glutinous (KDML105 and RD15) of closely related varieties† in some provinces of Thailand.

Province	RD6	RD15	KDML 105	Total KDML† area
		%		*(rai)*
North				
Chiang Rai (G)‡	87.1	10.4	2.5	860,210
Payao (G)	76.9	20.6	2.5	445,846
Lampang (G)	93.8	0.3	5.9	285,198
Lampoon (G)	88.4	1.4	10.2	105,131
Chiang Mai (G)	88.0	1.5	10.4	392,722
Mae Hong Son (NG)	5.4	-	94.6	40,783
Tak (NG)	25.4	2.2	72.4	92,572
Kamphaeng Phet (NG)	-	9.5	90.5	76,767
Sukhothai (NG)	34.4	13.5	52.1	183,238
Phrae (NG)	96.8	2.6	0.5	153,874
Nan (NG)	100.0	-	-	17,009
Uttaradit (NG/G)	45.2	-	54.8	87,084
Phitsanulok (NG)	35.8	-	64.2	145,563
Pichit (NG)	-	-	100.0	50,575
Nakon Sawan (NG)	36.1	-	63.9	185,852
Uthai Thani (NG)	-	-	100.0	14,522
Petchabun (NG)	36.4	-	63.6	320,768
Northeast				
Loei (G)	88.9	-	11.1	131,320
Nongbua Lampoo (G)	91.3	-	8.7	722,913
Udon Thani (G)	92.1	4.2	3.6	1,492,798
Nongkai (G)	90.7	4.0	5.3	1,048,475
Skol Nakorn (G)	90.9	-	9.1	1,272,770
Nakon Panom (G)	69.7	3.5	26.8	669,264
Mookdaharn (G)	95.6	-	4.4	323,626
Yasothorn (NG/G)	44.3	16.7	38.9	970,876
Amnaj Charoen (G/NG)	49.5	2.9	47.6	921,890
Ubon Ratchatani (G)	60.9	2.9	36.1	2,490,683
Srisaket (NG)	10.3	5.4	84.2	2,348,563
Surin (NG)	3.6	13.2	83.2	2,351,466
Burirum (NG)	17.9	9.5	72.6	2,053,461
Mahasarakam (G)	74.6	8.5	16.9	1,534,349
Roi-et (G)	56.5	3.8	39.7	2,168,133
Kalasin (G)	83.3	-	16.7	950,823
Khon Kaen (G)	85.2	1.8	13.0	1,769,046
Chayapoom (G/NG)	45.7	8.7	45.6	900,344
Nakon Rachasima (NG)	20.5	6.4	73.1	2,036,127

† Combined area under nonglutinous KDML 105 and RD15 and glutinous RD6. Both RD 6 and RD 15 are derived from KDML105 by mutation with radiation.
‡ Province where glutinous (G) and non-glutinous (NG) rice is main staple.

The arrival of markets may mean that farmers' choice of varieties will be determined elsewhere as well as locally. The market is not necessarily always an enemy of diversity but may actually help to enhance it. It has been shown that income elasticity of demand for special quality grains may be higher than the income elasticity of demand for the cereal itself (Pingali et al., 1997). For example, an increasing interest in blue corn, waxy Hmong corn or hand milled hill rice in city markets may add an economic incentive for certain groups of farmers to maintain their traditional varieties. Thus special quality rice such as Basmati rice from Pakistan and India, Jasmine rice from Thailand and traditional japonica rice in Korea and Japan are fetching premium prices. Many different kinds of rice, with variously different pericarp pigmentation, can now be seen the rice retail market throughout Thailand. Some hilltribe farmers in Northern Thailand now regularly sell their own hand milled hill rice and with the proceeds buy twice to four times as much rice from the lowland market. In Chiang Mai some farmers find ready market and good prices for special rice that are fed to prized fighting cocks.

Within this broader picture, niche diversity may still be found because of numerous different variations and combinations of the socio-economic conditions of individual farm households. Some poor farmers in the North and Northeastern regions of Thailand find the good eating quality of RD 6 to be a disadvantage in their household economics. They reasoned that because "it tastes so good, we tend to eat too much, so the harvest runs out before the next season crop is ripe". Farmers in some rainfed rice area in Chiang Mai choose to grow traditional tall varieties because they can also sell the straw as mulch for garlic, shallot and onion. Ethnic minority groups have special varieties that are preferred for home consumption. Traditional varieties are also kept for medicinal purpose, as "heirlooms" ("our mother/ grandmother said to keep this"), or even as "pet rice" ("we are not sure why we keep this, but we like it").

DIVERSITY IN THAILAND'S LOCAL RICE GENETIC SYSTEM

How much diversity remains in Thailand's local rice germplasm? Which processes contributed to genetic changes in the past, which of them are likely to continue to do so into the future? With only one fifth the country's rice land planted to traditional local varieties, many of the old varieties have clearly disappeared from farmers' field. However, planted area tells only partial and incomplete story, and similarly number of traditional varieties that are still grown. Most papers on *in situ* conservation refer to the names and types recognized by farmers. Diversity, by definition, is measurable by the statistical term of "variance". In applied genetics, it refers to the variance of "a gene" within a population. Thus the variance may be measured among alternative forms (polymorphism) of a gene (alleles) at individual gene positions on a chromosome (loci), among several loci, among individual plants in a population or among populations (Brown et al., 1990). Diversity may be estimated from variances of morphological or physiological expressions of the gene. With the advent of molecular genetics, we can now measure the variance of actual DNA sequences of a gene or a specific length of DNA (a DNA marker). In addition to quantifying diversity by measuring the variance of genes and DNA markers within and between populations, an understanding of the structure and dynamics of the diversity and their causation is crucial to the management

of crop genetic systems. Furthermore, "coancestry" of homologous genes in individuals and populations of local varieties, and the evolutionary forces affecting the whole genome may be learned from estimates of marker diversity (Brown, 1999). Such knowledge would inform decisions on which populations need to be conserved and what conditions are needed to enhance *in situ* conservation in the long run.

The structure of diversity

The *ex situ* collection of rice germplasm at the National Rice Genebank, which began in 1937, holds 24,000 entries. Among these there were 5,900 that did not share the same name (Vutiyano, 2000). Number of named varieties is misleading. Among the 6,000 entries that have so far been characterized by various morphological and some physiological traits (Vutiyano, 2001), varieties with the same name were often clearly different. Some generic names, especially by colour of the husk, e.g. red, white, yellow, and so on, are often given to very different rice from different parts of the country. *Luang On* (soft yellow) was one of the most popular names, it was borne by 32 different entries. Some of the other popular names were *Tong Ma Eng* ("gold that came by itself", 9 entries), *Kao Daeng* (16 entries for "red rice"), *Khao Tahaeng* (19), *Khao Puang* (17), *Luang* (18 entries for yellow), *Luang Thong* (24 entries for "golden yellow"), and *Luang Pratew* (12). A total of 34 populations of rice were collected from Rangsit and Ayuthya with the name *Pingaew.* Apart from obvious morphological (grain shape and size, grain quality) and physiological (some were deep water rice some were regular wetland rice) differences, DNA analysis of 36 SSLP (Simple Sequence Length Polymorphism) markers showed that all except 4 of the populations differed from one another by more than 50% (Vanavichit et al., 2001).

In contrast to "improved" varieties that come out of breeding programs, local varieties carry a great amount of genetic diversity within individual populations. Genetic diversity in both morphological traits and isozymes were observed in one population collected from the lowland and one from the upland of Chiang Mai (Oka, 1988). However, this aspect of diversity existing in local rice germplasm remains to be investigated. Studies of local germplasm often treat each named variety as genetically homogenous (e.g. Chitrakorn, 1995; Pankao, 1996; Chantaraprayoon, 1997). Variation in morphological traits such as the presence of awns, hull and pericarp colour and some physiological ones such as time of heading can be commonly observed in farmers' field. Some of the variation may have resulted from accidental and random mixing of seeds. Some farmers may also deliberately mix seeds from different varieties. Isozyme analyses of "admixtures" showed that some were indeed random mixtures of discreet types, but others exhibited continuous variations that indicated natural heterogeneity (Dennis, 1987). Some variations are recognized as useful by farmers, as long as they do not interfere with or may be useful in crop management or usage. *Bue Chomee* (wild fowl rice), one of the most popular varieties for highland paddy in Northern Thailand contains considerable variation in heading dates, but matures uniformly. Variation in dates of heading is valued for the flexibility provided against gall midge, one of the region's most prevalent insect pest that is most damaging at panicle initiation. A measure of yield stability is provided by those panicles that initiated at different times and so escaping damage. *Bue Chomee* also cooks uniformly. Most of the women farmers, who are usually responsible for cooking, insist that rice that are accidental or random mixtures are not acceptable because they cook badly, as

different types would require different length of time to cook. The within variety genetic diversity could be an important component of *in situ* conservation of rice genetic resources. At Chiang Mai University we are investigating within variety diversity of *Bue Chomee* and other popular local varieties such as *Bue Polo* (large grain rice), *Bue Hmong* (Hmong rice, responsive to improved condition in rotation with cabbage and other highly fertilised vegetables) that have arisen from farmers' selection.

The six major biophysical environments listed above may be a good starting place to conceptualize the structure of the genetic system of Thailand's native rice. Adaptation barriers, especially lethal ones, could indicate separation of genepools. An obvious example would be the limited chance of survival in low lying areas of the Central Plains for rice from other environments without flood tolerance and/or "floating ability", ability to keep up with rising flood water by stem elongation. Rice from the Central Plain sets seed poorly, producing largely empty grain, when grown in the North, and similarly when rice from the lowlands is grown in the highlands. Unlike natural species, crop plants not only have to survive a move into a new environment but must also be reasonably productive. Generally upland varieties will survive and produce seed under wetland conditions, and *vice versa*. However, varieties that are equally productive under dryland and wetland conditions are rare, *Sew Maechan* and *Kae Noi* are two known exceptions. Photosensitivity prevents most local populations from being grown in the dry season. Although many would flower in the longer days of April and May, most of them produce only a few panicles and are not sufficiently productive as dry season crop. Ecological, management, technological and other considerations may provide the basis for further differentiation of populations. Populations may also be very different if they had been separated for a very long time. Truly indigenous populations of lowland rainfed rice from the neighbourhood of the two prehistoric sites of earliest records of rice, the Spirit Cave in the North (Gorman, 1969) and Non Nok Tha in the Northeast (Solheim, 1972) might be expected to be very different. A group of glutinous rice from the Northeast has been found with grain shape that was clearly distinct from non-glutinous rice and glutinous rice from other parts of the country (Figure 2).

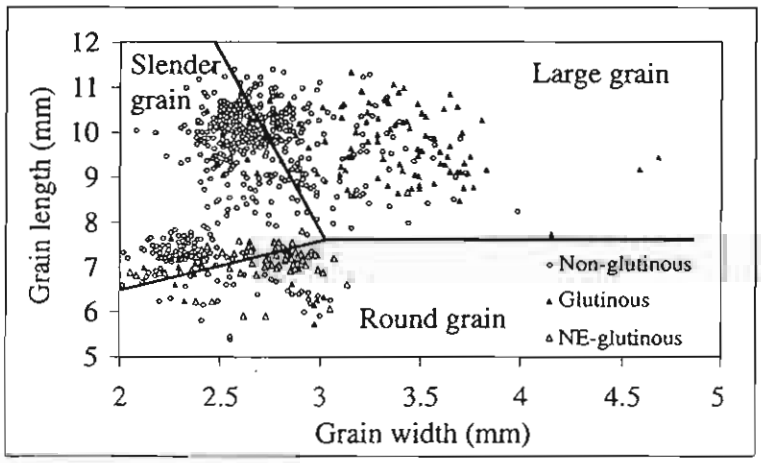

Figure 2. Distribution of grain size (length:width) of a set of glutinous rice germplasm from the Northeast (NE-glutinous) compared with non-glutinous (Non-glutinous) and glutinous (glutinous) rice from the rest of the country. The lines separating grain with different shapes were adapted from Oka (1988).
Source: Plotted with data from OAE (1998)

Genetic changes over time

Local use and management of rice germplasm has influenced local diversity of rice genetic resources in the past in two major ways. The first is by introduction of new genetic materials from elsewhere, and the second is by selection from existing diversity, from within populations as well as between them. Introduction of new germplasm from elsewhere was common even when it was not so easy to travel around the country (Rerkasem and Rerkasem, 1984). Information about promising varieties and seeds were disseminated by those with opportunities to travel such as traders, migrants and those visiting relatives. Rice brought from "home" is still grown by many recent migrants who arrived in Thailand in 1950's from China, Myanmar and Laos. The pace and range of new germplasm introduction has increased with "modern" development and improved communication and transportation in the past 50 years or so.

The establishment of the Rice Department in 1954, later renamed Rice Research Institute, has been instrumental in germplasm exchanges between different regions and introducing really "exotic" germplasm, from outside the country, including those that are products of modern plant breeding even incorporation of genes from wild relatives of rice. Some of these have lost their original official names and been incorporated into the "local germplasm". In Kamphaeng Phet, a very popular "local" variety known as *Cee See* turns out to be an early HYV imported from the Philippines named C4-63 introduced into Thailand in the mid 1960's. The name *Bue Kaset* is often encountered among local Karen rice names (*Bue* is rice in Karen). Close enquiries found that the name refers to many different kinds of rice, including HYVs, that have originated from governmental, non-governmental and foreign aid programmes that were identified with *Kaset*, a local euphemism for modern agriculture.

Examples of contributions from recent introductions into the local germplasm are provided by Supanburi 1 (released 1994) and Pathumtani 1 (released 2000). Supanburi 1, an HYV, is commonly grown in double cropping, irrigated area around Kanchanburi and Ratchaburi, northwest of Bangkok. It's breeder's code is SPR85163-5-1-1-2, and its pedigree is IR25393-57-2-3/RD23//IR27316-96-3-2-2///SPRLR77205-3-2-1-1/SPRLR79134-51-2-2 (Somrith and Chitrakorn, 2001). Supanburi 1 in farmers' field, however, appears to be genetically diverse and very different from the certified Supanburi 1 from the national Rice Research Institute. A "mixing in" with local germplasm, including wild rice, is suspected. This may have happened mechanically with the spread of combined harvesting, or genetically by geneflow through local wild rice (see below). Pathumtani 1, a semi-dwarf, non-photosensitive, aromatic rice, is a potential source of genes from *Oryza nivara*. The parentage of Pathumtanit 1 includes IR50, which had incorporated genes for resistance to grassy stunt virus from the wild rice, O. *nivara* (Chitrakorn, pers. comm.).

Rice is largely self-fertilizing. Even at the 0.03% › 0.1%, natural cross fertilization contributes significantly to geneflow between genotypes (e.g. Brown, 1957; Rea?o and Pham, 1998). A much greater extent of geneflow can be expected to be mediated by the cross-fertilizing wild rice (*Oryza rufipogon*) which is common throughout the country. Numerous observations have been made of "hybrid swarms" between wild and cultivated rice in Thailand (Oka and Chang, 1961; Morishima et al., 1984; Chitrakorn, 1995). Our survey of the Chiang Mai Valley in the early 1980's found that farmers were very much aware of these new forms

(Rerkasem and Rerkasem, 1984). Because of their largely weedy habit, most were avoided when panicles were selected for seed. But plants exhibiting hybrid vigor were sometimes observed (Morishima et al., 1984), they would presumably be progenitors of emerging new local varieties. The process of geneflow through wild rice is therefore likely to have played a crucial role in past evolution of local rice germplasm. This raises two other issues for future *in situ* conservation: (i) the emergence of weedy rice as serious weed and (ii) the possibility of "contamination" of local genepool by genetically modified rice.

Weedy rice, first noted in Malaysia in 1988, the Philippines in 1990 and Vietnam in 1994, is suddenly becoming a serious problem in the rice fields of Asia (Mortimer et al., 2000). In Thailand invasive populations of wild rice have been found in Kanchanaburi, Ratchaburi, Nakorn Nayok and several provinces in the Northeast in 2001 › 2002 (Chanya Maneechote, pers. comm.). The cause of this sudden invasiveness is still to be identified, and will probably vary from place to place. For example, the weedy rice in Malaysia has been shown, by means of DNA fingerprinting, to be very different from wild rice as well as the crop rice it had invaded, (Mortimer et al., 2000). On the other hand, several signs of introgression were exhibited in rice fields in Kanchanaburi, Thailand. Gene flow between species is suggested by the appearance of many domesticated traits (e.g. prolific reproductive capacity, lower dormancy, husk and pericarp colour, grain shape and size, grain quality, panicle type, awnlessness, shattering resistance and photoperiod response) in the wild population and wild traits (awns, stigma colour and exertion, grain type, pericarp colour, shattering, etc.) in the cultivated population. Where weedy rice has resulted from introgression between wild and crop rice, an obvious dilemma has been raised for *in situ* conservation of wild rice population. Heavy infestation can mean complete crop failure (Puckridge et al., 1988; Chin et al., 2000). The problem of weedy rice is a serious threat to rice production so that they have now become targets for eradication (Mortimer et al., 2000). The implication of wild rice eradication on the process of geneflow and diversity in cultivated rice should be carefully considered.

Anyone concerned about "contamination" of local *Oryza* genepool should be reminded that geneflow is an ongoing process that has been going on for a long time. Large scale introduction of "exotic" rice germplasm into rice fields in the country probably began about the same time as the Green Revolution. RD1, Thailand's own first HYV, a progeny of a cross between IR8 and Leuang Thong, was released in 1969 (Somrith and Chitrakorn, 2001). Other HYVs that followed had various foreign germplasm in their parentage, e.g. TN1 (Taiwan), Sigadis (Indonesia), C4-63 (early Philippines HYV), and various IRRI germplasm featuring wild rice in their pedigree. These "foreign" genes that have been incorporated into the local genepool for some 40 years have at least all come from within the species *Oryza sativa*, or its close relatives with the same genus *Orzya*. Introduction of transgeneic rice would mean potential for geneflow from transgenes from others species. An obvious cause for concern would be herbicide resistace in a genetically modified rice that could be incorporated into local wild rice populations.

THE NICHE FOR LOCAL RICE GENETIC DIVERSITY IN THAILAND'S FUTURE CROPPING SYSTEMS

According to Brown (1999) conservation of genetic resources may have any of the following aims, individually or together:

1. Conserving the maximum number of multilocus genotypes and mximum allelic richness;
2. Safeguarding the evolutionary processes that generate new multilocus genotypes; and
3. Improving the population performance and increasing the productivity in a defined range of local environments.

Some have suggested that to conserve local genetic resources it may be necessary to conserve the whole agricultural systems (Qualset et al., 1997). Agricultural systems, however, have always been changing and will continue to change. How then may the above conservation objectives be reached in agricultural systems that must change and evolve to meet the needs and opportunities of those who make a living from growing rice? Conflicts, and possible trade-offs, can occur between the conservation objectives. Indeed, modern plant breeding has done so well by the improvement of population performance and increasing productivity (objective 3). Its very success has led to the increased dominance of the few improved varieties and displacement of local germplasm, and thus threatening objectives 1 and 2 in the first place. There may also be conflicts between the conservation objectives, which may not be those of farmers' or local communities but driven by national needs and aspiration, and farmers' production and livelihood objectives.

Most ideal in conservation are those "win-win" situations in which local people are able to make a decent living while resources are being conserved. Understanding the agrodiversity of local rice genetic systems as presented above is expected enable such win-win situations involving local rice genetic resources to be identified, and the conditions for their success explained so that they may be encouraged in other locations. Furthermore trade-offs between the various sets of objectives, production vs. conservation and local vs. national, may be weighed and addressed.

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REFERENCES

Allard, R.W., and A.D. Bradshaw. 1964. Implication of Genotype-Environmental Interaction in Applied Plant Breeding. Crop Science 4: 503-508.

Almekinders, C., and W. De Boef. (eds). 2000. Encouraging Diversity. Intermediate Technology Publications, London.

Azmi, M., M.Z. Abdullah, B. Mislamah, and B.B. Baki. 2000. Management of weedy rice (*Oryza sativa* L.): the Malaysian experience. p. 91-96. In B.B. Baki, D.V. Chin and M. Mortimer (eds) Wild and Weedy Rice in Rice Ecosystem in Asia – A Review. IRRI Limited Proceedings No. 2. IRRI, Los Baños.

Bellon, M.R. 1996. The dynamics of crop infraspecific diversity: A conceptual framework at the farmer level. Economic Botany 50: 26-39.

Basilio, C., and M. Razon. 2000. The use of rice genetic resources by farmers in Nueva Ejica, The Philippines. p. 89-94. In C. Almekinders and W. De Boef (eds) Encouraging Diversity. Intermediate Technology Publications, London.

Brown, A.H.D. 1999. The genetic structure of crop landraces and the challenge to conserve them *in situ* on farm. p. 29-48. In S.B. Brush (ed) Genes in the Field. International Plant Genetic Resources Institute, Rome and International Development Research Centre, Ottawa.

Brown, F.B. 1957. Natural cross pollination in rice in Malaya. Malay Agric. J. 40: 264-267.

Brush, S.B. 1995. In situ conservation of landraces in centres of crop diversity. Crop Science. 35:346-354.

Brush S.B. (ed) 1999. Genes in the Field. International Plant Genetic Resources Institute, Rome and International Development Research Centre, Ottawa.

Chang, T.T. 1976. The origin, evolution, cultivation, dissemination, and diversification of Asian and African rices. Euphitica 25: 425-441.

Chang, T.T. 1989. Domestication and the spread of the cultivated rices. p. 408-417. In D.R. Harris and G.C. Hillman (eds) Foraging and Farming: the Evolution of Plant Exploitation. Unwin-Hyman, London.

Chantaraprayoon, P. 1997. Isozyme Differentiation and Yield Performance of Local Upland Rice. MS Agriculture (Agronomy) Thesis, Graduate School, Chiang Mai University, Chiang Mai, Thailand.

Chin, D.V., V.H. Tran, and L.V. Thiet. 2000. Weedy rice in Vietnam. p. 45-50. In B.B. Baki, D.V. Chin and M. Mortimer (eds) Wild and Weedy Rice in Rice Ecosystem in Asia – A Review. IRRI Limited Proceedings No. 2. IRRI, Los Baños.

Chitrakorn, S. 1995. Charaterization, evaluation and utilization of wild rice germplasm in Thailand. Pathum Thani Rice Research Center, Thailand Rice Research Institute, Bangkok.

Dennis, J.V. 1987. Farmer Management of Rice Variety Diversity in Northern Thailand. Ph.D. Thesis, Cornell University.

Fowler, C., and P. Mooney. 1990. Shattering: food, politics and the loss of genetic diversity. University of Arizona Press, Tucson.

Fu, Y., and A. Chen. 1999. Diversity of upland rice and of wild vegetables in Baka, Xishuangbanna, Yunnan. PLEC News and Views 12:15-19.

Gong, Z., H. Guo, K. Zhou, S. Yin, and F. Wu. 2001. The current status of upland rice varieties diversity in Xishuangbanna – a case study from Baka, Daka and Mannalong villages. Acta Botanica Yunnanica Supplement XIII: 178-186.

Gorman, C. 1969. Hoabinhian: a pebble-tool complex with early plant associations in Southeast Asia. Science 163:671-673.

Gypmantasiri, P., A. Wiboonpong, B. Rerkasem, I. Craig, K. Rerkasem, L. Ganjanapan, M. Titayawan, M. Seetisarn, P. Thani, R. Jaisaard, S. Ongprasert, T. Radanachaless, and G.R. Conway. 1980. An Interdisciplinary Perspective of Cropping Systems in the Chiang Mai Valley: Key questions for research. Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand.

Harris, D.R. 1989. An evolutionary continuum of people-plant interaction. p. 11-26. In D.R. Harris and G.C. Hillman (eds) Foraging and Farming: the Evolution of Plant Exploration. Unwin-Hyman, London.

Holden, J.H.W., W.J. Peacock, and J.T. Williams. 1993. Genes, Crops and the Environment. Cambridge University Press, Cambridge.

Kaosa-ard, M., and B. Rerkasem. 2000. The Growth and Sustainability of Agriculture in Asia. Asian Development Bank's Study of Rural Asia: Beyond the Green Revolution, Volume 2. Oxford University Press.

Louette, D. 1999. Traditional management of seed and genetic diversity: what is a landrace. p. 109-142. In S.B. Brush (ed) Genes in the Field. International Plant Genetic Resources Institute, Rome and International Development Research Centre, Ottawa.

Meng, E.C.H., J.E. Taylor, and S.B. Brush. 1998. Implications for the conservation of wheat landraces in Turkey from a household model of varietal choice. p. 127-143. In M. Smale (ed) Farmers, Gene Banks and Crop Breeding: Economic Analyses of Diversity in Wheat, Maize, and Rice. Boston: Kluwer Academic Press and CIMMYT.

Morishima, H., S. Sano, and H.I. Oka. 1980. Observations on Wild and Cultivated Rices and Companian Weeds in the Hilly Area of Nepal, India and Thailand. Report of a Study Tour in Tropical Asia, 1979. Mishima, Japan: National Institute of Genetics

Mortimer, M., S. Pandey, and C. Piggin. 2000. Weedy rice: approaches to ecological appraisal and implications for research priorities. p. 97-105. In B. Baki, D.V. Chin, and M. Mortimer (eds) Wild and Weedy Rice in Rice Ecosystem in Asia – A Review. IRRI Limited Proceedings No. 2. IRRI, Los Baños.

Oka, H.I. 1988. Origin of Cultivated Rice. Japan Scientific Society Press and Elsevier.

OAE. 1998. Report on the Survey of Main Season Rice, 1996/97 Season. Agricultural Statistic Document No. 9/1998.

Pankao, P. 1996. Isozyme and Yield Differntiation of Rice Varieties Grown by Karen Community. MS Agriculture (Agronomy) Thesis, Graduate School, Chiang Mai University, Chiang Mai, Thailand.

Pingali, P.L., M. Hossain, and R.V. Gerpacio. 1997. Asian Rice Bowls: The Returning Crisis? CAB International, Wallingford, United Kingdom.

Pingali, P.L., and M. Smale. 2000. "Agriculture Industrialized". In S.A. Levin (ed) Encyclopedia of Biodiversity, Vol. 1. Academic Press: San Diego CA, USA.

Pretty, J.N. 1995. Regenerating Agriculture. Joseph Henry Press, Washington D.C.

Puckridge, D.W., L. Chankasom, P. Vonsaroj, P. Thongbai, and S. Chinawong. 1988. Effect of tillage and sowing methods on control of wild rice. p 593-598. In Proceedings of the 1987 International Deepwater Rice Workshop. Manila (Philippines): International Rice Research Institute.

Qualset, C.O., A.B. Damania, A.C.A. Zanatta, and S.B. Brush. 1997. Locally based crop plant conservation. In N. Maxted, B.V. Ford-Lloyd and J.G. Hawkes (eds) Plant Genetic Conservation, the In Situ Approach. London: Chapman and Hall.

Reaño, R., and J.L. Pham. 1998. Does cross-pollination occur during seed regeneration at the International Rice Genebank? Int. Rice Res. Note 23:5-6

Reid, W.V., S. Laird, C. Meyer, R. Gamez, A. Sittenfeld, D. Janzen, M. Golling, and C. Juma. 1993. Biodiversity Prospecting: Using Resources for Sustainable Development. World Resource Institute, Washington D.C.

Rerkasem, B., and K. Rerkasem.1984. The agroecological niche and farmer selection of rice varieties in the Chiangmai Valley, Thailand. p. 303-311. In A.T. Rambo and P.E. Sajise (eds) An Introduction to Human Ecology Research on Agricultural Systems in Southeast Asia. Los Banos: University of the Philippines at Los Banos.

Rerkasem, K., B. Rerkasem, M. Kaosa-ard, C. Roonruangsee, S. Jesdapipat, B. Shinawatra, and B. Wijukprasert. 1994. Assessment of Sustainable Highland Agricultural Systems. Bangkok: Thailand Development Research Institute (TDRI).

Rerkasem, K., N. Yimyam, C. Korsamphan, C. Thong-ngam, and B. Rerkasem. 2002. Agrodiversity Lessons in Mountain Land Management. Mountain Research and Development 22:4-9

Smale, M. (ed) 1998. Farmers, Gene Banks and Crop Breeding. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Solheim, W.T. 1972. An earlier agricultural revolution. Sci. Am. 226: 34-41.

Somrith, B., and S. Chitrakorn. 2001. Development of Thai rice varieties. p. 29-76. In Science, Technology and Thai Rice. BIOTEC and NSTDA. Public Relations Office, National Science and Technology Development Agency, Bangkok.

Suthi, C. 1985. Highland agriculture: from better to worse. p. 107-142. In J. McKinnon and B. Vienne (eds) Hill Tribes Today. White-Lotus, Bangkok and Paris.

Vanavichit, A., S. Tragoolrung, and T. Toojinda. 2001. Biotechnology and rice improvement. p. 79-121, In Science, Technology and Thai Rice. BIOTEC and NSTDA. Public Relations Office, National Science and Technology Development Agency, Bangkok.

Vuthiyano, C. 2000. Traditional Thai Rice Varieties. Thai Rice Research Institute, Bangkok.

Vuthiyano, C. 2001. Plant Germplas Database: Rice. Thai Rice Research Institute, Bangkok.

Zhu, Y., H. Chen, J. Fan, Y. Wang, Y. Li, J. Chen, S. Yang, L. Hu, J. Leung, T.W. Mew, P.S. Teng, Z. Wang, and C.C. Mundt. 2000. Genetic diversity and disease control in rice. Nature 406: 718 – 722.

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Overcoming wheat sterility problem with boron efficiency

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Key words: boron, deficiency, durum, efficiency, sterility, triticale, wheat

Abstract

Grain set failure, due to boron (B) deficiency-induced sterility of the anthers and pollen, is a cause of yield loss in many wheat growing areas of Asia. This paper illustrates a range of genotypic variation in the response to B that can be found in the wheat germplasm, and offers a solution in selection and breeding for boron efficiency.

Introduction

Boron (B) deficiency is a cause of yield loss in many of the world's wheat growing areas, from Brazil's irrigated varzeas (flood plains) of Sao Paulo and Minas Gerais (da Silva and de Andrade, 1980) and Cerrados (da Silva and de Andrade, 1983), to India's states of West Bengal, Assam, Orissa and along the Indo-Nepal border (Tandon and Naqvi,1992), Nepal (Misra *et al.*, 1992), China (Li *et al.*, 1978) and Bangladesh (Reuter, 1987; Rerkasem, 1995). Boron deficiency depresses wheat yield through an adverse effect on male fertility, and so depressing grain set and seed yield. There are also reports of genotypic variation in the response to B in wheat (Jamjod *et al*, 1992; Rerkasem and Loneragan, 1994; Subedi *et al.*, 1997). Selecting for B efficiency has been suggested as one cost effective solution to the problem of yield loss due to boron deficiency in farmers' wheat crops (Rerkasem and Jamjod, 1997a).

Materials and methods

Three sets of bread wheat (29IBWSN, 4HTWYT, 17ESWYT) and one each of durum (28IDYN) and triticale (28ITYN) genotypes from the International Centre for Wheat and Maize Improvement (CIMMYT) were screened in a sand culture without added B, in duplicated blocks at Chiang Mai in Thailand, with boron efficient wheat Fang 60 as local control. Entries from the last four sets were also sown in single rows in duplicated blocks soil with 0.1, 0.2 and 0.3 mg hot water soluble B kg^{-1} in the field. The effect of boron deficiency was measured with the Grain Set Index (GSI) from 10-20 randomly selected ears (Rerkasem and Loneragan 1994).

Results and discussion

In sand culture without added B in which Fang 60 set grain normally (GSI > 85%), the mean GSI (±SD) for 4HTWYT was 57.2±22.7, 17ESWYT 42.7±26.5, 29ISWYN 19.3±27.0, 28IDYN 10.6±16.6, 28ITYN 17.9±23.9.

Rated on the basis of GSI in sand culture without added B (Anantawiroon *et al* 1997) entries were found to be largely B inefficient (Table 1). All of the durum entries were inefficient, so were all of the triticale except one that was moderately efficient. The frequency for B efficiency was slightly higher among the bread wheat, being the highest in 4HTWYT followed by 17ESWYT and 29IBWSN. In the soil with 0.1 mg HWSB kg^{-1} mean GSI of the different B efficiency classes generally followed those in the sand culture, and grain set was increased with increasing soil B (Table 2). For durum, triticale and some of the very inefficient bread wheat, however, soil B had to be increased to 0.3 mg HWSB kg^{-1} before the GSI would exceed 85% (data not shown). Potential usefulness of the germplasm in low B soils areas in Bangladesh, Nepal, India, China and other countries has been severely limited by this very high frequency of extreme B inefficiency.

Table 1. Frequency distribution (%) of bread wheat, durum and triticale genotypes in four efficiency classes, by GSI in sand culture without added B[a]

Nursery or trial[b]	Number of entries	Frequency (%) VI[a]	I	ME	E
29IBWSN	409	77.3	20.0	2.2	0.5
4HTWYT	49	10.2	51.0	34.7	4.0
17ESWYT	49	20.4	67.3	10.2	2.0
28IDYN	49	87.8	12.2	0	0
28ITYN	49	51.1	46.9	2.0	0

a) VI = very inefficient (GSI ≤ 20%), I = inefficient (GSI = 21-70%), ME = moderately efficient (GSI 71-85%), E = efficient (GSI > 85%)

b) From CIMMYT, Mexico: the 29th International Bread Wheat Screening Nursery; 4th High Temperature Wheat Yield Trial, 17th Elite Selection Wheat Yield Trial, 28th International Durum Yield Nursery, 28th International Triticale Yield Nursery.

With B efficiency, as in Fang 60 and efficient genotypes identified in the 4HTWYT, normal grain set can be achieved in low B soil without B fertiliser. It is likely that genetic factor(s) involved in B efficiency are available in initial bread wheat populations used to generate the CIMMYT germplasm. The parentage of Fang 60, the most B efficient wheat found so far, can be frequently encountered in the pedigrees of CIMMYT wheat (Skovmand *et al* 2000). However, an increase in the frequency of efficient genotypes in the germplasm may be possible only if B efficiency is specifically included as a breeding objective.

For many wheat growing areas on low B soils that are still unfamiliar with fertiliser use, B efficient genotypes offer a more effective solution that is embodied in the seed so there is little problem with technology transfer. It may be prudent, however, to examine how B efficiency and sensitivity to B toxicity are related. It has been demonstrated that B efficiency is not simply the mirror image of sensitivity to B toxicity (Rerkasem and Jamjod 1997b). Sensitivity to B toxicity in wheat is controlled largely through B exclusion (Nable *et al* 1997). While the mechanistic explanation of B efficiency remains to be worked out, there is a possibility there may be some link between the mechanism controlling B efficiency and those controlling sensitivity to B toxicity.

The CIMMYT germplasm consists of superior genetic materials that have incorporated desirable characteristics such as high yielding capability and resistance to important diseases. It therefore promises well for the solution to the boron deficiency-induced sterility problem that some boron efficient genotypes were identified in the wheat germplasm. This together with the predominance of boron inefficiency in the germplasm tested so far have led us to suggest that, for wheat improvement programmes serving large tracks of low B soils found in other wheat growing regions as well as Asia, (a) screening for B efficiency would be extremely worthwhile in germpalsm evaluation and (b) increasing the frequency of B efficiency should be included as a breeding objective.

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References

Anantawiroon P, Subedi K and Rerkasem B *In* Developments in Plants and Soil Sciences 76. pp. 101-104. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Da Silva AR and de Andrade JMV 1980 Embrapa, Centro de Pesquisa Agropecuária dos Cerrados - CPAC. Circular Técnica no. 2. 69 p.

Da Silva AR and de Andrade JMV Pesq. agropec. bras., Brasilia 18, 593-60.

Jamjod S, Mann CE and Rerkasem B 1992 *In* Wheat Special Report 11. Eds. CE Mann and B Rerkasem. pp. 79-82. CIMMYT, Mexico.

Li BH, Li WH, Kui MC, Chao WS, Jem HP, Li CR, Chu WJ and Wang CL 1978 J Northeastern Agric Coll 3, 1-19.

Misra R, Munankarmi RC, Panday SP and Hobbs PR 1992 *In* Wheat Special Report 11. Eds. CE Mann and B Rerkasem. pp. 65-71. CIMMYT, Mexico.

Nable RO, Banuelos GS and Paull JG 1997 Plant Soil 198, 181-198.

Rerkasem B 1995 *In* Proceedings No. 72. pp. 8-12. Australian Centre for International Agricultural Research, Canberra.

Rerkasem B and Loneragan JF Agron J 86, 887-890.

Rerkasem B and Jamjod S 1997a. Euphytica 96, 257-262.

Rerkasem B and Jamjod S 1997b Plant Soil 193, 169-180.

Reuter DJ 1987 SAGRIC International.

Skovmand B, Mackay MC, Lopez C and McNab A (Eds.) 2000 On Compact Disk. CIMMYT, Mexico.

Subedi KD, Budhathoki CB, Subedi M, GC YD 1997 Plant Soil 188, 249-256.

Tandon JP and Naqvi SMA 1992 *In* Wheat Special Report No. 11. Eds. CE Mann and B Rerkasem. pp. 76-78. CIMMYT, Mexico.

Table 2. Boron responses in bread wheat, durum and triticale in different B efficiency classes (GSI, % ± SE)

B efficiency class	4HTWYT		17ESWYT		28IDYN		28ITYN	
Soil B[a] mg kg^{-1}	0.1	0.2	0.1	0.2	0.1	0.2	0.1	0.2
Very inefficient	9.0±6.6	70.5±17.8	35.0±10.9	63.2±15.6	11.9±9.7	54.7±12.7	16.9±1.3	62.5±7.3
Inefficient	40.2±11.4	82.2±16.0	48.0±17.2	84.9±10.3	12.6±7.9	72.3±4.9	31.2±2.1	80.6±2.7
Moderately efficient	55.2±15.1	86.3±7.9	70.0±14.4	90.6±2.5	NE	NE	53.9±6.7	84.5±12.7
Efficient	89.2±10.0	95.4±2.6	71.5±5.4	92.8±3.2	NE	NE	NE	NE
Fang 60	94.3±4.1	98.5±2.2	85.5±5.3	99.0±1.1	98.3±1.4	100.0±0.0	91.3±8.8	98.2±5.3

a) Hot water soluble B. HWSB

b) NE = no entry

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Grain iron concentration in Thai rice germplasm

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Key words: dryland, grain iron concentration, rice, wetland

Abstract

Iron deficiency anemia can be found world wide, especially in Asia. Preschool children and pregnant women are the most commonly affected. For most people, more than 50 percent of their iron intake comes from cereals. Rice, which is the staple food in Thailand, has the lowest iron concentration among the cereals. A solution to iron deficiency may be found by increasing iron concentration in the rice grain. In this experiment, we measured grain iron concentration in 38 Thai rice genotypes grown under wetland and well-drained conditions. The grain iron concentration was found to vary with genotype and water conditions, from 12-25 mg Fe kg^{-1} in unhusked rice and 7-19 mg Fe kg^{-1} in brown rice. Grain iron concentrations under dryland condition was higher than that under wetland condition for about half of the genotypes, and in the other half of the genotypes there was no effect of water condition on the grain iron.

Introduction

Iron (Fe) deficiency anemia is a worldwide problem. In Asia, some 60–70% of pre-school children and pregnant women are reported to be affected (IFPRI, 1999). For most people, more than half of their daily Fe intake comes from cereals (Juliano, 1993). This has recently been confirmed by a detailed study in Mindanao, the Philippines (Senadhira et al., 1998). Virtually everyone, rich as well as poor, gets about half of their Fe intake from cereals.

Rice is the staple food for most people in Asia as well as Thailand, but unfortunately it has the reputation for having the lowest Fe concentration in grain among the cereals (Senadhira *et al.*, 1998). On the other hand, a study at IRRI has found genotypic variation in grain Fe concentration ranging from 10–22 mg Fe kg^{-1} (Senadhira et al., 1998). So it has been suggested that increasing Fe concentration of rice grain may offer a means to increase daily Fe intake, and hence help to reduce the incidence of Fe deficiency anemia. This study sets out to evaluate the level of grain Fe in standard varieties of Thai rice, and also to explore the range of grain Fe that can be found in local Thai rice cultivars.

Materials and methods

This experiment evaluated a total of 38 rice genotypes from Thailand under two soil conditions, in three replications. Eight rice plants were grown in each black plastic bag containing 8 kg of soil (San sai series). The soil conditions were waterlogging (W+, the black plastic bag was submerged in a 20 L container of water, the soil surface was submerged under 10 cm of water) and dryland (W0, well-drained soil). Basal fertiliser was applied at the rate of 0.23 g N pot^{-1}, 0.23 g P_2O_5 pot^{-1} and 0.23 g K_2O pot^{-1} one week after transplanting, and 0.34 g N pot^{-1}, 0.34 g P_2O_5 pot^{-1} and 0.34 g K_2O pot^{-1} four weeks later. Iron concentration was determined in mature grain either as unhusked (whole grain with palea and lemma intact) and brown rice (palea and lemma removed) by dry-ashing and atomic absorption spectrometry (Emmanuel *et al.*, 1984).

Results

A wide range of grain Fe concentration was found among the 38 genotypes of Thai rice. The Fe concentration in unhusked rice in W+, the normal wetland condition, ranged from 12 to 23 mg Fe kg-1. Six genotypes were between 16-19 mg Fe kg-1. Most of the remainder ranged between 13-16 mg Fe kg-1. In brown rice the grain Fe ranged between 7 and 15 mg Fe kg-1, which was lower than in unhusked. Most of the genotypes had less than 10 mg Fe kg-1 (Table 1a). In W0 (well-drained soil) the Fe concentration in unhusked rice ranged from 14-24 mg Fe kg-1. Two genotypes had more than 22 mg Fe kg-1. Most of the genotypes ranged between 16-22 mg Fe kg-1. In brown rice the grain Fe ranged between 8-19 mg Fe kg-1. Nine genotypes were between 13-19 mg Fe kg-, and most of the remainder less than 13 mg Fe kg-1 (Table 1b). There was no correlation between Fe concentration in unhusked and brown rice in either W+ (R2=0.26) or W0 (R2=0.29).

Grain Fe concentrations in brown rice in W+ correlated with that in W0 (R^2=0.53) (Figure 1). From Figure 1 it may be seen that genotypes tended to have higher grain Fe concentration in W0 than in W+.

Discussion

Northern Thailand has been designated as one of the centers of diversity of *Oryza sativa* (Chang, 1976). That a diverse range of grain Fe concentration has been found in local rice cultivars is to be expected. Most standard Thai cultivars are, however, among the lowest in grain Fe, e.g. KDML105 and RD 6 had < 10 mg Fe kg^{-1} in both dryland and wetland conditions. On the other hand, in some traditional upland cultivars, e.g. CMU122, CMU123, especially high grain Fe, > 16 mg Fe kg^{-1}, was obtained under dryland condition, in which they are normally grown. It is surprising that the grain Fe concentration of rice grown under dryland condition should be the same as or even higher than that grown under wetland condition. When soils are flooded, the concentration of Fe^{2+} in the

soil solution increases to several times that in aerated soil (Ponnamperuma, 1972). Rice plants growing in flooded soil have been reported to contain Fe at many times those growing in well-drained soil (Beyrouty *et al.*, 1994). In this study, however, we have found that grain Fe concentration of rice growing in flooded soil either was the same as or less than those growing in well-drain soils. Clearly, Fe concentration in the rice grain is not directly related to total Fe in the whole plant.

Table 1. Number of rice genotypes with various grain Fe concentrations in unhusked rice and brown rice under wetland and dryland conditions.

mg Fe kg^{-1}	Number of rice genotypes	
	Unhusked	Brown
(a) Wetland condition		
< 10	0	27
10.1 – 13.0	6	8
13.1 – 16.0	25	3
16.1 – 19.0	6	0
19.1 – 22.0	0	0
> 22	1	0
Total	38	38
Mean ± SD	14.7±1.9	8.7±1.7
Range	12–23	7-15
(b) Dryland condition		
< 10	0	15
10.1 – 13.0	0	14
13.1 – 16.0	5	8
16.1 – 19.0	14	1
19.1 – 22.0	17	0
> 22	2	0
Total	38	38
Mean ± SD	18.8±2.4	11.1±2.6
Range	14-24	8-19

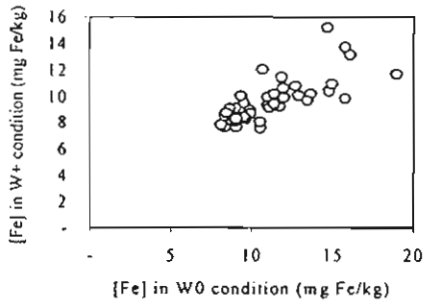

Figure 1. The relationship between Fe concentrations in brown rice of 38 cultivars grown under W0 and W+ conditions (R^2=0.53)

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References

Beyrouty CA, Grigg BC, Norman RJ and Wells BR 1994 Plant Nutr. 17, 39-55.

Chang TT 1976 Euphytica 25, 425-441.

De Datta KS 1981 Principles and practices of rice production. The International Rice Research Institute. Los Banos, Philippines.

Emmanuel D, Dell B, Kirk G, Loneragan J, Nable R, Plaskett D and Webb M 1984 Manual of Research Procedures. First edition. Plant nutrition research group school of environmental and life science. Murdoch University, Australia.

International Food Policy Research Institute 1999 World Food Prospects: Critical Issue for the Early Twenty-first Century. October. Washington, D.C.

Juliano BO 1993 FAO Food and nutrition series No. 26, Rome.

Ponnamperuma FN 1972 Adv. Agron. 24, 29-96.

Senadhira D, Gregorio G and Graham RD 1998 Paper presented at the International Workshop on Micronutrient Enhancement of Rice for Developing Countries, September 3, 1998. At Rice Research and Extension Center, Stuttgart, Arkansas.

Social Challenges for the Mekong Region

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Biotechnology and agriculture

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Raging debates for and against genetically modified (GM) crops compel me to begin with a declaration about my beliefs regarding biotechnology. It is as foolish to be blindly for genetically modified crops as to be unthinkingly against them. Much more than just the genetically modified crops, biotechnology (Box 1) is a powerful, Promethean tool that can help us manage plants, animals, microbes and other life forms and processes including human health, for our benefits. The arrival of genetically modified crops in the region is inevitable, regardless of national policies and protests by guardians of public interest. To ask if genetically modified crops would be good or bad for the Mekong Region is as pointless as asking if fire is good or bad. However, if not handled properly biotechnology may cause much more serious harm than the original Promethean gift. It would be more relevant to question where the weakness lies in the Mekong Region's capacity to: 1) forestall any adverse impact that may arise from genetically modified crops; and 2) reap full benefits from biotechnology, and what it will take to fill this gap within the next 30 years.

Among readers of this paper will be many who ask if biotechnology will help poor farmers eg. those in the Mekong Region who have been left behind by the Green Revolution and other technological gains in the last 30 years. In this chapter[1] I begin by examining some answers to this question. I then review local capacity for biotechnology and other essential sciences, followed by an examination of the status of local plant genetic resources, and potential impact of biotechnology and intellectual property laws. In the final section I suggest some opportunities for addressing current shortcomings.

Will biotechnology help poor farmers in the Mekong countries?

A simple answer to this might be in the cost of investment for different kinds of biotechnology. The cheapest is tissue culture, which is already being used widely in orchid and mushroom culture in the region. It is also used as a tool in plant breeding eg. in anther and embryo culture. At the other end of the scale, it

[1] Research at the Chiang Mai University Plant Nutrition laboratory is funded by Thailand Research Fund, McKnight Foundation, USAID and the European Union. I would also like to specifically recognise contributions from Dr Sansanee Jamjod, Dr Chanya Maneechote and Professor Barbara Schaal on some of the information and ideas on genetic diversity and gene flow presented in this chapter.

long run, as it acts as a strong disincentive for private investment in crop improvement.

The alarm about potential ecological risks from genetically modified crops has been raised by some scientists (Altieri 2000). Such concerns include resistance that can develop in major pest species and effects of substances that are products of transgenic modification (eg. *Bt* and other toxins) on non-target organisms through the whole trophic chain down to soil insects and micro-organisms. However, since fire was discovered, no technology has ever been absolutely without risk. It is important that these ecological risks are not considered in isolation. For example, the ecological risks of *Bt* cotton should be weighed against the ecological, human health and economic cost of pesticide use. By banning commercial planting of *Bt* cotton, Thailand has also missed an opportunity to monitor and weigh tradeoffs among all of these risks under local conditions.

Although genes are costly to identify and develop, once isolated they can be used in many different crops and ways. For example the *Bt* gene, which confers resistance to other insects as well as cotton bollworm, can also be used to protect against particular insect pests in corn, cabbage, rice and soybean. Once a gene is incorporated into a crop species, plant breeders without capacity for DNA technology may use the gene by the old fashioned methods of manual cross fertilisation and backcrossing. The more widely a gene is used, the more thinly can its research and development costs be spread. The licensing of *Bt* cotton from Monsanto in China could hold valuable lessons for other Mekong Region countries.

The case made against genetically modified crops is often based on a wide range of reasons associated with individual cases, and rarely on the actual problem specific to biotechnology. The safety record of the introduction of and experimentation with genetically modified crops in the region has indeed been miserable. The case made against *Bt* cotton in Thailand was based on accusations of a dereliction of duty on the part of the director general of the Department of Agriculture; a hard driving American culture of lobbying; the questionable ethics of politicians, public servants and scientists; and predatory business practices (Biothai website). These may indeed be important issues in the safe and equitable use of biotechnology in the region, in the way that bad driving is a major cause of traffic accidents throughout the Mekong Region. To make these into indictments against biotechnology, however, is like saying cars must be banned altogether because we cannot get anyone to follow traffic laws, traffic police can be bribed, and the poor pedestrians and cyclists are more often killed than the rich in big expensive cars. On the other hand, some may consider nuclear energy to be a more appropriate analogy. While there are still so many uncertainties associated with risks from biotechnology, local regulatory as well

Commercially developed *Bt* cotton and other genetically modified crops offer at least one option through which poor farmers may choose to solve their food security problems. The obstacle to farmers taking this option is not the price of genes that their private owners will extract, but rather, public dissent. It would take a particularly inept seed company to set up prices that are beyond the reach of growers or to push a technology that will bankrupt them. If unconstrained by public dissent, Cambodia, Lao PDR, Myanmar and Vietnam can simply choose to follow China's example in adopting biotechnology if they so choose. Genetically modified crops may also be transferred from China, as has already happened with hybrid rice in Vietnam. Public rejection of food made from genetically modified crops in importing countries in Asia and Europe is an important constraint for the whole region.

Private seed companies, however, will not of their own volition pay attention to potential adverse impacts of genetically modified crops. There is also no profit motive for private investment in many of the crops and problems that are important to local farmers eg. rice and cassava. Furthermore, there are questions regarding use and conservation of the region's valuable local plant genetic resources. Transfer of technology from outside the region, including the international agricultural centres, will always make a valuable contribution. However, for reasons that will be made clear later, the Mekong Region will have to increasingly rely on its own public research capacity. To fully exploit the potential of biotechnology and to forestall any adverse impacts on human health and the environment requires an agricultural research capacity far beyond modern biotechnology with its focus on genes and DNA.

Uniqueness of the Mekong Region's problems

The Mekong Region is unique and we cannot just sit and wait for knowledge to be transferred from richer places.

The region contains the centres of diversity of many valuable crop species, including fruit (mango, banana, citrus), vegetables (the eggplants, gingers), orchids and timber species as well as sugar cane and the all important rice and its wild relatives. Many problems specific to these primary gene pools are overlooked or ignored by others in developed countries. The issue of genetic erosion of wild maize is important to Mexico, its native land, but is of no particular concern in the USA or Europe where it is not a native.

Furthermore, many aspects of the problems of these native species require close monitoring. Hybrid swarms, resulting from crosses between cultivated and wild rice, are common in the region. These are probably the primary source of diversity in local rice germplasm; new local varieties continue to be selected from these by farmers. They are also likely to give us clues to the dynamics of the local *Oryza* (the rice genus) gene pool. However, these hybrids closely

rather uneven in the Mekong Region. A minimal capacity is indicated in Cambodia, Myanmar and Lao PDR by the absence of these countries from regional collaborations such as the Asian Rice Biotechnology Network, Asian Maize Biotechnology Network, and so on. Vietnam participates in the Asian Rice Biotechnology Network. It has shown interest in biotechnology, but investment has so far been relatively limited (ADB 2001).

China's enthusiasm for biotechnology is matched by its investment in research funding through programmes such as the National Program on High Technology Development (known as the 863 programme) and the National Program on Basic Research in which agricultural biotechnology is a major component (Quifa Zhang 2000). Capacity building has been served by special grants in the 863 programme to promote research by young scientists. National Key Laboratories have been established in the general areas of agricultural biotechnology, crop genetics and breeding in north, central and south China. The labs have been well equipped to conduct biotechnology and molecular biology research. Opportunities, facilities and support for biotechnology research are also provided by the Ministry of Agriculture, Ministry of Education and Chinese Academy of Sciences. The strength in biotechnology achieved by China can also be gauged by the number of internationally competitive grants Chinese scientists have won (eg. three or four of the new projects in the highly competitive Collaborative Crop Research Programme of the McKnight Foundation have been won by China). The list of crops on which biotechnology research has been conducted in China include rice, wheat, corn, cotton, tomato, potato, cucumber, papaya and tobacco. Most of these are important in Yunnan, papaya and tobacco are indeed uniquely so. From this, and also research papers from Yunnan that have begun to appear in internationally refereed journals (*Nature*, *Theoretical and Applied Genetics*, *Genetica* etc.), it might be concluded that Yunnan, being carried along with the rest of China, is probably most advanced in terms of biotechnology capacity in the Mekong Region.

Held up as the success story of biotechnology in a developing country is Thailand's happy experience with a diagnosis for viral pathogens of shrimps with a DNA probe. This has been credited with saving the country's shrimp farming at least US$1 billion since 1996. The country's commitment to biotechnology is evident in the establishment of the National Centre for Genetic Engineering and Biotechnology (Biotec) in 1991. Biotec spends 30% of its research and development (R&D) budget on in-house research, and 70% is allocated to designated research projects conducted by universities and research institutions in the country. Biotec's current focuses are on shrimp farming; technology for the utilisation of cassava; rice (disease resistance and genome project); dairy (reproductive technology); genetic engineering (from disease resistant papaya and tomato, *Bt* cotton, to drought tolerant and aromatic rice); DNA finger printing (pathogens); and supporting trade with DNA diagnoses (to comply with

combination with a no-tillage practice that together protect the soil surface and draw nitrogen from the atmosphere.

Factors limiting crop production on the acid soils in the mountains of the Mekong Region, however, cannot be assumed to be the same as those in South America. They may also differ from place to place in the region, as do opportunities for a solution. Traditional rice varieties grown by Karen farmers in northern Thailand can yield reasonably well at a pH as low as four with a very low level of available essential nutrients such as phosphorus. Acid tolerance and phosphorus efficiency genes are apparently already available amongst the local rice germplasm of the Mekong Region's uplands. A research question that could help improve food security for people who live on some of these acid upland soils might be why the Karen rice that can often yield up to 4 ton/ha, sometimes yields only 0.8 ton/ha on neighbouring fields.

Currently lacking in the Mekong Region is capacity in the basic sciences needed to define such local problems, and to evaluate potential solutions. Recent progress in seemingly abstract understanding of boron nutrition in plants illustrates this second point. Soils low in boron are widespread from Yunnan to northern Myanmar, northern and northeastern Thailand and probably throughout northern Lao PDR and Vietnam. Nutrient management of crops produced on these soils would greatly benefit if we knew which of the major crop species (annuals and trees, mango, longan, lychee, durian, mangosteen, papaya, rambutan, jack fruit, tamarind etc as well as pioneer forest species), are able to recycle boron from their old leaves. This, in turn, rests on a basic understanding of boron mobility in the phloem of these local species.

The usefulness of such basic knowledge became evident when a group at the University of California at Davis showed that genetic modification to confer the ability to produce boron transporting sugar-alcohols has also enabled the plant to recycle boron accumulated in old tissues via the phloem and re-use it to build new leaves, flowers and fruit when supply from the soil runs out at critical times. But this research applies to temperate species such as apple, almond and walnut, and is not applicable to our important species such as durian, mango, lychee and longan. There is no alternative to getting to know our own crops on a case by case basis. Of course we could wait until someone else in Australia, California or South America conducts this basic research. But this comes with the risk of the Mekong Region losing its comparative advantage in its local crops to sub-tropical regions in these developed countries.

The situation with rice quality is another example of a bottleneck in crop improvement that biotechnology can help to correct. Rice quality used to be the preoccupation of just Thailand, but is now also worrying Vietnam and China sitting on their million ton mountains of unsold hybrid rice, and no doubt other Mekong Region countries too as their stock of unsold rice begins to grow.

The progress has been sufficient for direct technology transfer from elsewhere, notably in the case of maize, rice and also the intensive livestock and feed industry. Unfortunately capacity for basic science related to agriculture that is needed to make the fullest use of biotechnology, as well as to forestall potential dangers, is still extremely limited.

The allocation of Royal Golden Jubilee PhD scholarships provides a rough measure of this in Thailand. Only 76 of these scholarships, allocated nation-wide on the basis of the professors' proven record in research, were won by agricultural science between 1998 and 2001. This is compared with the 400 that went to medical and biological science, and almost 300 to the physical sciences and engineering combined. The achievement of agricultural science becomes even smaller in proportion to the number and size of agricultural science faculties in the whole country. The focus on technology transfer may explain the direction taken by agricultural research in the past 30 years. While sufficient for the particular purpose, it also means that capacity for basic, mechanistic, explanatory research in agricultural sciences is much more limited than in the medical, biological and physical sciences. In the meanwhile it has become abundantly clear that farmers are much more effective in conducting adaptive research than young agricultural science graduates. Countless on-farm adaptive experiments are being conducted each season by farmers all over the region.

Poor linkages between researchers and farmers

Another critical weakness in agricultural research that will undermine biotechnology's effectiveness, is the link between agricultural research and farmers and farming in the region. This on-the-ground intelligence failure may be even more destructive than those wrongly targeted missiles. Farming systems, participatory research, participatory plant breeding, and numerous other ideas and methods aiming to improve researchers' recognition of the need to understand and respond to on-farm conditions have come and gone without much real, lasting effect. In part this is because the region's (much the same in many other places the world over) agricultural research has been entirely supply driven. There are limited mechanisms to assess research results for relevance to farmers' real needs as well as scientific and technical quality. The team from Mahidol University and Chulalongkorn University that worked on the DNA probe for shrimp pathogens was made up of laboratory based scientists who probably had never heard of participatory research and similar jargons. They are, however, world-class biologists, who worked on a clearly defined problem in close collaboration with the shrimp industry and its farmers (Morakot Tanticharoen 1999).

Indeed, real differences can be made even by old-fashioned soil science and plant nutrition, backed up by dependable laboratory analyses, as demonstrated by a group at King Mongkut Institute of Technology in Thailand, working closely

incentives to researchers is not beyond reach in this region. The questions are how might some of this strength be redirected towards agriculture and also whether it is a realistic goal for the Mekong Region as a whole to aim towards.

Possible adverse impacts of biotechnology

Genetically modified crops are generally the focus of safety concerns related to biotechnology. The concerns focus on two areas: (a) food safety and (b) the environment, specifically genetic erosion.

Food safety

The concern about food safety related to genetically modified crops is still largely based on fear of the unknown. Evidence of poisoning from food made from genetically modified crops is specific to certain compounds and groups of people and is actually quite rare. It would be most informative and useful to compare the frequency of cases of critical food poisoning from genetically modified crops and other forms of contamination, including 'natural' (from fungi, insects, and herbal 'medicines') as well as 'unnatural' ones (eg. methylated spirit, pesticides and prescription drugs). A recent United Kingdom updated review of genetically modified plants for food use and human health (The Royal Society 2002) pointed out that 'novel foods' (including so-called health food and organically grown food) should be subjected to the same stringent nutritional safety assessment that is currently applied to infant formulas.

Three other conclusions of this Royal Society review may interest those readers of this book concerned about the safety of foods derived from GM crops.

Firstly: *"The allergenic risks posed by GM food plants are in principle no greater than those posed by conventionally derived crops or by plants introduced from other areas of the world."*

Secondly: *"Plant viral DNA sequences are commonly used in the construction of the genes inserted into GM plants, and concern has been expressed about this. Having reviewed the scientific evidence we conclude that the risks to human health associated with the use of specific viral DNA sequences in GM plants are negligible."*

Thirdly: *"One concern associated with GM foods is the possibility that genes introduced into GM plants might become incorporated into the consumer's genetic make-up. Since the Royal Society's 1998 report various papers have been published on the topic. The results need to be viewed in the context of a normal diet, which for humans and animals comprises large amounts of DNA. This DNA is derived not only from the cells of food sources, but also from any contaminating microbes and viruses. Given the very long history of DNA consumption from a wide variety of sources, we conclude that such consumption*

contributions in precisely measuring the extent of diversity that exists, its geographic and ecological structure and dynamics. However, support from other sciences for basic understanding of plants and their environment (agronomy, biochemistry, botany, ecology, ecophysiology, evolution and genetics, as well as economics and the social reasons for germplasm conservation), will be essential.

Box 2 Why is genetic diversity important?

Conventional plant breeding teaches that genes associated with low yield potential in landraces can be eliminated through pure line selection. Data from the 1950s from Thai Rice Department supporting this have been cited (Oka 1988). But Oka went on, *"However, the yield data have come from experiments under the same cultural management (optimal condition of experimental stations - BR) for a few years and provide no information on yield stability under changing environments. Gene diversity in populations would bring about population buffering to stabilise yield, as discussed by Allard and Bradshaw (1964)"*. Indeed, a preliminary study at Chiang Mai University suggests that a very high degree of genetic diversity within a local rice variety called *Bue Chamee* (wild fowl rice, in Karenese) may be the reason for its success in highland paddies over a wide area in the mountains of northern Thailand with a highly diverse biophysical and socioeconomic environment. Characterisation of local genetic diversity, with morphological and physiological analysis, aided by modern biotechnology, will enable the true value of genetic diversity to be precisely measured. It may then be possible to select for productivity without sacrificing diversity.

Many consider the erosion of the *Oryza* genetic system in the Mekong Region to have begun with releases of modern varieties which replaced numerous older varieties with only a few new ones. The modern varieties are products of modern plant breeding. They include promising local varieties that have been through pure line selection to make them genetically homogenous; they also include high yielding varieties and hybrid rice. In Thailand high yielding varieties are still grown on a relatively limited scale. However, Thailand has taken to 'improving' local elite varieties since the 1950s. Through pure line selection, a local Thai elite variety from Bangkhla near Bangkok named Khao Dawk Mali became KDML105, which gave rise to RD15 and a glutinous rice RD6 through mutation breeding. These three varieties together account for 60% the country's main season planting, and more than 90% of the rice area in many provinces (OAE 1998). The three are, unfortunately, genetically very close. In the early 1990s a blast epidemic decimated tens of thousand hectares of this presumably homogenous population of the KDML 105 stock in several provinces. This genetic homogenisation of local traditional cultivars continues in Thailand, and is now being repeated with local rice germplasm in Lao PDR (CGIAR website) and possibly Myanmar.

research team from Chiang Mai University has since found other similarly genetically diverse populations in other parts of Thailand.

Gene flow from introduced germplasm into wild populations would have been going on ever since rice varieties were moved around the region (eg. with the different waves of migrations from southern China into Myanmar, Thailand, Lao PDR and Vietnam and various maritime, river and over-land trade traffics that plied the whole of the region, including mountainous areas long before colonial time). The introduction of GM rice and the advent of modern biotechnology, however, add some new and potentially dangerous elements to the local rice-wild rice genetic system.

This involves the process of gene 'transformation' ie. introduction of the 'exotic' or 'trans' genes from other species. The *Bt* gene that confers tolerance to insect damage comes from a bacterium called *Bacillus thuringiensis*. Other GM crops often contain genes transferred from micro-organisms, including viruses. A potential danger lies in the possibility of genetic interactions of these transgenes with other major genetic systems, from the crop species and its wild relatives to pests, pathogens and weeds and beneficial insects, micro-organisms such as nitrogen fixing bacteria and mycorrhizal fungi, and various other life forms down the trophic chain. The simplest scenario for such genetic interactions would be an 'escape' of a gene for resistance to specific herbicides from GM rice into wild rice. The biotechnology method of embryo culture and embryo rescue has also enabled 'wide-crosses' (ie. hybridisation between rice and other species of the *Oryza* genus) to be made. Wild rice is increasingly used as a source of disease resistance and other useful genes (eg. cytoplasmic male sterility for hybrid rice). For example, Pathumthani 1, one of Thailand's new non-photosensitive, aromatic rices, contains some wild rice genes.[5] The genetic barrier between cultivated rice and its wild relatives is likely to be much reduced in cultivated rice varieties that have incorporated wild rice genes. A recent study by the USA National Research Council concluded that gene migration between the transgenic crop and wild populations of squash in the United States could pose an environmental risk (NRC 2000). This risk is associated with introgression of the transgenes (of the transgenic yellow squash, Freedom II, which is resistant to watermelon mosaic virus 2 and zucchini yellow mosaic virus) into wild populations.

Even more recent is a report of definitive evidence of transgenic contamination in local maize germplasm in the remote mountainous region of Sierra Norte de Oxaca, in Mexico (Quist and Chapela 2001). In this native germplasm, the researchers found weak but clear evidence of p-35S, a promoter from the cauliflower mosaic virus, widely used in transgenic crops, presence of the

[5] Songkran Chitrakorn, personal communication.

International and national legal framework

The Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS)[6] has obliged developing countries to enact laws to confer ownership rights to intellectual and tangible property which are products of creativity, invention, and know-how (intellectual property) as well as biological materials and devices (tangible property). Means of protecting such property that have been applied to plant germplasm include patents, trade secrets, trademarks and geographic marks and appellation origin.

The last one allows legal rights to make and market certain kinds of products to certain geographical regions only, eg. Champagne from the region by that name in France, and similarly for Bordeaux, Burgundy, Cognac, Scotch, and so on. This is currently applied to wine and spirits only, but there are signs that agreements are being reached in the World Trade Organisation (WTO) to extend the application to food and other agricultural products as well. On the plus side for the Mekong Region, it will mean that no one else will be allowed to export Thai rice or Yunnan ham. But there will also be a down side, in that the Mekong Region will no longer be able to export Basmati and Japanese rice, or other products claimed by other geographical regions.

The UPOV (International Union for the Protection of New Varieties of Plants) Convention of 1961 established the principle that an improved variety can be legally owned by the breeder. The Convention is concerned with protecting the results of conventional plant breeding, so is generally believed to have the full weight of the seed industry behind it. The *Exceptions of the Breeder's Right (Article 15)* allows plant breeders to use without restriction protected varieties in the production of new varieties. In its 1991 revision the UPOV Convention was brought in line with contemporary technological developments. In particular, it extended protection to 'essentially derived varieties' in an attempt to strengthen protection for plant breeders who initially develop a new and distinct variety against others who merely make derivations from the initial work. Article 14, 5(c) says that *"Essentially derived varieties may be obtained for example by the selection of a natural or induced mutant, or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, backcrossing or transformation by genetic engineering"*.

Many developed countries have enacted legislation to protect plant breeders' rights in new varieties which complies with the UPOV Convention. For example, Australia's *Plant Variety Right Act 1987* broadly followed the criteria for protection agreed under UPOV, and its *Plant Breeders' Rights Act 1994*

[6] One of the trade agreements made in the General Agreement on Tariffs and Trade (GATT), known as the Uruguay Round, which concluded on December 15, 1993. In specific reference to the protection of plant varieties, the TRIPS Agreement *"requires that protection be provided either by patents or by an effective* sui generis *system or a combination of both"* (Article 273 (b)).

- the protection of intellectual property rights of plant breeders in order to provide incentives for research and development of new plant varieties, based on the principles of biological safety and food security; and
- the protection of the rights of farmers and local communities to share in the benefits from development of new varieties based on 'their' traditional varieties, in order to provide incentives to communities to conserve traditional and local plant varieties.

However, for all its high minded intentions, the law still lacks a mechanism for implementation two years after its enactment. Among the obstacles for the NPVP 2542's streamlined implementation, especially for communities' rights, is genetic variation within populations of local rice varieties. For various biological, ecological and management reasons, local rice varieties are often not genetically uniform, but are mixtures of several genotypes. Variations are sometimes obvious and can be distinguished visually, others are less obvious and can be distinguished only with special tests. Yet, like other laws originally designed to protect plant breeders' rights, the application of NPVP 2542 is based on the plant variety's uniformity and its ability to 'breed true' ie. all primary traits are maintained in succeeding generations. A community or communities can claim ownership to a particular local variety only if they can show that it is genetically uniform and breeds true. The NPVP 2542 law will therefore not allow claims to many local rice varieties in the region. It would be unrealistic, and logistically and financially impossible, to expect public institutions such as the Department of Agriculture to bear the burden of such proof.

The drive to apply the NPVP 2542 to communities' rights to germplasm is often based on the fear of 'biopiracy' by multi-nationals. However, all who champion the property rights of local communities should be made aware of potential conflicts among local communities in the application of the NPVP 2542. Local germplasm has historically been an 'open source' resource, and many have contributed to development and conservation of local varieties. There is also a potential confusion in the use of names for local varieties, which is often the only handle for local recognition of a variety. The same name is sometimes used for different varieties, and one variety may be known by different names in different locations. Again it would be unrealistic and costly to put the burden of proof of ownership on a public institution such as the Department of Agriculture. Neither will it be adequate to simply post property right claims at the district or sub-district office (as is now common practice with land ownership claims) and expect other communities to register their objection.

It is as yet unclear if the objectives of the NPVP 2542 will be realised by the application of the law. But enforcement of the law can help prevent piracy of privately developed crop varieties as well as GM crops such as *Bt* cotton.

Thai Rice Research Institute. Some of this variation may have come from the original farmers' heterozygous population at Bangkla that had not been through pure-line selection.

This genetic variation within the population has apparently not done Thailand's internal high quality rice market and its US$ 300 million a year export any harm. It however complicates the application of NPVP 2542, and weakens the protection of its local germplasm. For a start the Thai Rice Research Institute will have to determine which of its many breeders' versions of KDML105 is the 'real' one that is protected by the law. NPVP 2542 may even encourage a form of biopiracy. A private person or company can simply select any of the numerous forms of local variety that have been grown by some farmers for a long time. Strict enforcement of the law would force these farmers to pay a licensing fee to the owner of the registered 'new variety', who would also be legally empowered to sell the variety or take it out of the country.

Patent and plant breeders rights laws in developed countries (eg. Australia, Europe, Japan and the USA) all empower individuals and corporations to poach at will from the common pool of plant genetic resources, including the part that is being 'held in trust' by CGIAR centres.[8] According to the tradition of germplasm sharing, farmers and communities may not object sharing with others even in far away countries. The problem arises because original users of the common genetic resource can now be 'fenced out' by the new legal owners empowered by the various property right laws.

Yet another aspect of the property right problem is highlighted by the issue of rice quality in Thailand. Like any other rice producing country, Thailand badly needs R&D to improve acceptability to farmers of new 'improved' rice varieties released from its breeding programmes, to make better use of all of the new inventions and improvements in rice genetics, including higher yields, more β-carotene, more iron and zinc etc. Furthermore it will enable rice breeders to explore other definitions of 'quality' to expand the country's market opportunity, and lessen dependence on just the one single quality type of Thai Hom Mali. Apart from reducing the risk in the market, this will also mitigate the risk in the production system which is currently too dependent on a very narrow gene pool of KDML105 and its mutant sisters, RD6 and RD15.

In order to do this, as discussed above, Thailand will need capacity in biochemistry, genetics (the old-fashioned Mendellian kind as well as molecular) and ecophysiology. Skills need to be acquired, tools (gas chromatography, various spectrometry and electron microscopy, and so on) mastered to enable various quality characteristics to be quantified, and screening procedures

[8] As referred to elsewhere, several CGIAR members, including IRRI, have been involved in recent disputes about germplasm ownership.

Harnessing biotechnology for the Mekong Region: the next 30 years

Building local technical capacity

Pre-fabricated GM crops and modern biotechnology alone are definitely not going to be enough to enable the Mekong Region to reap the full benefits of biotechnology. And without some local capacity for understanding the key functions and processes in the region's important plants, animals and microbes, the GM crops that will inevitably arrive will pose a real threat. The question is whether the Mekong Region can develop the necessary local capacity to handle these threats and make the most of what biotechnology has to offer.

My answer to this is *"Why not?"*. If the capacity for agricultural science in the region has been somewhat limited, it was surely not because of any lack of aptitude but more because there has been no real demand for, and so no investment in, good research. Now suddenly Thailand and Yunnan are awash with public money chasing good research, and Vietnam most probably will soon follow. On the evidence of our record, good and useful agricultural science will not simply just happen because of all this money.

The region is as much in need of capacity in agricultural research management as in technical capacity. A number of regional and relatively long term (say, 10 to 15 years) projects on well chosen agricultural problems could explore and teach how this could be done. The next generation of scientists and professors will be part of this process by making graduate programmes, especially already established and well funded ones like Thailand's Royal Golden Jubilee PhD programme, and perhaps the equivalent in China and Vietnam, an integral part of this effort. Provisions for graduate level training in Thailand, Vietnam and Yunnan for individuals from Cambodia, Lao PDR and Myanmar in the first 10 to 15 years would help towards building local capacity for graduate training, and thus perpetuate such capacity in these countries afterwards. Carefully matched collaboration with advanced labs in developed countries can also make valuable contributions. Issues of intellectual property will need to be addressed in any collaboration in general, and on germplasm specifically.

Intellectual and tangible property rights

The current climate of suspicion and mistrust surrounding the property rights relating germplasm and research findings are an obstacle to collaboration and a distraction for working scientists. Some standard air-tight memoranda of agreement for sharing biological materials, information and trade secrets for the purpose of germplasm research would help calm nerves and remove the fears that are now holding up much needed collaboration. However, there is no alternative to researchers in public institutions, including universities, having some basic intellectual property rights capacity. Innovations often simply

regional or provincial licensing agreement, along with their efficacy over space and time. Such experimentation could also explore research management capacity that will have to deal with not only the technical agronomic, biological and ecological side of GM crops, but also how to manage conflicting interests of different stakeholders, from farmers, consumers and NGOs who have appointed themselves guardians of public safety.

Looking after native germplasm

Clearly rice needs careful biotechnology management to safeguard the species' native gene pool. This could also teach lessons on the management of gene pools of other native species. Evidence of gene migration in *Oryza* discussed above clearly points to a danger from GM rice. It appears that how a GM rice hybridises with local wild rice and how their progenies behave could be important criteria that must be determined before any GM rice can be released into the field in the Mekong Region. Understanding phylogeography of local cultivated and wild rice populations on the other hand will help with gene pool management at the national level. For example, stringent standards for releasing GM rice might need to be enforced only in regions with genetically unique rice populations and not everywhere.

Concluding remarks

The case of rice and wild rice raises the possibility that the whole gene pool of a native crop species and its wild relatives may become contaminated and decimated through gene migration. Much has been achieved in transfer of biotechnology to Asia in the last 10 years or so. But in the Mekong Region there is still insufficient capacity to adapt biotechnology for local use and to forestall any potential harmful impacts. There are no 'widely adapted' GM crops nor safety protocols that can be simply and safely transferred from elsewhere. The region needs to develop its own regulatory and local scientific capacity to cope with biotechnology. Such technical capacity is, however, dependent on a local capacity for managing agricultural science research that can find the fine balance between understanding and application.

References

ADB (2001) *Agricultural Biotechnology, Poverty Reduction, and Food Security*. Asian Development Bank, Manila.

Allard RW and Bradshaw AD (1964) 'Implication of genotype-environmental interaction in applied plant breeding', *Crop Science* 4, 503-508.

Altieri MA (2000) 'Executive summary', in Proceedings of International Workshop on the Ecological Impacts of Transgenic Crops. University of California, Berkely, 2-4 March.

Benjavan Rerkasem and Kanok Rerkasem (1984) 'The agroecological niche and farmer selection of rice varieties in the Chiangmai valley, Thailand' in AT Rambo and

Thai Rice Research Institute (1990) 'Introducing 59 improved varieties of Thai rice and temperate cereals'. Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok.

The Economist, 'A survey of technology and development', 10 November 2001.

The Royal Society (2002) 'Genetically modified plants for food use and human health - an update', Policy Document 4/02. London.

Social Challenges for the Mekong Region

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Uplands land use

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The uplands of mainland Southeast Asia stretch from Myanmar to Vietnam and include parts of Cambodia, Lao PDR, Thailand and neighbouring Yunnan Province of China. The uplands, defined here as lands more than 600 metres above sea level, encompass about half of the total land area of the Mekong Region. The uplands are home to many diverse ethnic groups and include pockets characterised by rapid population growth and severe poverty. Significant land use changes are taking place, many of which are leading to tensions within and between upland communities, and between 'upstream' and 'downstream' watershed communities. Food security remains one of the major challenges for upland communities and the governments in the region, but the changing socioeconomic landscape has seen other related concerns emerge.

Agricultural production for subsistence, traditionally dominated by shifting cultivation, is rapidly being replaced or added to by other forms of land use. There are still some communities which rely almost totally on the produce from their subsistence agricultural system. However, many more are now involved in some type of commercial production. New enterprises and land uses are providing new opportunities, but also creating tensions and conflicts. These conflicts arise from internal and external pressures, including government policies. In this chapter I discuss some concerns, focusing on: 1) tensions between crop production, commercialisation and ecosystem/biodiversity conservation; 2) relevance and effectiveness of public policies; and 3) conflicts.

Although I do make some reference to all parts of the Mekong Region, I draw most of my examples from northern Thailand, Vietnam and Lao PDR. At the end of the chapter I put forward a series of questions for policy makers and researchers. Interventions aiming to support poor and marginalised people in the uplands should address these questions, and of course others which are beyond the scope of this particular chapter.

Crop production, commercialisation and ecosystem/biodiversity conservation

A number of factors contribute to the tension between crop production and protection of ecosystems and biodiversity at local and watershed levels. These include the complex relationships between internal and external factors such as population levels and growth, migration, state land tenure policies, customary tenure arrangements, commercialisation, social obligations within traditional

Table 1 Ethnic minority groups in the Mekong Region (by country)

Country	*Number of ethnic groups*	*Population (million people)*		*% Total*
		Minorities	*Total*	
Cambodia	36	0.31	9.45	3.3
Lao PDR	47	2.01	4.88	41.2
Myanmar	>12	>6.8	46.55	14.6
Thailand	10	0.79	58.27	1.4
Vietnam	53	9.88	73.81	13.4
Yunnan	25	13	42	31
Total	183	32.79	234.96	14

Sources: Derived from various texts (Ma Yin 1989, World Resources Institute 1994, Kampe 1997). The data include some ethnic minority people not resident in uplands.

Large-scale infrastructure development projects, such as construction of the Nam Ngum dam in Lao PDR or the Hoa Binh dam in Vietnam, have also displaced people and affected land use in surrounding areas. For example, between 50–60,000 people from the Muong and Tai minorities were displaced by the huge Hoa Binh project whose reservoir extends 230 kilometres back from the dam wall on the Song Da (Black) River, flooding about 200 km^2 of forests and farmland (Hirsch 1998).

There has been a large increase in capital intensive and land extensive monocropping in the uplands. In some parts of the region, this is not a new phenomenon. For example, following the end of the civil war in China in 1949, the communist government promoted the transformation of large parts of the Yunnan uplands. Rubber[1], sugarcane, tea trees and other plantation crops were introduced or vastly expanded. Opium cultivation was totally eradicated. Credit, inputs and terracing earthworks were initially subsidised as part of this transformation. Plantations were seen as 'modern' agriculture replacing the 'primitive' shifting agriculture practised by the area's ethnic minorities, such as the Hani, Jino, Lisu and other smaller groups. Masses of Han Chinese were relocated to work in the collective plantations. Many Han migrants settled in the southernmost counties of Xishuangbanna and Simao in the Lancang Jiang valley (Upper Mekong watershed) and the Red River valley, areas bordering on Myanmar, Lao PDR and Vietnam (Chapman 1991, Kanok Rerkasem 1999). Part of the Yunnan transformation also saw many upland communities move to lower elevations, when land was available, to get involved in wet rice cultivation. During this period, the introduction of hybrid rice – via the Green Revolution – promoted large increases in yields. Due to recent decentralisation to increasingly

[1] Rubber plantations are usually located lower than 600 metres above sea level, hence their impact above that height is indirect.

evaluated. There is concern that intensive farming systems, driven by business goals, are linked to increased destruction of natural forests, biodiversity reduction and general land and water degradation associated with soil erosion, soil fertility depletion, water pollution etc..

The commercial production of high value crops, vegetables and fruit trees also requires substantial water in the dry season. Sprinkler-fed irrigation is economically feasible and relatively easy to install. Large expansion of commercial production in the uplands will increase water use in upstream watersheds and reduce water availability downstream. The extent of future impacts of this problem has yet to be quantified but conflicts over upstream water use are increasing, especially in Thailand. Vietnam's upland landscapes are also changing with the advent, for example, of large areas of coffee monocultures and less degrading tree crops such as persimmon, apricot, plum and lychee.

It must be remembered that Thailand uplands are relatively well-connected to markets, aided by large-scale infrastructure development, especially highway networks. Other parts of the region, such as parts of the Vietnam uplands, are far less well-connected which obviously disadvantages producers by giving them fewer practical options. A separate basic issue is that all the commercial systems, reliant on external markets, are vulnerable to unpredictable market prices. At the time of writing, the troubles facing coffee producers dealing with a world-wide glut offer a harsh lesson that getting the agronomy right is only part of the battle. These problems have diminished the potential of the alternative crops to improve the general livelihoods for the upland ethnic minority populations.

It has been recognised for some time that the promotion of cash crops in the Mekong Region, especially subtropical and temperate species of vegetables, fruit and cut flowers, has to be considered in relation to international trade agreements administered by the World Trade Organisation (WTO), and large-scale infrastructure development such as highway networks and international river transport (TDRI 1994). There will be strong market competition for these commodities. For example, China, which is the biggest producer of these crops and boasts a more favourable climate and lower costs of production, could successfully take over the Mekong Region markets and jeopardise development efforts in the promotion of cash crops on a wider scale. Of the Mekong Region countries, Vietnam is next in line to join the WTO. Nevertheless, with or without trade agreements, legal and 'illegal' trade in these crops has already spread throughout the border areas of the region.

There are many proposals for governments in the region to develop collaborative programmes for large scale production of major cash crops and forest plantations. Some private sectors and agribusiness companies have already

exploitation for timber production. Each has been used to restrict people's access to forested areas.

In Vietnam, a policy push for 'non-shifting' agriculture was adopted in the 1980s aiming to fundamentally change the dominant type of farming system by an estimated 2.9 million people from 400,000 ethnic minority families in 34 mountainous provinces (Sargent et al. 1991). This has involved a comprehensive socioeconomic development programme for ethnic minorities, as well as for forest protection and restoration. State funds have been directly channelled to projects in the form of cash payments and interest-free loans to households contracted to protect and restore forests. It is claimed that at least 600,000 people in 378 communes have stopped shifting cultivation, with some 140,000 hectares coming under permanent crop production. A mid 1990s study reported an increase in forest cover of 47,000 hectares of newly planted forests and 70,900 ha of tree plantations for industry (Le Duy Hung 1995). Agroforestry development is also promoted with support from government to sustain local livelihoods based on multiple sources of products (Do Dinh Sam et al. 1997). The greatest incentive in this programme was a revised land law providing up to 50 years tenure for land users investing in commercial tree crops. However, no funds were made available for land improvements. Consequently, rehabilitating degraded forest has proven very difficult.

Table 2 Extent of land under shifting cultivation in mountain areas of the Mekong Region

Country	*Area (10^3 ha)*			*% forest used for shifting cultivation*
	Land	*Forest*	*Shifting cultivation*	
Cambodia	17,652	12,163	n.a.	n.a.
Lao PDR	23,080	13,173	400	3.04
Myanmar	65,774	28,856	181	0.63
Thailand	51,177	12,735	400	3.14
- N. Thailand	*16,966*	*7,523*	*400*	*5.32*
Vietnam	32,536	8,312	3,500	42.1
Yunnan/China	39,410	9,533	130	1.36
Total	229,629	84,772	>4,611	5.44

Sources: (Fujisaka 1991, Lovelace 1991, Do Dinh Sam 1994, Banerjee 1995, FAO 1995, TDRI 1997)

The beneficiaries of the programme have turned out to be the lowland majority ie. the ethnic Vietnamese, or Kinh groups. Limited funds and prejudice against ethnic minority cultures have been the major constraints to extending this kind of programme to remote mountain communities. A further weakness of this programme is the lack of community participation and local initiative. As the

Box 1 Understanding traditional land use

There is still much to be learnt about traditional land uses, such as shifting cultivation. Even the terminology and classification of shifting cultivation remain to be clarified as knowledge about shifting cultivation increases. For example, the 'pioneer' form of shifting cultivation was classified as the most destructive form in terms of forests and natural regeneration processes (Grandstaff 1980). Others see it as a system with a very long fallow period (Kunstadter and Chapman 1978, Chunthaboon Sutthi 1996). As productive land for cultivation is diminishing, the so-called 'pioneer' shifting cultivators turn to rotational practice with managed fallow. A practical taxonomy of shifting cultivation may have significant value for the design and development of alternatives to shifting cultivation. Above all, a shifting cultivation community tends to practise a mosaic pattern of land use eg. the Tay of northern Vietnam incorporate wet rice fields, homegardens, fish ponds, livestock, tree gardens, swiddens, managed fallow and forests. The whole production system may be referred to collectively as a composite swidden (Rambo 1996, Rambo and Le Trong Cuc 1998).

Mosaic patterns of land use are quite common in mountain areas of Southeast Asia. This production approach can be found even in a former pioneer shifting cultivation community (TDRI 1994). A study in the Hmong village of Pah Poo Chom in Chiang Mai Province of northern Thailand has shown that after eradication of opium growing, these former pioneer swiddeners have completely turned to alternative cash crops, cabbage and lychee in particular. On the surface, the village land use system is very simple and dominated by a few cash crops, but household subsistence is derived from numerous additional livelihood activities. These include the production of upland rice from small swidden fields and the distribution of harvests from some 52 species of non-rice swidden crops grown along field edges and in homegardens (maize, waxy corn, sweet sorghum as well as many local vegetables and root crops).[4] Another part of the mosaic is the sale of local livestock and collection of minor forest products from community-managed forests or household agroforestry plots. These mosaic patterns of land use are also now being classed as agroforestry landscapes (Thomas 2002).

Forest cover

Forest cover policies are obviously connected to shifting cultivation policies. Governments in Vietnam and Yunnan are hoping to increase forest cover by increasing incentives such as long term land allocation to individual households. These various experiments provide opportunities for the regional exchange of lessons and experiences to improve the situation.

In Vietnam the government has been paying cash to encourage upland farmers to plant and manage forest trees. Public funds have underpinned initiatives such as Programme 327, which was established in 1992 to provide state loans for agricultural and forest development on degraded lands and for forest protection

[4]Urban migration and off-farm employment in general have increasingly been incorporated into the livelihood strategies of upland peoples, but these factors are beyond the scope of this chapter.

population pressure. However, this strategy shifts the pressure to traditional upland populations and the environment. In Vietnam, the government had plans to move five million lowlanders to the uplands in the 1970s and 1980s. In Dak Lak province alone, resettlement policies brought in more than 300,000 people, mostly northerners, between 1976 and 1996. During the same period, however, spontaneous migration brought in about another 350,000 people. The results were deforestation, water scarcity and conflict between resource users (Ahmad 2000). The upland plateau of middle Vietnam may be capable of carrying a greater population, but systems need developing which are more economically and ecologically sustainable. The massive transfer of lowland populations with inappropriate practices of non-traditional shifting cultivation may upset the traditional practices that continue to exist in mountain areas, threatening local livelihoods and environmental well being. For example, land competition would threaten the practice of long fallow in rotational shifting cultivation. Shorter fallows lead to severe land degradation and impede natural forest regeneration.

In Lao PDR, despite a sparse upland population, the government still adopted a policy of relocation from the hills for many reasons eg. forest protection, improved access to government support and services, prevention of illicit crop growing, promoting sedentary settlement and so on. In Palaveck of Muang Hom district near Xieng Khuang, massive numbers of Lao Soung (highland ethnic minorities, in this case principally Hmong) have moved down to the valley floors for wet rice cultivation as an alternative to traditional shifting cultivation with opium. Development of paddy terraces was encouraged with government incentives eg. land tenure, agricultural tax breaks, extension services and infrastructure development (roads, small-scale irrigation etc.). Other government policies, especially large infrastructure development such as dams and national highways, also drove many upland communities to relocate. In the Nam Ngum area, large populations of Lao Thueng (midland ethnic minorities) have resettled along the roadside with the high expectation of earning alternative income. In the case of dam construction, where villagers are moved from the reservoir sites and often receive little support from the government, the settlers resume shifting cultivation in the hills above the reservoir, with consequences for the reservoir's sedimentation load.

In the early 1960s, Thailand also adopted resettlement schemes for hill tribes in the north. The concept of resettlement was to develop the remote upland peoples. A few *Nikhom Chao Kao* (resettlement areas for hill tribes) were established to bring hundreds of thousands of upland people to lowland sites. The Thai experience of resettlement was not successful, however, and most people could not remain in the *nikhom* areas, fleeing instead to join relatives returning to their original villages in the mountains (Chupinit Kesmanee 1987-8). The problems were due to inadequate support for subsistence, unsuitable sites for farming, a lack of necessary infrastructure and so on. But today, resettlement of

Box 3 The effort to reduce opium production in northern Thailand

Thirty years of experience have now been gained from an enormous effort by Thailand's government, with assistance from international donors and agencies, at a cost of more than US$206 million (Renard 2001) to eradicate opium production. The initial emphasis in northern Thailand was on eradicating illicit opium cultivation, promoting border security and pursuing social integration. In 1972, an opium eradication campaign was promoted through a development strategy of crop replacement. A wide range of cash crops (both annuals and perennials) was introduced to replace income from opium. Infrastructure, road construction in particular, was developed extensively to support large scale production of cash crops for external markets. In 1983 the Office of Narcotics Control Board and the United Nations Funds for Drug Abuse Control jointly identified some 72 major opium-producing areas in northern provinces to target large scale highland development projects. This Area Planning Approach helped to target development efforts to about 60% of the entire area of opium growing in the country (ONCB 1983). As a consequence, opium production in northern Thailand declined sharply from over 145 tons in 1965 to about 30 tons in the 1980s and a further drop to the insignificant level of an average of 14.5 tons for the past five years, 1995–2000 (Kanok Rerkasem 1999, ONCB 2001). At the same time the importance of traditional land use has also declined.

Traditional shifting cultivation is now very rare and permanent cropping has become the major type of land use. Long-fallow shifting cultivation is constrained from exceeding a seven-year cycle and many former opium growers have turned to production of cash crops and commercial fruit trees for external markets. Taken together with the population increase and other external forces, competition for land and natural resources is increasing. Sustainability of land use has become a critical problem and social conflict in land use within upland communities and between the upland and the lowland communities has increased.

Throughout the long development effort in the uplands of northern Thailand, the approach evolved from the initial implementation of the crop replacement approach in the early 1970s to integrated rural development from the 1970s to late 1980s and a participatory approach from 1990 up to the present. Despite some of the negative consequences, development in northern Thailand has been offered as an 'Alternative Development Model' for neighbouring countries like Lao PDR, Myanmar and Vietnam for opium eradication projects (UNDCP 2000). Regional collaboration in upland development projects would indeed benefit from careful evaluation of this Thai experience. Many initiatives are being proposed, including the United Nations International Drug Control Programme (UNDCP) Regional Cooperation on the Eradication of Illicit Drug Crops and Alternative Development and the World Agroforestry Centre's Global Project on Alternatives to Slash and Burn (ASB).

Traditional shifting cultivation is becoming rare in the region, especially Yunnan in China, Vietnam and northern Thailand. In Mae Chaem and Mae Sarieng of northern Thailand, for example, the fallow period of the former long-fallow shifting cultivation systems of the Karen and Lua people has now been reduced to five years or less. Without external inputs, the productivity of upland rice of

This issue of land security in Thailand is one of the main areas for concern in highland development and political debates. Thailand is unique in the region for its refusal to grant land rights to ethnic minorities and hence many highlanders reside in areas which are claimed by the state. There are virtually no tenure arrangements. Traditional land tenure arrangements should be taken into account in order to promote sustainable use of land and forest protection. Karen in Thailand, for example, are known to have strong community control over land use and land allocation to member households, following customary rules and regulations (Prasert Trakarnsupakorn 1997). Protected areas such as headwater and utility forests are community owned and managed. Land for shifting cultivation and fallow fields are shared and allocated to individual households in the cropping phase. Only paddy lands are privately owned. Without recognition of the value of these traditional land tenure practices, land disputes and conflict over land resources designated for other uses often arise between the villages and other stakeholders, especially forestry department rangers.

The Thailand situation differs from other countries in the Mekong Region, where local administration and social integration of ethnic minorities is more advanced (Chayan Vaddhanaphuti 1996). With little progress made in the past, there have been many land disputes and conflicts at various scales, including within single communities, between communities, as well as between communities and government agencies. Instead of enabling communities to play a role in forest and watershed protection, intervention has involved increased imposition of state control to maintain the functions of ecosystems and biodiversity through strict nature preservation. This then puts pressure on village land by appropriating it for other uses such as forest conservation and reforestation and aggravates land disputes in local communities. Consistent with the on-going processes of decentralisation and innovation required by the 1997 Constitution, present forestry policies make mention of local participation and the roles of community organisations (eg. *tambon* administration organisations at the subdistrict level). However, it is questionable whether such concepts have been absorbed into the institutional culture of forestry and other departments. In the non-governmental sector, participatory land use and local watershed management are becoming popular approaches to conflict resolution, land use planning, monitoring and evaluation at the local level (Uraivan Tan Kim-yong 1990, Prasong Jantakad and Carson 1998). Over 30 NGOs are using such approaches with almost 500 villages, forming networks in the northern provinces to participate in the process of local and national campaigns for land use rights and a community forestry bill (Table 3).

On the operational side, participatory approaches are being used in Thailand to empower communities to reduce land disputes between villages in local watersheds. The process of public participation in policy is still in the project phase and the state has yet to put it into effect on a large scale. It remains to be

economic and political factors (eg. majority and state prejudice against minorities, insurgency concerns, drug-related issues etc). Lowland communities often blame upland people for land and forest degradation, and especially a decline in water volume, which they attribute to both shifting cultivation and extensive cash crop production. Lowlanders also criticise uplanders for chemical pollution in natural streams. While in some instances such claims might be true, the wholesale stereotyping of upland land use practices is unwarranted. Depletion of lowland water may be associated with increasing water use in the uplands due to different types of land use, including state forest plantations with high water requirements, but it is also associated with a dramatic increase in dry season production (and water demand) by lowlanders. Shifting cultivation includes many types of land and forest management and some types are inappropriate. But upland farming also includes a diversity of traditional land and forest management practices that check soil erosion, sustain fertility and crop productivity with biological processes and conserve natural biodiversity in village land use systems. Whatever the roots of the tension, resolving land and forest degradation will mean eliminating misconceptions about land degradation and biodiversity protection with specific reference to upland communities.

Policy intervention questions

Interventions that could support the poor and marginalised people of the uplands are raised here in the form of key questions.

Are there examples within the region of successful cases of upland management where people can make a living while protecting the environment and biodiversity?

Examples of 'best practice' in land management in the Mekong Region uplands are claimed frequently with very little systematic investigation and documentation. Much is known about farmers' management and conservation of biodiversity but good analysis of this indigenous management is much harder to find. That is, the literature is rich in description but poor in critical analysis. I therefore propose that case studies are needed to analyse both successful and unsuccessful land management practices so as to help identify useful strategies and necessary conditions for success. The conditions may focus on biophysical, technical as well as institutional aspects of management systems. The results could then be used in the design and development of alternative upland management policies. It would also be useful to have a utilitarian taxonomy of the traditional land management practices of shifting cultivators across the region.

An interdisciplinary approach would be appropriate for the above field-based study. A range of appropriate tools and methods should also be chosen for field investigation as well as analysing the results. Many tools and methods are now available eg. participatory appraisal (Chambers 1983, Pretty et al. 1995,

Lessons learned from the case studies proposed above could be exchanged through community interaction, cross-country visits and regional training sessions.

How can government agencies play a significant role in building, fostering and supporting local capacity and community organisation at the grassroots level, and its effective interaction with other levels?

For future development to be consistent with a 'people's participation' paradigm, government agencies, development workers and other relevant actors will have to shift from implementing a conventional top down approach to more interactive approaches at various levels of administrative structures and social organisation. This will involve:

- Providing effective, transparent and accountable mechanisms linking local (community) resource management to provincial, prefecture and district governments and governmental agencies involved in formulating and implementing policies at national and other relevant levels.
- Increasing technical capacity for appraisal, monitoring and evaluation, including appropriate information 'feedback' mechanisms and channels for two-way flows of information.
- Coordinating the sharing and 'wise use' of common resources, including equitable distribution of costs and benefits among villages sharing watersheds and between upland and lowland villages.
- Incorporating and promoting land management and sustainable livelihood systems of rural communities, taking into account the management of environmental services and biodiversity-rich ecosystems.

Research questions to support sustainable land use

Several critical research questions about sustainable land use in the Mekong Region uplands remain to be answered. The following questions focus on issues related to upland land use in transition. The research area covers a wide spectrum of land use, from traditional shifting cultivation to permanent fields. Various questions will need to deal with institutional aspects, while others may be purely technical.

1. How can the productivity of upland rice and associated crops be maintained with shorter fallow cycles?
2. How can cash crops be grown, especially on acidic steep land, with minimum soil erosion?
3. How can productivity of upland crops be maintained or improved with minimum use of water and agricultural chemicals?

1. How may the six countries learn from each other's developmental lessons in upland management, including successful and unsuccessful experiences?
2. What is the real local cost of 'exporting' cash cropping to marginal land in other countries within the region? There are many proposals for governments in the region to develop collaborative programmes for large-scale production of major cash crops and forest plantations. Some private sectors and agro-industrial companies have already conducted such programmes on a commercial basis. Examples include maize and soybean production for Thailand in Lao PDR and Myanmar, eucalyptus plantations for the Chinese pulp industry in Thailand, rubber plantations for China in the Wa area of Myanmar, and so on.
3. What impact will major Mekong Region infrastructure development (eg. roads, bridges and Mekong River traffic) have on upland land use sustainability?
4. How may the devastating effects of regional trade in minor forest products be minimised, and the extraction process be managed sustainably? Can some of the species be domesticated and properly managed?

References

Achara Rakyuthitham (2000) 'Community networks: peoples' participation in natural resource and watershed management', report of a Northern Development Foundation seminar. Chiang Mai.

Ahmad A (2000) 'An institutional analysis of changes in land use pattern and water scarcity in Dak Lak province of Vietnam', in papers presented at Nordic conference on Institutions Livelihoods and the Environment: Change and Response in Mainland Southeast Asia. Copenhagen, 27-29 September 2000.

Banerjee AK (1995) 'Rehabilitation of degraded forests in Asia', Technical Paper 270. World Bank, Washington DC.

Boonta Suepradit and Achara Rakyuthitham (1999) 'Three decades of community forestry in the State of confusion of the Thai society'. Northern Development Foundation, Chiang Mai.

Brookfield H (2001) *Exploring Agrodiversity*. Columbia University Press, New York.

Chambers R (1983) *Rural Development: Putting the Last First*. Longman, Essex.

Chambers R (1997) *Whose Reality Counts? Putting the Last First*. Intermediate Technology, London.

Chapman EC (1991) 'The expansion of rubber in southern Yunnan, China', *Geographical Journal* 157, 36-44.

Chapman EC, Bounthong Bouanhom and Hansen PK (eds.) (1998) *Upland Farming Systems in the Lao PDR: Problems and Opportunities for Livestock*. ACIAR Proceedings 87, Australian Centre for International Agricultural Research, Canberra.

Chayan Vaddhanaphuti (1996) 'Present situation of indigenous people in Thailand' in *Cultural Values and Sustainable Resource Management*. UNESCO Office, Jakarta, 21-25.

Narit Yimyam, Kanchanaporn Lodkaew and Kanok Rerkasem (2001) 'Nutrient cycling through *Macaranga denticulata* in rotational shifting cultivation', in CMUPNlab Working Paper 1: Report of a Workshop on Regeneration Ecology and management for Degraded Landscape and Forest Ecosystems. Chiang Mai University, Chiang Mai, 81-94.

ONCB (1983) 'Master plan for opium poppy cultivating regions of Thailand'. Office of Narcotics Control Board and United Nations Fund for Drug Abuse Control, Bangkok.

ONCB (2001) 'Opium cultivation and eradication report for Thailand: 2000-2001'. Office of Narcotics Control Board, Bangkok.

Prasert Trakarnsupakorn (1997) 'The wisdom of the Karen in natural resource conservation' in D McCaskill and K Kampe (eds.) *Development or domestication? Indigenous Peoples of Southeast Asia*. Silkworm Books, Chiang Mai, 219-236.

Prasong Jantakad and Carson S (1998) 'Community based natural resource management from villages to inter-village network: a case study of Pang Ma Pha district, Mae Hong Son province, northern Thailand', in papers presented at a World Bank Community Based Natural Resource Management Workshop. World Bank, Washington DC, 10-14 May 1988, 14.

Pretty J, Guijt I, Thompson J and Scoones I (1995) *Participatory Learning and Action: A trainer's guide*. Methodology Series, International Institute for Environment and Development, London.

Rambo AT (1995) 'Defining highland development challenges in Vietnam: some themes and issues emerging from the conference' in AT Rambo, R Reed, Le Trong Cuc and MR DiGregorio (eds.) *The Challenges of Highland Develoment in Vietnam*. East-West Centre, Honolulu, xi-xxvii.

Rambo AT (1996) 'The composite swiddening agroecosystem of the Tay ethnic minority of the northwestern mountains of Vietnam' in *Proceedings of a Symposium on Montane Mainland Southeast Asia in Transition*. CMU Consortium,Chiang Mai University, Chiang Mai, 69-89.

Rambo AT and Le Trong Cuc (1998) 'Some observations on the role of livestock in composite swidden systems in northern Vietnam' in EC Chapman, Bounthong Bouanhom and PK Hansen (eds.) *Upland Farming Systems in the Lao PDR - Problems and Opportunities for Livestock. ACIAR Proceedings No. 87*. 71-78.

Renard RD (2001) *Opium Reduction in Thailand 1970-2000: A Thirty Year Journey*. Silkworm Books for United Nations International Drug Control Programme, Chiang Mai.

Riethmuller R (1988) 'Differentiation and dynamics of land-use in mountain valley environment: a case study of a new colonisation area in the upper Mae Nam Pa Sak catchment area, Thailand' in W Manshard and WB Morgan (eds.) *Agricultural Expansion and Pioneer Settlements in the Humid Tropics*. United Nations University, Tokyo, 74-94.

Sargent C, Palmer J and Morrison E (1991) 'Setting Priorities for Research in the Land Use Continuum in Vietnam. National Seminar Proceedings.' International Institute for Environment and Development, London.

Stocking MA and Murnaghan N (2001) *Handbook for the Field Assessment of Land Degradation*. Earthscan Publications, London.

Boron Nutrition of Crops and Genotypic Variation in Boron Efficiency

Boron nutrition of crops

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1. CROP RESPONSES TO LOW BORON

Adverse effects of boron (B) deficiency on physiological processes are associated with both vegetative growth and reproductive growth (Dell and Huang, 1997). The vegetative processes reportedly affected by B deficiency are root and leaf growth, vascular differentiation and assimilate partitioning; reproductive ones include flower and gametes development, fertilisation and fruit growth. Boron responses of crops in farmers' fields, however, can be very different from those that interest plant nutritionists and physiologists. The adverse effect of B deficiency on individual physiological processes is relevant to farmers only when it also affects crop productivity, i.e. its economic return. Such effects may be associated with whole plant responses or directly involved in the formation of the quantity of yield (e.g. fruit and grain set) and/or quality of the product.

1.1 Physiological vs. Field Responses

Field responses to B application have been documented on 132 crops in 80 countries (Shorrocks, 1997). However, not all of the physiological responses to low B documented are encountered in field grown crops on low B soils. Two of the most rapid response to B depletion or deficiency are inhibition or cessation of root (Bohnsack and Albert, 1977; Dugger, 1983;

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Marschner, 1995, Shelp, 1993, Dell and Huang, 1997) and leaf elongation (e.g. Kirk and Loneragan, 1988, Noppakoonwong et al., 1993, Huang et al., 1996). However, reports of such effects of B deficiency from the field are extremely rare. Some of these responses do not lend themselves readily to field observation. Some of the physiological responses are also less relevant to whole plant and crop response than others.

Some of the physiological processes are less sensitive to B deficiency than others. Without detectable effect on vegetative growth, B deficiency may cause yield losses in field grown wheat through grain set failure (Rerkasem and Loneragan, 1994). The B level sufficient in meeting demand for vegetative growth in wheat can be insufficient for its anther and pollen development (Rerkasem et al 1997a). In barley, the level of B that is limiting to grain set may also depress the number of spikelets spike^{-1} (Jamjod and Rerkasem, 1999), while tillering may actually be promoted (Ambak and Tadano, 1991). In oilseed rape reproductive growth has been found to be more sensitive to B deficiency than vegetative growth, with root growth even less sensitive than above ground vegetative plant parts (Asad, 1998). For B deficiency to become limiting to root growth, external B had to drop to about half the level that was limiting to above ground vegetative growth. Thus long before B deficiency can become severe enough to limit above ground vegetative or root growth, field grown crops may have already failed through the adverse effect on reproductive growth and seed yield.

1.2 The B Limiting Step

Differential sensitivity to B deficiency may also be found among individual steps of each developmental stage. The most sensitive, which might be called "the B limiting step(s)", will be the one(s) through which whole plant response and crop performance are limited by B deficiency. Adverse effects of B deficiency on reproductive growth have been reported to be associated with male sterility in many cereal species. Cross pollination experiments have established that male fertility is the B limiting step in wheat (Rerkasem et al., 1993; Rerkasem and Jamjod, 1997a). While B deficiency also causes male sterility in maize, the B limiting step may be pollen germination which is dependent on B concentration in the stigma or silk rather than male fertility (Vaughan, 1977, Agarwala et al., 1981). For barley, it is yet unclear that B deficiency depresses grain set primarily through pollen germination as well as causing male sterility. However, the same level of B deficiency that depresses grain set has also been reported to depress the number of spikelet spike^{-1} at the same time (Jamjod and

Rerkasem, 1999). Thus, barley grain yield may be depressed by B deficiency through its compounding effects on at least two B limiting steps.

The adverse effect of B deficiency on reproductive development may be related to higher demand for B in reproductive tissues or difficulties in supplying B to them, or both. In those crop species in which B is immobilised in older tissues, reproduction may fail due to B deficiency even while large amounts of B is present in the whole plant (Brown and Shelp, 1997). When the old B can be recycled to supply elevated demand for reproduction, plant B could be more efficiently used, as has been demonstrated in a gene transfer experiment in tobacco (Brown et al., 1999). Phloem loading, transport and utilisation of B for reproduction were all enhanced as the result of the sorbitol production activated by the introduced gene. The management of crop B nutrition may be made more efficiently, by means of genetic manipulation or fertiliser management, if the B limiting step can be identified.

1.3 The Timing of Boron Sensitive Events

In addition to their relative sensitivity to B deficiency, the relevance of the B response of certain physiological processes to whole plant response, and thus that of crop productivity, may also be dependent on the chronological order of their occurrence. Boron deficiency during early growth, e.g. adversely affecting germination and seedling growth, may have a direct bearing on final seed yield quite independently of how other physiological processes respond to B. In China, survival of transplanted oilseed rape seedlings may sometimes be depressed by B deficiency, an effect that can closely correlate ($R^2 = 0.77$) with seed yield (Xue et al, 1998). The seed of grain legumes containing insufficient B when sown in low B soils may grow into abnormal seedlings (Rerkasem et al, 1990, Rerkasem et al, 1997a). These abnormalities during early growth, which include the absence of the entire epicotyl, no growth after unifoliate leaves, ragged trifoliate leaves, or arrested apical growth accompanied by premature lateral branching, may have a long lasting effect that is reflected in a depression of seed yield. Sensitivity to B deficiency of male gametogenesis is especially important to yield response to B in cereals, e.g. wheat (Rerkasem et al, 1993) and barley (Jamjod and Rerkasem, 1999). The adverse effect of B on male fertility is less relevant in those species in which B deficiency causes the loss of flower buds or whole flowers before anthesis. A typical symptom of B deficiency in field grown sunflower is the corky and brittle peduncle that develops into a horizontal break that can result in the loss of the whole flower head (Fernandez et al., 1985, Rerkasem, 1986). Similarly, flower

buds in B deficient black gram may begin to shed as soon as they are formed (Rerkasem et al., 1987a).

1.4 Boron and Quality

Apart from quantity of yield, the B limiting step in crop production may be associated with the quality and therefore price of the harvested crop, i.e. seed and fruit. A specific symptom of B deficiency that has been known for a long time is the hollow heart in peanut (Harris and Brolmann,1966). Boron deficiency has to be severe enough to cause at least 40 percent of hollow heart to have any effect on seed yield, but in some markets a marked reduction in price can result from only one or two percent of hollow heart. Percentage hollow heart has been found closely correlated to infection by the Aflatoxin causing fungus, *Aspergillus flavus*, (Rerkasem et al., 1988), although it is still unclear if this is a specific association with the low seed B status or a secondary one of damaged seed in general. The adverse effect of low seed B on germination, found at < 10 mg B kg^{-1} in green gram (Bell et al., 1989) and soybean (Rerkasem et al., 1997a), can be expected in other species. The management of B for fruit production is complicated by the different effects of B on yield and various quality characteristics. For example, the B level that has no effect on fruit number or yield may be limiting fruit size and shelf life in avocado (Smith et al., 1997).

The case of apple in Yunnan in south-western China (Dong et al., 1997) illustrates the complex situation of B nutrition in fruit trees for which optimum B levels may be quite different for yield and various quality characteristics, which are also different from those associated with other physiological responses. To manage for optimum apple production in China, orchards with Golden Delicious at 7 x 7 m spacing generally try to keep about 400 fruit per mature tree, thinning excess fruit by hand as necessary. The effect of B deficiency in causing fruit abscission is of no consequence as long as it does not leave fewer than 25% of the total fruit set. The low B that causes about 75% fruit drop, however, is likely to be also limiting to fruit size. Applying B increases fruit size and sugar:acidity ratio, but beyond a certain level this may have adverse effects on other quality characteristics including a loss of fruit firmness and overshooting the market preferred sugar:acidity ratio.

2. OVERCOMING BORON DEFICIENCY IN FARMER'S CROP

On most agricultural soils, it should be possible to correct the problem of B deficiency with an application of 1-2 kg B ha^{-1}. Incidences of B deficiency that continue to occur in farmers' fields throughout the world clearly indicate inaccessibility of this simple and relatively inexpensive solution to many farmers. The on-farm management problem associated with B deficiency is related to the difficulty of diagnosis and the management of B fertilisers.

2.1 Diagnosis

Although various methodologies for diagnosing B deficiency have been available for a long time (Bell, 1997), affected crops in farmers' fields are rarely diagnosed as such. Few farmers in the developing world are aware of soil and plant analyses as a means to determine if crop nutrition is the yield limiting factor. For those who happen to have the knowledge, supporting logistics that would enable samples to be properly collected, analysed and results interpreted and returned in good time are virtually nonexistent. Exceptions are industrial crops such as rubber and large oil palm estates and timber plantations. Quality control of analytical standard is another common problem in labs that are in operation. Furthermore only a few labs in parts of the developing world where B deficiency is a problem are set up to conduct B analyses in soil or plant, although the equipment and other costs involved are relatively inexpensive. Using visual symptoms that are distinctive and specific to B deficiency for diagnosis can be effective and cost very little. The hollow heart symptom in peanut has been successfully used to map areas prone to B deficiency in Thailand, percentage of seed with hollow heart in a crop used to indicate the severity of deficiency (Rerkasem et al 1987b). The major bottleneck is getting such information through to farmers and farm advisors. Booklets or postcards containing distinct and specific symptoms of a few crops common to the area, e.g. peanut, papaya, mango will do for many tropical countries, that can be made widely available may go a long way towards alleviating B deficiency in farmers' crops.

In addition to all the difficulties above, another obstacle to overcoming B deficiency in farmers' crops is related to its highly variable nature. Year to year variation in crop B responses due to climatic conditions is well known, and continues to be reported in the literature (e.g. Xue et al, 1998). Compounding this variability is the wide range of genotypic variation in the response to low B that can be found in many species of the world's major

food crops (see below for more detail discussion on the topic). Incidences of B deficiency observed in farmers' field in one year may not be confirmed next year when the weather becomes less dry, less humid or less cold, or farmers switch back to older varieties known to be unaffected in the same way. The final verification of B deficiency diagnosis with fertiliser trials can also be rendered erroneous by B contamination in the basal fertilisers used. Many formulae of compound fertilisers and macro-nutrients in Asia have be found to contain large amounts of B (Bell et al, 1990).

2.2 Management of Boron Fertiliser

Brazil, Bangladesh, China, Nepal and Thailand are some of the countries where B deficiency has been identified on broad national or regional scale. Among these, the only country where B fertiliser is routinely applied to farmers' crops is Brazil. Brazilian farmers on low B soils are required to include B in their fertiliser management package as a condition for securing farm loans. In the other countries, incidences of B deficiency continue to be common among farmer's crops. Boron fertiliser is applied only occasionally, mostly to high valued crops. For example, tobacco fertiliser in Northern Thailand and Yunnan in south-western China have contained B for many decades. In Thailand foliar B application is routine in vegetables production and orchards of tropical fruits, e.g. durian, rambutan and mangosteen, even in areas where B deficiency has never been diagnosed such as near Bangkok and in the South (Sumitra Poovarodom, pers comm). For high value crops, this trend to apply B as a preventive as well as a corrective measure, can also be found in other countries.

For many important field crops, e.g. wheat and pulses, however, the uncertainty of diagnosis combined with the uncertainty of return means that B deficiency may continue to be an important cause of yield loss in many parts of the world. Breeding and selecting for B efficiency may offer a solution. In the next section this paper will examine potential and limitation of genotypic variation in B efficiency as a means for overcoming B deficiency, and also other implications of genotypic variation in B efficiency in crop B nutrition.

3. GENOTYPIC VARIATION IN BORON EFFICIENCY

In many crop species, genotypes growing on the same soil may be found affected differently by B deficiency. Such genotypic variation in the

response to low B has been reported in monocotyledons and dicotyledons, herbaceous plants and trees, field crops, vegetables, fruits and timber species (Rerkasem and Jamjod, 1997b). Nutrient efficiency has been defined as the ability of a genotype to grow and yield well in soils too deficient for a standard genotypes (Graham, 1984). The practical interest in B efficiency in crop introduction and breeding program is, however, to eliminate genotypes that are less B efficient than existing materials as well as to identify those that may be even more efficient. Furthermore, for B it is generally the newly introduced germplasm that are adeversely affected by deficiency when older established genotypes are not (Rerkasem and Jamjod, 1997b, Anatawiroon et al., 1997, Srivastava et al., 2000). Many authors have successfully evaluated genotypes for B efficiency based simply on their performance in low B relative to the performance in B sufficiency (e.g. Xue et al., 1998, Stangoulis et al., 2000). It appears that B efficiency could be defined either without reference to standard genotypes, as these authors have done, or with standard genotypes that can be either more B efficient or inefficient, or preferably both.

Genetic diversity of B efficiency can mean a difference between complete crop failure and normal yield in some crop species. In bread wheat the most efficient genotypes will set grain and yield normally in soils in which the most inefficient set no grain at all (Rerkasem and Jamjod, 1997a). Similarly for lentil, Nepalese landraces named 'Simal' and 'Simrik' yielded 1.2 t/ha of grain on a soil in which a very large proportion of introduced germplasm was so adversely affected by B deficiency that they yielded nothing at all (Srivastava et al., 2000). Another crop species with almost as large differences between the most B inefficient and efficient is black gram (Rerkasem, 1991). In other species, e.g. oilseed rape (Xue et al., 1998, Stangoulis et al, 2000), green gram (Rerkasem, 1991), sunflower (Blamey et al., 1984), and barley (Jamjod and Rerkasem, 1999), the differences may not be quite so large. However, even in such species B efficiency can mean a difference between a crop that is an economic failure or success. Selecting for B efficiency therefore offers a simple means by which yield and economic loss due to B deficiency can be prevented, especially in those crops in which B fertiliser application is for some reason not feasible.

4. CROP BREEDING AND IMPROVEMENT FOR LOW BORON SOILS

Yield and economic losses are the obvious outcome for growing B inefficient crop varieties on low B soils. In addition, B inefficiency in

introduced germplasm can be a major obstacle to crop improvement. For example, in China the introduction of high quality cultivars of oilseed rape, low in either or both of erucic acid and glucosinolates, have led to severe yield losses due to their extreme B inefficiency (Yang et al., 1993). Similarly, 82% of a lentil germplasm, numbering almost 500 entries, introduced into Nepal for the purpose of improving local lentil production, were found to be extremely inefficient compared with local landraces (Srivastava et al., 2000). Our own evaluation of CIMMYT germplasm also found very high frequencies of B inefficiency in bread wheat, and also durum and triticale that are distributed widely throughout the wheat growing world (Table 1).

Table 1. Frequency distribution (%) of boron inefficiency in bread wheat, durum and triticale genotypes by GSI in sand culture without added B[a]

Nursery or trial[b]	Number of entries	Frequency (%)		
		Inefficient	Moderately efficient	Efficient
29IBWSN	409	97.3	2.2	0.5
4HTWYT	49	61.2	34.7	4.0
17ESWYT	49	87.7	10.2	2.0
28IDYN	49	100.0	0.0	0.0
28ITYN	49	98.0	2.0	0.0
28ITYN	49	98.0	2.0	0.0

a) Inefficient, GSI = 0-70%; Moderately efficient, GSI = 71-85%; Efficient, GSI > 85%, with Fang 60 as B efficient standard.

b) From CIMMYT, Mexico: the 29th International Bread Wheat Screening Nursery; 4th High Temperature Wheat Yield Trial, 17th Elite Selection Wheat Yield Trial, 28th International Durum Yield Nursery, 28th International Triticale Yield Nursery.

Source: Adapted from Rerkasem and Jamjod (2001)

When the soil on which a crop breeding and improvement program is carried out is diagnosed with B deficiency, a common course of action is to apply B fertiliser over the whole station. For lentil in Nepal, it has been suggested that evaluation of introduced germplasm should be conducted on soils in which B is not limiting (Srivastava et al., 2000). However, unless B deficiency as the limiting factor for a particular crop species has also been removed from farmers' fields, screening for B efficiency should be essential at some stage before materials selected for superior agronomic characteristics reach the farmer's field. Evaluating for B efficiency can greatly enhance the cost effectiveness of crop improvement and breeding program serving soils prone to B deficiency, especially in those species in which genotypic variation in the response to low B in the soil is very large as found in wheat and lentil. Such screening would ensure that B inefficient genotypes that are certain to fail in farmers' fields are eliminated before they

reach costly yield trials and on-farm evaluation. With all of our knowledge and understanding on the subject, it would indeed be a pity if farmers' crops should fail just because newly released, supposedly "improved", varieties happen to B inefficient.

Where B efficiency already exists, increasing the frequency in germplasm would be a simple matter of including B efficiency as one of the breeding objectives. The parentage of B efficient Fang 60 and Sonora 64 are actually very common among the pedigrees of CIMMYT wheat (Skovmand et al 2000). The relatively high frequency of B efficiency in the 4HTWYT in Table 1 is therefore not surprising. As we have seen in Thailand's wheat improvement program (Rerkasem and Jamjod, 1997), unintended selection pressure can quickly lead to increases in the frequency of B efficiency. A similar selection pressure clearly does not exist for the rest of the germplasm. Boron efficiency is not one of the breeding objectives of this major international breeding program at CIMMYT, and international yield trials and nurseries in Table 1 are intended for a wide range of environments most of which do not have B deficiency as a limiting factor. However, a B inefficiency frequency of 90% to almost 100% would definitely be a constraint to the potential usefulness of the germplasm on low B soils.

5. CONCLUSION

There are numerous observations and reports of physiological responses to B deficiency in plants. Not all of these are equally relevant to whole plant responses in the field and productivity of farmers' crops. The key to understanding crop B nutrition is the B limiting step, through which whole plant response and crop performance are limited by B deficiency. The management of crop B nutrition may be made more efficiently, by means of genetic manipulation or fertiliser management, if the B limiting step can be identified. The evidence of a wide range of genotypic variation in B efficiency in major crop species has two implications to crop production on low B soils. Firstly, economic success and failure for the particular crop that individual farmers grow will depend on the degree of B efficiency of the crop varieties grown. Secondly, a crop breeding and improvement program will have failed if farmers are constrained from adopting newly released, supposedly improved crop varieties because of their B inefficiency. It is encouraging that work on B efficiency now goes on in major crop species such as wheat, lentil and other pulses and oilseed rape on low B soils such as Nepal, Bangladesh, and China. The problem of B deficiency in these and

other crops on low B soils will not be overcome unless crop improvement objectives specifically include B efficiency or B fertiliser is applied.

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REFERENCES

Agrawala, S. C., Sharma, P. N., Chatterjee, C. and C. P. Sharma, 1981, Development and ezymatic change during pollen development in boron deficiency wheat plants. *J Plant Nutr* 3: 329-336.

Ambak, K. and Tadano, T., 1991, Effect of micronutrient application on the growth and occurrence of sterility in barley and rice in a Malaysian deep peat soil. *Soil Sci.Plant Nutr.* 37: 715-724.

Anatawiroon, P., Subedi, K., and Rerkasem, B., 1997, Screening wheat for boron efficiency. *In* Boron in Soils and Plants. Eds. R.W. Bell and B. Rerkasem, Kluwer Academic Publishers, Dordrecht, pp. 101-104..

Asad, A., 1998, External and Internal Boron Requirements of Plants Using Boron Buffered Solution Culture. Ph.D. Thesis. Murdoch University.

Bell, R.W., 1997, Diagnosis and prediction of boron deficiency for plant production. *Plant Soil* 193: 149-168.

Bell, R.W., Rerkasem, B., Keerati-Kasikorn, P., Phetchawee, S., Hiranburana, N., Ratanara, T.S., Pongsakul, P., and Loneragan, J.F., 1990, Mineral Nutrition of Food Legumes in Thailand, with particular reference to micronutrients. ACIAR Tech Rep No. 16, 52 p. Australian Centre for International Agricultural Research, Canberra, Australia.

Bohnsak, C.W. and Albert, L.S., 1977, Early effects of boron deficiency on indoleacetic acid oxidase levels of squash root tips. *Plant Physiol.* 59: 1047-1050.

Blamey, F.P.C., Vermuelen, W.J., and Chapman, J., 1984, Inheritance of boron status in sunflower. *Crop Sci.* 24: 43-46.

Brown, P.H., Bellaloui, N., Hu, H., and Dandekar A., 1999, Transgenically enhanced sorbitol synthesis facilitates phloem boron transport and increases tolerance of tobacco to boron deficiency. *Plant Physiol.* 119: 17-20.

Brown, P.H., and Shelp, B.J., 1997, Boron mobility in plants. *Plant Soil* 193: 85-101.

Dell, B., and Huang, L., 1997, Physiological response of plants to low boron soils. *Plant Soil* 193: 103-120.

Dong, R.H., Noppakoonwong, R.N., Song, X.M., and Rerkasem, B., 1997, Boron and fruit quality of apple. In *Boron in Soils and Plants*, R.W.Bell and B.Rerkasem, eds., Kluwer Academic Publishers, Dordrecht, pp. 125-130..

Dugger, W.M., 1983, Boron in plant metabolism. In *Encyclopedia of Plant Physiology, New Series, vol. 15B.* A. Läuchli, and R.L. Bieleski, eds., Springer-Verlag, Berlin, pp. 626-650.

Fernandez, P.G., Baudin, C.G, Esquinas, T. M., and Vara, J.M.M., 1985, Boron deficiency in sunflower in Spain. Proc XI Int. Sunflower Conf. pp. 1-8. Borax Holdings Ltd., London.

Graham, R.D., 1984, Breeding for nutritional characteristics in cereals. *Adv. Plant Nutr.*1: 57-102.

Harris, H.C., and Brolmann, J.B., 1966, Comparison of calcium and boron deficiencies of the peanut II. Seed quality in relation to histology and viability. *Agron. J.* **58**: 578-582.

Huang, L., Ye, Z., and Bell, R.W., 1996, The importance of sampling immature leaves for the diagnosis of boron deficiency in oilseed rape (*Brassica napus* cv. Eureka). *Plant Soil* **183**: 187-198.

Jamjod, S. and Rerkasem, B., 1999. Genotypic variation in responses of barley to boron deficiency. *Plant Soil* **215**: 65-72.

Kirk, G.J. and Loneragan, J.F., 1988, Functional boron requirement for leaf expansion and its use as a critical value for diagnosis of boron deficiency in soybean. *Agron. J.* **80**: 758-762.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Second Edition. Academic Press, London. 889pp.

Noppakoonwong, R.N., Bell, R.W., Dell, B., and Loneragan, J.F., 1993, An effect of light on the B requirement for leaf blade elongation in black gram (*Vigna mungo*). *Plant Soil* **155/156**: 413-416.

Rerkasem, B., 1986, Boron deficiency in sunflower and mungbean at Chiang Mai. *J. Agric. (CMU)* **2**: 163-172.

Rerkasem, B., 1991, Comparison of green gram (*Vigna radiata*) and black gram (*Vigna mungo*) in boron deficiency. In *Mungbean 90, Proceedings of the Mungbean Meeting 90.* Bangkok Office of Tropical Agricultural Research Centre, Japan, pp. 167-174.

Rerkasem, B., Bell, R.W., and Loneragan, J.F., 1990, Effects of seed and soil boron on early seedling growth of black and green gram (*Vigna mungo* and *Vigna radiata*. In: *Plant Nutrition – Physiology and Application.* M.L Van Beusichem, ed., Kluwer Academic Publishers, Dordrecht. pp. 281-285.

Rerkasem, B., Bell, R.W., Lordkaew, S. and Loneragan, J.F., 1997 a. Relationship of seed boron concentration to germination and growth of soybean (*Glycine max). Nutrient Cycling in Agroecosystems* **48**: 217-223.

Rerkasem, B., Netsangtip, R., Bell, R.W., Loneragan, J.F., and Hiranburana, N., 1987a, Comparative species responses to boron in a Typic Tropaqualf in Northern Thailand. *Plant Soil* **106**: 15-21.

Rerkasem, B., and Jamjod, S., 1997a, Boron deficiency induced male sterility in wheat (*Triticum aestivum* L.) and implications for plant breeding. *Euphytica* **96**: 257-262.

Rerkasem, B., and Jamjod, S., 1997b, Genotypic variation in plant response to low boron and implications for plant breeding. *Plant Soil* **193**: 169-180.

Rerkasem, B. and Jamjod, S., 2001, Overcoming wheat sterility problem with boron efficiency. Proceedings of the 14th International Plant Nutrition Colloquium. July 27 – August 3 2001, Horst et al., eds., Hannover, Germany.

Rerkasem, B. and Loneragan, J. F., 1994, Boron deficiency in two wheat genotypes in a warm, subtropical region. *Agron. J.* **86**: 887-890.

Rerkasem, B., Lordkaew, S. and Dell, B., 1997b, Boron requirement for reproductive development in wheat. *Soil Sci. Plant Nutr.* **43**: 953-957.

Rerkasem, B., Netsangtip, R., Bell, R.W. and Loneragan, J.F., 1987b, Hollow-heart symptom in groundnut used to survey for boron deficiency in Thailand. *International Arachis Newsletter* **2**: 11-12.

Rerkasem, B., Netsangtip, R., Lordkaew, S. and Cheng, C., 1993, Grain set failure in boron deficient wheat. *Plant Soil* **155/156**: 309-312.

Rerkasem, B., Srichuwong, S., Lodkaew, S., Tongrod, W. and Pridisripipat, S., 1988, Boron deficiency and infection by *Aspergillus* in peanut kernels. In *Proceedings of the 7th National Groundnut Conference.* A Patanothai, ed. Khon Kaen University, Thailand, pp 394-398.

Shelp, B.J., 1993, Physiology and biochemistry of boron in plants. *In* Boron and Its Role in Crop Production. Ed. U C Gupta. pp. 53-85. CRC Press, Boca Raton, FA, USA.

Shorrocks, V.M., 1997, The occurrence and correction of boron deficiency. *Plant Soil* **193**: 121-148.

Skovmand, B., Mackay, M.C., Lopez, C. and A. McNab (Eds), 2000, *Tools for the New Millenium*. On CD ROM, CIMMYT, Mexico.

Smith, T.E., Hetherington, S.E., Asher, C.J., Stephenson, R.A., 1997, Boron deficiency of avocado. Effects on fruit size and ripening. In *Boron in Soils and Plants*, R.W.Bell and B. Rerkasem, eds., Kluwer Academic Publishers, Dordrecht, pp. 135-138.

Srivastava, S.P., Bhandari, T.M.S., Yadav, C.R., Joshi, M., and Erskine, W., 2000, Boron deficiency in lentil: yield loss and geographic distribution in a germplasm collection. *Plant Soil* **219**: 147-151.

Stangoulis, J.C.R., Grewal, S.H., Bell, R.W., and Graham, R.D., 2000, Boron efficiency in oilseed rape: I. Genotypic variation demonstrated in field and pot grown *Brassica napus* L. and *Brassica juncea* L. *Plant Soil* **225**: 243-251.

Vaughan, A.K.F., 1977, The relation between the concentration of boron in the reproductive and vegetative organs of maize plants and their development. *Rhod. J. Agric. Res.* **15**: 163-170.

Xue, J., Lin, M., Bell, R.W., Graham, R.D., Yang, X., and Yang, Y., 1998, Differential response of oilseed rape (*Brassica napus* L.) cultivars to low boron supply. *Plant Soil* **204**: 155-163.

Yang, Y., Xue, J., Ye, Z. and Wang, K., 1993, Responses of rape genotypes to boron application. *Plant Soil* **155/156**: 321-324.

Boron Efficiency in a Wheat Germplasm from Bangladesh

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1. INTRODUCTION

Wheat production in Bangladesh has grown from about 32,000 tons from 60,000 ha in 1961 to almost 2 million tons from 800,000 ha by the year 2000. Wheat has contributed significantly to the country's food security. Bangladesh wheat crop, however, often suffers from the problem of grain set failure. Boron (B) deficiency has been identified as one major cause of this problem. Soils on which wheat is grown in Bangladesh commonly contain 0.1-0.3 mg hot water soluble B kg^{-1} (HWS B), at which B deficiency has been shown to cause grain set failure through male sterility (Li et al 1978; Rerkasem and Loneragan 1994). On the other hand, wheat genotypes have been shown to respond differently to low B (Rerkasem and Jamjod, 1997). This study evaluated a set of wheat varieties and advanced breeding lines from Bangladesh national breeding program to assess their response to B in two experiments conducted at Chiang Mai University, Thailand.

2. MATERIALS AND METHODS

Experiment 1 compared three Bangladeshi wheat varieties (Gourab, Kanchan, and Sourav) with two B inefficient (SW 41 and E 12) and one B efficient (Fang 60) standard genotypes in a sand culture at 4 levels of added

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B (0, 0.1, 0.3 and 10 μM), in three replicates. Experiment 2 evaluated 37 released varieties and advanced breeding lines of wheat from Bangladesh national wheat programme, in duplicate blocks, in the sand culture with 0 and 10 μM of added B (B0 and B10) and in a low B soil (0.1 mg HWS B kg^{-1}) in the field. Also included in the experiment were the three B efficiency checks from experiment 1. In sand culture, plants were grown in freely drained earthenware pots (Ø 30 cm, 30 cm deep) containing washed river quartz sand. The pots were watered twice daily with 1 liter of nutrient solution (1000 μM $CaCl_2$, 250 μM $MgSO_4$, 500 μM KH_2PO_4, 10 μM FeEDTA, 250 μM K_2SO_4, 1 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.1 μM $CoSO_4$, 0.1 μM Na_2MoO_4 and 5 mM KNO_3) with the varying levels of B. The pots were flushed with water once every 4-5 weeks to wash out excess salt. In the field, entries were sown in duplicate blocks, each entry in one meter row with 0.25 m spacing between rows. At maturity the B effect was assessed on grain set and yield components in the main stem from all plants in pots and from ten randomly selected ears from the field.

3. RESULTS AND DISCUSSION

In sand culture without added B, Kanchan and Gourab had similar Grain Set Index (GSI, Rerkasem and Loneragan, 1994) as the B inefficient SW41 and E-12 at about 20% compared with 59 % in Sourav and 89 % in the B efficient Fang 60 (Tab. 1).

Table 1. Effect of boron on grain set (GSI, %) in three major wheat varieties from Bangladesh compared with B efficient (Fang 60) and inefficient (SW 41, E-12) checks

Variety/ Genotype	Boron level (μM) 0	0.1	0.3	10
Kanchan	19.7aA	82.1bcBC	83.1bA	84.0bA
Gourab	22.6aA	86.8bC	91.9bBC	91.1bAB
Sourav	58.8aB	88.6bC	93.9bC	91.4bAB
Fang 60 (E)[a]	89.1aC	92.7aD	88.aAB	93.9aB
SW 41 (I)	20.7aA	67.8bA	84.7cAB	88.1cAB
E-12 (VI)	14.7aA	74.9bAB	84.9cAB	86.8cAB
F-test	Genotype **	Boron **	G x B **	

Differences (by LSD $p < 0.05$) in same row indicated by lowercase letters and in same column by uppercase letters. ** significant at $p < 0.01$
a) E = efficient, I = inefficient, VI = very inefficient

Without added B, the GSI of 37 varieties and advanced breeding lines from Bangladesh ranged from 4% to 55% while it was 82% in Fang 60, 30% in SW 41 and 17% in E-12. Increasing B in the nutrient solution to 10 μM

increased GSI to 80% to 90% in most genotypes. The GSI in B0 of the germplasm correlated well with the GSI in B0 relative to B10 (Fig. 1). As previously suggested (Anantawiroon et al 1997), the inclusion of B efficient and inefficient checks enable germplasms to be evaluated for B efficiency in low B in the absence of B sufficiency control. Based on their GSI in sand culture without added B, out of 37 genotypes from Bangladesh, 6 may be considered very inefficient, 28 inefficient and 3 moderately inefficient no genotype was even moderately efficient (Tab. 2). The B inefficiency of the germplasm was confirmed in the field.

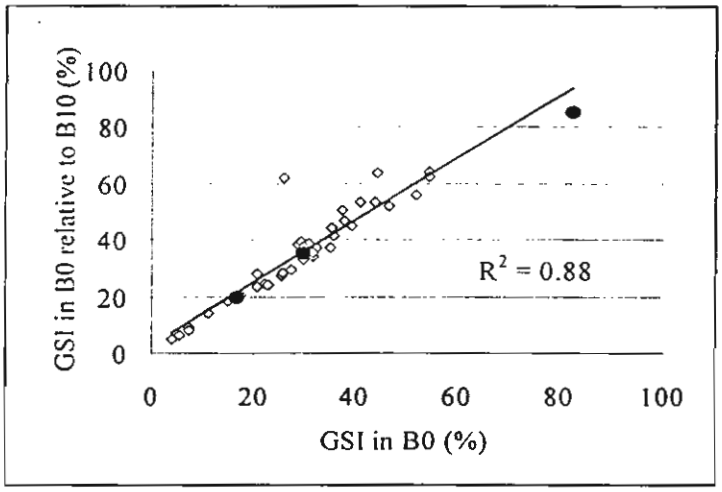

Figure 1. GSI in B0 and GSI in B0 relative to B10 for a wheat germplasm from Bangladesh. Solid circles are, from top, B efficient (Fang 60), and B inefficient (SW 41 and E-12) checks

The two common Bangladeshi varieties, Gourab and Kanchan, were in the same B inefficient class as SW 41. More than 90% of the germplasm tested, which contained released varieties and advanced breeding lines from Bangladesh, was also in this same B inefficient class. The remainders, including the standard variety Sourav were only slightly less inefficient. For an area with widespread low B soils where B fertilizer is still rarely applied in farmers' field, it seems that breeding and selecting for B efficiency would be desirable, especially since genetic sources for B efficiency already exist. It is known that B fertilizer is sometimes applied on station where breeding programs are conducted, to enable germplasm evaluation without the yield potential being limited by B. However, for genotypes destined for low B soils where B fertilizer is not used by farmers, evaluation of B efficiency would be essential some time before advanced breeding lines reach on-farm trials.

Table 2. Frequency distribution of boron efficiency in a wheat germplasm from Bangladesh and their response to boron

Boron Efficiency class[a]	GSI (%) in B0	Number of entries	Mean GSI (%) in each class[b]		
			B0	B10	Field[c]
Very inefficient	0-20	7	9.8	84.5	28.2
Inefficient	21-50	27	31.7	83.5	47.0
Moderately inefficient	51-70	3	53.7	88.4	62.0
Moderate efficient	71-85	0	ne	ne	ne
Efficient	>85	0	ne	ne	ne
Fang 60 (Efficient)			82.5	97.1	84.3
SW 41 (Inefficient)			29.9	85.5	33.8
E-12 (Very Inefficient)			16.8	85.5	38.8

a) Rerkasem and Jamjod (1997) b) ne = no entry c) Soil with 0.1 mg HWS B kg^{-1}

4. CONCLUSION

A wheat germplasm from Bangladesh evaluated for B efficiency has been found to be largely inefficient. Considering the widespread occurrence of low B soils in the country, we suggest that boron efficiency should be included as one of the wheat breeding objectives.

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REFERENCES

Anatawirron, P., Subedi, K.D., and Rerkasem, B. 1997, Screening wheat for boron efficiency. In: *Boron in Soils and Plants*. R.W. Bell and B. Rerkasem, eds. Kluwer Academic Publishers, The Netherlands, pp. 101-104.

Li, B.H., Li, W.H., Kui, M.C., Chao, W.S., Jern, H.P., Li, C.R., Chu, W.J., and Wang, C.L., 1978, Studies on cause of sterility of wheat. *Journal of Northeastern Agricultural College* 3: 1-19.

Rerkasem, B., and Jamjod, S., 1997 Genotypic variation in plant response to low boron and implications for plant breeding. *Plant Soil* 193: 169-180.

Rerkasem, B., and Loneragan, J.F., 1994, Boron deficiency in two wheat genotypes in a warm, subtropical region. *Agron. J.* 86: 887-890.

The Effect of Boron on Pollen Development in Two Wheat Cultivars (*Triticum aestivum* L., cv. 'Fang 60' and 'SW 41')

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1. INTRODUCTION

Boron deficiency causes male sterility in wheat but sensitivity differs among cultivars. In previous studies, a B- inefficient cultivar (cv. SW41) growing in sand culture at low B supply produced pollen that appeared normal at vacuolated young microspore stage, but by anthesis, had become deformed and empty containing no starch (Rerkasem et al., 1997). It has been suggested that the adverse effect of B deficiency may be related to the B requirement during the critical phase of anther development surrounding pollen meiosis: the period from premeiotic interphase through meiosis to late tetrad (Rawson, 1996; Huang et al., 2000). This study was to test the effect of short term B deficiency on pollen quality of B efficient and inefficient wheat cultivars to determine whether different sensitivities to B deficiency during critical stages of pollen microsporogenesis might explain the known cultivar differences in B efficiency.

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2. MATERIALS AND METHODS

Seed of wheat (Fang 60-B efficient and SW 41-inefficient: see Rerkasem et al. 1997) were imbibed in aerated 2 mM $CaSO_4$ solution for 24 hours in the dark at 25°C. Seedlings were then transferred into trays containing 8 L 1/3 strength nutrient solution with 10 μM H_3BO_3 and (give final conc in 8L) MES (2-[N-Morpholino]ethanesulfonic acid) solution and pH was adjusted to 6.0 ± 0.2 everyday with 1 M KOH or 10 % H_2SO_4. Four days after germination, uniform seedlings were transferred to pots containing 5 L of complete nutrient solution with adequate B (10 μM). Nutrient solution was continuously aerated with filtered air and the dry weight increment of extra plants was used to calculate the amount of nutrients for maintaining nutrient supply with programmed nutrient addition (Asher and Blamey, 1987). Seedling roots were rinsed in three changes of 5 mM $CaSO_4$ solution in order to remove B adsorbed on the root surface before transplanting. Two uniform plants per pot were transferred into the B treatments: either low B (0.1 μM B, -B) or adequate B (10 μM B, +B) during the critical stage of pollen development (premeiotic to late tetrad). Pollen developmental stages were identified by dissecting extra plants and staining the microspores with DAPI (4'-6-Diamidino-2-phenylindole·2HCl, Sigma Lot 104F-0542) and examining them under a UV-fluorescence microscope (Vergne et al., 1987). After 5 days of treatment, plants were transferred back to adequate solution B supply (10 μM) and harvested at anthesis. Anthers were collected and fresh pollen examined for viability by the fluorochromatic (FCR) test (Heslop-Harrison et al, 1984) and absence or presence of nuclei by DAPI. Starch accumulation in pollen was assessed by the iodine (KI/I_2) test.

3. RESULTS AND DISCUSSION

Withholding B for 5 days depressed pollen viability at anthesis in the B-inefficient wheat cultivar (cv. SW 41) by 40-70 % (Fig. 1). In contrast to previous reports, starch accumulation in both cultivars was not affected by the temporary B deficiency (Tab. 1). Furthermore, the pollen of SW41 in B- also appeared to differ from SW41 in B+ and Fang 60 in B- and B+ in two other respects. Many of the pollen of SW41 in B- remained attached in pairs and their mitotic nuclei were fewer (Fig. 2).

The cultivar SW41 was more sensitive to B deficiency during the critical stage of microsporogenesis than Fang 60. B deficiency during meiosis has been previously shown to inhibit anther elongation and severely depressed pollen viability (Huang et al., 2000). In SW 41, B deficiency decreased B

content in anthers (Rerkasem et al., 1997). It is possible that the adverse effect of pollen development is caused by inadequate supply of B to the ear and anthers. Rerkasem and Loneragan (1994) and Rerkasem et al. (1997) could not detect any difference in flag leaf and whole ear B concentrations between tolerant and susceptible cultivars and Subedi et al. (1999) even found that a tolerant cultivar had lower B in the flag leaf. Therefore, it is unclear whether cultivars differ in B demand or ability to deliver B into the ear. However, B deficient Fang 60 and SW 41 did not differ in their pattern of B partitioning after flag leaf emergence onwards (Subedi et al., 1999). This contradicts a conclusion drawn by Rawson (1996) that the tolerant genotypes can utilise previously stored B when uptake is limited during the critical reproductive stage. Therefore, the mechanism for B efficiency is still unclear.

Unlike in previous reports (e.g. Li et al., 1978 and Da Silva and da Andrade, 1980), this study found the effect of low B on pollen viability without any effect on starch accumulation. Starch accumulation was not sensitive to B withdrawal in the 5 days during premeiotic to late tetrad. In wheat, starch is normally visible about 12 to 24 h after pollen grain mitosis I and the microspore was packed with numerous starch at mitosis II (Bennett et al., 1973). In this study, the B supply would have been restored during starch accumulation. It is unclear what role B plays in starch accumulation, if any. The presence of starch obviously does not indicate viable pollen. On the other hand, it is interesting that inviable pollen can continue to accumulate starch.

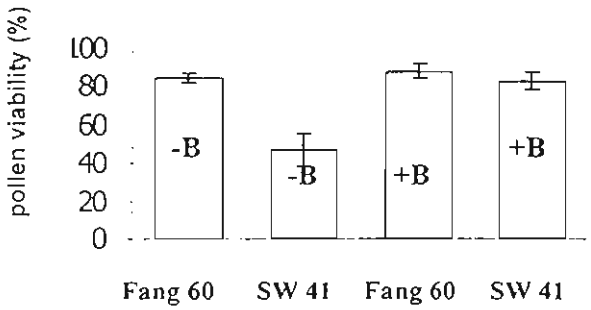

Figure 1. The effect of short term B deficiency on pollen viability (%) in two wheat cultivars by fluorochromatic (FCR) test.

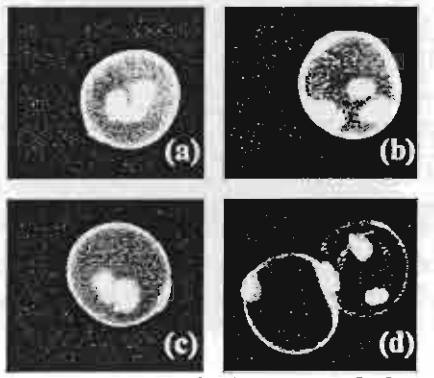

Figure 2. The pollen at anthesis by DAPI test; (a) +B, Fang 60: (b)+B, SW 41; (c) –B, Fang 60; (d)–B, SW 41.

Table 1. Reaction to KI/I_2 staining for starch in the pollen of two wheat cultivars at anthesis.

Cultivar	Boron treatment	
	- B	+ B
Fang 60	+++	+++
SW 41	+++	+++

+++ = most pollen were stained black.

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REFERENCES

Asher, C.J., and Blamey, F.P.C., 1987, Experimental control of plant nutrient status using programmed nutrient addition. *J. Plant Nutr* **10**: 1371-1380.

Bennett, M.D., Rao, M.K., Smith, J.B., and Bayliss, M.W., 1973, Cell development in the anther, the ovule and the young seed of *Triticum aestivum* L. var. Chinese Spring. *Phil. Trans. Royal Soc. London Ser. B* **266**: 39-81.

Da Silva, A.R., and da Andrade, J.M.V., 1983, Influence of micronutrients on the male sterility of upland wheat and on rice and soybean yields in red-yellow latosol. *Pesquisa Agropecuaria Brasileira, Brasilia* **18**: 593-601.

Heslop-Harrison, J., Heslop-Harrison, Y., and Shivahna, K.R., 1984, The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. *Theor.Appl. Genet.* **67**: 367-375.

Huang, L., Pant, J., Dell, B., and Bell, R.W., 2000, Effects of Boron Deficiency on Anther Development and Floret Fertility in Wheat (*Triticum aestivum* L. Wilgoyne). *Ann. Bot.* 85: 493-500.

Li, W.H., Kui, M.C., Chao, W.S., Jern, H.P., Li, C.R., Chu, W.J., and Wang, C.L., 1978, Studies on cause of sterility of wheat. *J. Northeast. Agric. College* **3**:1-19.

Rawson, H.M., 1996, The developmental stage during which boron limitation causes sterility in wheat genotypes and the recovery of fertility. *Aust. J.Plant Phys.* **23**: 709-717.

Rerkasem, B., and Loneragan, J.F., 1994, Boron deficiency in two wheat genotypes in a warm, subtropical region. *Agron. J.* **86**: 887-890.

Rerkasem, B., Lordkaew, S., and Dell, B., 1997, Boron requirement for reproductive development in wheat. *Soil Sci.Plant Nutr.* **43**: 953-957.

Subedi, K.D., Gregory, P.J., and Gooding, M.J., 1999, Boron accumulation and partitioning in wheat cultivars with contrasting tolerance to boron deficiency. *Plant Soil* **214**: 141-152.

Vergne, P., Delvallee, I., and Dumas, C., 1987, Rapid assessment of microspore and pollen development stage in wheat and maize using DAPI and membrane permeabilization. *Stain Technol* **62**: 299-304.

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22 Intensification and diversification of land use in the highlands of northern Thailand[1]

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NORTHERN THAILAND covers an area of approximately 171 000 km² and its 17 provinces are characterized by long mountain ridges and narrow valleys. Four major rivers, the Ping, Wang, Yom and Nan, flow southward and are the major tributaries of the country's biggest waterway, the Chao Phraya. The area shares borders with Laos and Myanmar, and contacts with neighbouring countries in the Mekong subregion can be traced back as far as 1050, the *Thai Era of Lan Na* (Penth, 2000). The population is now close to one million, made up of several main ethnic minority groups, including Karen[2], Hmong, Lahu, Lisu, Yao, Akha, H'tin, and other small minority groups such as Lua, Khamu, Shan and Yunnanese (Haw) Chinese (National Security Council and National Economic and Social Development Board, 1993; Department of Public Welfare, 1995). Virtually all of these people make a living by growing rice for subsistence and other crops for cash income, which in the days before the 1970s and 1980s included the opium poppy (*Papaver somniferum*). Originally, crop production activities were based on two broad groups of traditional shifting cultivation land-use systems, termed rotational and pioneer shifting cultivation (Kunstadter et. al., 1978; Grandstaff, 1980). Rotational shifting cultivators typically settled in one place, and grew crops in a rotation involving 1 year of cropping and 5–10 years of fallow. Pioneer shifting cultivators were migratory. Crops were grown on land cleared from mature forests, and the whole village would pick up and move to a new site after a few years of continuous cropping. Traditionally, opium was the major cash crop of the pioneer shifting cultivators. This system, which may or may not have been practised in its classic form, had been particularly severely abused for its destructive impact on biodiversity and the soil.

The land-use systems have undergone marked changes since 1960. In this chapter, we describe these changes in general, and provide examples from four villages to highlight some positive and negative aspects of the new systems.

The highlands in the context of national policy

Development efforts of the Thai government began in the highlands in the 1970s. With support from various international assistance schemes, they were

directed at eradicating opium poppy cultivation. Central to these efforts were attempts to develop alternative cash crops. The first highland development master plan was initiated in 1983 with assistance from the United Nations Fund for Drug Abuse Control (UNFDAC). It targeted areas and groups involved in opium poppy cultivation (Office of the Narcotic Crops Control Board and UNFDAC, 1983). A second master plan of a similar nature followed in 1988. An important element of these master plans was the coordination of a large number of development activities initiated and supported by several international agencies and bilateral assistance agreements, and implemented as numerous 'highland development projects'.

These largely externally funded projects, which lasted until the late 1990s, helped to direct considerable public investment into the highlands in the form of road building, schools, health services and electrification, as well as the transfer of agricultural technology. Currently public investment for development comes from the Royal Thai Government. There has been a national master plan for highland community development, the environment and narcotic crop control for a period of five years from 1997 to 2001. Apart from this, the highlands receive public investment allocation on the same basis as the rest of the country. Support for development in the highlands is now at a much lower level than in the 30 years before 1990. The exceptions are a handful of villages that continue to receive substantial financial, technical and marketing assistance for their cash cropping through the Royal Project, which is partly funded privately through the Royal Project Foundation and partly publicly from a budget allocation to the Ministry of Agriculture and Cooperatives.

Establishment of permanent villages

Traditionally villages were highly mobile. Pioneer shifting cultivators moved in search of new forests after 5–10 years of continuous cropping. The villages of rotational shifting cultivators also split to establish new settlements when the population grew too large to be accommodated by the existing land. By the mid-1970s, however, movement had virtually stopped. Many Hmong, Lisu, Lahu and Akha villages became permanently settled in the 1960s or earlier. They acknowledged the increasing difficulty of finding new forests to clear. To settle, they frequently bought developed wet-rice land and the associated technology, including the irrigation system, from lowland Thai or Karen farmers. Apparently opium production was sufficiently productive to allow at least some highland farmers to accumulate enough wealth to buy irrigated wet-rice land and invest in commercial crop production.

The trend towards permanent settlement was reinforced by national policies instituted since the 1960s. Originally very few people in the mountains who belonged to any of the ethnic minority groups were recognized as

citizens of Thailand.[3] Permanent settlement is still required as a first step towards official recognition and eventually to Thai citizenship. Citizenship has been granted to only about one-third of the population. Provision of health and education services, roads and electricity offered further incentives to settle permanently. There was also pressure through the national conservation and reforestation policy. Although the highlands had always been regarded by law as national property, they had until relatively recently been treated as a free good. In the past 40 years large areas of the highlands have been designated watershed areas, national parks, forest and wildlife reserves, with strict enforcement of conservation laws. All of these factors combined to make village movement and setting up of new settlements virtually impossible.

New cropping systems

New cropping systems have developed with permanent settlement. Thanks to strict enforcement of drug control laws, opium poppy cultivation has almost disappeared. To meet demand for home consumption by older addicts some small areas of cultivation remain, but these are well hidden. Wet-rice is grown by all ethnic groups, often in small highland valleys. Where dry season irrigation is available, rice may be followed by another crop, usually soybean or vegetables. Areas suitable for wet-rice are keenly sought after, but the amount of relatively flat land with sufficient water supply is limited. Cultivation on the slopes is still widespread, and much of it is on very steep gradients. Some land is cropped annually, some with two or three years fallow, and occasionally with the original full cycle of 5–10 years fallow. Upland rice, maize and various other food and cash crops are grown. The very short fallow periods, and sometimes lack of a fallow period, are associated with low yields and heavy weeding requirements. Farmers are reluctant to apply costly fertilizers and pesticides to subsistence-crops, but do use them on high-value cash crops such as cabbages, coffee, tomato, potato, ginger, lettuce and flowers. Furthermore, all of the high-value crops that are grown in the dry season are irrigated by a system of sprinkler irrigation fed gravitationally from mountain streams and springs. These raise another set of problems.

Sustainability problems of cropping intensification

Improved national transportation, rising incomes in Bangkok and other cities, and a temperate environment, combine to create special opportunities for crop production in the highlands. The cooler climate provides an advantage for the production of temperate fruits, vegetables and flowers. During the monsoon season, vegetable production in the highlands has far fewer problems with insect damage than in the lowlands and there is better surface

drainage on the slopes. Lychees are harvested much earlier and fetch very high prices. Research to find alternatives to opium and to evaluate new crop species and types began in the 1970s, and has continued with increasing commercial interests and initiatives. The two most recent additions are potato production to supply the fast-growing demand of manufacturers of potato products, and hybrid maize seed production. Commercial seed producers have discovered that the mountains provide the ideal conditions for isolation of populations to prevent unintended cross-fertilization between breeding lines.

All of the new cash crops are subject to wild price fluctuations. Downturns in prices threaten the food security of poor village families who have converted completely to cash cropping. The new crops require heavy fertilizer and pesticide applications. Intensive cultivation with a bare soil surface during the wet season contributes to soil erosion and has led to sedimentation behind dams and weirs and in paddy fields. There are also downstream hydrological consequences. Expansion of irrigated cropping in the highlands has been blamed for many mountain streams and springs running dry in the dry season. Conflicts have erupted between highland and lowland communities on these issues. In the Mae Taeng Irrigation Project of the Royal Irrigation Department, for example, the decline of dry season stream flow during the five months December to April from 1972 to 1991 led to an overall reduction of flow of 60.8 million m^3 over the 19 years, averaged at 3.2 million m^3 per year (Thailand Development Research Institute, 1995). In 1993 an ugly confrontation broke out between an upstream Hmong community in Paklouy village and lowland farmers in Chom Thong district of Chiang Mai valley who had their water supply for irrigation dry up (Benjasilaraks and Silaraks, 1999; Rakyuthitham, 2000). Few of the accusations and counter accusations are substantiated by actual measurements, and it is not certain how the problem of upstream and downstream conflict can be resolved in a near future.

Against this general background, the highlands are nevertheless a place of much diversity in both the environment and how farmers and communities respond to new challenges and opportunities. The case studies below, covering people with diverse ethnic backgrounds and contrasting traditional land-management systems, illustrate local innovation and adaptability. The villages are located in Chiang Mai and Mae Hong Son provinces (Figure 22.1). Two of these, Loh Pah Krai and Pah Poo Chom, are former opium-growing, pioneer shifting cultivator villages. The other two, Mae Rid Pagae and Tee Cha, are Karen, who are traditionally sedentary.

Loh Pah Krai – from opium to wet-rice and home gardens

This Lahu village had been a typical pioneer shifting cultivator village before the villagers settled at Mae Ai, north of Chiang Mai, in the mid-1960s. Within

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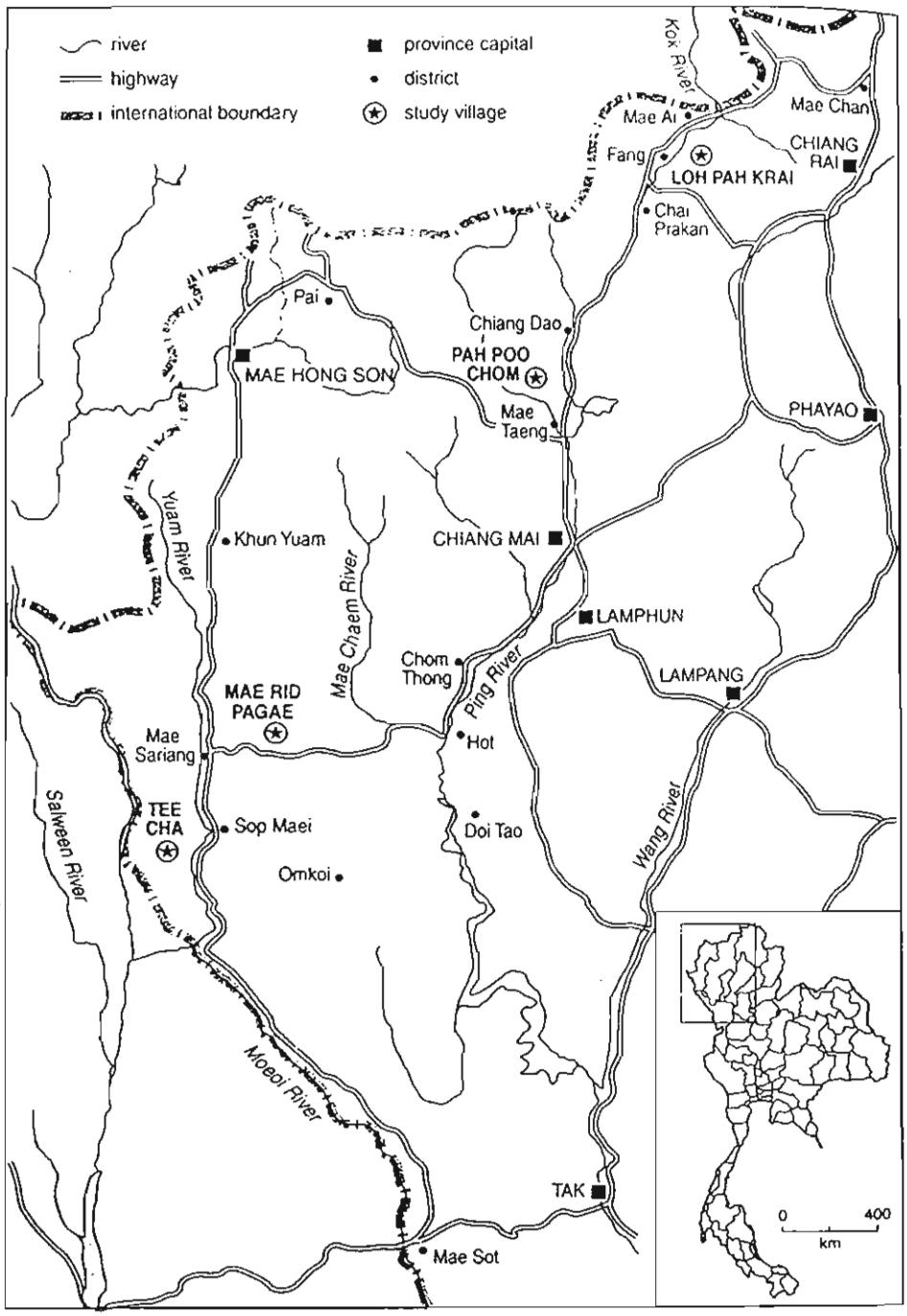

Figure 22.1 *Location of the case study villages in Chiang Mai and Mae Hong Son provinces, Thailand*

the life-time of some of the older members, the village had moved through and, in their own words, 'eaten up several forests' in Chiang Rai and Chiang Mai provinces. To settle permanently, the village bought a sizeable tract of irrigated rice land from some lowland Thais. This was not simply a transfer of land ownership. Along with the land, the Lahu farmers also secured the technological and management skills associated with wet-rice cultivation and the irrigation system. By the mid-1990s they had adopted a double crop system of rice and soybean in the wet-rice fields, and developed an effective scheme for sharing scarce dry season water with downstream villages as well as within the village.

When they first settled, the village was also growing some upland rice and maize on the slopes in short two or three year rotations. By the early 1990s most of the upland fields were cropped every year, often with double-cropping systems of maize or upland rice followed by a grain legume, such as soybean, payee (*Lablab purpureus*) or one of the *Vigna* species. A number of fields within ten minutes walk from the village houses had been developed into home gardens. Some 33 cultivated species were identified in one of the gardens (Table 22.1). Many of the species were grown for sale outside the village, but others provided a year-round supply of food, herbs, spices and animal feed. The food crops were readily shared within the village. Some species were incorporated into vegetative contour conservation strips, for example, lemongrass (*Cymbopogon citratus*), pigeonpea (*Cajanus cajun*) and cha-om (*Acacia pennata* subsp. *insuavis*).

Pah Poo Chom – many ways to biodiversity utilization and conservation

Pah Poo Chom is a Blue Hmong village situated in Mae Taeng watershed, north of Chiang Mai. The villagers settled on a mountain ridge at 940 m above sea level in 1963, and by 1970 most of the surrounding forests had been cleared and cropped with rice, maize and opium. According to the Tribal Research Centre of the Department of Public Welfare, the village was in a stage of extreme poverty, crop yields were low, and opium addicts accounted for 80 per cent of the adult male population and also included some women and even young children (Oughton, 1970; Oughton and Imong, 1970). Based on evidence from this village and many other highland villages, imminent collapse was predicted for highland cropping systems in the 1960s and 1970s (see Keen, 1972; Walker, 1975; Cooper, 1984). Since the early 1990s Pah Poo Chom has been transformed. Cash cropping has been adopted in a major way, but successful management of its biological diversity has also contributed to food security, income generation and conservation of biological diversity.

Table 22.1 Crop species found in a home garden belonging to a farmer from Loh Pa Krai, Mae Ai District, Thailand

Crop type	*Common name*	*Scientific name*
Introduced tree crops	Bamboo	*Dendrocalamus asper*
	Lychee	*Litchi chinensis*
	Santol	*Sandoricum koetjape*
	Mango	*Mangifera indica*
	Jackfruit	*Artocarpus heterophyllus*
	Tamarind	*Tamarindus indica*
New crops grown for cash	Adzuki bean	*Vigna angularis*
	Soybean	*Glycine max*
	Payee	*Lablab purpureus*
	Ginger	*Zingiber officinale*
	Green gram	*Vigna radiata*
Local plants	Cha-om	*Acacia pennata* subsp. *insuavis*
	Banana	*Musa sapientum*
	Papaya	*Carica papaya*
	Jujube	*Ziziphus jujuba*
	Upland rice	*Oryza sativa*
	Maize	*Zea mays*
	Sugarcane	*Saccharum officinarum*
	Sweet sorghum	*Sorghum vulgare*
	Ma Kua	*Solanum* spp.
	Chilli pepper	*Capsicum* spp.
	Pineapple	*Ananas comosus*
	Pumpkin	*Cucurbita moschata*
	Wax or white gourd	*Benincasa cerifera*
	Cowpea, several types	*Vigna unguiculata*
	Pigeonpea	*Cajanus cajun*
	Mustard green	*Brassica* spp.
	Taro	*Colocasia* spp.
	Pak Ped	*Vernonia silhetensis*
	Lemongrass	*Cymbopogon citratus*
	Sweet potato	*Ipomoea batatas*
	Tobacco	*Nicotiana tabacum*
	Sesame	*Sesamum indicum*

Source: Rerkasem et al. (1995)

Lychee trees and sprinkler-irrigated vegetables, principally cabbage, have become the main source of cash income. Villagers market most of their vegetables in Chiang Mai, carrying them in their own pick-up trucks. Agricultural land now accounts for only one-quarter of the village land. The balance is made up of natural forests, two parts conservation forest and one part utility forest dominated by bamboos, especially *Dendrocalamus* and *Bambusa* species (Figure 22.2). The largest number of species was found in the conservation forests, followed by the utility forests, the home gardens, agroforest edges between fields and lychee/vegetable intercrops (Table 22.2).[4] A large proportion of the species in each Land-use Stage are used. Harvesting bamboo shoots for sale is an important source of income especially for poorer villagers. Traditional crops that were part of the opium and upland rice swid-

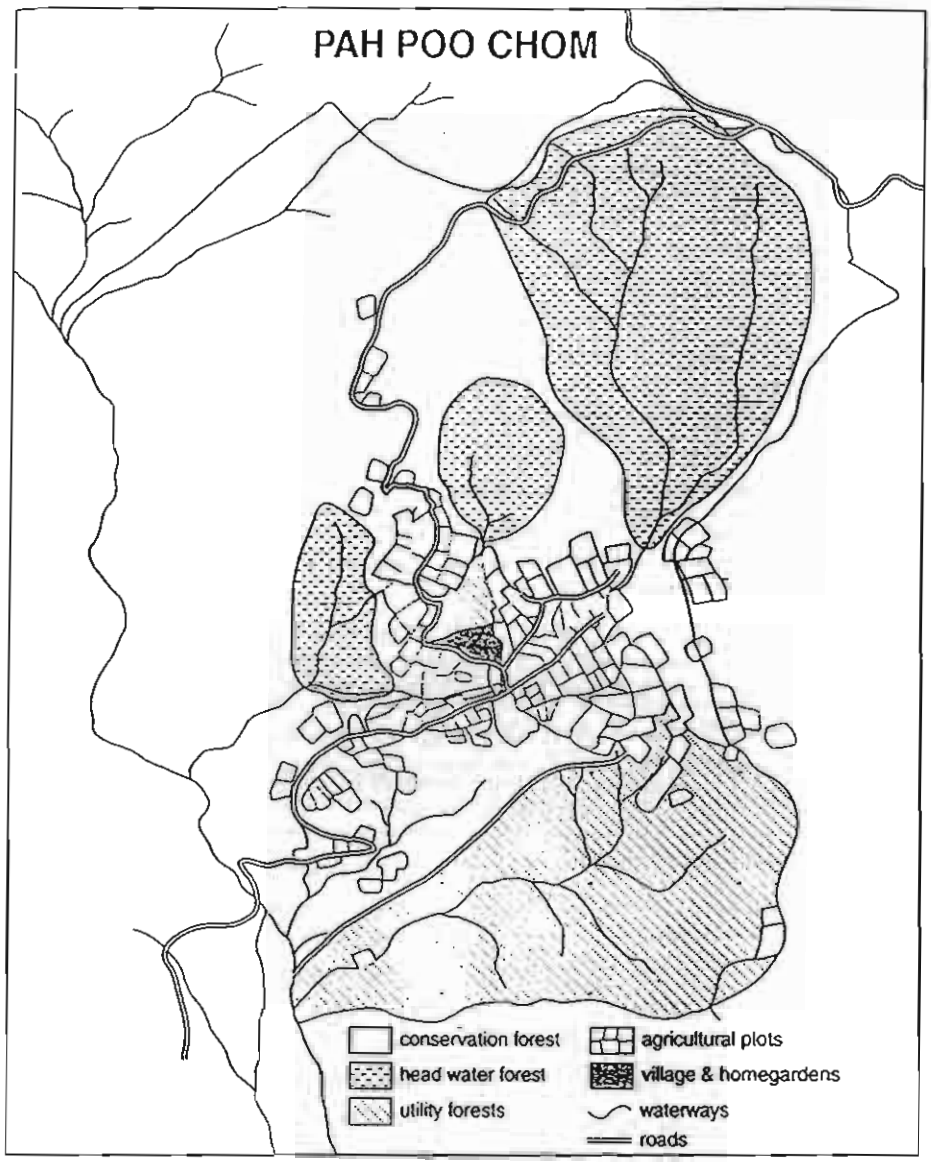

Figure 22.2 *Land-use map of Pah Poo Chom, Thailand*

dens have been conserved and incorporated into new cropping systems. The wild, semi-domesticated and traditional crops and vegetables from Pah Poo Chom (see, for example. Table 22.3) contribute significantly to village food security. They also find ready demand among the urban Hmong community around the Chiang Mai market. A traditional Hmong waxy or glutinous corn is now popular in the city market. Several clumps of a special bamboo are

managed by one old man, who crafts them into the Can, a traditional Hmong musical instrument. Sold for 3000–4000 baht[5] each, several of these are made each year and are sometimes exported to Hmong communities in Laos.

Table 22.2 Number of plant species in various Land-use Stages and Field Types of Pah Poo Chom village, Thailand

Land-use Stage/Field Type	*Number of Species**		
	Total	*Used*	*% Used*
Conservation forests (10 × 10 m)	152	133	87.5
Utility forests (10 × 10 m)	135	110	81.5
Bamboo dominant	89	82	92.1
Agroforest edges (total in the 3 sample plots)	89	68	76.4
Wild mango dominated (10 × 10 m)	33	27	81.8
Wetter area (10 × 30 m)	18	14	77.8
Patch close to village, near road (20 × 50 m)	63	46	73.0
Home gardens (total in two gardens)	68	57	83.8
Garden 1 (30 × 30 m)	45	38	84.4
Garden 2 (25 × 30 m.)	45	34	75.6
Lychee/vegetable intercrops (10 × 10 m)	34	12	35.3
Upper slopes	19	12	63.2
Lower slopes	12	5	41.7

*Numbers of species do not add up to the total in each category and grand total because some species occurred in more than one sample.
Source: Field Survey (1999)

Table 22.3 Useful plant species and their numbers in one semi-cultivated field (5 × 10 m) in Pah Poo Chom, Thailand

Species with common or local name			*Number of plants in sample*
Domesticated	*Zea mays*	Waxy corn (Kaopode in Hmong)	300
	Allium ascalonicum	Shallot	1150
	Brassica juncea	Leaf mustard (Pak-kahd in Hmong)	250
	Cucurbita moschata	Pumpkin	4
	Coriandrum sativum	Coriander	5
	Ipomoea batatas	Sweet potato	3
	Litchi chinensis	Lychee seedlings	1
Semi-domesticated	*Momordica* sp.	Wild bitter gourd	20
	Solanum torvum	Susumber	1
Wild herbaceous	*Crassocephalum crepidioides*	Lum Phasi	1
	Amaranthus viridis	Amaranth	1
	Asytasiella neesiana	Edible fern	11
	Phrynium capitatum	Tong Sard for food wrapping	9 clumps
	Musa acuminata	Wild banana	22 hills
	Zingiberaceae sp.	Kong: edible fruit, leaves for lining rice storage container, fibre for rope making	5 hills
	Total 15 species		1783

Source: Field Survey (2000)

Mae Rid Pagae – cash cropping improving food security

Mae Rid Pagae is a Skaw Karen village at 1200 m above sea level, some four hours by road from Chiang Mai. In the past, the limited irrigated wetland and 'sustainable' rotational shifting cultivation provided enough food for the population only in some years. There were sometimes bad years in which production fell short and many had to walk to the nearby town of Mae Sariang to seek work. As the population grew and the national conservation policy limited expansion of crop production into forest land, the problem of food security worsened. The luxury of adequate rice production with long fallow rotation in traditional shifting cultivation was impossible and farmers had to find viable alternatives to support their livelihood.

Cabbage production began in the early 1980s. Currently, visiting traders buy direct from farmers and truck the crop to Bangkok and beyond. However, it is not a monoculture of cabbage that has been adopted by Mae Rid Pagae. Instead, new cropping systems have evolved, incorporating traditional components and the cabbage. In the irrigated fields, cabbage is grown with irrigation in the dry season following the wet-season rice. On the slopes, upland rice and cabbage are grown in alternate years. The problem of sharply fluctuating prices has not eased, but rice yield has been greatly boosted by the incorporation of cabbage. This is probably due to the residual organic and inorganic fertilizers used in cabbage production, and the effect of clean-weeding of the cabbage in reducing weed infestation of rice. Farmers' reports of rice yield having doubled or tripled have since been confirmed in crop-cutting surveys.

The village also has the advantage of a well-structured soil that is less susceptible to erosion than many others. Vegetative contour strips have been adopted, incorporating weed species such as *Chromolaena odorata*, to check water flow down the slopes. However, farmers do acknowledge that they have begun to receive complaints from downstream villages about perceived water contamination with pesticides. In practice, the use of pesticides is limited to the dry season crops that are grown in areas with supplementary irrigation only.

Tee Cha – Pada fallow, a local innovation

In 1999 the population of Tee Cha numbered 148 in 41 households. The Pwo Karen village, established more than 200 years ago, is situated almost on the Myanmar border in Mae Hong Son province. It is one of a few villages where rotational shifting cultivation is still apparently sufficiently productive to meet food security needs (Figure 22.3). A good forest cover, with numerous uses and services, dominates land use. Being relatively isolated, lack of access to the market limits cash cropping. The cropping system in Tee Cha is

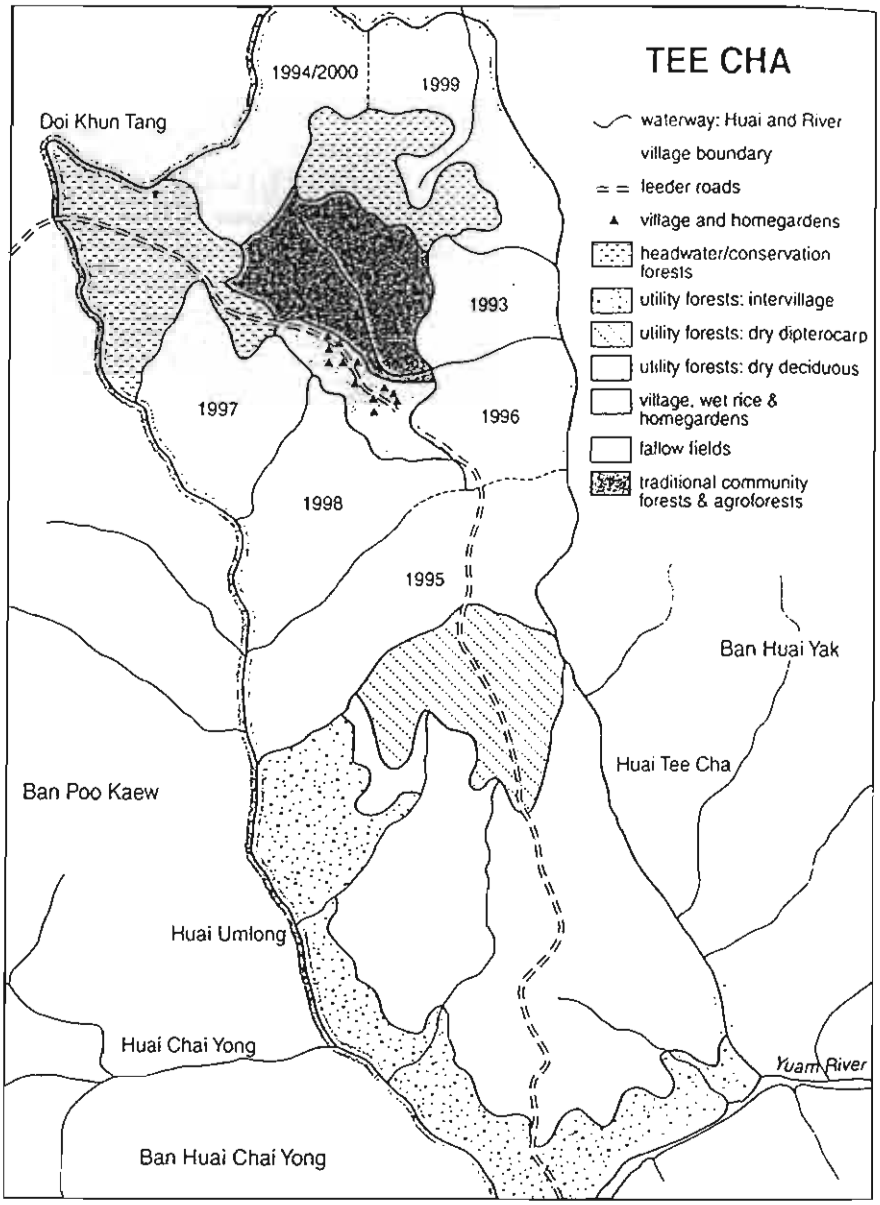

Figure 22.3 *Land-use map of Tee Cha, Thailand*

predominantly subsistence and is managed partly on a communal basis. Upland rice is grown in rotation with six years of fallow. The fallow management, mainly controlling fire and restricting the use of the regenerating forest, felling and burning the mature fallow, and the allocation of land-use rights, are communally organized. Managing the rice crop is an individual

enterprise for each farm household, although there is much sharing and exchanging of germplasm and labour.

The seven-year cycle where land is cropped with upland rice every seventh year, is much shorter than the traditional ten-year or more cycle that used to be common in the region. Farmers attribute sustainability of the shorter cropping rotation to the dominance of Pada (*Macaranga denticulata* Muell. Arg.) during the fallow years. The fallow-enriching properties are recognized by other ethnic groups of northern Thailand, the H'tin and Khamu, who call it Teen Tao (Tong Tao in northern lowland Thai), and by forest ecologists (Whitmore, 1982). Pada is a small tree of the Euphorbiaceae family and it begins to flower and produce seed after three years. In the cropping year, Pada seedlings emerge as a thick carpet among the rice, presumably from a seed bank that accumulated during the previous three years. Farmers manage Pada in a number of ways. The seedlings are not considered weeds, so are not destroyed during hand weeding of the rice. However stands may be thinned if they become too dense and seedlings may be transplanted to areas with poor establishment.

The observations of farmers about the tree's value is corroborated by field observations. Preliminary measurements indicate that Pada trees play a major role in nutrient cycling of the cropping system. Nutrients such as nitrogen, phosphorus and potassium have been found to accumulate in the biomass of Pada-dominated fallow after the sixth year in much greater amounts than in Pada-less fallow after ten years (Zinke et al., 1978). A good stand of Pada, which reaches almost over the farmers' head by the time of rice harvest, is associated with an upland rice yield that is about twice the yield with few or no Pada. Attempts to establish Pada in neighbouring villages where it does not occur naturally, however, have so far been unsuccessful.[6]

Adapting to change

Since the 1960s land use in the highlands of Northern Thailand has undergone dramatic change due to external and internal pressures, which have included national conservation and highland development policies, population growth and rising expectations and aspirations of the villagers themselves. Intensive land use has replaced traditional shifting cultivation but some villagers, by using local innovations, have been better able to adjust their land-use systems to cope with the impact of change on food security and the environment.

Many other successful cropping systems and practices can be found in other villages throughout the mountains of northern Thailand. These observations belie the general belief that intensification of agriculture in the mountains is not sustainable and inevitably leads to yield decline and

degradation. To understand how some farmers and villages in these difficult environments succeed, however, has required a holistic approach to the study of village land management that recognizes variations in time, space and the management units that exist in the agroecosystem. We conclude this chapter with three characteristic factors that have contributed to these farmers more successfully adapting to change.

First, the mountains are agroecosystems with great diversity. This diversity includes variability in:

- the physical environment, for example, soil properties and microclimates
- the social and economic context of the farming system
- the local management capacity that may range from the different agronomic skills and ability to learn of the individual farmers. to the community's capacity to manage common resources or interact effectively with the market or the provincial and national government.

Second, there is a great diversity of plant genetic resources available in these mountain villages, including domesticated, semi-domesticated and wild species that are little known to outsiders, but that have been incorporated into successful new cropping systems.

Third, the innovations that have given rise to new cropping systems would not have materialized without the farmers' intimate knowledge of the specific sets of physical and socio-economic conditions defining their particular environment and the knowledge of the plant genetic resources available to them.

Innovations and knowledge originating from the outside can become useful only when they happen to fit local conditions. Modern agriculture and its various associated sciences have a great potential to benefit these mountain farmers. The challenge is to identify how they can be made relevant to local needs and conditions. Since it will never be possible to completely characterize the local variability, the best thing is to work closely with farmers.

Chapter 17

1 PLEC began work in Amazonian Brazil in 1992, and work in Amapá, already begun under another project, was then incorporated into PLEC. The task reported here overlaps the period of the two projects.
2 For more complete discussions of the formation of the smallholder timber industry see Pinedo-Vasquez et al. (2001) and Sears et al. (2000).

Chapter 18

1 Translated by Liang Luohui, Managing Coordinator of PLEC.

Chapter 19

1 This was a student paper when it was written in the mid-1990s. Even though there have been some changes in the situation at Baka, and in the market for *Amomum villosum*, since that time, the paper is reprinted without substantial change.
2 55000 mu is approximately 3700 hectares; 1 ha is equivalent to 15 mu.

Chapter 20

1 We thank San Long, and Mi Ba for helping us in the field work. Liang Luohui has given invaluable assistance with finalization of the English version of this paper. Kevin Coffey advised on the use and interpretation of the indices employed.

Chapter 21

1 One hectare is equivalent to 15 mu.

Chapter 22

1 The authors wish to acknowledge support for part of the work reported in this chapter from UNU-PLEC and Thailand Research Fund
2 Karen, Hmong, Lahu, Lisu are some of the most common ethnic minority groups living in the mountainous areas of mainland Southeast Asia.
3 Although some groups, including the Karen, have been in Thailand for several hundred years, others are migrants within the twentieth century.
4 Use of these edges between fields is a particularly distinctive feature of the Pah Poo Chom system. A few are substantial, with natural as well as planted trees. The crops and other products obtained from them are mainly used for self-provisioning, and in this respect they constitute an important diversification of what is otherwise largely a commercial system of land use.
5 At the time of this research US$1 = Bt43.
6 Research into Pada currently being undertaken includes its biology of seed production and dormancy, its contribution to productivity of upland rice in the cropping system, its role in nutrient cycling and relationship with other key fallow species. The role of mycorrhiza and nitrogen-fixing endophytes in the nutrition of Pada is also being investigated. This work is supported by UNU-PLEC and Thailand Research Fund.

การตอบสนองของพันธุ์ข้าวไร่และข้าวนาสวนต่อสภาพดินขังน้ำและดินระบายน้ำดี

Response of upland and lowland rice cultivars to waterlogged and well-drained soil conditions

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Abstract : Rice in Thailand is mostly grown in rainfed lowland ecosystems, where water supply is variable during the growing season. However, there is limited understanding of how Thai rice cultivars adapt to changes in water regime. The objective of this work was to examine the responses of upland and lowland rice cultivars to waterlogged and well-drained soil conditions. Two upland (Sew Mae Jun and Kae Noi) and two lowland (Chainat 1 and KDML105) cultivars were compared in waterlogged (W+, the soil surface was submerged under 10 cm. of water) and well-drained (W0, water the plant everyday but not have water standing in the pot) soil, with three replications. There were separate pots for each harvested at 2, 4, and 8 weeks in which root and shoot length, root and shoot dry weight, total root volume, aerenchyma development and nutrient content were measured. Shoot and root growth of all cultivars grown in waterlogging throughout were higher than in drainage throughout, except Kae Noi. Shoot and root growth of Kae Noi in both of waterlogged and well-drained soil conditions were not different. Aerenchyma development of Sew Mae Jun and KDML 105 were not different in two water soils conditions, while Kae Noi was higher in W+ at 5 cm from the root tips. Nutrient contents of all cultivars were also generally higher in waterlogged soils. Nitrogen contents of upland rice cultivars in well-drained soil were higher than lowland rice cultivars. Moreover, nitrogen contents of upland rice cultivars were equal in both soil water conditions. Whereas, lowland rice cultivars in well-drained soil were 50% less than in waterlogged soil conditions. Phosphorus contents had the same response to soil water conditions as nitrogen contents. Specific nutrient uptake of root, Kae Noi and KDML 105 were equal in both of soil water conditions, while Sew Mae Jun and Chainat 1 in well-drained soil were higher than in waterlogged soil.

บทคัดย่อ : ระบบการปลูกข้าวในประเทศไทยส่วนใหญ่เป็นการปลูกข้าวแบบอาศัยน้ำฝน ซึ่งเป็นสภาพที่มีการเปลี่ยนแปลงของระดับน้ำในระหว่างฤดูเพาะปลูก แต่ความเข้าใจเกี่ยวกับการปรับตัวของพันธุ์ข้าวไทยต่อการเปลี่ยนแปลงของระดับน้ำยังมีอยู่ค่อนข้างจำกัด การศึกษานี้มีวัตถุประสงค์เพื่อตรวจสอบการตอบสนองของพันธุ์ข้าวไร่ และข้าวนาสวนต่อสภาพน้ำขัง ทำการศึกษาพันธุ์ข้าว 4 พันธุ์ คือ ชิวแม่จัน แกน้อย (พันธุ์ข้าวไร่) ชัยนาท 1 และขาวดอกมะลิ 105 (พันธุ์ข้าวนาสวน) ปลูกเปรียบเทียบในสภาพดินขังน้ำ (ขังน้ำสูงจากผิวดิน 10 ซม) และสภาพดินระบายน้ำดี (รดน้ำทุกวัน แต่ไม่มีน้ำขังที่ผิวดิน) ทำ 3 ซ้ำ แยกเก็บข้อมูลแต่ละกระถางที่อายุ 2 4 และ 8 สัปดาห์ โดยเก็บข้อมูลความยาวราก ความสูงต้น น้ำหนักแห้งราก น้ำหนักแห้งต้น ปริมาตรรากรวม การพัฒนาโพรงอากาศ และปริมาณธาตุอาหารในต้นข้าว จากการทดลองพบว่าการเจริญของต้นและรากข้าวทุกพันธุ์ที่ปลูกในสภาพดินขังน้ำเจริญเติบโตดีกว่าในสภาพดินระบายน้ำดี ยกเว้นข้าวพันธุ์แกน้อยมีการเจริญเติบโตในทั้งสองสภาพน้ำที่ไม่แตกต่างกัน สำหรับการพัฒนาโพรงอากาศในรากข้าวของพันธุ์ชิวแม่จันเหมือนกับข้าวพันธุ์ขาวดอกมะลิ 105 ซึ่งมีการสร้างปริมาณโพรงอากาศที่ไม่แตกต่างกันตามสภาพน้ำ ในขณะที่การพัฒนาโพรงอากาศที่ระยะ 5 เซนติเมตรจากปลายรากของพันธุ์แกน้อยสร้างโพรงอากาศมากกว่าพันธุ์อื่นๆที่ปลูกในสภาพเดียวกัน และพบว่าต้นข้าวที่ปลูกในสภาพดินขังน้ำมีปริมาณธาตุอาหารสะสมมากกว่าในดินระบายน้ำดี การสะสมปริมาณ

ไนโตรเจนของพันธุ์ข้าวไรที่ปลูกในสภาพน้ำไม่ขังมากกว่าพันธุ์ข้าวนาสวน โดยพันธุ์ข้าวไร่มีปริมาณไนโตรเจนสะสมเท่ากันทั้งที่ปลูกในสภาพน้ำขังและน้ำไม่ขัง ในขณะที่พันธุ์ข้าวนาสวนที่ปลูกในสภาพน้ำไม่ขังมีปริมาณไนโตรเจน น้อยกว่าถึง 50% ของต้นที่ปลูกในสภาพน้ำขัง ซึ่งปริมาณฟอสฟอรัส และโพแทสเซียมของพันธุ์ต่างๆมีการตอบสนองต่อสภาพน้ำเช่นเดียวกับปริมาณไนโตรเจน ข้าวพันธุ์แกน้อย และขาวดอกมะลิ 105 มีความสามารถจำเพาะของรากในการดูดธาตุอาหารที่เท่ากันไม่ว่าจะปลูกในสภาพน้ำขังหรือน้ำไม่ขังก็ตาม ในขณะที่พันธุ์ชิวแม่จันและชัยนาท 1 ที่ปลูกในสภาพน้ำไม่ขังมีความสามารถจำเพาะของรากในการดูดธาตุอาหารได้ดีกว่าที่ปลูกในสภาพน้ำขัง

คำนำ

การจำแนกพื้นที่ปลูกข้าวโดยใช้สภาพน้ำเป็นตัวแบ่งสามารถแบ่งได้เป็น นาชลประทานและนาอาศัยน้ำฝน พื้นที่ปลูกข้าวในระบบนาอาศัยน้ำฝน เป็นพื้นที่ที่ขาดระบบชลประทานและมักจะขาดการขังน้ำอย่างต่อเนื่องในระหว่างฤดูกาลเพาะปลูก ปริมาณและระยะเวลาในการได้รับน้ำของนาอาศัยน้ำฝน ซึ่งสาเหตุเหล่านี้ทำให้เกิดความเสียหายต่อผลผลิตข้าวได้ (Widawsky and O' Toole, 1990; Zeigler and Puckridge, 1995) ปริมาณฝนและธาตุอาหารมีผลต่อผลผลิตข้าวโดยตรงต่อกระบวนการทางสรีรวิทยาที่เกี่ยวข้องกับระยะการเจริญเติบโตทางลำต้นและใบและการติดเมล็ด สภาพดินที่มีการขังน้ำและดินไม่มีน้ำขังสลับกัน จะลดความเป็นประโยชน์ของธาตุอาหารต่างๆ ที่ข้าวจะดูดใช้ และทำให้มีธาตุอาหารต่ำ ซึ่งเป็นปัจจัยจำกัดศักยภาพการสร้างผลผลิต (Bell et at., 2001) ข้าวนาน้ำฝนปลูกได้ทั้งในสภาพนาสวนและนาไร่ ข้าวนาสวนเป็นการปลูกข้าวโดยใช้เมล็ดโดยตรง หรือ การย้ายกล้าไปปลูกในแปลงที่มีการเตรียมดินและมีน้ำเพียงพอ ข้าวไร่มักจะใช้เมล็ดปลูกโดยตรง และไม่มีการเตรียมแปลง ดินมีการระบายน้ำแบบธรรมชาติ โดยไม่มีน้ำขังที่ผิวหน้าดินเลย ประเทศไทยมีความหลากหลายของพื้นที่ปลูกข้าว และมีพันธุ์ข้าวมากมาย การทราบถึงความสามารถของพันธุ์ข้าวต่างๆมีการปรับตัวต่อสภาพน้ำที่มีการเปลี่ยนแปลงอย่างไรนั้น จะทำให้สามารถจัดการธาตุอาหารและหลักการในการปรับปรุงพันธุ์ข้าวให้มีความสามารถในการให้ผลผลิตที่สูงขึ้น ในนาน้ำฝนที่ควบคุมระดับน้ำไม่ได้

วัตถุประสงค์ของการวิจัย

การทดลองนี้มีวัตถุประสงค์เพื่อตรวจสอบการตอบสนองของการเจริญของต้นและรากข้าว รวมทั้งการสะสมปริมาณธาตุอาหารของพันธุ์ข้าวไร่ และข้าวนาสวนในสภาพดินขังน้ำและดินระบายน้ำดีในระยะ 8 สัปดาห์แรก

อุปกรณ์และวิธีการ

วางแผนการทดลองแบบ Factorial ศึกษา 2 ปัจจัย คือ พันธุ์และสภาพน้ำ โดยศึกษาพันธุ์ข้าว 4 พันธ์ แบ่งเป็นข้าวไร่ 2 พันธุ์ คือ ซิวแม่จัน และแกน้อย ข้าวนาสวน 2 พันธุ์ คือ ชัยนาท 1 และขาวดอกมะลิ 105 ปลูกทดสอบในสองสภาพน้ำ คือ ดินขัง (ขังน้ำสูงจากผิวหน้าดิน 10 เซนติเมตร) และดินที่มีการระบายน้ำดี (รดน้ำทุกวัน แต่ไม่มีน้ำขังที่ผิวดิน) ทำ 3 ซ้ำ ปลูกข้าวในกระถางดินเผาขนาดเส้นผ่านศูนย์กลาง 30 เซนติเมตร ลึก 30 เซนติเมตร ซึ่งบรรจุดินชุดสันทราย 5 กิโลกรัมต่อกระถาง ย้ายปลูกกล้าข้าวอายุ 7 วัน จำนวน 3 ต้นต่อกระถาง ปลูกพันธุ์ละ 24 กระถาง หลังย้ายปลูก 1 สัปดาห์ จึงเริ่มสภาพดินขังน้ำ และดินระบายน้ำดี ใส่ปุ๋ยไนโตรเจน 0.37 กรัมต่อกระถาง ฟอสฟอรัสและโพแทสเซียม 0.26 กรัมต่อกระถาง โดยแบ่งใส่ 2 ครั้ง คือ หลังย้ายปลูก 2 สัปดาห์ และใส่อีกครั้งหลังจากใส่ปุ๋ยครั้งที่ 1แล้ว 4 สัปดาห์ และทำการเก็บข้อมูลแยกแต่ละกระถางที่ระยะ 2 4 และ 8 สัปดาห์ โดยเก็บข้อมูลความยาวราก ความสูงต้น ปริมาตรรากรวม น้ำหนักแห้งราก น้ำหนักแห้งต้น ประเมินการพัฒนาโพรงอากาศ และการสะสมธาตุไนโตรเจน ฟอสฟอรัส และโพแทสเซียม

ผลการทดลอง

พันธุ์ข้าว 4 พันธุ์มีการตอบสนองต่อสภาพน้ำไม่ต่างกันในระยะ 2 สัปดาห์แรก พันธุ์ข้าวที่ปลูกในสภาพน้ำขังและระบายน้ำเริ่มแสดงความแตกต่างในน้ำหนักแห้งรากตั้งแต่สัปดาห์ที่ 4 (ตารางที่ 1) โดยพบว่าเมื่อปลูกในสภาพน้ำขัง ข้าวไร่ซิวแม่จันและแกน้อยมีน้ำหนักแห้งรากต่ำกว่าข้าวนาสวนชัยนาท 1 และขาวดอกมะลิ 105 แต่ในสภาพน้ำไม่ขังข้าวพันธุ์ซิวแม่จัน และข้าวนาสวนทั้ง 2 พันธุ์ มีน้ำหนักแห้งรากต่ำกว่าครึ่งหนึ่งของในสภาพน้ำขัง ที่แปลกไปคือพันธุ์แกน้อยที่มีน้ำหนักแห้งรากไม่ต่างกันตามสภาพน้ำ อิทธิพลของสภาพน้ำต่อพันธุ์ข้าวมีความแตกต่างชัดเจนขึ้นที่สัปดาห์ที่ 8 ในสภาพน้ำไม่ขัง (ตารางที่ 2) พันธุ์ที่มีน้ำหนักแห้งรากสูงสุดคือพันธุ์แกน้อย ปานกลางคือ ซิวแม่จันและขาวดอกมะลิ 105 ที่ต่ำสุดคือชัยนาท 1 เมื่อขังน้ำพันธุ์แกน้อยก็ยังมีน้ำหนักแห้งรากไม่ต่างไปจากไม่ขังน้ำ ในขณะที่น้ำหนักแห้งรากในสภาพน้ำขังในซิวแม่จันและข้าวนาสวน 2 พันธุ์ สูงกว่าในสภาพน้ำไม่ขังถึง 2-4 เท่า

นอกจากนี้ยังพบว่าสภาพน้ำมีอิทธิพลต่อพันธุ์ข้าวต่างกันในความยาวต้นและน้ำหนักแห้งต้น (ตารางที่ 3) และปริมาณธาตุอาหารในต้น (ตารางที่ 4) ที่ 8 สัปดาห์ในสภาพน้ำไม่ขังพันธุ์แกน้อยมีความยาวต้นสูงกว่าข้าวนาสวน 2 พันธุ์กับซิวแม่จัน และเมื่อขังน้ำแกน้อยก็ยังมีความยาวต้นไม่ต่างไปจากไม่ขังน้ำ ในขณะที่ข้าวอีก 3 พันธุ์ในสภาพน้ำขังมีความยาวต้นสูงกว่าในน้ำไม่ขังอย่างมีนัยยะสำคัญ นอกจากนี้ยังได้พบว่าพันธุ์แกน้อยกับชัยนาท 1 ยังรักษาความแตกต่างในการตอบสนองต่อสภาพน้ำในแง่ของน้ำหนักแห้งต้นด้วย กล่าวคือในสภาพน้ำไม่ขังแกน้อยมีน้ำหนักแห้งต้นสูงกว่าชัยนาท 1 และเมื่อชัยนาท 1 มีน้ำแห้งต้นสูงขึ้นอีก 1 เท่าตัวเมื่อขังน้ำ ในขณะที่น้ำหนักแห้งต้นในแกน้อยไม่แสดงการตอบสนองต่อการขังน้ำ เป็นที่น่าสังเกตุว่าสภาพ

น้ำมีผลต่อน้ำหนักแห้งต้นของข้าวไร่อีกพันธุ์หนึ่งคือชิวแม่จันคล้ายกับแกน้อย สำหรับขาวดอกมะลิ 105 มีน้ำหนักแห้งต้นในสภาพน้ำไม่ขังเท่ากับแกน้อย แต่ขาวดอกมะลิ 105 แตกต่างไปจากพันธุ์ข้าวไร่ตรงที่มีน้ำหนักแห้งต้นเพิ่มขึ้นถึงร้อยละ 60 เมื่อขังน้ำ และเมื่อพิจารณาน้ำหนักแห้งรวมทั้งต้นยิ่งทำให้เห็นถึงความแตกต่างระหว่างพันธุ์ที่ตอบสนองต่อสภาพน้ำได้ชัดเจนมากขึ้น โดยจะเห็นได้ว่าพันธุ์แกน้อย และชิวแม่จันมีน้ำหนักแห้งรวมทั้งต้นไม่แตกต่างกันทั้งที่ปลูกในสภาพดินขังน้ำและไม่ขังน้ำ ในขณะที่พันธุ์ข้าวนาสวน คือ ชัยนาท 1 และขาวดอกมะลิ 105 ที่ปลูกในสภาพขังน้ำมีน้ำหนักแห้งรวมทั้งต้นมากกว่าในสภาพไม่ขังน้ำถึงหนึ่งเท่าตัว และเมื่อเปรียบเทียบความสัมพันธ์ต่อสภาพน้ำของน้ำหนักแห้งรวมทั้งต้น จะเห็นได้ว่าพันธุ์แกน้อยที่ปลูกในสภาพน้ำไม่ขังมีความสามารถในการสร้างน้ำหนักแห้งรวมของทั้งต้นได้เท่าหรือดีกว่าในสภาพน้ำขัง ในขณะที่พันธุ์ชิวแม่จันที่ปลูกในสภาพน้ำไม่ขังก็มีความสามารถสร้างน้ำหนักแห้งรวมทั้งต้นได้ถึง 80% ซึ่งใกล้เคียงกับพันธุ์ขาวดอกมะลิ 105 ซึ่งเป็นพันธุ์ข้าวนาสวนที่สร้างน้ำหนักแห้งรวมทั้งต้นได้ 70% ในทางตรงกันข้ามข้าวพันธุ์ชัยนาท 1 ซึ่งเป็นพันธุ์ข้าวนาสวนเช่นเดียวกับพันธุ์ขาวดอกมะลิที่ปลูกในสภาพน้ำไม่ขังมีความสามารถในการสะสมน้ำหนักแห้งรวมทั้งต้นได้เพียงครึ่งหนึ่งของสภาพน้ำขัง (ตารางที่ 6) สำหรับความสามารถในการดูดธาตุอาหารจำเพาะของราก ข้าวพันธุ์แกน้อย และขาวดอกมะลิ 105 มีความสามารถจำเพาะของรากในการดูดธาตุอาหารที่เท่ากันไม่ว่าจะปลูกในสภาพน้ำขังหรือน้ำไม่ขังก็ตาม ในขณะที่พันธุ์ชิวแม่จันและชัยนาท 1 ที่ปลูกในสภาพน้ำไม่ขังมีความสามารถจำเพาะของรากในการดูดธาตุอาหารได้ดีกว่าที่ปลูกในสภาพน้ำขัง (ตารางที่ 5)อีกลักษณะหนึ่งที่สามารถใช้พิจารณาความสามารถของพันธุ์ที่ตอบสนองต่อสภาพน้ำได้ชัดเจน คือ สัดส่วนของรากต่อต้น โดยทุกพันธุ์มีสัดส่วนของรากต่อต้นในสภาพน้ำขังอยู่ระหว่าง 30-40% ยกเว้นพันธุ์ขาวดอกมะลิ 105 ที่มีสัดส่วนรากต่อต้นที่ 20% ทั้งในสภาพน้ำขังและน้ำไม่ขัง เช่นเดียวกับพันธุ์แกน้อยที่มีสัดส่วนรากต่อต้นไม่แตกต่างกันในทั้งสองสภาพน้ำ แต่พันธุ์แกน้อยมีความสามารถในการสร้างรากได้มากกว่าพันธุ์ขาวดอกมะลิ 105 ในขณะที่พันธุ์ชิวแม่จันและชัยนาท 1 ที่ปลูกในสภาพน้ำไม่ขังมีสัดส่วนรากต่อต้นเพียงครึ่งหนึ่งของในสภาพน้ำขัง

การทดลองนี้ยังพบว่า ข้าวทุกพันธุ์สามารถสร้างโพรงอากาศได้ทั้งที่ปลูกในสภาพดินขังน้ำและดินไม่ขังน้ำ ข้าวพันธุ์ชิวแม่จันมีการสร้างโพรงอากาศในปริมาณเท่ากับพันธุ์ขาวดอกมะลิ 105 โดยสามารถสร้างปริมาณโพรงอากาศได้เท่ากันทั้งสองสภาพ เช่นเดียวกับข้าวพันธุ์ชัยนาท 1 ที่ระยะ 8 สัปดาห์ที่มีการสร้างโพรงอากาศในปริมาณที่ไม่แตกต่างกันในทั้งสองสภาพน้ำ นอกจากนี้ที่ระยะ 5 เซนติเมตรจากปลายรากข้าวพันธุ์แกน้อยที่ปลูกในไม่ขังสภาพน้ำสร้างโพรงอากาศได้มากกว่าพันธุ์อื่นๆ และยังสร้างมากขึ้นเมื่อปลูกที่สภาพขังน้ำ (รูปที่ 1) โดยทั่วไปต้นข้าวที่อยู่ในสภาพขังน้ำสะสมธาตุอาหารไนโตรเจน ฟอสฟอรัส และโปแตสเซียมได้มากกว่าในสภาพน้ำไม่ขัง (ตารางที่ 4) แต่นอกจากนี้ยังได้พบว่าสภาพน้ำมีผลต่อการสะสมธาตุไนโตรเจน และฟอสฟอรัสต่างกันในพันธุ์ข้าวไร่และข้าวนาสวน กล่าวคือสภาพน้ำมีผลเพียงเล็กน้อยต่อการสะสมธาตุอาหารในข้าวไร่พันธุ์ชิวแม่จันและแกน้อย ในขณะที่ในสภาพน้ำไม่ขัง ข้าวนาสวนชัยนาท 1 สะสมฟอสฟอรัสในได้เพียงหนึ่งในสาม และขาวดอกมะลิ 105 สะสมได้เพียงครึ่งหนึ่งของเมื่อขังน้ำ

วิจารณ์และสรุปผลการทดลอง

การศึกษานี้ได้แสดงให้เห็นอย่างชัดเจนว่า ในข้าว 4 พันธุ์ที่ทำการศึกษา ไม่มีพันธุ์ใดมีการปรับตัวในสภาพน้ำไม่ขังดีกว่าสภาพน้ำขัง มีแต่บางพันธุ์เจริญเติบโตได้ดีพอกันในสภาพขังน้ำและไม่ขังน้ำ ในขณะที่บางพันธุ์เจริญเติบโตได้ดีกว่าในสภาพน้ำขัง และความแตกต่างระหว่างพันธุ์ข้าวในการตอบสนองต่อสภาพน้ำมิได้จำเพาะอยู่ระหว่างข้าวไร่และข้าวนาสวนเท่านั้น เมื่อพิจารณาจากการเจริญเติบโตของต้นและรากและการสะสมธาตุอาหาร ข้าวไร่พันธุ์แกน้อยนับว่ามีการปรับตัวเข้ากับสภาพน้ำไม่ขังได้ดีที่สุด และข้าวนาสวนพันธุ์ชัยนาท 1 ปรับตัวเข้ากับสภาพน้ำไม่ขังได้ต่ำที่สุด สำหรับข้าวนาสวนขาวดอกมะลิ 105 กับข้าวไร่ชิวแม่จันน่าจะอยู่ตรงกลาง เพราะในสภาพน้ำไม่ขัง สองพันธุ์นี้มีน้ำหนักแห้งรากและต้นสูงกว่าชัยนาท 1 และต่ำกว่าหรือเท่ากับแกน้อย

สภาพน้ำมีผลต่อภาวะธาตุอาหารในต้นข้าวสองทางคือ (1) ปริมาณธาตุอาหารที่เป็นประโยชน์ในสภาพน้ำขังจะสูงกว่าเมื่อน้ำไม่ขัง (Yoshida, 1981) (2) การทำงานดูดอาหารของรากที่อาจต่างกันในสภาพน้ำขังและไม่ขัง เมื่อพิจารณาจากปริมาณธาตุอาหารในต้น พบว่าในสภาพน้ำไม่ขังรากข้าวพันธุ์ไร่แกน้อยมิได้มีความสามารถดูดธาตุอาหารจำเพาะในการสะสมฟอสฟอรัสสูงไปกว่าพันธุ์อื่น (แกน้อย 0.56 mg ชิวแม่จัน 0.74 mg ชัยนาท 1 0.88 mg และ ขาวดอกมะลิ 105 0.80 mg ต่อกรัมน้ำหนักแห้งราก) (ตารางที่ 5) ความแตกต่างในการตอบสนองต่อสภาพน้ำของพันธุ์ข้าวที่วัดได้ จึงน่าจะมาจากความสามารถในการสร้างรากในสภาพไม่ขังน้ำ มากกว่าความสามารถจำเพาะในการดูดธาตุอาหารของรากของแต่ละพันธุ์ นอกจากนี้ความสามารถปรับตัวต่อสภาพน้ำไม่ขังของพันธุ์ข้าวอาจวัดได้จากสัดส่วนราก:ต้น (ROOT:SHOOT RATIO) (ตารางที่ 7) โดยเห็นได้ชัดเจนว่าข้าวไร่พันธุ์แกน้อยรักษาสัดส่วนราก:ต้น ไว้ที่ 0.30 ในสภาพน้ำไม่ขัง เทียบกับ 0.39 ในสภาพน้ำขัง เช่นกันในพันธุ์ขาวมะลิ 105 ที่มีสัดส่วนราก:ต้น ในสภาพน้ำขัง 0.23 ใกล้เคียงกับในสภาพน้ำไม่ขังคือ 0.21 แต่ข้าวนาสวนสำหรับเขตชลประทาน พันธุ์ชัยนาท 1 มีสัดส่วนราก:ต้น 0.36 ในสภาพน้ำขัง แต่ในสภาพน้ำไม่ขังมีเพียง 0.14 เป็นที่น่าสังเกตว่าข้าวพันธุ์ขาวดอกมะลิ 105 แม้จะเป็นข้าวนาสวน แต่ในสภาพน้ำไม่ขัง สามารถสร้างรากได้ดีพอๆกับพันธุ์ข้าวไร่ ความสามารถนี้อาจเป็นเหตุผลสำคัญอันหนึ่งที่ทำให้ข้าวพันธุ์ขาวดอกมะลิ 105 ซึ่งเป็นที่นิยมเพราะเป็นข้าวคุณภาพสูง สามารถปลูกในนาน้ำฝนเกือบทั่วประเทศ นับเป็นร้อยละ 23 ของพื้นที่ปลูกข้าวทั้งประเทศ และเมื่อรวมกับ กข 6 และ กข 15 ซึ่งปรับปรุงพันธุ์มาจากขาวดอกมะลิ 105 ด้วยการกลายพันธุ์โดยวิธีอาบรังสี ด้วยแล้วนับได้ถึงร้อยละ 54 ของพื้นที่ปลูกข้าวทั่วประเทศในปีการเพาะปลูก 2543/44 (OAE, 1998)

โดยสรุปการศึกษานี้ได้แสดงให้เห็นว่าพันธุ์ข้าวไทยมีการตอบสนองต่อสภาพน้ำต่างกัน โดยพันธุ์ที่พบว่าปรับตัวต่อสภาพน้ำไม่ขังได้ดีที่สุดคือข้าวไร่พันธุ์แกน้อย ตามด้วยพันธุ์ชิวแม่จัน ดีปานกลางคือข้าวพันธุ์ขาวดอกมะลิ 105 และได้น้อยที่สุดคือข้าวนาสวนพันธุ์ชัยนาท 1 ความสามารถในการสร้างรากและสัดส่วนราก:ต้น อาจเป็นตัวบ่งชี้ความสามารถปรับตัวของพันธุ์ข้าวต่อสภาพน้ำไม่ขังได้ดี

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เอกสารอ้างอิง

Bell, R. W., Ros, C. and Seng, V. 2001. Improving the efficiency and sustainability of fertiliser use in drought- and submergence-prone rainfed lowlands in Southeast Asia. In: Increased Lowland Rice Production in the Mekong Region. Pp. 155-169 . Eds S. Fukai. And J. Basnayake. Proceedings of an International Workshop, Vientiane, Laos. 30 October – 1 November 2000. ACIAR Proceeding 101.

OAE, 1998. Report on the Survey of Main Season Rice, 1996/97 Season. Agricultural Statistic Document No. 9/1998.

Widawsky, D.A., O'Toole, J.C., 1990. Prioritizing the rice biotechnology research agenda for Eastern India. The Rockefeller Foundation, New York.

Yoshida, S. 1981. Fundamentals of rice crop science. International Rice Research Institute. Los Banos, Philippines. 269 p.

Zeigler, R.S., Puckridge, D.W., 1995. Improving sustainable productivity in rice-based rainfed lowland systems of South and Southeast Asia. Feeding 4 billion people, The challenge for rice research in the 21st century. Geo J. 35, 307-324.

Table 1 Root dry weight of four rice cultivars when grown in waterlogging (W+) and well-drainage (W0) soil conditions at 4 weeks.

Cultivar	Root dry weight (g)	
	W+	W0
Sew Mae Jun	0.36 Ba	0.16 Bb
Kae Noi	0.28 Ba	0.30 Aa
Chainat 1	0.49 Aa	0.25 Ab
KDML 105	0.50 Aa	0.20 Ab
Mean	*0.41*	*0.23*
	F-test	$LSD_{0.05}$
	G^{ns}	-
	W^{*}	*0.07*
	$G \times W^{*}$	*0.13*

ns = non significant ($p<0.05$), * significant at $p < 0.05$. The difference between water treatments in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 2 Root dry weight, shoot dry weight and total dry weight of four rice cultivars when grown in waterlogging (W+) and well-drainage (W0) soil conditions at 8 weeks.

Cultivars	RDW (g/plant)		SDW (g/plant)		Total dry weight (g/plant)	
	W+	W0	W+	W0	W+	W0
Sew Mae Jun	3.86 Ba	2.07 Bb	13.0Aa	11.7Aa	16.68 Ba	13.77 Aa
Kae Noi	3.46 Ba	3.32 Aa	8.9 Ca	11.2 Aa	11.36 Ca	14.52 Aa
Chainat 1	5.53 Aa	1.26 Bb	15.6 Aa	8.9 Bb	21.13 Aa	10.16 Ab
KDML 105	3.92 Ba	2.16 Bb	17.5 Aa	11.1Ab	21.42 Aa	13.26 Ab
Mean	*4.19*	*2.20*	*13.76*	*10.73*		
	F-test	$LSD_{0.05}$	*F-test*	$LSD_{0.05}$	*F-test*	$LSD_{0.05}$
	G^{ns}	-	G^{ns}	-	G^{ns}	-
	W^{*}	*0.47*	W^{*}	*2.17*	W^{*}	*3.54*
	$G \times W^{*}$	*0.94*	$G \times W^{*}$	*4.34*	$G \times W^{*}$	*5.01*

ns = non significant ($p<0.05$), * significant at $p < 0.05$. The difference between water treatments in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 3 Shoot length of 4 rice cultivars when grown in waterlogging (W+) and well-drainage (W0) soil conditions at 8 weeks.

Cultivar	Shoot length (cm)	
	W+	W0
Sew Mae Jun	80.6 Ba	68.4 Ab
Kae Noi	73.2 Ca	67.9 Aa
Chainat 1	60.3 Da	51.9 Bb
KDML 105	92.2 Aa	63.3 Ab
Mean	*76.58*	*62.90*
	F-test	$LSD_{0.05}$
	G^{ns}	-
	W^{*}	*2.98*
	$G \times W^{*}$	*5.96*

ns = non significant ($p<0.05$), * significant at $p < 0.05$. The difference between water treatments in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 4 Nitrogen, phosphorus, and potassium contents (mg/plant) of four rice cultivars when grown under waterlogged and well-drained soil conditions for 8 weeks.

Cultivars	N contents (mg)		P contents (mg)		K contents (mg)	
	W+	W0	W+	W0	W+	W0
Sew Mae Jun	6.04 Aba	5.34 Aba	2.48 BCa	1.54 Aa	2.79	2.54
Kae Noi	5.16 Ba	6.56 Aa	2.28 Ca	1.82 Aa	2.21	2.38
Chainat 1	6.60 Aba	3.38 Bb	3.38 ABa	1.10 Ab	3.14	1.82
KDML 105	8.16 Aa	4.28 ABb	3.52 Aa	1.54 Ab	3.64	2.38
Mean	*6.49*	*4.89*	*2.92*	*1.50*	*2.95 a*	*2.28 b*
	F-test	$LSD_{0.05}$	*F-test*	$LSD_{0.05}$	*F-test*	$LSD_{0.05}$
	G^{ns}	-	G^{ns}	-	G^{ns}	-
	W^{*}	*1.14*	W^{*}	*0.48*	W^{*}	*0.53*
	$G \times W^{*}$	*2.29*	$G \times W^{*}$	*0.96*	$G \times W^{ns}$	-

ns = non significant ($p<0.05$), * significant at $p < 0.05$. The difference between water treatments in the same row isindicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 5 Nutrient uptake (mg /g root DW) in 4 rice cultivars under waterlogged and well drained soil conditions for 8 weeks.

Cultivars	N uptake (mg/g RDW)		P uptake (mg/g RDW)		K uptake (mg/g RDW)	
	W+	W0	W+	W0	W+	W0
Sew Mae Jun	1.57 Ab	2.57 ABa	0.64	0.74	0.73 Ab	1.22 Aa
Kae Noi	1.49 Aa	2.02 Ba	0.66	0.56	0.65 Aa	0.73 Ba
Chainat 1	1.19 Ab	2.70 Aa	0.61	0.88	0.57 Ab	1.46 Aa
KDML 105	2.05 Aa	2.11 ABa	0.89	0.80	0.92 Aa	1.19 Aa
Mean	*1.58*	*2.35*	*0.70*	*0.75*	*0.72*	*1.15*
	F-test	$LSD_{0.05}$	*F-test*	$LSD_{0.05}$	*F-test*	$LSD_{0.05}$
	G^{ns}	-	G^{ns}	-	G^{ns}	-
	W^{*}	*0.34*	W^{ns}	-	W^{*}	*0.20*
	$G \times W^{*}$	*0.68*	$G \times W^{ns}$	-	$G \times W^{*}$	*0.40*

ns = non significant ($p<0.05$), * significant at $p < 0.05$. The difference between water treatments in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

Table 6 Root-shoot ratio and Relative response to water of four rice cultivars when grown in waterlogging (W+) and well-drainage (W0) soil conditions at 8 weeks.

Cultivar	Root-Shoot Ratio		Relative response to water	
	W+	W0	W0/W+	
Sew Mae Jun	0.30 ABa	0.18 Bb	0.82 B	
Kae Noi	0.39 Aa	0.30 Aa	1.17 A	
Chainat 1	0.36 Aa	0.14 Bb	0.48 C	
KDML 105	0.23 Ba	0.21 ABa	0.69 BC	
Mean	*0.32*	*0.21*	*0.79*	
	F-test	$LSD_{0.05}$	*F-test*	$LSD_{0.05}$
	G^{*}	*0.072*	G^{*}	*0.29*
	W^{*}	*0.051*		
	$G \times W^{*}$	*0.10*		

ns = non significant ($p<0.05$), * significant at $p < 0.05$. The difference between water treatments in the same row is indicated by lower case letters. The difference between cultivars in the same column is indicated by upper case letters.

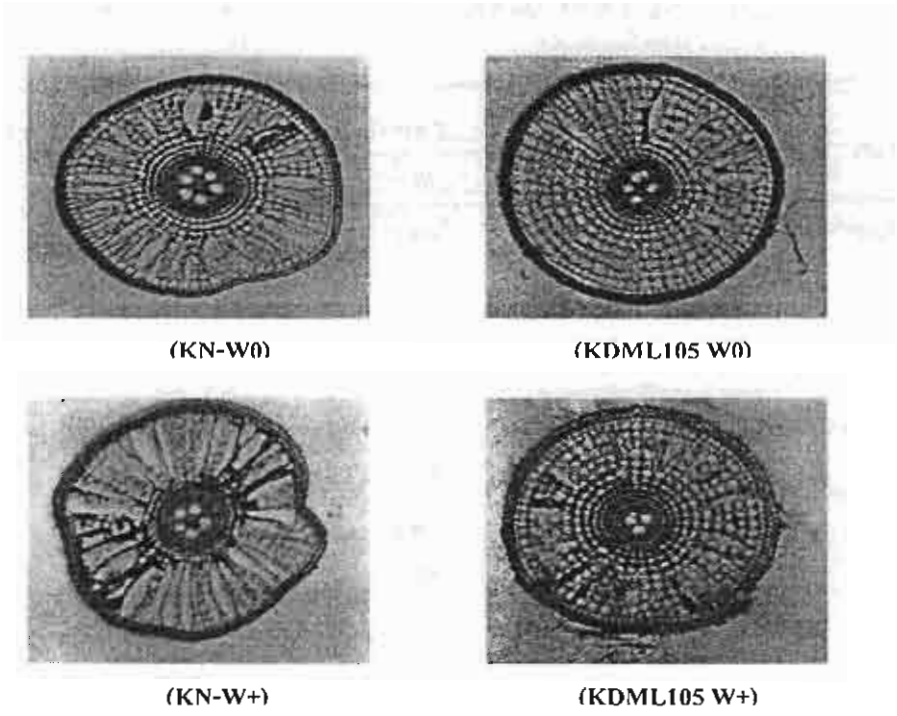

Figure 1 Aerenchyma appearance of Kae Noi (KN) and KDML 105 in waterlogged (W+) and well-drained soil condition at 5 cm from the terminl root.

เปรียบเทียบการตอบสนองต่อการขาดธาตุโบรอนในข้าวบาร์เลย์และข้าวสาลี
Comparative Response to Boron deficiency in Barley and Wheat

Keywords: Barley, Boron deficiency, Boron efficiency, Wheat

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Abstract

To determine if boron (B) deficiency, commonly reported to depress grain set in wheat, has the same effect in barley, two experiments compared three wheat and three barley genotypes at various B levels in sand culture. Plants were grown with varied levels of added B, from 0 to 10 μM. Without added B, the genotypes ranged in Grain Set Index (GSI) from 0 to 93 % for wheat and 0 to 67 % for barley. Boron concentration of the ear and flag leaf at boot stage in wheat and barley correlated ($r = 0.8 – 0.9$, $p < 0.01$) with the effect of B on GSI. Grain set was the only response to low B, also measurable in decreased number of grains ear^{-1} and grains $spikelet^{-1}$, in wheat. In barley, B deficiency also depressed the number of spikelets ear^{-1} by 23 to 75 % and induced a "rat-tail" symptom of terminal spikelet degeneration. There was a weak correlation between ear and flag leaf B and the effect of B on ear size in barley ($r = 0.47$ and 0.37, respectively, $p < 0.1$). In some barley genotypes, the low B level that depressed grain set sometimes also delayed ear emergence and depressed the number of ears $plant^{-1}$ but sometimes increased tillering. These results demonstrate that the phenotypic response to low B is more complex in barley than wheat. Different strategies may be required for managing B nutrition and different approaches for selecting B efficient genotypes in the two species.

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บทคัดย่อ

รายงานการขาดโบรอนของข้าวบาร์เลย์มีความขัดแย้งกันอยู่ บางรายงานพบว่าเมื่อขาดโบรอนข้าวบาร์เลย์มีน้ำหนักแห้งฟางลดลง ในขณะที่บางรายงานพบว่าน้ำหนักแห้งฟางเพิ่มขึ้น เพื่อยืนยันให้เห็นผลกระทบจากการขาดโบรอนทั้งการเจริญเติบโตทางลำต้นใบและการแพร่ขยายพันธุ์ที่แตกต่างกัน จึงเปรียบเทียบการตอบสนองต่อระดับโบรอนในข้าวสาลีและข้าวบาร์เลย์ โดยทำการทดลองในกระถางทราย ในฤดูปลูก 2541/2542 ที่ภาควิชาพืชไร่ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ ผลจากการขาดโบรอนทำให้ข้าวบาร์เลย์และข้าวสาลีติดเมล็ดลดลง ข้าวบาร์เลย์ยังได้รับผลกระทบจากการขาดโบรอนในการเจริญเติบโตทางลำต้นและใบด้วย โดยข้าวบาร์เลย์จะมีขนาดรวงลดลง การออกรวงช้า มีจำนวนหน่อและน้ำหนักแห้งสูง ต่างจากข้าวสาลีที่ไม่พบอิทธิพลจากการขาดโบรอนในลักษณะดังกล่าว ดังนั้นอาการขาดโบรอนของข้าวบาร์เลย์อาจมีกลไกของการขาดที่มีความซับซ้อนมากกว่าข้าวสาลี ความแตกต่างระหว่างข้าวบาร์เลย์และข้าวสาลีรวมทั้งความแตกต่างในระหว่างพันธุ์ต่อการตอบสนองต่อการขาดโบรอน ทำให้การจัดการเกี่ยวกับธาตุโบรอนเป็นไปได้อย่างเหมาะสม รวมทั้งใช้เป็นแนวทางในงานปรับปรุงพันธุ์ เพื่อคัดเลือกหาพันธุ์ที่ทนทานต่อการขาดโบรอนต่อไป

คำนำ

โบรอนเป็นจุลธาตุที่มีความจำเป็นสำหรับการเจริญเติบโตและการพัฒนาของพืช Shorrock (1997) รายงานว่า มีพื้นที่ที่มีปัญหาดินขาดโบรอนแพร่กระจายอยู่ทั่วโลก และพื้นที่ในประเทศไทยก็มีรายงานการขาดโบรอนด้วยเช่นกัน (เบญจวรรณ และคณะ, 1989; เพิ่มพูน, 2540) เนื่องจากโบรอนเป็นโมเลกุลที่ไม่มีขั้ว ดังนั้นจึงเกิดการชะล้างได้โดยง่ายโดยเฉพาะในบริเวณพื้นที่ที่มีโครงสร้างของเนื้อดินหยาบ (Wilson *et al.*, 1951) และมีฝนตกชุก (Gupta, 1979) การปลูกพืชในพื้นที่ซ้ำๆ กันเป็นเวลานานก็เป็นสาเหตุหนึ่งที่ทำให้ดินขาดโบรอนได้ (Rerkasem & Rerkasem, 1991) ดังนั้นปัญหาดินขาดโบรอนจึงเป็นสาเหตุสำคัญประการหนึ่งที่ทำให้ผลผลิตของพืชลดลง ความต้องการโบรอนเพื่อการเจริญเติบโตและการพัฒนาของพืชแต่ละชนิดแตกต่างกัน ทำให้ปริมาณโบรอนในพืชแต่ละชนิดมีความแตกต่างกัน (Bergmann, 1992) ในพืชใบเลี้ยงคู่จะต้องการโบรอนในปริมาณที่สูงกว่าพืชใบเลี้ยงเดี่ยว อย่างไรก็ตามถึงแม้ว่าในธัญพืชซึ่งเป็นพืชใบเลี้ยงเดี่ยวจะมีความต้องการโบรอนต่ำ แต่ก็มีรายงานการขาดโบรอนด้วย (เบญจวรรณ และศันสนีย์, 2532; Simojoki, 1972; Li *et al.*, 1978 da Silva and de Andrade, 1983; Sthapit, 1988; Amak and Tadano, 1991) เช่นใน ข้าวสาลี (*Triticum aestivum* L.) และข้าวบาร์เลย์ (*Hordeum vulgare* L.) แต่ในรายงานการขาดโบรอนที่ผ่านมา พบว่าในข้าวบาร์เลย์ยังมีความขัดแย้งกันอยู่ โดย Ambak and Tadano (1991) พบว่า การขาดโบรอนทำให้การเจริญเติบโตทางลำต้นเพิ่มสูงขึ้นในขณะที่ Jamjod and

Rerkasem (1999) รายงานว่าลดลง การศึกษาทดลองในครั้งนี้ จึงมีวัตถุประสงค์เพื่อหาความแตกต่างระหว่างธัญพืชทั้งสองชนิดนี้ในการตอบสนองต่อการขาดโบรอน เพื่อที่จะสามารถใช้เป็นแนวทางในการจัดการเกี่ยวกับธาตุอาหารโบรอนได้อย่างเหมาะสม และสามารถใช้เป็นพื้นฐานในงานปรับปรุงพันธุ์ต่อไป

อุปกรณ์และวิธีการ

ดำเนินการวิจัยที่ภาควิชาพืชไร่ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ ระหว่างเดือนตุลาคม พ.ศ. 2541 ถึงเดือนมิถุนายน พ.ศ. 2543 โดยแบ่งเป็น 2 การทดลอง คือ การทดลองแรก ใช้ข้าวบาร์เลย์และข้าวสาลีอย่างละ 3 พันธุ์ ข้าวบาร์เลย์ได้แก่พันธุ์ BRB 9, BCMU 96-9 และพันธุ์ CMBL 92029 ข้าวสาลี ได้แก่พันธุ์ Fang 60, SW 41 และ Tatiara ส่วนการทดลองที่สอง ใช้พันธุ์ BRB 9 (ข้าวบาร์เลย์) และพันธุ์ SW 41 (ข้าวสาลี) ปลูกในกระถางดินเผาขนาดเส้นผ่าศูนย์กลาง 30 ซม. ลึก 30 ซม. บรรจุด้วยทรายแม่น้ำ ใช้สารละลายธาตุอาหารดัดแปลงมาจากสูตรของ Broughton and Dilworth (1971) ด้วยการเพิ่มไนโตรเจนในอัตรา 5 mM ใช้รดร่วมกับโบรอนระดับต่างๆ กัน คือ 0, 0.1, 0.3 และ 0.5 μM B (การทดลองแรก) 0 และ 10 μM B (การทดลองที่สอง) วันละ 2 ครั้งๆ ละ 1 ลิตร เช้ากับเย็น แบ่งการเก็บตัวอย่างเป็น 2 ครั้ง ครั้งที่ 1 เก็บเมื่อพืชเข้าสู่ระยะตั้งท้องเต็มที่ (full boot) และนำไปวิเคราะห์หาความเข้มข้นโบรอนในเนื้อเยื่อส่วนรวงและใบธง โดยนำตัวอย่างไปอบที่ อุณหภูมิ 80 องศาเซลเซียส นาน 48 ชั่วโมง และนำไปวิเคราะห์หาความเข้มข้นของโบรอนในเนื้อเยื่อพืชโดยวิธีของ Lohse (1982)

การบันทึกผล

บันทึกอายุการออกรวง, จำนวนหน่อ/ต้น, จำนวนรวง/ต้น, จำนวนช่อดอกย่อย/รวง (ขนาดรวง), น้ำหนักแห้ง, จำนวนเมล็ด/รวง, น้ำหนักเมล็ด/กระถาง และดัชนีการติดเมล็ด (Grain Set Index; GSI) ในข้าวสาลี จะนับ 2 ดอกแรกของ 10 ช่อดอกย่อยตรงกลางรวง (Rerkasem and Loneragan, 1994) และนับ 10 ช่อดอกย่อยตรงกลางรวง (Jamjod and Rerkasem, 1999)

ผลการทดลองทั้งหมดวิเคราะห์สถิติโดยวิธีการวิเคราะห์ความแปรปรวน (Analysis of Variance) และเปรียบเทียบความแตกต่างระหว่างสิ่งทดลองโดยวิธี LSD (Least Significant Difference) ที่ระดับความเชื่อมั่น 95%

ผลการทดลอง

อิทธิพลของการขาดโบรอนต่อการให้ผลผลิตและการเจริญเติบโตของข้าวบาร์เลย์และข้าวสาลี

การขาดโบรอนกระทบต่อการติดเมล็ดของข้าวบาร์เลย์และข้าวสาลี ความรุนแรงของการขาดโบรอนแตกต่างกันในระหว่างพันธุ์และระหว่างพืช เมื่อไม่ใส่โบรอน (B0) ข้าวบาร์เลย์และข้าวสาลีมีดัชนีการติดเมล็ด 12.5-32.2 % และ 0.2-94.8 % ตามลำดับ เมื่อเพิ่มระดับโบรอน การติดเมล็ดของทุกพันธุ์จะเพิ่มขึ้นด้วย การเพิ่มระดับโบรอนขึ้นไปจนถึง 5 μM (B5) ไม่พบว่ามีข้าวบาร์เลย์หรือข้าวสาลีพันธุ์ใดเลยที่สามารถติดเมล็ดได้เกิน 90 % ยกเว้นข้าวสาลีพันธุ์ Fang 60 ซึ่งติดเมล็ด > 90 % ทั้งที่ใส่และไม่ใส่โบรอน (ตารางที่ 1)

ระดับโบรอนที่ทำให้ดัชนีการติดเมล็ดของข้าวสาลีลดลง ไม่มีผลทำให้จำนวนช่อดอกย่อยของข้าวสาลีลดลง โดยมีจำนวนช่อดอกย่อยต่อรวงอยู่ระหว่าง 13-15 ช่อดอกย่อย ในขณะที่ข้าวบาร์เลย์มีความแตกต่างระหว่างพันธุ์เนื่องจากผลของการขาดโบรอน จำนวนช่อดอกย่อยต่อรวงของพันธุ์ BCMU 96-9 และ CMBL 92029 มีจำนวนต่ำสุดเมื่อปลูกที่ B0 และเพิ่มขึ้นเมื่อเพิ่มระดับโบรอน แต่พันธุ์ BRB 9 พบว่า ที่ระดับ B0 ถึง B5 จำนวนช่อดอกย่อยต่อรวงไม่แตกต่างกัน โดยจะมีจำนวน 8.9-10 ช่อดอกย่อยต่อรวง (ตารางที่ 2)

ผลของการขาดโบรอนทำให้ข้าวบาร์เลย์ (BRB 9) และข้าวสาลี (SW 41) มีจำนวนเมล็ดต่อรวงต่ำและทำให้ผลผลิตลดลง เมื่อปลูกที่ B0 ข้าวบาร์เลย์และข้าวสาลีมีผลผลิต (กรัม/กระถาง) 5.2 และ 1.3 กรัม และเมื่อเพิ่มระดับโบรอนเป็น B10 ผลผลิตเพิ่มขึ้นเป็น 67.2 และ 82.6 กรัม ตามลำดับ นอกจากจำนวนช่อดอกย่อยต่อรวงของข้าวบาร์เลย์ที่ลดลงแล้ว ยังพบอิทธิพลของการขาดโบรอนในระยะการเจริญเติบโตทางลำต้นและใบของข้าวบาร์เลย์ด้วย โดยที่ B0 ข้าวบาร์เลย์จะมีจำนวนหน่อและน้ำหนักแห้งเพิ่มขึ้น นอกจากนี้ ที่ B0 การออกรวงของข้าวบาร์เลย์จะช้ากว่าที่ B10 (ตารางที่ 3)

ความเข้มข้นของโบรอนในเนื้อเยื่อใบธงและในรวง

พันธุ์ข้าวบาร์เลย์และข้าวสาลีดูดโบรอนขึ้นไปสะสมในส่วนของใบธงและรวงได้แตกต่างกัน โดยที่ B0 ข้าวบาร์เลย์พันธุ์ BRB 9 มีโบรอนในใบธงสูงที่สุดคือ 13.2 mg/kg รองลงมาคือพันธุ์ CMBL 92029 มี 6.9 mg/kg และพันธุ์ CMU 96-9 มีต่ำที่สุดคือ 4.6 mg/kg ในข้าวสาลีพันธุ์ Fang 60 มีความเข้มข้นโบรอนในใบธงสูงที่สุดคือ 9.3 mg/kg รองลงมาคือพันธุ์ SW 41 มี 7.5 mg/kg และพันธุ์ Tatiara มีต่ำที่สุดคือ 3.8 mg/kg ส่วนความเข้มข้นของโบรอนในรวง พบว่า ข้าวบาร์เลย์พันธุ์ CMU 96-9 มีความเข้มข้นของโบรอนในรวงสูงที่สุดคือ 9.3 mg/kg รองลงมาคือพันธุ์ BRB 9 มี 6.6 mg/kg และพันธุ์ CMBL 92029 เป็นพันธุ์ที่มีโบรอนอยู่ต่ำที่สุดคือ 4.1 mg/kg ในข้าวสาลีพันธุ์ Fang 60 และ SW 41 มีความเข้มข้นของโบรอนในรวงไม่แตกต่างกันคือ 8.5 และ 7.8 mg/kg ส่วนพันธุ์ Tatiara มีต่ำที่สุดคือ 4.9 mg/kg (ตารางที่ 4)

Table 1. Response in Grain Set Index (%) in barley and wheat to boron supply in sand culture (Experiment 1).

Species/genotype	Added B (μM)			
	0	0.1	0.3	5.0
Barley				
BRB 9	32.2 aD	57.5 bD	67.6 cC	76.7 dC
BCMU 96-9	23.4 aC	24.8 aC	28.4 aB	54.6 bB
CMBL 92029	12.5 aB	13.5 aB	22.0 bB	41.6 cA
Wheat				
Fang 60	94.8 aF	94.4 aF	98.7 aE	98.5 aD
SW 41	67.3 aE	74.6 bE	77.4 bcD	81.9 cC
Tatiara	0.2 aA	1.9 aA	13.0bA	61.2 cB
Effects	Genotype	Boron	G x B	
F-test	**	**	**	

Differences (by LSD $p < 0.05$) in the same row are indicated by different lowercase letters and in the same column by different uppercase letters. ** significant at $p < 0.01$

Table 2. Response in the number of spikelets ear^{-1} in barley and wheat to boron supply in sand culture (Experiment 1).

Species/genotype	Added B (μM)			
	0	0.1	0.3	5.0
Barley				
BRB 9	8.9 aA	9.9 aA	10.0 aA	9.2 aA
BCMU 96-9	20.2 aE	25.6 cE	24.0 bE	24.2 bE
CMBL 92029	13.0 aB	15.0 bCD	15.9 bD	17.6 cD
Wheat				
Fang 60	14.3 aC	14.2 aBC	14.6 aBC	14.4 aBC
SW 41	13.0 aB	13.7 aB	13.9 aB	14.0 aB
Tatiara	15.7 aD	15.8 aD	15.4 aCD	15.6 aC
Effects	Genotype (G)	Boron (B)	G x B	
F-test	**	**	**	

Differences (by LSD $p < 0.05$) in the same row are indicated by different lowercase letters and in the same column by different uppercase letters. ** significant at $p < 0.01$

Table 3. Response of vegetative and reproductive growth in wheat (SW 41) and barley (BRB 9) to boron supply in sand culture (Experiment 2).

	Added B (μM B)				G x B
	0	10	0	10	
	Wheat (SW 41)		**Barley (BRB 9)**		
Shoot dry weight (g pot^{-1})	65.4 c	67.2 c	40.4 b	29.6 a	*
Tillers plant^{-1}	12.0 a	9.6 a	32.4 b	15.5 a	**
Day of ear emergence	56.9 c	57.3 c	49.1 b	45.8 a	*
Spikes plant^{-1}	7.2 a	6.4 a	18.8 c	13.1 b	*
Spikelets spike^{-1}	17.6 bc	19.0 c	8.9 a	16.9 b	*
Grains spike^{-1}	0.1 a	44.5 d	2.7 b	16.7 c	*
Grain yield (g pot^{-1})	1.3 a	82.6 c	5.2 a	67.2 b	*
Grain Set Index (%)	0.3 a	97.7 c	24.6 b	98.0 c	*

F-test of genotype by boron interaction, significant level: * $p < 0.05$, ** $p < 0.01$. Differences between B levels in each species are indicated by different lowercase letters (by LSD $p < 0.05$)

Table 4. Boron concentration (mg B kg^{-1}) in ears and flag leafs at booting of barley and wheat genotypes grown in sand culture at four levels of boron (Experiment 1).

Species/genotype	Added B (μM)			
	0	0.1	0.3	5.0
	Boron concentration (mg B kg^{-1}) in flag leafs			
Barley				
BRB 9	13.2 aD	13.4 abD	14.6 bcE	15.6 cBC
BCMU 96-9	4.6 aA	5.4 abA	6.5 bB	8.7 cA
CMBL 92029	6.9 aB	7.0 aB)	7.7 aB)	7.7 aA
Wheat				
Fang 60	9.3 aC	10.9 bC	12.1 cD	16.2 dC
SW 41	7.5 aB	7.7 aB	9.3 bC	14.3 cB
Tatiara	3.8 aA	4.1 aA	4.5 aA	8.1 bA
	Boron concentration (mg B kg^{-1}) in ears			
Barley				
BRB 9	6.6 aB	9.5 bC	12.3 cD	12.8 cE
BCMU 96-9	9.3 bD	8.5 abBC	8.2 aB	9.3 bC
CMBL 92029	4.1 aA	5.0 abA	5.7 bcA	6.1 cA
Wheat				
Fang 60	8.5 aCD	8.8aBC	9.9 bC	10.4 bD
SW 41	7.8 aC	8.2 aB	10.9 bC	10.9 bD
Tatiara	4.9 aA	5.4 aA	6.4 bcA	7.3 bB

Effects	Genotype (G)	Boron (B)	G x B
F-test (ear)	NS	**	**
F-test (flag leaf)	**	**	**

Differences (by LSD $p < 0.05$) in the same row are indicated by different lowercase letters and in the same column by different uppercase letters. NS = not significant, $p < 0.05$. ** significant at $p < 0.01$

วิจารณ์ผลการทดลอง

พบความแตกต่างระหว่างข้าวบาร์เลย์และข้าวสาลีและระหว่างพันธุ์ของแต่ละชนิด ในการตอบสนองต่อการขาดโบรอน เปอร์เซ็นต์การติดเมล็ดซึ่งใช้ดัชนีการติดเมล็ดเป็นตัวชี้วัด มีความสัมพันธ์กับค่าความเข้มข้นของโบรอนในใบธงและในรวง โดยมีค่าความสัมพันธ์ (r) 0.8-0.9 แต่เฉพาะข้าวสาลีพันธุ์ Tatiara ซึ่งอ่อนแอต่อการขาดโบรอน จะมีดัชนีการติดเมล็ดและความเข้มข้นโบรอนในรวงและใบธงต่ำ และเมื่อเพิ่มระดับโบรอน การติดเมล็ดและความเข้มข้นโบรอนก็เพิ่มขึ้นเช่นกัน ในขณะที่พันธุ์ SW 41 และ Fang 60 มีความเข้มข้นของโบรอนในรวงไม่แตกต่างกัน แต่พันธุ์ SW 41 ติดเมล็ดต่ำกว่าพันธุ์ Fang 60 ข้าวบาร์เลย์เมื่อปลูกที่ B0 พันธุ์ BCMU 96-9 มีความเข้มข้นโบรอนในรวงสูงกว่าพันธุ์ BRB 9 แต่ติดเมล็ดต่ำกว่า การใช้ค่าความเข้มข้นโบรอนในใบธงและในรวง เพื่อแยกความแตกต่างระหว่างพันธุ์จึงไม่สามารถทำได้ เช่นเดียวกับ Rerkasem and Lonergan (1994) ที่ไม่สามารถแยกความแตกต่างระหว่างข้าวสาลีพันธุ์ SW 41 และ Sonora 64 ว่าพันธุ์ไหนมีประสิทธิภาพมากกว่ากันโดยการใช้ค่าความเข้มข้นของโบรอนในเนื้อเยื่อเป็นตัวบ่งชี้ Rerkasem *et al.* (1997) พบว่า ข้าวสาลีพันธุ์ SW 41 มีความเข้มข้นโบรอนในส่วนของเกสรตัวผู้และส่วนของเกสรตัวเมียสัมพันธ์กับการติดเมล็ด ถ้าความเข้มข้นสูง การติดเมล็ดจะสูงด้วย การผสมเกสรเกิดขึ้นได้อย่างสมบูรณ์เมื่อมีความเข้มข้นของโบรอนในส่วนของเกสรตัวผู้ (anther) และส่วนของเกสรตัวเมีย (carpel) 10 และ 8 mg/kg DW ตามลำดับ ดังนั้น เพื่อบ่งชี้ความแตกต่างระหว่างพันธุ์ที่แน่นอนกว่านี้อาจจำเป็นต้องใช้เนื้อเยื่อส่วนที่ทำหน้าที่เกี่ยวข้องกับการสืบพันธุ์ของพืชโดยตรง เช่นส่วนของเกสรตัวผู้ หรือเกสรตัวเมีย

งานทดลองของ Huang *et al.* (2000) พบว่า ข้าวสาลีที่มีความเข้มข้นโบรอน 1 mg/kg DW จะไม่แสดงความผิดปกติทางด้านการเจริญเติบโตในระยะสร้างต้นและใบ แต่ในการทดลองนี้ข้าวบาร์เลย์เมื่อปลูกที่ B0 มีความเข้มข้นของโบรอนในเนื้อเยื่อสูงเช่นเดียวกับข้าวสาลี แต่ขนาดรวงกลับลดลง จึงอาจจะเป็นไปได้ว่า ข้าวบาร์เลย์มีความสามารถในการดูดโบรอนเข้าไปในรวงที่สร้างและพัฒนาอยู่ภายในกาบใบและเป็นส่วนที่ไม่มีการคายน้ำได้ไม่ดีเท่ากับข้าวสาลีหรืออาจจะมีความต้องการโบรอนสำหรับพัฒนาการของรวงมากกว่าข้าวสาลี และการขาดโบรอนยังทำให้ปรากฏอาการ rat-tail หรือว่าหางหนู ซึ่งมีลักษณะเป็นปอยสีขาว ขึ้นบริเวณปลายรวงของข้าวบาร์เลย์คล้ายคลึงกับอาการขาดคอปเปอร์ (Snowball and Robson, 1983) ที่ B0 ข้าวบาร์เลย์จะแตกหน่อเพิ่มขึ้น แสดงว่าโบรอนไม่เป็นปัจจัยจำกัดในการสร้างหน่อ จำนวนหน่อที่เพิ่มขึ้นและการติดเมล็ดที่ลดลงแสดงให้เห็นว่า ความต้องการโบรอนในการสร้างผลผลิตหรือการติดเมล็ดจะสูงกว่าความต้องการสำหรับการเจริญเติบโตทางลำต้นและใบ ลักษณะของการแตกหน่อที่เพิ่มขึ้นอาจจะเกี่ยวข้องกับการรักษาสมดุลของฮอร์โมนภายในต้นพืช เช่นเดียวกับในถั่วลันเตาที่มีการแตกตาข้างเพิ่มขึ้นเมื่อขาดโบรอน โดย Li *et al.* (1997) ให้เหตุผลว่าการขาดโบรอนไปจำกัดการเจริญของส่วนยอด

ทำให้ปริมาณ IAA ที่สร้างจากส่วนยอดลดลง อิทธิพลการข่มของตายอดที่มีต่อตาข้างจึงลดลง ทำให้พืชแตกตาข้างเพิ่มขึ้น

สรุปผลการทดลอง

จากผลการศึกษานี้ พบความแตกต่างระหว่างข้าวบาร์เลย์และข้าวสาลีในการตอบสนองต่อการขาดโบรอน โดยผลกระทบจากการขาดโบรอนทำให้ขนาดรวงและการติดเมล็ดของข้าวบาร์เลย์ลดลง ขณะที่ในข้าวสาลีเฉพาะการติดเมล็ดเท่านั้นที่ลดลง ทำให้สามารถกำหนดระยะเวลาและความเหมาะสมสำหรับการจัดการใส่ธาตุอาหารโบรอนให้แก่พืชทั้งสองชนิด โดยข้าวบาร์เลย์จำเป็นที่จะต้องให้ธาตุอาหารโบรอนอย่างเพียงพอตั้งแต่เริ่มปลูกหรือก่อนปลูก และจะต้องให้อย่างเพียงพอต่อเนื่องไปจนถึงความต้องการในระยะผสมเกสร ส่วนในข้าวสาลีนั้น การใส่ธาตุอาหารโบรอนในระยะแรกอาจจะไม่จำเป็นแต่จะจำเป็นภายหลังจากที่มีการสร้างรวงและการพัฒนารวงที่อยู่ภายในกาบใบสมบูรณ์แล้ว วิธีการปลูกพืชเพื่อทดสอบความสามารถในการทนต่อการขาดโบรอนด้วยวิธีการนี้ ยังสามารถใช้เป็นข้อมูลพื้นฐานสำหรับงานปรับปรุงพันธุ์ เพื่อคัดเลือกหาพันธุ์ที่ทนต่อการขาดโบรอนและมีประสิทธิภาพในการนำโบรอนไปใช้ได้สูง

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References

เบญจวรรณ ฤกษ์เกษม, รจเร เนตรแสงทิพย์, สิทธิชัย ลอดแก้ว, พิมลรัตน์ ทองรอด, สุพร ปรีดิศรี พิพัฒน์, สาวิต มีจุ้ย, ณรงค์ ผลวงษ์. 2531. การสำรวจอาการเมล็ดกลวงในถั่วลิสงเพื่อเป็นการบ่งชี้การขาดโบรอนในภาคเหนือ. รายงานการสัมมนางานวิจัยถั่วลิสง ครั้งที่ 7.

เบญจวรรณ ฤกษ์เกษม และ ศันสนีย์ จำจด. 2532. การแก้ไขปัญหารวงลีบเนื่องจากการขาดธาตุโบรอนในข้าวสาลีและข้าวบาร์เลย์. วารสารดินและปุ๋ย. 11, 200-209.

เพิ่มพูน กีรติกสิกร. 2537. ผลงานวิจัยธาตุอาหารเสริมกับพืชตระกูลถั่วที่เป็นอาหารในภาคตะวันออกเฉียงเหนือ. วารสารดินและปุ๋ย. 11:155-167.

Ambak, K. and T. Tadano. 1991. Effect of micronutrient application on the growth and occurrence of sterility in barley and rice in a Malaysian deep peat soil. Soil Sci. Plant Nutr. 37,715-724.

Bergmann, W. 1992. Nutritional Disorders of Plants-Development, Visual and Analytical Diagnosis. Fischer Berlag, Jena.

Broughton, W. J. and M. J. Dilworth. 1971. Control of leghaemoglobin synthesis is snake beans. Biochem. J. 125,1075-1080.

da Silva, A. R. and J. M. V. de Andrade. 1983. Influence of micronutrients on the male sterility on upland wheat and on rice and soybean yields in red-yellow latosos. Pesg. Agropec bras. Brasilla, 18, 593-601.

Gupta, U. C. 1979. Boron nutrition of crops. Adv.Agron.31:273-307.

Huang, L., J. Pant, B. Dell and R. W. Bell. 2000. Effect of boron deficiency on anther development and floret fertility in wheat (*Triticum aestivum* L. 'Wilgoyne'). Annals of Botany. 85,493-500.

Jamjod, S. and B. Rerkasem. 1999. Genotypic variation in response of barley to boron deficiency. Plant Soil 215,65-72.

Li, C. J., H. Y. Yuan, Y. G. Zhang and F. S. Zhang. 1997. Growth of lateral buds versus changes of endogenous indoleacetic acid and zeatin/zeatin riboside content in pea plants grown under boron deficiency. In Boron in Soils and Plants. Eds. R. W. Bell and B. Rerkasem. pp. 179-182. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Li, W. H., M. C. Chao, N. S. Jerm, C. R. Li, W. J. Chu and C. L. Wang. 1978. Study on cause of sterility of wheat. J. Northeastern Agri. College, 31-19.

Lohse, G. 1982. Microanalytical azomethine-H method for boron determination in plant tissues. Commun. Soil Sci. Plant Anal. 13,127-134.

Martens, D. C. and D. T. Westermann. 1991. Fertilizer applications for correcting micronutrient deficiencies. In Micronutrients in Agriculture. 2nd ed. J.J. Mordtvedt, F.R. Cox, L.M. Shuman and R. M. Welch. pp. 549-592. SSSA Book Series no. 4. SSSA, Madison, WI.

Rerkasem, B. and J. F. Loneragan. 1994. Boron deficiency in two wheat genotypes in a warm, subtropical region. Agron.J. 86,887-890.

Rerkasem, B., S. Lordkaew and B. Dell. 1997. Boron requirement for reproductive development in wheat.Plant nutrition. pp. 69-73.

Rerkasem, B. and K. Rekasem. 1991. A decline of soil fertility under intensive cropping in northern Thailand. In: Soil Management for Sustainable Rice Production in the Tropics, Int. Board for Soil Res and Management Monograph No.2, pp. 315-328. IBSRAM, Bangkok.

Shorrocks, V. M. 1997. The occurrence and correction of boron deficiency. Plant and Soil. 193,121-148.

Simojoki, P. 1972. Tuloksia ohran boorilannoituskokeista. Ann. Agric. Fenn. 11, 333-341.

Snowball, K. and A. D. Robson. 1983. Symptoms of Nutrient Deficiencies. Department of Soil Science and Plant Nutrition. Institute of Agriculture, University of Western Australia.

Sthapit, B. R., K. D. Subedi, T. P. Tiwari, S. L. Chaudhary, K. D. Joshi, B. K. Dhital and S. N. Jaisi. 1989. Studies on causes of wheat sterility in the hill, Tar and Terai of Nepal. Seminar paper No. 5/89. Paper presented at the winter crops seminar, National Wheat Development Programe, Bhairhawa, Nepal.

Wilson, C. M., R. L. Lovvorn. and W. W. Woodhouse. 1951. Movement and accumulation of water soluble boron within the soil profile. Agron. J. 43, 363-367.

การตอบสนองต่อโบรอนในถั่วพุ่ม

Boron Response in Cowpea

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Abstract

Legume species are economically important in Thailand. Some species are tolerant to boron (B) deficiency, e.g. soybean and green gram. Boron deficiency affects seed quality in some species e.g. peanut, green gram and black gram. However, there is a lack of information on B response of cowpea (*Vigna unguiculata*). Two experiments were conducted in sand culture with two cowpea cultivars (Cowpea 1 and Cowpea 2) grown at Agronomy Department, Faculty of Agriculture, Chiang Mai University from August 2001 to April 2002. In the first experiment, two cowpea cultivars were compared in sand culture with 3 levels of added B (0, 1 and 10 μM B referred to B0, B1 and B10 respectively) to the nutrient solution. In both cultivars, in B0, B deficiency was adversely affected by flowering and no pod was produced. When B was increased to B1, both cowpea cultivars flowered and set pods normally. Further increase B level to B10 had no significant effect on seed yield and the yield components. However, seed B concentration was increased by the*[N1]* B increase. From 4 mg B/kg at B1, the seed B concentration was increased 4 times at B10. In the second experiment, seeds from the first experiment (seed from B1 = SB1, seed from B10 = SB10) were sown in two levels of added B (0 and 10 μM B referred to B0, B10 respectively). External B level had no effect on germination percentage of the cowpea, while seed B had a slight effect. SB1 seeds of both cultivars averaged 95.3% germination compared with 99.3% from SB10. When grown in B0, SB1 seeds from both cultivars produced seedlings that averaged 22.1% abnormal compared with almost none from SB10 seeds. When grown in B10, however, almost all seedlings were normal. These results have shown that B deficiency depressed seed yield in cowpea by adversely affecting flower development and pod set. At the level of B that had no effect on seed yield, low level of external or soil B may depress seed B concentration which may then in turn adversely affect seed quality in term of germination and seedling growth when sown in low B soil.

บทคัดย่อ

พืชตระกูลถั่วสำคัญของประเทศไทย บางชนิดทนต่อการขาดโบรอน เช่น ถั่วเหลือง และถั่วเขียว บางชนิดไม่ทนต่อการขาดโบรอน เช่น ถั่วเขียวผิวดำ การขาดโบรอนมีผลต่อคุณภาพเมล็ดในถั่วบางชนิด เช่น ถั่วลิสง ถั่วเขียว ถั่วดำ แต่ยังไม่มีข้อมูลว่าถั่วพุ่ม (*Vigna unguiculata* spp. *unguiculata*) มีการตอบสนองต่อโบรอนเป็นแบบใด การศึกษานี้จึงได้ทำการทดสอบการตอบสนองต่อโบรอนในถั่วพุ่ม 2 พันธุ์ โดยทำการทดลองในกระถางทรายในเดือนสิงหาคม พ.ศ. 2544 ถึง เมษายน พ.ศ. 2545 ที่ภาควิชาพืชไร่ คณะเกษตรศาสตร์

การทดลองที่ 1 ปลูกถั่วพุ่ม 2 พันธุ์ (Cowpea 1 และ Cowpea 2) ให้โบรอนในสารละลายธาตุอาหาร 3 ระดับ คือ 0, 1 และ 10 μM 3 ซ้ำ พบว่าที่ระดับโบรอน 0 μM ถั่วพุ่มทั้ง 2 พันธุ์ ล้มเหลวในพัฒนาการของดอกและการติดฝัก แต่ไม่พบว่าถั่วมีการติดฝักและผลผลิตเพิ่มขึ้นต่อไปอีกเมื่อเพิ่มโบรอนในอาหารเป็น 10 μM อย่างไรก็ตามการเพิ่มโบรอนในอาหารจาก 1 μM เป็น 10 μM ทำให้ปริมาณโบรอนในเมล็ดในถั่วทั้งสองพันธุ์มีโบรอนในเมล็ดเพิ่มขึ้นถึง 4 เท่าจาก 4 mg B/kg ที่ 1 μM

การทดลองที่ 2 นำเมล็ดถั่วที่ได้จากการทดลองที่ 1 เพาะในทรายที่มีการให้โบรอน 2 ระดับ (0 และ 10 μM) 3 ซ้ำ พบว่าในทรายที่ใส่โบรอน 0 μM เมล็ดที่มีโบรอนในเมล็ดต่ำมีเปอร์เซ็นต์การงอกเฉลี่ย 95.3 เปอร์เซ็นต์ ในขณะที่เมล็ดที่มีโบรอนในเมล็ดที่สูงกว่ามีเปอร์เซ็นต์การงอกเฉลี่ย 99.3 เปอร์เซ็นต์ โดยพันธุ์ Cowpea 2 มีเปอร์เซ็นต์การงอกของเมล็ดสูงกว่าพันธุ์ Cowpea 1 เล็กน้อย นอกจากนี้เมื่อนำไปปลูกในทรายที่มีการใส่โบรอน 0 μM เมล็ดที่มีโบรอนต่ำ (4 mg B/kg) ให้ต้นอ่อนสมบูรณ์เพียง 77.9 เปอร์เซ็นต์ ในขณะที่เมล็ดที่มีโบรอนสูงกว่า (15-18 mg B/kg) ให้ต้นอ่อนสมบูรณ์เกือบ 100 เปอร์เซ็นต์ แต่ไม่พบอิทธิพลของโบรอนในเมล็ดต่อความสมบูรณ์ของต้นอ่อนเมื่อปลูกในทรายที่รดด้วยโบรอนที่ 10 μM โดยเมล็ดที่มีโบรอน 4-18 mg B/kg ให้ต้นอ่อนสมบูรณ์เกือบ 100 เปอร์เซ็นต์ ทั้งหมด

คำนำ

ปัญหาการขาดธาตุโบรอนในพืชปลูกนั้นเป็นปัญหาที่พบได้ในหลายประเทศ (Gupta, 1979; Shorrocks, 1997) และได้พบภาวะขาดโบรอนแพร่หลายในจังหวัดภาคเหนือตอนบน และบางจังหวัดในภาคเหนือตอนล่าง รายงานผลการทดลองได้แสดงถึงผลของการขาดโบรอนในการลดผลผลิตถั่วเขียว ถั่วเขียวผิวดำ ถั่วเหลือง และถั่วลิสง การลดคุณภาพเมล็ด ในด้านความงอก และความผิดปกติของต้นอ่อน ปัจจัยที่พบว่ามีผลต่อการสนองต่อโบรอนในถั่วได้แก่ ฤดูกาล ชนิดของพืช และพันธุ์พืช ถั่วเขียวผิวดำที่ปลูกฤดูหนาวจะขาดโบรอนรุนแรงกว่าที่ปลูกในฤดู (เบญจวรรณ, 2537)

การขาดโบรอนของพืชมีผลกระทบต่อระยะเจริญพันธุ์มากกว่าระยะการเจริญเติบโต ดังนั้นผลผลิตอาจลดลงมากโดยที่พืชมิได้แสดงอาการขาดโบรอนทางต้นและใบ เนื่องจากการขาดธาตุนี้

ก่อให้เกิดผลเสียหายในช่วงการออกดอกติดผลและการพัฒนาของเมล็ดมากเป็นพิเศษ (Noppakoowong et al., 1997) ในถั่วเหลืองผลผลิตเมล็ดลดลงเนื่องจากการขาดโบรอน มีความสัมพันธ์กับลักษณะต้นที่เตี้ยลง และการลดลงของจำนวนข้อทั้งหมด จำนวนข้อที่ติดฝัก จำนวนเมล็ด/ฝัก และน้ำหนักแต่ละเมล็ดด้วย ทำให้เกิดฝักลีบและลดจำนวนเมล็ดต่อฝัก (Rerkasem et al., 1993) อาการขาดโบรอนที่เกี่ยวข้องโดยตรงกับการสร้างผลผลิตในถั่วเขียวผิวดำคือ การร่วงของช่อดอกและความล้มเหลวของการติดฝัก ในถั่วลิสงอาการเมล็ดกลวง (hollow heart) เป็นอาการจำเพาะเนื่องจากการขาดโบรอน (Harris and Brolman, 1966) ซึ่งถือเป็นลักษณะของเมล็ดด้อยคุณภาพ ส่วนในถั่วเหลืองการขาดโบรอนทำให้เกิดแผลในเมล็ดมีลักษณะเป็นรอยบุ๋มตรงใบเลี้ยง (cotyledon)

การขาดโบรอนทำให้เมล็ดถั่วบางชนิดมีความงอกและความสมบูรณ์ของต้นอ่อนลดลง เมล็ดถั่วเขียวผิวดำที่มีปริมาณโบรอนน้อยกว่า 6 มก.โบรอน/กก. จะมีเปอร์เซ็นต์การงอกต่ำและในจำนวนเมล็ดที่งอกมีเปอร์เซ็นต์ผิดปกติของต้นอ่อนสูง (Bell et al., 1989) แสดงถึงอิทธิพลของการขาดโบรอนที่อาจมีผลต่อการเจริญเติบโต และการรอดชีวิตของต้นอ่อน (embryo) ในเมล็ดในระยะการสร้างเมล็ดก่อนถั่วเหลืองแก่

Vigna unguiculata ถูกจำแนกออกเป็น 2 กลุ่มใหญ่ๆ กลุ่มแรก คือ ถั่วฝักยาว (spp. *sesquipedalis*) ซึ่งเป็นพืชผักที่สำคัญ กลุ่มที่สอง คือ Cowpea (spp. *unguiculata*) (Duke, 1981) ซึ่งยังแบ่งตามลักษณะต้นได้ 2 กลุ่ม คือ กลุ่มที่มีทรงลำต้นเป็นพุ่มตรง มีความสูง 1-3 ฟุต และกลุ่มที่มีรูปทรงลำต้นเลื้อยอาจมีลำต้นยาวถึง 15 ฟุต ถั่วพุ่ม มีแหล่งปลูกที่สำคัญคือ สระบุรี นครราชสีมา ขอนแก่น นครสวรรค์ เชียงใหม่ ลำปาง เป็นต้น (ศูนย์ศึกษาค้นคว้าและพัฒนาเกษตรกรรมภาคตะวันออกเฉียงเหนือ, 2537) หลายจังหวัดในภาคเหนือ (เบญจวรรณ, 2537) และภาคตะวันออกเฉียงเหนือ (เพิ่มพูน, 2537) มีรายงานว่าพบพื้นที่ที่ขาดโบรอน ซึ่งอาจเป็นข้อจำกัดในการสร้างผลผลิตและคุณภาพเมล็ด

วัตถุประสงค์ของการศึกษา

วัดอิทธิพลของโบรอนต่อการสร้างผลผลิตและคุณภาพเมล็ดพันธุ์ของถั่วพุ่ม

อุปกรณ์และวิธีการทดลอง

การทดลองที่ 1 ศึกษาอิทธิพลของโบรอนต่อการสร้างผลผลิตและความเข้มข้นโบรอนในเมล็ดถั่วพุ่ม

จัดสิ่งทดลองแบบ Factorial 2 ปัจจัย ในแผนการทดลองแบบ Complete Randomize Design โดยใช้ถั่วพุ่ม 2 พันธุ์ (Cowpea1, Cowpea2) ปลูกในกระถางบรรจุทรายโดยให้สารละลายโบรอน 3 ระดับ คือสารละลายที่มีธาตุอาหารพืชครบยกเว้นโบรอน 0 μMB (B0) และสารละลายธาตุอาหารพืชดัดแปลงจาก Broughton and Dilworth (1971) ที่มีโบรอน 1 และ 10 μMB (B1, B10) 4 ซ้ำ ปลูกกระถางละ 5 หลุม หลุมละ 2 เมล็ด หลังจากนั้น 7 วันถอนแยกให้เหลือหลุมละ 1 ต้น ทุกวันเช้า-เย็น กระถางละ 1 ลิตรเมื่อถึงระยะสุกแก่เก็บทุกต้น นับจำนวนฝักต่อกระถาง จำนวนเมล็ดต่อฝัก น้ำหนักเมล็ดต่อกระถาง น้ำหนักเมล็ด และวัดความเข้มข้นโบรอนในเมล็ด

การทดลองที่ 2 ศึกษาผลของโบรอนต่อการงอกของเมล็ด

จัดสิ่งทดลองแบบ Factorial 3 ปัจจัย ในแผนการทดลองแบบ Complete Randomize Design นำเมล็ดที่ได้จากการทดลองที่ 1 ของถั่วทั้ง 2 พันธุ์ เมล็ดจากที่ปลูกในทรายที่ให้สารละลาย 1 μMB (SB1) และ 10 μMB (SB10) เพาะความงอกในกระบะทรายที่มีสารละลายโบรอน 2 ระดับ คือ รด สารละลายที่มีธาตุอาหารพืชครบยกเว้นโบรอน 0 μMB (B0) และ สารละลายธาตุอาหารพืชที่มีโบรอน 10 μMB (B10) 4 ซ้ำ ๆ ละ 20 หลุม หลุมละ 1 ทุกวัน เช้า-เย็น เมื่อครบ 14 วัน นับจำนวนต้นที่งอกและจำนวนต้นอ่อนผิดปกติ *[N2]*

การวิเคราะห์ข้อมูล

วิเคราะห์ความแปรปรวนของข้อมูล (AOV) ตามแผนการทดลองแบบ Factorial in Complete Randomize Design ตรวจสอบความแตกต่างของค่าเฉลี่ยโดยใช้ LSD ที่ระดับความเชื่อมั่น 95%

ผลการทดลองและวิจารณ์

ได้มีรายงานความแตกต่างในการตอบสนองต่อโบรอนในถั่วหลายชนิด อาทิถั่วลิสง (Keerati-kasikorn *et al*, 1993) ถั่วเขียวและถั่วเขียวผิวดำ (อยุธย์, 2545) และถั่วเหลือง (ณัฐวุฒิ, 2546) การศึกษานี้พบว่าถั่วพุ่มทั้งสองพันธุ์มีการตอบสนองต่อโบรอนคล้ายกันเมื่อไม่มีการให้โบรอน (B0) ถั่วพุ่มจะแสดงอาการขาดที่ใบคือใบหงิกมีลักษณะเป็นตะปุ่มตะป่ำ และเมื่อถึงระยะการเจริญพันธุ์ อาการขาดโบรอนในถั่วพุ่มที่พบนี้คล้ายกับอาการในถั่วเขียวและถั่วเหลืองในรายงานที่อ้างถึงใน

เบื้องต้นจะเกิดการร่วงของดอก เกิดการล้มเหลวของการติดฝัก ทำให้ถั่วพุ่มทั้งสองพันธุ์ไม่สามารถสร้างผลผลิตได้

เมื่อมีการให้โบรอนในอาหาร 1 μMB ต้นถั่วไม่แสดงอาการขาดโบรอนอีกต่อไป และการติดฝักเพิ่มและให้ผลผลิตเมล็ดเพิ่มขึ้น โดยพันธุ์ Cowpea1 จะให้น้ำหนักเมล็ดเฉลี่ยสูงกว่าคือ 62.2 กรัมต่อกระถาง ในขณะที่พันธุ์ Cowpea2 ให้น้ำหนักเมล็ดเฉลี่ยเพียง 36.4 กรัม แต่การเพิ่มโบรอนต่อไปอีกเป็น 10 μMB ไม่ทำให้การติดฝักหรือสร้างผลผลิตเมล็ดเพิ่มขึ้นอีก (Table 1) จำนวนเมล็ดต่อฝักของถั่วทั้งสองพันธุ์ไม่ตอบสนองต่อโบรอน โดย Cowpea1 และ Cowpea2 มีจำนวนเมล็ดต่อฝักอยู่ระหว่าง 12-13 และ 10-11 เมล็ดต่อฝัก ตามลำดับ (Table 2) น้ำหนัก 100 เมล็ดของถั่วทั้งสองพันธุ์ก็ไม่มีการตอบสนองต่อโบรอนที่ใช้ปลูกเช่นกัน โดยน้ำหนัก 100 เมล็ดเฉลี่ยของ Cowpea1 และ Cowpea2 คือ 11.7 และ 18.2 กรัม (Table 3) ผลผลิตเมล็ดต่อกระถางของถั่วทั้งสองพันธุ์ไม่ตอบสนองต่อโบรอน แต่ Cowpea1 ก็ให้ผลผลิตที่สูงกว่า คือ 62.2 กรัม ในขณะที่ Cowpea2 ให้ผลผลิต 36.4 กรัม (Table 4) อย่างไรก็ตามปริมาณโบรอนในเมล็ดในถั่วทั้งสองพันธุ์ เพิ่มขึ้นเมื่อเพิ่มโบรอนในอาหาร จาก 4 mg B/kg ที่ 1 μMB เป็น 15-18 mg B/kg ที่ 10 μMB (Table 5)

ได้มีผู้พบว่าปริมาณโบรอนในเมล็ดต่ำอาจมีผลกระทบต่อคุณภาพเมล็ดถั่วหลายชนิด (อยุธย์ 2545; Bell et al., 1989) ซึ่งโดยทั่วไปอิทธิพลของโบรอนในเมล็ดจะถูกลบล้างด้วยโบรอนในดินหรือวัสดุปลูกอื่นได้เพียงบางส่วน จากการศึกษานี้พบว่าโบรอนในทราย ที่ใช้ปลูกไม่มีผลต่อการงอกของเมล็ด แต่เป็นโบรอนในเมล็ดที่มีผลต่อการงอกของเมล็ด โดยถั่วพุ่มทั้งสองพันธุ์ มีเปอร์เซ็นต์การงอกเฉลี่ย 99.2 เปอร์เซ็นต์ เมื่อมีโบรอนในเมล็ดสูง และมีเปอร์เซ็นต์การงอกเฉลี่ย 95.5 เปอร์เซ็นต์ เมื่อมีโบรอนในเมล็ดต่ำ (Table 6)

จนเมล็ดที่งอกเจริญเติบโตเป็นต้นอ่อน การที่ต้นอ่อนจะเจริญเติบโตเป็นปกติได้นั้น ขึ้นอยู่กับปริมาณโบรอนภายนอก โดยที่ระดับโบรอนสูง (B10) ไม่มีความแตกต่างกันของการเจริญเติบโตของต้นอ่อน ถึงแม้ว่าเมล็ดที่นำมาเพาะนั้นจะมีโบรอนในเมล็ดต่ำก็ตาม แต่เมื่อนำเมล็ดที่มีโบรอนในเมล็ดต่ำมาเพาะในทรายที่ไม่มีการให้โบรอนจะพบว่าถั่วพุ่มทั้งสองพันธุ์จะได้ต้นอ่อนที่สมบูรณ์ลดลง จาก 100 เปอร์เซ็นต์ เป็น 75 เปอร์เซ็นต์ (Table 7) การที่เมล็ดที่มีโบรอนต่ำมีเปอร์เซ็นต์ความงอกลดลง สอดคล้องกับบทบาทสำคัญของโบรอนต่อการสร้างผนังเซลล์ (Matoh 1997) ซึ่งมีผลสืบเนื่องคือการยับยั้งของการแบ่งเซลล์ (Dell and Huang, 1997) ซึ่งอาจเกิดขึ้นตั้งแต่ในระยะแบ่งเซลล์ในคัภพะ (embryo) หลังจากการผสมเกสร จึงทำให้เมล็ดตายไปตั้งแต่ยังอยู่บนต้นแม่ และไม่อาจแก้ไขได้ด้วยการให้โบรอนในช่วงกำลังงอก ซึ่งสอดคล้องกับรายงานเรื่องเดียวกันในถั่วเขียว (Bell et al, 1989) ในการศึกษานี้ได้ทำการวัดความงอก 2 เดือนหลังการเก็บเกี่ยว เนื่องจากอิทธิพลของการขาดโบรอนที่พบเกี่ยวกับความแข็งแรงของต้นอ่อน สิ่งที่ควรศึกษาต่อไปน่าจะเป็นอิทธิพลของปริมาณโบรอนในเมล็ดต่อคุณภาพในการเก็บรักษาของเมล็ด

สรุป

การขาดโบรอนอย่างรุนแรงในถั่วพุ่มคล้ายกับอาการในถั่วเขียว ถั่วเขียวผิวดำ และถั่วเหลืองที่ได้มีผู้รายงานไว้ โดยจะแสดงออกที่ใบในระยะการเจริญเติบโต และเมื่อถึงระยะการสร้างผลผลิตจะแสดงออกถึงการล้มเหลวในการสร้างดอกและฝัก ส่วนการขาดโบรอนเพียงเล็กน้อยนั้นถึงแม้ว่าจะไม่ทำให้เกิดผลกระทบต่อการสร้างผลผลิต แต่จะไปแสดงออกเมื่อนำเมล็ดพันธุ์ไปปลูกในที่ที่มีโบรอนต่ำแล้วทำให้การงอกของเมล็ดลดลง และได้ต้นอ่อนที่สมบูรณ์ต่ำ แต่ก็สามารถแก้ไขได้โดยการให้โบรอนจากภายนอก

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เอกสารอ้างอิง

ณัฐวุฒิ สุขคำภา. 2546. ผลของโบรอนในถั่วเหลือง 3 พันธุ์. ปัญหาพิเศษวิทยาศาสตร์บัณฑิต (เกษตรศาสตร์) สาขาวิชาพืชไร่มหาวิทยาลัยเชียงใหม่.

เบญจวรรณ ฤกษ์เกษม. 2537. โบรอนในการผลิตถั่วในภาคเหนือ. ดินและปุ๋ย. 16: 130-154.

เพิ่มพูน กีรติกสิกร. 2537. ผลงานวิจัยธาตุอาหารเสริมกับพืชตระกูลถั่วที่เป็นอาหารในภาคตะวันออกเฉียงเหนือ. วารสารดินและปุ๋ย. 16: 155-167.

ศูนย์ศึกษาค้นคว้าและพัฒนาเกษตรกรรมภาคตะวันออกเฉียงเหนือ. ถั่วพุ่ม (Cowpea). ขอนแก่น : สำนักงานปลัดกระทรวงเกษตรและสหกรณ์. 2537

อยุธย์ คงปั้น. 2545. ความแตกต่างทางพันธุกรรมของการตอบสนองต่อการขาดโบรอนในถั่วเขียวผิวมันและถั่วเขียวผิวดำ. วิทยานิพนธ์วิทยาศาสตร์ มหาบัณฑิต (เกษตรศาสตร์) สาขาวิชาพืชไร่มหาวิทยาลัยเชียงใหม่.

Bell, R.W., McLay, L., Plaskett,D., Dell, B., and Loneragan, J.F. 1989. Germination and vigour of black gram (Vigna mungo (L.) Hepper) seed from plants grown with and without boron. Aust. J. Agric. Res. 40: 273-279.

Broughton, W.J. and Dillworth, M.J. 1971. Control of leghaemoglobin synthesis is snake bean. Biochem. J. 125: 1075-1080.

Dell, B. and Huang, L. 1997. Physiological response of plants to boron. Plant and Soil 193:103-120.

Duke, James A. 1981. Handbook of Legumes of World Economic importance. USA: A Division of Plemum Publishing Corporation.

Gupta, U.C. 1979, Boron nutrition of crops. Adv. Agron. 31:273-307.

Harris, H.C., and Brolman, J.B. 1966. Comparison of calcium and boron deficiencies of the peanut. Seed quality in relation to histology and viability relationship. Agron. J. 58: 578-582.

Matoh, T. 1997. Boron in plant cell wall Plant and Soil 193:59-70

Noppakoonwong, R.N., Bell, R.W., Dell, B.,and Loneragan, J.F. 1993. An effect of light on the B requirement for leaf blade elongation in black gram (Vigna mungo). Plant Soils. 155/156: 413-416.

Rerkasem, B, Bell, R.W., Lodkaew, S., and Loneragan, J.F. 1993. Boron deficiency in soybean (Glycine max L. Merr.), Peanut (Arachis hypogaea),and black gram (Vigna mungo L. Hepper): symptomsin seeds and differences among soybean cultivars in susceptibility to boron deficiency. Plant Soils 150: 150.

Rerkasem, B. 1990. Comparison of green gram (Vigna radiata) and black gram (Vigna mungo) in boron deficiency. In Mungbean 90, Proceedings of the Mungbean Meeting 90. Bangkok Office of Tropical Agricultural Research Centre, Japan, pp. 167-174.

Rerkasem, B., Bell, R.W., and Loneragan, J.F. 1990. Effects of seed and soil boron on early seedling growth of black and green gram (Vigna mungo and Vigna radiata). Plant Nutrition-Physiology and Application. M.L Van Beusichem, Ed., Kluwer Academic Publishers, Dordrecht. pp. 281-285.

Shorrocks, V.M. 1997. The occurrence and correction of boron deficiency. Plant and Soil 193, 121-148.

Table 1 Number of pods pot^{-1} of two cowpea cultivars grown in tree levels of added B.

	Cowpea1	Cowpea2	Mean
B0	0	0	0
B1	39	21	30.0
B10	49	17	33.0
Mean	44.0A	19.0B	31.5
	G	B	GxB
F-test	**	ns	ns
$LSD_{0.05}$	10.5	-	-

Table 2 Number of seeds pod^{-1} of two cowpea cultivars grown in tree levels of added B.

	Cowpea1	Cowpea2	Mean
B0	0	0	0
B1	12	10	11.0
B10	13	11	12.0
Mean	12.5A	10.5B	11.5
	G	B	GxB
F-test	*	ns	ns
$LSD_{0.05}$	1.8	-	-

ns = non significant

* = significant at $p < 0.05$

** = significant at $p < 0.01$

Table 3 100 seeds weight (g) of two cowpea cultivars grown in tree levels of added B.

	Cowpea1	Cowpea2	Mean
B1	13.0	18.1	15.6
B10	10.4	18.2	14.3
Mean	11.7A	18.15B	14.95
	G	B	GxB
F-test	**	ns	ns
$LSD_{0.05}$	1.62	-	-

Table 4 Seed yield (g pot^{-1}) of two cowpea cultivars grown in tree levels of added B.

	Cowpea1	Cowpea2	Mean
B0	0	0	0
B1	59.1	37.2	48.15
B10	65.3	35.6	50.45
Mean	62.20A	36.40B	49.30
	G	B	GxB
F-test	**	ns	ns
$LSD_{0.05}$	10.5	-	-

Table 5 B concentration (mgB kg^{-1}) of two cowpea cultivars grown in two levels of added B.

	Cowpea1	Cowpea2	Mean
B1	3.6C	4.0C	3.8
B10	18.4A	15.3B	16.9
Mean	11.0	9.7	10.3
	G	B	GxB
F-test	ns	**	*
LSD	-	1.85	2.16

ns = non significant

* = significant at $p < 0.05$

** = significant at $p < 0.01$

Table 6 Seed germination (%) of two cowpea cultivars grown in two levels of added B.

Levels of B (μM)	Seed source (μM)	Cowpea1	Cowpea2	Mean
B0	SB1	93.1	96.9	95
	SB10	98.8	100	99.4
B10	SB1	93.1	98.1	95.6
	SB10	99.4	98.8	99.1
Mean		96.1B	98.5A	97.3
	SB1	93.1	97.5	95.3b
	SB10	99.1	99.4	99.3a

	G	B	SB	GxB	GxSB	BxSB	GxBxSB
F-test	*	ns	**	ns	ns	ns	ns
$LSD_{0.05}$	2.17	-	2.17	-	-	-	-

Table 7 Normal Seedling (%) of two cowpea cultivars grown in two levels of added B.

Levels of B (μM)	Seed source (μM)	Cowpea1	*Cowpea2*	Mean
B0	SB1	75.8	80.1	77.9B
	SB10	100.0	99.4	99.7A
	Mean	87.9	89.8	88.8
B10	SB1	97.8	99.3	98.6A
	SB10	100.0	99.4	99.7A
	Mean	98.9	99.4	99.2
	SB1	91.2	92.9	92.1
	SB10	99.3	99.4	99.3

	G	B	SB	GxB	GxSB	BxSB	GxBxSB
F-test	ns	**	**	ns	ns	**	ns
$LSD_{0.05}$	-	7.2	7.2	-	-	10.18	-

ns = non significant * = significant at $p < 0.05$ ** = significant at $p < 0.01$

เสนองานสัมนาวิชาการคณะเกษตรศาสตร์ วันที่ 27 มิย. 2546

การจัดการความหลากหลายของต้นปะดะในไร่หมุนเวียนของกะเหรี่ยงโปว์ ในภาคเหนือของประเทศไทย

Agrobiodiversity management of Pada (*Macaranga denticulata* (Bl.) Muell. Arg.) in Traditional Shifting Cultivation of Pwo Karen in Northern Thailand.

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บทคัดย่อ

การทำไร่หมุนเวียนของกะเหรี่ยงโปว์ บ้านทิชะ ต. สบเมย อ. สบเมย จ. แม่ฮ่องสอน ซึ่งมีระยะรอบของการหมุนเวียนลดลงจาก 10-15 ปี เหลือเพียง 7 ปี โดยมีต้นปะดะเป็นพืชเด่นในช่วงของการทิ้งแปลง จากการสังเกตของเกษตรกร พบว่าหากบริเวณใดที่มีต้นปะดะขึ้นอยู่เป็นจำนวนมากก่อนที่จะตัดถางป่า เพื่อการปลูกข้าวไร่ จะทำให้ผลผลิตข้าวไร่ที่ปลูกในบริเวณนั้นสูงตามไปด้วยเกษตรกรบ้านทิชะจึงมีการจัดการเพื่อให้ในไร่หมุนเวียนช่วงของการทิ้งแปลงมีต้นปะดะขึ้นอยู่อย่างหนาแน่น การศึกษานี้เป็นการประเมินผลของต้นปะดะต่อระบบการผลิตข้าวไร่ของกะเหรี่ยงโปว์ ผลการศึกษาพบว่าในบริเวณที่มีต้นปะดะหนาแน่นมาก หลังจากที่มีการทิ้งแปลงไว้ 6 ปี จะมีการสะสมของน้ำหนักแห้งของป่าเหล่ารวมทั้งสิ้นสูงถึง 42.7 ตันต่อเฮกตาร์ และมีปริมาณการสะสมของธาตุอาหารสูงถึง 535 กิโลกรัมของไนโตรเจน 38 กิโลกรัมของฟอสฟอรัส 254 กิโลกรัมของโพแทสเซียม 132 กิโลกรัมของแคลเซียม และ 46 กิโลกรัมของแมกนีเซียม สำหรับในช่วงฤดูการปลูกข้าวไร่พบว่าผลผลิตข้าวไร่แปลงที่มีต้นปะดะหนาแน่นมากจะให้ผลผลิตข้าวสูงถึง 3.04 ตันต่อเฮกตาร์ แต่ผลผลิตข้าวไร่ในสภาพที่มีต้นปะดะน้อยจะมีค่าเพียง 1.15 ตันต่อเฮกตาร์ ผลการศึกษานี้แสดงถึงความสำคัญของต้นปะดะที่สามารถฟื้นฟูผลผลิตข้าวไร่ในสภาพที่มีระยะรอบของการหมุนเวียนสั้นให้มีความยั่งยืนในระบบของการผลิตข้าวไร่

คำสำคัญ: การทำไร่หมุนเวียน ป่าเหล่า น้ำหนักแห้งของพืช

Abstract

The rotational shifting cultivation of Pwo Karen communities in Sop Moei of Mae Hong Son province was decreased rotate cycling from 10-15 to 7 years. They had found the beneficial effect of a pioneer bush species: *Macaranga denticulata* (Bl.) Muell. Arg., on rice yields in their tradition shifting cultivation. The effects are readily observable in patches where the trees grow vigorously and evenly in dense stands before cutting and burning for cultivation. Local people know the plant well with the name "Pada" in Karen. Karen

farmers in Sop Moei have been able to develop management practice and enable pada to evenly establish with dense canopy in the fallow period plots of their rotational shifting cultivation. This study was aiming to assess the positive effect of pada on upland rice in rotational shifting agriculture. The result incepted that by the end of 6 years fallow regrowth, the total amount of fallow biomass accumulate could be as high as 42.7 $t.ha^{-1}$. This provides the total amount of 535 kg N, 38 kg P, 254 kg K, 132 kg Ca and 46 kg Mg ha^{-1}. During cropping period, yield of upland rice after dense population of pada was up to 3.04 $t.ha^{-1}$, which was three times higher than those in the sparse population. Against this background the important of pada effective fallow enriched species maybe addressed and its contribution to sustain productivity of upland rice productivity should be examination.

Keywords: rotation shifting cultivation, fallow regrowth, biomass

บทนำ

การทำการเกษตรของเกษตรกรชาวเขาบนที่สูง โดยเฉพาะอย่างยิ่งเพื่อการยังชีพ เช่น การปลูกข้าว หรือพืชไร่บางชนิด จะมีวิธีการทำ 2 วิธีด้วยกันคือ การทำไร่เลื่อนลอย (pioneer shifting cultivation) และการทำไร่หมุนเวียน (rotational shifting cultivation) (Rerkasem, and Rerkasem, 1994) การทำไร่หมุนเวียนจะเป็นการทำไร่อย่างถาวร ซึ่งไร่ของเกษตรกรจะอยู่รอบ ๆ หมู่บ้าน ในประเทศไทยเกษตรกรบนที่สูงที่มีการทำการเกษตรแบบนี้ได้แก่ กระเหรี่ยง หรือ ลั๊วะ โดยจะมีรอบการหมุนเวียนในการทำการเกษตรและทิ้งแปลง(rotate cycling)ประมาณ 10 – 15 ปี ในขณะที่การทำไร่เลื่อนลอยนั้น จะเป็นการทำการเกษตรแบบไม่ถาวร จะมีการย้ายพื้นที่ไปเรื่อย ๆ ขึ้นอยู่กับความอุดมสมบูรณ์ของดิน กลุ่มเกษตรกรที่ทำการเกษตรแบบนี้ได้แก่ ม้ง อีก้อ เย้า และลีซอ เป็นต้น

สำหรับกระเหรี่ยงโปว์บ้านทิชะ ต.สบเมย อ.สบเมย จ.แม่ฮ่องสอน ได้มีการทำการเกษตรแบบไร่หมุนเวียนในการผลิตข้าวไร่ และในช่วง 15 ปีที่ผ่านมาระยะเวลาการฟื้นฟูป่าเหล่า (fallow phase) ซึ่งเป็นพื้นที่ที่ละทิ้งเพื่อให้พืชได้มีการฟื้นตัวขึ้นมาใหม่ลดลง โดยเกษตรกรสังเกตุพบว่าพืชชนิดหนึ่งคือ ต้นปะคะ (*Macaranga denticulata* (Bl.)Muell. Arg.) ขึ้นอยู่บริเวณใดเป็นจำนวนมาก ก่อนที่จะทำการตัดถางไร่เพื่อปลูกข้าวไร่ จะทำให้ผลผลิตของข้าวไร่ที่ปลูกในบริเวณนั้นสูงตามไปด้วย ดังนั้นเกษตรกรจึงได้มีการพัฒนาการจัดการเพื่อให้ในแปลงป่าเหล่าของตนเองมีต้นปะคะนี้ขึ้นอยู่หนาแน่นกว่าไม้ชนิอื่น ปัจจุบันระยะเวลาในการหมุนเวียนของการทำไร่ใช้ระยะเวลารวมทั้งสิ้นเพียง 7 ปี โดยมีช่วงของการทิ้งแปลงเพียง 6 ปี เท่านั้น

ในด้านพฤษศาสตร์ของต้นปะคะ เป็นไม้ไม่ผลัดใบ อยู่ในตระกูล Euphorbiaceae มีขนาดต้นเล็ก จนถึงปานกลาง มีลำต้นตั้งตรง และเมื่อโตเต็มที่สูงได้ถึง 18-19 เมตร และมีขนาดเส้นรอบวงได้ถึง 40 ซม. มีลำต้นตั้งตรง (Gardner *et. al.* , 2000) ปะคะมีผลขนาดเล็กโดย 1 ผลจะมี 3 พู ในแต่ละพูจะมี 1 เมล็ด เมื่อผลยังดิบจะมีสีเขียว แต่เมื่อแก่จะมีสีเขียวอ่อนจนกระทั่งสีน้ำตาล เมล็ดมี

เปลือกหุ้มแข็งขนาดเล็ก เฉลี่ย 2.4*2.3*2.2 มม. ผลจะมีลักษณะเหนียวสามารถติดไปกับสัตว์ที่ขนาดใหญ่ เช่น วัว ควาย ได้ทำให้มีการกระจายตัวได้ดี สำหรับการงอกของเมล็ดและการตั้งตัวของต้นกล้ายังมีผลการศึกษาน้อยมาก ต้นปะคะพบว่ากระจายตัวขึ้นอยู่ในความสูงจากระดับน้ำทะเลตั้งแต่ 525 ถึง 1370 เมตร ขึ้นไป ต้นปะคะนั้นมีอยู่ด้วยกันหลากหลายชนิด ตัวอย่างเช่น ในแอฟริกา มี 80 ชนิด สำหรับในเขตร้อนทางตะวันออกพบถึง 200 ชนิด สำหรับนักนิเวศวิทยา ต้นปะคะจัดอยู่ในพืชประเภท pioneer species ที่ขึ้นอยู่ใน secondary forests (Whitmore, 1982) แต่ไม่ใช่ปะคะทุกชนิดที่จะขึ้นเป็น pioneer species ในบ้านทิชะเกษตรกรพบว่า มี 3 ชนิด แต่มีเพียง *M. denticulata* เท่านั้น ที่ช่วยในการทำให้ข้าวไร่มีผลผลิตเพิ่มขึ้น ในขณะที่อีก 2 ชนิดซึ่งได้แก่ *M. gigantea* และ *M. Kurzii* ไม่มีผลต่อผลผลิตของข้าวไร่

จากข้อสังเกตเบื้องต้นที่กล่าวมา จึงทำให้เกิดคำถามเชิงวิชาการซึ่งจะนำไปสู่การศึกษาผลของต้นปะคะในแปลงป่าเหล่าที่มีต่อผลผลิตของข้าวไร่ โดยศึกษาว่าต้นปะคะมีผลผลิตข้าวไร่ในระบบหมุนเวียนที่ระยะเวลาของช่วงในการทิ้งแปลงต่าง ๆ อย่างไร และอะไรเป็นข้อจำกัดในการจัดการกับต้นปะคะในแปลงป่าเหล่าของเกษตรกร

วิธีการศึกษา

การศึกษานี้ใช้แปลงเกษตรกรเป็นพื้นที่ในการศึกษา แบ่งออกเป็นหลายส่วน โดยเริ่มแรกทำการศึกษาถึงชนิดไม้ที่ขึ้นในแปลงป่าเหล่าที่มีอายุ 6 ปีซึ่งพร้อมที่จะทำการแผ้วถาง เพื่อทำไร่สำหรับปีการเพาะปลูก 2543 และเผาในเดือนเมษายน 2543 ศึกษาใน 3 แปลงแต่ละแปลงมีขนาด 20 ม * 20 ม ส่วนต่อมาศึกษาเปรียบเทียบระหว่างแปลงที่มีต้นปะคะขึ้นอยู่อย่างหนาแน่นและแปลงที่มีต้นปะคะขึ้นอยู่กระจัดกระจาย เก็บ 3 ซ้ำ ขนาดของแปลงที่เก็บคือ 10 ม * 10 ม ชั่งน้ำหนักสดพืชในแปลงแยกออกเป็นส่วนของปะคะ พืชอื่น ๆ (รวมกัน) และเศษซากพืช จากนั้นสุ่มตัวอย่างเพื่อนำไปอบในตู้อบ (อุณหภูมิ 70°c นาน 24 ชม.) เพื่อหาค่าน้ำหนักแห้ง

สำหรับการศึกษาถึงกระทบผลผลิตของข้าวไร่ปี 2543 ทำโดยการสุ่มพื้นที่ 6 แปลง(ขนาด 2 ม * 5 ม)จากแปลงของเกษตรกร (นายนพพร) เพื่อศึกษาผลกระทบของความหนาแน่นของต้นปะคะต่อน้ำหนักแห้ง ผลผลิตและองค์ประกอบของผลผลิตของข้าวไร่ โดยใน 3 แปลงมีปะคะจำนวนมาก และ 3 แปลงมีต้นปะคะจำนวนน้อย สุ่มเก็บตัวอย่างจำนวน 2 ครั้งจากต้นข้าวที่อายุ 30 วันหลังปลูกจำนวน 10 ต้นต่อ 1 ตัวอย่าง และก่อนการเก็บเกี่ยว ตัวอย่างที่ได้นำไปวิเคราะห์หาปริมาณธาตุอาหาร ได้แก่ N, P, K, Ca และ Mg ตรวจนับองค์ประกอบของผลผลิตข้าวไร่ และผลผลิตของข้าวไร่ และเก็บผลผลิตจากแปลงเกษตรกรรายอื่น ๆ เพิ่มอีก 5 ราย ซึ่งมีขนาดพื้นที่เก็บตัวอย่างเท่ากับ 1 ม * 1 ม โดย 4 แปลงจะเปรียบเทียบผลผลิตของข้าวไร่ในสภาพแปลงที่มีต้นปะคะขึ้นอยู่ต่างกัน ส่วนอีก 1 แปลงจะเปรียบเทียบระหว่างแปลงที่มีการทิ้งแปลงช่วงระยะที่ต่างกัน (3 ปีและ 6 ปี)

ผลการทดลอง

1. ช่วงของการทิ้งแปลง (Fallow phase)

(1) การกระจายตัวของต้นปะคะและไม้ชนิดอื่น

ในช่วงการทิ้งแปลงของการทำไร่หมุนเวียนของเกษตรกร จะมีการกระจายตัวของต้นปะคะแตกต่างกันมากตามอายุของป่าเหล่า จากการศึกษาพบว่า การกระจายตัวของต้นปะคะ ในช่วงเริ่มแรกของการทิ้งแปลงจะมีจำนวนต้นปะคะเฉลี่ย 49,350 ต้นต่อเฮกตาร์ ในขณะที่เมื่ออายุของการทิ้งแปลงได้ 6 ปี จำนวนต้นปะคะเฉลี่ยเหลือเพียง 2,600 ต้นต่อเฮกตาร์ (ตารางที่ 1) ส่วนจำนวนของต้นไม้ชนิดอื่น ๆ หลังจากการทิ้งแปลงไว้ 6 ปี ในแปลงที่มีปะคะขึ้นอยู่หนาแน่น (dense area) พบว่ามีจำนวนต้นไม้ชนิดอื่นๆเพียง 1 ใน 4 ของจำนวนต้นปะคะเท่านั้นสำหรับในแปลงที่มีต้นปะคะขึ้นอยู่น้อย (sparse area) นั้นพบว่ามีไม้ชนิดอื่น ๆ มากกว่าต้นปะคะ ถึง 2 เท่า (ตารางที่ 2)

(2.) มวลชีวภาพและปริมาณธาตุอาหารที่สะสม

เมื่อทิ้งแปลงให้มีอายุ 6 ปี ก่อนที่จะมีการตัดถางและเผาสำหรับแปลงที่มีต้นปะคะขึ้นอยู่เป็นจำนวนมาก พบว่าจะมีการสะสมมวลชีวภาพเป็นน้ำหนักแห้งรวมสูงถึง 42.7 ตันต่อเฮกตาร์ (ตารางที่ 3) ซึ่งจะเป็นน้ำหนักแห้งที่ได้จากต้นปะคะถึง 2 เท่าของน้ำหนักอื่นๆ รวมทั้งหมด ในขณะที่แปลงที่มีการกระจายตัวของต้นปะคะน้อยจะมีการสะสมน้ำหนักแห้งที่ได้จากต้นปะคะอยู่เพียง ¼ ของน้ำหนักแห้งทั้งหมด ส่วนน้ำหนักแห้งที่เหลือส่วนมากจะได้จากพืชชนิดอื่นๆ

สำหรับในด้านปริมาณธาตุอาหารรวมที่สะสมในต้นพืชนั้น ซึ่งได้แก่ธาตุ N, P, K, Ca และ Mg จะมีมากหรือน้อยขึ้นอยู่กับปริมาณของน้ำหนักแห้งที่สะสมด้วย (ตารางที่ 4) ในแปลงที่มีต้นปะคะอยู่เป็นจำนวนมากจะพบว่ามีปริมาณธาตุอาหารที่สะสมทุกธาตุสูงกว่าอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับแปลงที่มีต้นปะคะอยู่น้อย ซึ่งอาจจะมีผลส่งไปถึงปริมาณของผลผลิตของข้าวไร่ในช่วงของการปลูกข้าวไร่ด้วย

2. ช่วงของการปลูกพืช (Cropping phase)

(1) ผลผลิตของข้าวไร่

ผลของความหนาแน่นของต้นปะคะที่มีต่อผลผลิตข้าวไร่นั้น พบว่าในแปลงที่มีต้นปะคะมากในช่วงการทิ้งแปลงนั้นจะทำให้ได้ผลผลิตโดยเฉลี่ยสูงถึง 3.04 ตันต่อเฮกตาร์ ซึ่งจะมากเกือบ 3 เท่าของแปลงที่มีต้นปะคะขึ้นอยู่อย่างกระจัดกระจาย(1.15 ตันต่อเฮกตาร์)ในช่วงทิ้งแปลง ในขณะเดียวกันผลผลิตของแปลงที่มีช่วงของการทิ้งแปลงสั้น(นายทองดี 3 ปี) พบว่าจะได้ผลผลิตเพียง 0.74 ตันต่อเฮกตาร์ ซึ่งจะต่ำมากเมื่อเปรียบเทียบกับแปลงที่มีการช่วงการทิ้งแปลงตามปกติคือ 6 ปี ส่วนในด้านของน้ำหนักฟางก็จะพบในทำนองเดียวกันคือในแปลงที่มีต้นปะคะมากก็ทำให้ได้น้ำหนักฟางสูงถึง 2.74 ตันต่อเฮกตาร์ ในขณะที่ฟางที่ได้จากแปลงที่มีปะคะน้อยมีเพียง 1.19 ตันต่อ

เฮกตาร์ และเมื่อมีการทิ้งแปลงไว้เพียง 3 ปีจะได้น้ำหนักฟางน้อยมากเพียง 0.72 ตันต่อเฮกตาร์ (แปลงนายทองดี) ดังตารางที่ 5

สำหรับในด้านองค์ประกอบของผลผลิตซึ่งเก็บจากแปลงของนายนพพร พบว่าในแปลงที่มีต้นปะคะจำนวนมากจะมีจำนวนรวงต่อต้นสูงกว่าแปลงที่มีต้นปะคะจำนวนน้อยถึง 42.3 % ต่างกันอย่างมีนัยสำคัญทางสถิติ (ตารางที่ 6) อย่างไรก็ตามในส่วนของจำนวนต้นต่อกอนั้นจะไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ ส่วนผลของจำนวนรวงที่แตกต่างกันนั้นอาจจะมาจากความล้มเหลวในการสร้างรวงของต้นข้าวที่อยู่ในแปลงที่มีต้นปะคะน้อยก็ได้ ในด้านน้ำหนัก 1000 เมล็ด ไม่พบความแตกต่างระหว่างแปลงที่มีต้นปะคะจำนวนมากหรือมีต้นปะคะจำนวนน้อย (ตารางที่ 6)

(2) การสะสมปริมาณธาตุอาหารของต้นข้าว

เมื่อเก็บเกี่ยวข้าวไร่ในระยะสุกแก่และนำมาวิเคราะห์ปริมาณธาตุอาหารที่สะสมในส่วนต่างๆ ของต้นข้าว ตามตารางที่ 7 พบว่า ต้นข้าวที่เก็บเกี่ยวจากแปลงที่มีปะคะจำนวนมากจะมีการสะสมของธาตุอาหารทุกชนิดทั้งในส่วนของข้าวเปลือก และฟางข้าว มากกว่าที่ได้จากแปลงที่มีต้นปะคะจำนวนน้อย โดยเฉพาะอย่างยิ่งธาตุไนโตรเจนที่ได้จากแปลงที่มีปะคะจำนวนมาก โดยส่วนของข้าวเปลือกจะได้ 34.1 กิโลกรัมไนโตรเจนต่อเฮกตาร์และในฟางมี 13.6 กิโลกรัมไนโตรเจนต่อเฮกตาร์ ในขณะที่ธาตุอาหารที่วิเคราะห์ได้จากแปลงที่มีต้นปะคะน้อยในส่วนของข้าวจะมีเพียง 13.9 กิโลกรัมไนโตรเจนต่อเฮกตาร์ ซึ่งจะมีการนำเอาเฉพาะส่วนที่เป็นเมล็ดข้าวเปลือกเท่านั้นที่ออกไปหลังจากการเก็บเกี่ยว ส่วนที่เหลือซึ่งได้แก่ฟางข้าวจะมีการทิ้งไว้ในแปลงเพื่อให้สลายตัวกลับลงสู่ดินต่อไป (ตารางที่ 7)

(3) การหมุนเวียนของธาตุอาหาร

เมื่อพิจารณาจากตารางที่ 4 ซึ่งจะแสดงถึงปริมาณธาตุอาหารที่สะสมและให้แก่ระบบ โดยมาจากต้นพืชที่ทำการตัดและเผาหลังจากทิ้งแปลงได้ 6 ปี และตารางที่ 7 แสดงถึง ปริมาณธาตุอาหารที่ต้นข้าวดูดไปสะสมไว้ และมีการนำออกไปจากระบบ เมื่อนำมาเปรียบเทียบกันจะพบว่ามีเพียงปริมาณเล็กน้อยเท่านั้น และเมื่อนำเอาข้อมูลมาเปรียบเทียบกันจะแสดงได้ ดังตารางที่ 8 โดยแปลงที่มีปริมาณต้นปะคะมากพบว่าจะมีปริมาณของธาตุอาหารเพียงเล็กน้อยเท่านั้นที่ถูกนำออกจากระบบไปคือ 8.9 25.5 และ 19.9 % ของธาตุ ไนโตรเจน ฟอสฟอรัส และโพแทสเซียม ตามลำดับ

สรุปและข้อเสนอสำหรับงานวิจัยในอนาคต

ในการจัดการไร่หมุนเวียนของกระเหรี่ยงโปว์ บ้านทิชะ โดยมีปะคะเป็นพืชเด่นในระบบ พบว่าจะให้ผลผลิตดีกว่าการทำไร่หมุนเวียนแบบดั้งเดิมของลั๊วะ บ้านป่าแป จังหวัดแม่ฮ่องสอน โดยที่ลั๊วะจะมีรอบวงจรในการใช้ไร่หมุนเวียนยาวนานถึง 10 ปี ซึ่งจากการศึกษาของ Zinke *et al.* (1978) และ Sabhasri (1978) พบว่าน้ำหนักของพืชที่สะสมหลังจากทิ้งแปลงได้ 4 ปี 7 ปี และ 10 ปี

มีค่าเท่ากับ 26.3 27.7 และ 63.1 ตันต่อเฮกตาร์ตามลำดับ ในขณะที่น้ำหนักแห้งของพืชที่สะสมหลังจากที่ทิ้งแปลงได้ 6 ปี ของกระเหรี่ยงบ้านทิชะ ในแปลงที่มีต้นปะคะมากจะให้สูงถึง 42.7 ตันต่อเฮกตาร์ โดยจะสูงกว่าน้ำหนักแห้งที่สะสมเมื่อทิ้งแปลงได้ 7 ปี ของลั๊วะ ประมาณ 60%

สำหรับในด้านของการสะสมปริมาณธาตุอาหาร เมื่อเปรียบเทียบระหว่างของกระเหรี่ยงบ้านทิชะที่มีการทิ้งแปลงได้ 6 ปี กับของลั๊วะบ้านป่าแปที่มีการทิ้งแปลงได้ 7 ปี พบว่าปริมาณธาตุอาหารในแปลงที่มีต้นปะคะจำนวนมากของกระเหรี่ยงโดยเฉพาะอย่างยิ่งธาตุอาหารหลัก คือ ไนโตรเจน ฟอสฟอรัส และโพแทสเซียม สูงถึง 535 38 และ 254 กิโลกรัมต่อเฮกตาร์ ตามลำดับ ในขณะที่การสะสมธาตุอหารในแปลงของลั๊วะจะได้ปริมาณธาตุอาหารคือ ไนโตรเจน ฟอสฟอรัส และโพแทสเซียม เพียง 143 16 และ 179 กิโลกรัมต่อเฮกตาร์ ตามลำดับ ดังต่อไปนี้

	Nutrient Content (kg/ha)			References
	N	P	K	
Lua system of Pa Pae: 7-year regrowth	143	16	179	Zinke *et. al.*,1978
Karen system of Tee Cha: 6-year regrowth	535	38	254	

โดยทั่วไประยะรอบของการทำไร่หมุนเวียนโดยมีต้นปะคะเป็นพืชเด่นในระบบต้องการรอบหมุนเวียนอย่างน้อยเพื่อรักษาผลผลิตข้าวไร่ในระดับที่ค่อนข้างสูงถึง 7 ปี แต่เมื่อหากลดระยะเวลาทิ้งแปลงลงอาจจะส่งผลกระทบต่อจำนวนประชากรต้นปะคะ และการสะสมน้ำหนักแห้งโดยรวมของป่าเหล่า ทำให้โอกาสการสะสมปริมาณธาตุอาหารลดลง ซึ่งจะสอดคล้องกับการศึกษาที่พบว่าเมื่อลดระยะของการทำไร่หมุนเวียนลงให้เหลือเพียง 4 ปี จะทำให้ผลผลิตของข้าวลดลงเป็นจำนวนมาก (แปลงนายทองดี ตารางที่ 5)

ผลของความหนาแน่นของต้นปะคะในช่วงของการทิ้งแปลงจะส่งผลให้ผลผลิตของข้าวไร่เพิ่มขึ้น โดยแปลงที่มีปะคะจำนวนมากจะทำให้ได้ผลผลิตสูงถึง 3.04 ตันต่อเฮกตาร์ ในขณะที่แปลงที่มีปะคะจำนวนน้อยจะทำให้ได้ผลผลิตเพียง 1.15 ตันต่อเฮกตาร์ (ตารางที่ 5) ซึ่งถ้าเปรียบเทียบกับผลผลิตข้าวไร่ของชาวเขาในจังหวัดทางภาคเหนือ ซึ่งจะมีค่าในช่วง 0.87–1.85 ตันต่อเฮกตาร์ หรือ เฉลี่ยเพียง 1.18 ตันต่อเฮกตาร์ เท่านั้น (Keen, 1972 และ Kunstadter and Chapman, 1978)

ถึงแม้ว่าการศึกษานี้ สามารถบ่งชี้ความแตกต่างของผลผลิตข้าวไร่ที่แตกต่างกันระหว่างแปลงที่มีความหนาแน่นของต้นปะคะต่างกัน แต่ความสัมพันธ์ระหว่างต้นปะคะกับข้าวไร่จำเป็นที่จะต้องค้นหาต่อไปในอนาคต การกระจายตัวของต้นปะคะและการเปลี่ยนแปลงจำนวนประชากรของต้นปะคะเปรียามเทียบกับต้นไม้ธรรมชาติชนิดอื่นๆ จำเป็นที่จะต้องติดตามเพื่อเป็นกุญแจที่สำคัญ ในการจัดการป่าเหล่าให้รอดพ้นจากการเปลี่ยนแปลงสู่สภาพเสื่อมโทรมที่มีไม้พุ่มขนาดเล็ก

หรือวัชพืชเป็นพืชเด่น ที่สำคัญที่สุดจากการศึกษานี้ ด้านปริมาณธาตุอาหารของป่าเหล่าที่มีต้นปะคะเป็นพืชเด่น มีการสะสมค่อนข้างสูงภายในระยะเวลาเพียง 6 ปี การศึกษาถึงที่มาและการหมุนเวียนของธาตุอาหารในระบบไร่หมุนเวียนที่มีปะคะเป็นพืชเด่น คาดว่าจะเป็นกุญแจที่สำคัญที่จะรักษาความยั่งยืนของผลผลิตข้าวไร่ในระบบไร่หมุนเวียนและการฟื้นตัวของป่าเหล่า

References

Gardner, S., Sidisunthorn, P. and Anusarnsunthorn, V. 2000. Forest Trees of Northern Thailand. A field guide. Bangkok: Kobfai publishing Project. 560 p.

Keen, F. G. B.,1972. Upland Tenure and Land Use in North Thailand. Bangkok: Siam Communication Ltd for the SEATO Cultural Programmer. 172 p.

Kerby, J., Elliott, S., Maxwell, J. F., Blakesley, D. and Anusarnsunthorn, V. 2000. Tree Seeds and Seedlings for Regenerating Forests in Northern Thailand. Chiang Mai:Forest Restoration Research Unit (FORRU), Chiang Mai University. 151 p.

Kunstadter, P. and Chapman, E.C. 1978. Problems of shifting cultivation and economic development in northern Thailand. In: P. Kunstadter, E.C. Chapman and S. Sabhasri (eds.) Farmers in the Forests: Economic Development and Marginal Agriculture in Northern Thailand. Pp. 3-23. Honolulu: The University Press of Hawaii for East-West Center.

Rerkasem, K.and Rerkasem, B. 1994.Shifting Cultivation in Thailand Its current situation and dynamics in the context of highland development. Faculty of Agriculture Chiang Mai University Thailand. 107 p.

Sabhasri, S., 1978. Effects of forest fallow cultivation on forest production and soil. In: P. Kunstadter, E.C. chapman and S. Sabhasri (eds.) Farmers in the Forests: Economic Development and Marginal Agriculture in Northern Thailznd. Pp. 160-184. Honolulu: The University Press of Hawaii for East-West Center.

Whitmore, T. C. 1982. On pattern and process in forests. In: E. I. Newman (ed.) The Plant Community as Working Mechanism: Special Publication of the British Ecological Society Produced to a Tribute to A.S. Watt. Pp. 45-59. Oxford :Blackwell Scientific Publication.

Zinke, P. J., Sabhasri, S. and Kunstadter, P.1978. Soil fertility aspects of the lua'forest fallow system of shifting cultivation. In: P. Kunstadter, E.C. Chapman and S. Sabhasri (eds.) Farmers in the Forests: Economic Development and Marginal Agriculture in Northern Thailand. Pp. 134-159. Honolulu: The University Press of Hawaii for East-West Center

Table 1. Number of *M. denticulata* in monitoring plots.

Stage of Fallow	Number of *M. denticulata* (plants/ha)		
	Dense Area	Sparse Area	Average
Beginning of Fallow Regeneration	66,000	32,700	49,350
End of 6-year Fallow Regeneration	4,200	1,000	2,600

Table 2. Number of plants in the fallow at the 6-years of regeneration

Abundance of *Macaranga* in fallow	Number of plants (plants/ha)		Total
	M. denticulata	**Other species**	
Dense area	4200	1220	5420
Sparse area	1000	2090	3090

Table 3. Distribution of biomass and productivity of fallow at the completion of 6 years of regeneration.

Composition of 6- year Fallow	Above Ground Biomass (t/ha)		
	Dense Area of *Macaranga*	Sparse Area of *Macaranga*	Average
M. denticulata	22.2	9.4	15.8
Other species	14.1	21.6	17.9
Litters	6.4	4.7	5.6
Total	42.7	35.7	39.9

Table 4. Distribution of fertility elements in 6 – year fallow regeneration.

Composition of 6 – year Fallow	Nutrient Content (kg/ha)									
	Dense Area of *M. denticalata*					Sparse Area of *M. denticulata*				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
M. denticulata	289	21	97	72	10	131	6	32	21	6
Other species	191	12	118	118	23	297	18	85	36	6
Litters	56	5	38	38	13	59	4	14	16	10
Total	535	38	254	254	132	488	134	132	72	22

Table 5. Effects of *Macaranga* in fallows on subsequent productivity of upland rice.

Farmer	*Macaranga* Abundance	Rice yield (t/ha)			Harvest
		Grain	Straw	Total dry weight	Index (%)
Niporn	Dense	2.51	2.35	4.86	51.6
	Sparse	0.83	0.97	1.80	46.1
Naechae	Dense	4.53	3.80	8.33	54.4
	Sparse	1.56	1.75	3.31	47.1
Suyo	Dense	2.99	3.29	6.28	47.6
	Sparse	1.14	1.08	2.22	51.4
Pho	Dense	3.24	2.56	5.80	55.9
	Sparse	1.37	1.40	2.77	49.5
Chaemon	Dense	2.80	2.48	5.28	53.0
	Sparse	1.50	1.20	2.70	55.5
Mean	Dense	3.04	2.74	5.78	52.6
	Sparse	1.15	1.19	2.34	49.1
Thongdee	dense	0.74	0.72	1.46	50.8

Note: Data from Noporn's plot were taken 10m x 10m quadrats with three replicates in different areas of *M. denticulata abundance*. Crop cutting survey was carried out in other farmers, plots with smaller quadrat (lm x lm). All plots are 6-years after regrowth accept Thongdee plot. Thongdee field is a young fallow of only 3-year after regrowth.

Table 6. Effects of *Macaranga* in fallows on plant establishment and yield components of upland rice.

Yield Components	Abundance of *Macaranga* in Fallow		Significant Level
	Dense Area	Sparse Area	
Number of hills/m^2	6.2	5.5	.05
Number of Plants/hill	3.0	2.7	NS
Number of tillers/plant	4.6	3.8	NS
Number of panicles/plant	4.1	2.6	.05
- % panicle bearing tillers	89.1	28.4	
1000 seed weight (g)	29.14	19.10	NS

Note: Number of hills were measured from a total area of $10m^2$. Statistical significant differences are indicated by LSD at 0.05% and NS for "not significant"

Table 7. Nutrient uptake of upland rice at maturity

Attributes	Amount of Nutrient Uptake (kg/ha)									
	Dense Area of *M. denticulata*					Sparse Area of *M. denticulata*				
	N	**P**	**K**	**Ca**	**Mg**	**N**	**P**	**K**	**Ca**	**Mg**
Grain	34.1	7.6	13.1	3.1	3.4	13.9	3.3	5.4	1.1	1.4
Straw	13.6	2.1	62.9	8.1	3.3	7.3	1.0	16.1	3.5	1.5
Total	47.7	9.7	76.0	11.1	6.7	21.2	4.3	31.5	4.6	2.9

Table 8. Proportion of nutrient uptake as percentage of total nutrient content in 6 year fallow.

Shifting Cultivation Cycle	Nutrient Content/Uptake (kg/ha)				
	N	P	K	Ca	Mg
Total content in fallow	53.5	3.8	25.4	13.2	46
Total uptake in rice	47.7	9.7	76.0	11.1	6.7
Percentage of total Content in fallow	8.9	25.5	19.9	8.4	14.5

การคัดเลือกพันธุ์ข้าวไทยภายใต้สภาพขาดธาตุเหล็กโดยวัดปริมาณคลอโรฟิลล์ในใบ

Screening Thai Rice Genotypes under Iron Deficiency by Leaf Chlorophyll

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Abstract : Wetland rice does not suffer from iron deficiency because of high availability of iron in submerged soil. Iron deficiency is a problem in upland rice and also rice grown without irrigation (non submerged soil). Besides, iron deficiency is a widespread problem in calcareous soil (pH 7.3-8.5) because of low availability of iron in the soil and inadequate uptake. Iron deficiency depresses chlorophyll synthesis (causing iron deficiency chlorosis) resulting from the damage of chloroplast structure. Therefore, Rice genotypes with high chlorophyll content was tolerant to iron deficiency. This experiment, setup to screen Thai rice genotypes which are tolerant to iron deficiency by measuring chlorophyll accumulation in the leaf. Thirty nine rice genotypes and KDML 105 (local check) were grown in sand culture in concrete boxes. The sand was watered twice daily with complete nutrient solution without iron. Measurement of leaf chlorophyll was made with a chlorophyll meter (SPAD 502) sixty days after sowing. Data were analysed by t-test. The results showed that five rice genotypes, Siw-Lao, Jon-Dang, PRE87003-1-3-1-1, RD 10 and Hom-Pitsanulok 1 had the highest SPAD value ($p<0.05$) suggesting that they were least affected by, i.e. most tolerant to iron deficiency. The tolerant to iron deficiency in these genotypes will b further investigated by comparing their iron uptake and utilization efficiency.

บทคัดย่อ : ธาตุเหล็กมีความสำคัญต่อการเจริญเติบโตของพืช เมื่อพืชขาดธาตุเหล็กจะมีผลทำให้โครงสร้างของคลอโรพลาสต์ได้รับความเสียหายจึงส่งผลให้มีการสังเคราะห์คลอโรฟิลล์ลดน้อยลง ซึ่งเป็นสาเหตุทำให้เกิดภาวะพร่องคลอโรฟิลล์ขึ้น จึงเป็นสาเหตุทำให้การสังเคราะห์แสงของพืชลดลง ดังนั้นในการทดลองนี้จะทำการคัดเลือกพันธุ์ข้าวไทยที่ทนทานต่อการขาดธาตุเหล็ก โดยใช้ปริมาณคลอโรฟิลล์ในใบเป็นตัวคัดเลือก ซึ่งพันธุ์ข้าวที่มีปริมาณคลอโรฟิลล์ในใบสูงจะเป็นพันธุ์ที่ทนต่อการขาดธาตุเหล็ก โดยในการทดลองใช้พันธุ์ข้าวไทย 39 พันธุ์ ปลูกเปรียบเทียบกับพันธุ์ขาวดอกมะลิ 105 ปลูกในกระบะทราย รดด้วยสารละลายที่ขาดธาตุเหล็ก แล้ววัดปริมาณคลอโรฟิลล์ในใบด้วยเครื่อง SPAD 502 chlorophyll meter เมื่ออายุได้ 60 วัน แล้ววิเคราะห์ข้อมูลทางสถิติด้วย t-test จากการทดลองพบพันธุ์ข้าว 5 พันธุ์ได้แก่ พันธุ์ซิวลาว พันธุ์จอนแดง พันธุ์ PRE 87003-1-3-1-1 พันธุ์ กข 10 และพันธุ์หอมพิษณุโลก 1 มีปริมาณคลอโรฟิลล์ (SPAD value) มากกว่าพันธุ์ขาวดอกมะลิ 105 โดยมีค่า $p < 0.05$ แต่อย่างไรก็ตามจากผลการทดลองนี้วิธีดังกล่าวเป็นการคัดเลือกพันธุ์ในขั้นต้น จะทำให้สะดวกต่อการปรับปรุงพันธุ์ที่มีสมรรถภาพในการใช้เหล็กในข้าว

Index words : Iron deficiency, Rice

คำนำ

เหล็กเป็นธาตุที่จำเป็นต่อการเจริญเติบโตของพืช การขาดธาตุเหล็กจะมีผลต่อการสร้างผลผลิตของข้าวโดยจะมีผลกระทบต่อโครงสร้างของคลอโรพลาสต์และทำให้การสังเคราะห์โปรตีนของข้าวลดลง ซึ่งมีผลต่อการสังเคราะห์แสงของข้าวทำให้ผลผลิตของข้าวลดลง สำหรับข้าวนาสวน(ปลูกในสภาพน้ำขัง) มักจะไม่มีปัญหาในการขาดธาตุเหล็กเนื่องจากในสภาพน้ำขังมีธาตุเหล็กอยู่ในรูปที่พืชดูดไปใช้ได้ (Fe^{2+}) ในปริมาณสูง แต่ข้าวที่ปลูกในประเทศไทยส่วนใหญ่ประมาณ 75% ปลูกอยู่นอกพื้นที่ชลประทานซึ่งไม่สามารถที่จะควบคุมน้ำได้ และไม่มีน้ำขังในพื้นที่นาโดยเฉพาะช่วงเวลาที่ฝนทิ้งช่วง การขาดธาตุเหล็กอาจเกิดขึ้นได้ ในสภาพดินแห้งของนาน้ำฝนอีกทั้งในข้าวไร่ และพื้นที่นาที่มีดินเป็นด่าง หรือพื้นที่นาที่เป็นดินเนื้อปูน (calcareous soil) ที่มี pH สูงๆ (pH อยู่ในช่วง 7.3-8.5) ในพื้นที่ที่มีภูเขาหินปูนทำให้ความเป็นประโยชน์ของเหล็กในดินต่ำ และไม่เพียงพอต่อการดูดใช้ หรือเพราะลดขบวนการเคลื่อนย้ายและขบวนการ metabolism ของธาตุเหล็กที่ถูกชักนำโดยไอออนตัวอื่นๆ (Foy, 1983) ซึ่งจะทำให้ข้าวขาดธาตุเหล็กได้ด้วย

มีรายงานว่าในพันธุ์พืชอาจมีสมรรถภาพในการใช้ธาตุเหล็กต่างกันในพืชหลายชนิด อาทิ เช่น ถั่วเหลือง ถั่วchickpea ข้าวโพด รวมทั้งข้าวด้วย (Neue et al., 1990) ดังนั้นความทนทานต่อการขาดธาตุเหล็ก น่าจะเป็นลักษณะทางพันธุกรรมที่เป็นประโยชน์อย่างหนึ่งโดยเฉพาะสำหรับข้าวที่ปลูกในพื้นที่ที่ขาดธาตุเหล็ก

ดังนั้นการคัดเลือกพันธุ์ที่มีความทนทานต่อการขาดเหล็กจะช่วยเป็นแนวทางในการปรับปรุงพันธุ์เพื่อช่วยในการเพิ่มปริมาณผลผลิตของข้าวให้มากกว่าเดิมหรือลดความเสียหายที่เกิดจากการขาดธาตุเหล็กในข้าว ซึ่งน่าจะเป็นแนวทางหนึ่งในการช่วยแก้ปัญหาดังกล่าวข้างต้น

อุปกรณ์และวิธีการทดลอง

ใช้พันธุ์ข้าวไทยทั้งหมด 39 พันธุ์ และใช้พันธุ์ขาวดอกมะลิ 105 เป็นพันธุ์ตรวจสอบปลูกในทรายปลูกเป็นแถวแถวละหนึ่งพันธุ์ ระยะปลูก 2 x 2 cm. ในแต่ละกระบะจะปลูกพันธุ์ตรวจสอบทุกกระบะ รดด้วยสารละลายธาตุอาหารสูตรของ Kimura B ที่ขาดธาตุเหล็ก (ความเข้มข้นของ Fe 0 ppm.) ทำการวัดปริมาณคลอโรฟิลล์ในใบด้วยเครื่อง SPAD 502 chlorophyll meter (มีหน่วยเป็น SPAD unit) เมื่ออายุได้ 60 วัน แล้ววิเคราะห์ข้อมูลทางสถิติด้วย t-test เพื่อเปรียบเทียบพันธุ์ข้าวไทยในสภาพการขาดธาตุเหล็ก

ผลการทดลอง

พันธุ์ข้าวที่ตอบสนองต่อการขาดธาตุเหล็กมีปริมาณ SPAD value ที่แตกต่างอย่างมีนัยสำคัญกับพันธุ์ตรวจสอบ ($p<0.05$) ซึ่งพบว่า พันธุ์ข้าว 5 พันธุ์ ที่มีปริมาณ SPAD value สูงกว่าพันธุ์ตรวจสอบ (ตารางที่ 1) ได้แก่ พันธุ์ชิวลาว ($p=0.049$), พันธุ์จอนแดง ($p=0.038$), พันธุ์ กข 10 ($p=0.041$) และพันธุ์หอมพิษณุโลก 1 ($p=0.025$)

วิจารณ์ผลการทดลอง

จากการทดลองนี้พบว่าข้าว 5 พันธุ์ ได้แก่ พันธุ์ชิวลาว, พันธุ์จอนแดง, พันธุ์ กข 10 และพันธุ์หอมพิษณุโลก 1 เป็นพันธุ์ที่คาดว่าน่าจะเป็นพันธุ์ที่ทนทานต่อการขาดธาตุเหล็ก โดยต้านทานต่อภาวะพร่องคลอโรฟิลล์ เพราะฉะนั้นการต้านทานต่อภาวะพร่องคลอโรฟิลล์จากการขาดธาตุเหล็ก ในพันธุ์ข้าวดังกล่าว จะช่วยลดปัญหาที่เกิดจากการขาดธาตุเหล็ก เนื่องจากเหล็กมีความสำคัญกับคลอโรพลาสต์ เมื่อพืชขาดเหล็กจะมีผลกระทบต่อโครงสร้างของคลอโรพลาสต์ ทำให้เกิดภาวะพร่องคลอโรฟิลล์ในใบพืช (Terry and Abadia, 1986) ซึ่งเมื่อพืชมีการสร้างคลอโรฟิลล์น้อยลง จะมีผลกระทบต่อการสังเคราะห์แสงของพืชทำให้การสังเคราะห์แสงได้น้อยลง (Terry, 1980) แต่อย่างไรก็ตามในการทดลองนี้เป็นเพียงการคัดเลือกพันธุ์ในขั้นต้นเท่านั้น ในอนาคตควรมีการศึกษาว่าพันธุ์ที่ได้จากการคัดเลือกที่เป็นพันธุ์ทนทานต่อการขาดธาตุเหล็กในการทดลองนี้ มีกลไกใดที่ทำให้เกิดความทนทานต่อการขาดธาตุเหล็ก

กิตติกรรมประกาศ

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เอกสารอ้างอิง

Foy, C D 1983 The physiology of plant adaptation to mineral stress. Iowa State J. Res. 57: 355-319.

Neue, H.U., R.S. Lantin, M.T.C. Cayton and N.U Autor, 1990. Screening of rice for adverse soil tolerance. In Genetic Aspects of Plant Mineral Nutrition. (N.El Bassam, M. Dambroth and B.C. Loughman, eds.); pp.523-531. Kluwer Academic, Dordrecht

Terry, N. and J. Abadia. 1986. Function of iron in chloroplasts. J. Plant Nutr.9:609-646.

Terry, N. 1980. Limiting factors in photosynthesis I. Use of iron stress to control photochemical capacity in vivo. Plant Physio. 65: 114-120.

Table 1 SPAD value of 39 rice genotypes when grown in iron deficiency (sand culture)

Variety	SPAD value	P value for comparison with Check
Dang-Hom	22.10b	0.912^{ns}
Siw-Loa	32.13a	0.049*
Kow-Dang	25.60b	0.723^{ns}
Kow-Fer-Hi	27.63b	0.270^{ns}
# 024 URN 16	17.40b	0.592^{ns}
# 052 URN 44	26.93b	0.374^{ns}
# 039 URN 31	30.83b	0.101^{ns}
# 027 URN 19	26.20b	0.347^{ns}
Check	22.70b	
# 015URN 07	28.80b	0.157^{ns}
Jon-Dang	31.37a	0.038*
SPT 84051	26.60b	0.377^{ns}
PRE 87003-1-3-1-1	30.83a	0.040*
Sew-Mea-Jun	25.93b	0.532^{ns}
Booa-Pa-Tor	22.67b	0.917^{ns}
Hom-Pu-Pan	22.20b	0.955^{ns}
Check	21.87b	
Booa-Ka	27.17b	0.179^{ns}
Joa-Loong 11	22.13b	0.937^{ns}
RD 10	27.47a	0.041*
RD 6	23.03b	0.874^{ns}
Basmati 5854	27.03b	0.212^{ns}
Hom-Nang-Fa	20.30b	0.380^{ns}
RD 27	23.63b	0.770^{ns}
Neaw-Moong-Piy	23.57b	0.750^{ns}
Check	22.53b	

Table 1 Continued.

Variety	SPAD value	P value for comparison with Check
Dok-Prow	28.00b	0.385 ns
RD 15	23.33b	0.496 ns
KDML (LR 02)	28.57b	0.109 ns
SPTLR 84051	27.07b	0.743 ns
RD 6D-20G-27	25.33b	0.719 ns
KDML 105	27.30b	0.527 ns
Chai-Nat 1	27.03b	0.771 ns
Hom-Pitsanulok 1	29.30a	0.025*
Neaw-U-Bon 2	23.93b	0.383 ns
Check	26.43b	
Madhuka	25.30b	0.886 ns
Neaw-San-Pa-Tong	26.03b	0.755 ns
Milagrosa	23.53b	0.703 ns
Kow-Koowpaokdom (MaeTang)	22.57b	0.266 ns
Dang-Konkan	29.20b	0.188 ns
Ja-Pu-Pu	22.03b	0.147 ns
Ja-Nae-Nea	28.90b	0.224 ns
Check	24.83b	

ns not significant, * significant at $p < 0.05$

Means within a column the same lowercase letter do not differ significantly at 5% with LSD.

การเคลื่อนย้ายโบรอนในถั่วเขียว

The mobility of boron in green gram

(*Vigna radiata* (L.) Wilczek)

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Abstract: Differences in mobility of boron (B) among species should have a significant bearing on the susceptibility to B deficiency especially in non-transpiring organs and affect the strategies for control of B deficiency. Remobilization of boron from old to younger tissues has been reported to vary among plant species, being essentially immobile in many species and completely mobile in others. This study aimed to verify B mobility in green gram plants (*Vigna radiata* (L.) Wilczek cv. Kampaengsan 1). Plants were first grown for 25 days in sand culture with 10 μM B (B10, sufficient) and 0.5 μM B (B0.5, deficient) added to nutrient solution. After that, the sand in all of pots was washed by running water through for 20 minutes twice in one day, 6 hours apart. Boron treatments were applied to the pots in 3 replicates, as follows, B10 for the first 25 days followed by B0.5 for the following 30 days (B10/B0.5), with the same B10 (B10/B10) and B0.5 (B0.5/B0.5) throughout the experiment. Plants were harvested at day 25 and day 55. The main stem of harvested plants was partitioned into the growing point (all parts above the youngest fully expanded leaf), the youngest fully expanded leaf blades (YFEL), the stems + petioles below YFEL, the rest of the leaf blades of the main stem (old leaf blades), the rest of the main stem, roots and their seeds. At 55 days, dry weight of vegetative parts (leaf blades, stems + petioles roots and nodule) were not different between treatments B10/B10, B10/B0.5 and B0.5/B0.5 but seed yields was significant lower in B10/B0.5 and even lower in B0.5/B0.5. B content in younger parts (growing point, YFEL, stems + petioles below YFEL) was much lower than old leaf. However, B content in YFEL and seeds of B10/B0.5 plants were higher than B0.5/B0.5. These suggested most B was accumulated in old parts whereas a little B could be mobilized to newly expanded leaf (YFEL) and seeds of B10/B0.5 plants. It was enough for seed development of these plants that were 64% while in B0.5/B0.5 were 20 % of maximum yields. It is not clear about source and route of those B and need to examine in the further research.

บทคัดย่อ: ความสามารถในการเคลื่อนย้ายโบรอนในต้นพืชมีผลต่อประสิทธิภาพการใช้โบรอน โดยเฉพาะอย่างยิ่งการขนส่งธาตุอาหารไปยังส่วนที่มีการคายน้ำน้อย และส่งผลกระทบต่อวิธีการที่ใช้ในการแก้ไขปัญหาการขาดโบรอน ได้มีรายงานเกี่ยวกับความแตกต่างกันในระหว่างชนิดพืชในความสามารถเคลื่อนย้ายโบรอนจากเนื้อเยื่อที่แก่ไปยังเนื้อเยื่อส่วนที่อ่อนกว่า ซึ่งในพืชส่วนใหญ่ไม่มีการเคลื่อนย้ายโบรอน แต่พบว่าในพืชบางชนิดสามารถเคลื่อนย้ายโบรอนได้ การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจสอบการเคลื่อนย้ายของโบรอนในถั่วเขียวพันธุ์กำแพงแสน 1 (*Vigna radiata* (L.) Wilczek cv. Kampaengsan 1) ทำการทดลองโดยปลูกถั่วเขียวใน sand culture และรดด้วยสารละลายธาตุอาหารที่ใส่โบรอน 10 μM (B10) และ 0.5 μM (B0.5) เป็นเวลา 25 วัน หลังจาก

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นั้นล้างทรายในกระถางโดยเปิดให้น้ำไหลผ่านทราย 2 ครั้ง (เช้า – เย็น) ครั้งละ 20 นาที จากนั้นแบ่งกรรมวิธีในการทดลองเป็น (1) ให้โบรอน 10 μM ใน 25 วันแรก หลังจากนั้นเปลี่ยนมาให้สารละลายธาตุอาหารที่มีโบรอน 0.5 μM เป็นระยะเวลา 30 วัน (B10/B0.5) (2) ให้โบรอน 10 μM ตลอดการทดลอง B10/B10) และ (3) ให้โบรอน 0.5 μM ตลอดการทดลอง (B0.5/B0.5) เก็บตัวอย่างพืชเมื่ออายุได้ 25 วัน และ 55 วัน นำมาวิเคราะห์หาปริมาณโบรอนในแต่ละส่วนของลำต้นหลัก ดังนี้ (1) ยอดที่เจริญใหม่ (ส่วนที่อยู่เหนือใบอ่อนที่สุดที่ขยายตัวเต็มที่: growing point) (2) ใบอ่อนที่สุดที่ขยายตัวเต็มที่ (youngest fully expanded leaf blades: YFEL) (3) ลำต้นและก้านใบของ YFEL (4) ใบส่วนที่เหลือทั้งหมด (รวมเรียกว่าใบแก่: old leaf blades) (5) ลำต้นหลักและก้านใบที่เหลือทั้งหมด (old stem + petioles) (6) ราก และ (7) เมล็ด ผลการทดลองพบว่า เมื่อพืชอายุ 55 วัน น้ำหนักแห้งในส่วนใบ ลำต้น ราก และปมราก ของต้นถั่วเขียวในแต่ละกรรมวิธีมีค่าต่างกันเพียงเล็กน้อย แต่ต้นที่ได้รับ B10/B0.5 มีผลผลิตเมล็ดถั่วเขียวลดลงเพียง 36 % ขณะลดลงถึง 81 % ในต้นที่ได้รับโบรอน 0.5 μM ตลอดการทดลอง และพบว่าปริมาณโบรอนใน ยอดที่เจริญใหม่ ใบอ่อนที่สุดที่ขยายตัวเต็มที่ ลำต้นและก้านใบของ YFEL ของต้นที่ได้รับโบรอนใน 30 วันหลัง (B10/B0.5 และ B0.5/B0.5) มีค่าน้อยกว่าในส่วนที่แก่ (ใบแก่ และลำต้นหลักและก้านใบที่เหลือทั้งหมด) อย่างไรก็ตามต้นถั่วเขียวที่ได้รับ B10/B0.5 มีปริมาณโบรอนในเมล็ด และใบอ่อนที่สุดที่ขยายตัวเต็มที่ สูงกว่าต้น B0.5/B0.5 จากผลการทดลองแสดงให้เห็นว่า พืชมีการสะสมโบรอนในระยะแรก (B10/B0.5) ของการทดลองในส่วนที่มีอายุมาก แต่พบว่ามีโบรอนจำนวนหนึ่งสามารถเคลื่อนย้ายไปยังใบอ่อนที่สุดที่ขยายตัวเต็มที่ และเมล็ด แม้ว่าจะเป็นปริมาณโบรอนที่น้อยมาก แต่ก็สามารถทำให้ผลผลิตเมล็ดของถั่วเขียวมากกว่าต้นที่ขาดโบรอนตลอดการทดลองถึง 3.5 เท่า ซึ่งจากการทดลองนี้ยังไม่ทราบแน่ชัดถึงแหล่งที่มา และเส้นทางลำเลียงของโบรอนดังกล่าว ซึ่งจะได้ทำการศึกษาต่อไป

Index words: Green gram, Boron, Remobilization ถั่วเขียว โบรอน การเคลื่อนย้ายย้อนกลับ

คำนำ

พืชดูดซึมธาตุอาหารโดยราก และลำเลียงพร้อมกับน้ำในน้ำเลี้ยงไซเลม (xylem sap) ไปยังส่วนที่อยู่เหนือดิน ธาตุอาหารเหล่านี้อาจเคลื่อนย้ายไปยังน้ำเลี้ยงโฟลเอ็ม (phloem sap) หรือถูกส่งออกไปเก็บรักษาไว้ในราก ลำต้น หรือเซลล์ใบ ซึ่งธาตุอาหารที่เก็บไว้ในเนื้อเยื่อต่างๆ อาจเคลื่อนย้ายย้อนกลับทางโฟลเอ็มไปยังส่วนอื่นของพืชที่มีอัตราการคายน้ำต่ำกว่า เช่น ยอดที่เพิ่งเจริญ เนื้อเยื่อที่เกี่ยวข้องกับการสืบพันธุ์ (Smith and Loneragan, 1997)

ธาตุอาหารพืชทุกธาตุสามารถเคลื่อนย้ายได้ในทางไซเลม แต่สำหรับการเคลื่อนย้ายทางโฟลเอ็มจะแตกต่างกันไป มีตั้งแต่สามารถเคลื่อนย้ายได้มากไปจนถึงไม่สามารถเคลื่อนย้ายได้ การพิจารณาถึงการเคลื่อนย้ายของธาตุอาหารทางโฟลเอ็ม หรือที่เรียกว่าการเคลื่อนย้ายย้อนกลับสามารถทำได้หลายวิธี ได้แก่ การวิเคราะห์หาความเข้มข้นของธาตุนั้นในน้ำเลี้ยงโฟลเอ็มโดยตรง การติดตามการเคลื่อนย้ายโดยใช้ไอโซโทป การสังเกตจากการพัฒนาอาการขาดธาตุ และการเปรียบเทียบปริมาณธาตุอาหารในส่วนต่างๆ ของพืช หรือในใบพืชที่อายุต่างกัน (Marschner, 1995)

ธาตุโบรอน (B) เป็นธาตุที่แตกต่างจากธาตุอาหารพืชชนิดอื่น พบว่าความสามารถในการเคลื่อนย้ายทางโฟลเอ็มมีความแตกต่างกันในระหว่างชนิดพืช (Brown and Shelp, 1997) ในพืชส่วนใหญ่ไม่มีการเคลื่อนย้ายทางโฟลเอ็ม Hu and Brown (1994) พบว่า อาการขาดโบรอนในต้นกล้าของ squash (*Cucurbita sp.*) เกิดขึ้นทันทีหลังจากที่ถูกย้ายไปยังสารละลายธาตุอาหารที่ไม่มีโบรอน นอกจากนี้ยังพบว่าต้นมะเขือเทศ (*Lycopersicon esculentum*) ที่ปลูกในสภาพที่มีโบรอนมากเกินพอเกิดอาการเป็นพิษของโบรอนโดยแสดงอาการเหลืองที่ปลายใบและขอบใบก่อน ซึ่งในส่วนดังกล่าวมีความเข้มข้นโบรอนสูงกว่าส่วนอื่นด้วย และเมื่อย้ายต้นมะเขือเทศไปไว้ในสารละลายธาตุอาหารที่ไม่มีโบรอน พบว่าอาการขาดโบรอนจะเกิดขึ้นในใบอ่อนและใบที่ยังไม่เจริญเต็มที่ (Oertli, 1993)

อย่างไรก็ตาม ได้มีรายงานที่บ่งชี้ถึงความสามารถในการเคลื่อนย้ายโบรอนทางโฟลเอ็ม ยกตัวอย่าง เช่น Hanson (1991) ได้ทดลองโดยให้โบรอน (500 มิลลิกรัมต่อลิตร) โดยการฉีดพ่นทางใบแก่แอปเปิล (*Malus domestica* Borkh.) สาลี่ (*Pyrus communis* L.) พลัม (*Prunus domestica* L.) และเชอรี่ (*Prunus ceasus* L.) ซึ่งพบว่าปริมาณโบรอนในใบที่ถูกฉีดพ่นลดลงอยู่ในระดับที่ใกล้เคียงกับใบที่ไม่ได้รับการพ่นโบรอน และพบว่าบริเวณตาใบ (buds) มีความเข้มข้นโบรอนสูงที่สุด จากการทดลองของ Delgado *et al.* (1994) พบว่าการให้โบรอนทางใบแก่ต้นมะกอกฝรั่ง (*Olea europaea* L.) ในระยะออกดอก ทำให้กิ่งที่ติดดอกและผล มีความเข้มข้นโบรอนเพิ่มขึ้น ทั้งในส่วนของแผ่นใบ ก้านใบ เปลือกไม้ รวมทั้งดอกและผลด้วย นอกจากนี้ยังได้มีรายงานว่าโบรอนสามารถเคลื่อนย้ายได้ในโฟลเอ็มในรูปที่รวมตัวกับน้ำตาลแอลกอฮอล์ (sugar alcohol) ที่ชื่อว่าซอร์บิทอล (sorbitol) ในแอปเปิล สาลี่ พลัม เชอรี่ (Brown and Hu, 1996) คื่นช่ายฝรั่ง และท้อ (Hu *et al.*, 1997)

ในการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจสอบการเคลื่อนย้ายของโบรอนในถั่วเขียว พันธุ์กำแพงแสน 1 ซึ่งได้มีรายงานว่าเป็นพันธุ์ที่ค่อนข้างทนต่อการขาดโบรอน (เบญจวรรณ, 2537) ซึ่งความรู้ความเข้าใจเกี่ยวกับความสามารถในการเคลื่อนย้ายโบรอนจะเป็นประโยชน์ต่อการแก้ไขปัญหาการขาดโบรอนต่อไป

อุปกรณ์และวิธีการ

ทดลองที่ภาควิชาพืชไร่ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ โดยปลูกถั่วเขียวพันธุ์กำแพงแสน 1 (*Vigna radiata* (L.) Wilczek cv. Kampaengsan 1) ในกระถางบรรจุทราย (เส้นผ่าศูนย์กลาง 50 เซนติเมตร สูง 50 เซนติเมตร) จำนวน 15 กระถาง ปลูกโดยวิธีหยอดเมล็ด 10 เมล็ดต่อกระถาง แล้วปลูกเชื้อไรโซเบียมโดยใส่ผงเชื้อลงไปโดยตรงในหลุมปลูก (ใช้เชื้อไรโซเบียมสำหรับถั่วเขียว จากกองปฐพีวิทยา กรมวิชาการเกษตร) หลังจากเมล็ดงอก รดด้วยสารละลายธาตุอาหาร

(ประกอบไปด้วย 1000 μM $CaCl_2.2H_2O$, 500 μM KH_2PO_4, 250 μM $MgSO_4.7H_2O$, 250 μM K_2SO_4, 10 μM $C_6H_5O_7Fe.H_2O$, 1 μM $MnSO_4.H_2O$, 0.5 μM $ZnSO_4.7H_2O$, 0.2 μM $CuSO_4.5H_2O$, 0.1 μM $CoSO_4.7H_2O$, 0.1 μM $NaMoO_4.2H_2O$ ดัดแปลงจาก Broughton and Dilworth, 1971) ที่ใส่โบรอน 2 ระดับ คือ 10 μM (B10, sufficient) จำนวน 9 กระถาง และ 0.5 μM (B0.5, deficient) จำนวน 6 กระถาง และเมื่อพืชอายุ 10 วัน ถอนแยกเหลือกระถางละ 5 ต้น

เก็บตัวอย่างพืชครั้งที่ 1 (H1) ก่อนระยะที่พืชออกดอก (25 วัน หลังจากเมล็ดงอก) โดยเก็บตัวอย่างจากต้นที่ได้รับโบรอนทั้ง 2 ระดับ (B10 และ B0.5) ระดับละ 3 กระถาง หลังจากนั้นล้างทรายในกระถางที่เหลือโดยเปิดน้ำให้ไหลผ่านทราย 2 ครั้ง (เช้า – เย็น) ครั้งละ 20 นาที และแบ่งกรรมวิธีในการทดลองเป็น (1) ให้โบรอน 10 μM ใน 25 วันแรก จากนั้นเปลี่ยนมาให้สารละลายธาตุอาหารที่มีโบรอน 0.5 μM เป็นระยะเวลา 30 วัน (B10/B0.5) (2) ให้โบรอน 10 μM ตลอดการทดลอง B10/B10) และ (3) ให้โบรอน 0.5 μM ตลอดการทดลอง (B0.5/B0.5) โดยแต่ละกรรมวิธีประกอบไปด้วยต้นถั่วเขียว 3 กระถาง เก็บตัวอย่างพืชอีกครั้งเมื่อพืชอายุได้ 55 วัน (H2)

นำตัวอย่างพืชที่เก็บมาอบในตู้อบอุณหภูมิ 80 °C เป็นเวลา 48 ชั่วโมง และนำไปบด จากนั้นนำไปวิเคราะห์หาความเข้มข้นโบรอนตามวิธีของ Lohse (1982) ในแต่ละส่วนของลำต้นหลักดังนี้

- ยอดที่เจริญใหม่ (ส่วนที่อยู่เหนือใบอ่อนที่สุดที่ขยายตัวเต็มที่: growing point)
- ใบอ่อนที่สุดที่ขยายตัวเต็มที่ (youngest fully expanded leaf blades: YFEL)
- ลำต้นและก้านใบของ YFEL
- ใบส่วนที่เหลือทั้งหมด (รวมเรียกว่าใบแก่: old leaf blades)
- ลำต้นหลักและก้านใบที่เหลือทั้งหมด (old stem + petioles)
- ราก (ไม่รวมปมราก)
- เมล็ด

ผลการทดลอง

ผลของโบรอนต่อการเจริญเติบโตและผลผลิต

น้ำหนักแห้งของพืชเมื่ออายุ 25 วัน (H1) ที่ได้รับโบรอนทั้ง 2 ระดับ (B10, B0.5 μM) มีค่าไม่แตกต่างกันทางสถิติ (Table 1.) ทั้งน้ำหนักในแต่ละส่วนของพืช และน้ำหนักแห้งรวมทั้งต้น เมื่อพืชเจริญเติบโตไปจนถึง 55 วัน (H2) ผลผลิตเมล็ดของต้นถั่วเขียวที่ได้รับโบรอน 0.5 μM ใน 30 วันหลัง (B10/BB0.5 และ B0.5/B0.5) ลดลงเมื่อเทียบกับต้นที่ได้รับ B10 ตลอดการทดลอง แต่พบว่าในต้น B10/B0.5 ให้ผลผลิตเมล็ดมากกว่า B0.5/B0.5 ประมาณ 3.5 เท่า นอกจากนี้ยังพบว่าน้ำหนักแห้งในส่วนใบ ก้านใบ ลำต้น ราก และปมราก ในแต่ละกรรมวิธีมีค่าต่างกันเพียงเล็กน้อย

ความเข้มข้นและปริมาณโบรอนในส่วนต่างๆ ของพืช

เมื่อพืชอายุได้ 25 วัน (H1) ความเข้มข้นโบรอนในตัวอย่างพืชทุกส่วนของต้นที่ได้รับโบรอน 0.5 μM มีค่าใกล้เคียงกัน ขณะที่ในพืชที่ได้รับโบรอน 10 μM มีความเข้มข้นโบรอนสูงที่สุดในส่วนยอดที่เจริญใหม่ และต่ำที่สุดในราก (Table 2.)

ในการเก็บตัวอย่างพืชครั้งที่ 2 (55 วัน) ความเข้มข้นโบรอนในทุกส่วนของพืชลดลงอย่างเห็นได้ชัดในทุกกรรมวิธี แต่พบว่าความเข้มข้นโบรอนในใบแก่ของต้นที่ได้รับโบรอน 10 μM ใน 25 วันแรก (B10/B10 และ B10/B0.5) ยังคงสูงอยู่เมื่อเทียบกับส่วนอื่นในต้นเดียวกัน ขณะที่ต้นที่ได้รับโบรอน 0.5 μM ตลอดการทดลองมีความเข้มข้นโบรอนแตกต่างกันไม่มากนักในแต่ละส่วน โดยมีค่าความเข้มข้นโบรอนสูงที่สุดในราก นอกจากนี้ยังพบว่า ความเข้มข้นโบรอนในใบอ่อนที่สุดที่ขยายตัวเต็มที่ และลำต้นและก้านใบของใบอ่อนที่สุดที่ขยายตัวเต็มที่ของต้น B10/ B0.5 มีค่าสูงกว่าส่วนดังกล่าวของต้น B0.5/ B0.5 (Table 2.)

เมื่อพิจารณาถึงปริมาณโบรอนที่อยู่ในส่วนต่างๆ ของพืช (Table 3.) ต้นถั่วเขียว B10 ที่อายุได้ 25 วันสะสมโบรอนทั้งต้นมากกว่าต้น B0.5 ประมาณ 2 เท่า โดยเฉพาะอย่างยิ่งในเนื้อเยื่ออ่อน (ยอดที่เพิ่งเจริญใหม่ และ ใบอ่อนที่สุดที่ขยายตัวเต็มที่) ทั้งนี้เมื่อพืชอายุได้ 55 วัน ต้นถั่วเขียวที่ได้รับโบรอน 10 μM ตลอดการทดลอง (B10/B10) ยังคงมีการสะสมโบรอนในทุกส่วนของพืชได้มากกว่าต้นที่ได้รับโบรอน 0.5 μM ใน 30 วันหลัง (B10/B0.5 และ B0.5/B0.5) โดยที่ปริมาณโบรอนจะมีอยู่มากในส่วนที่แก่ (ใบแก่ และลำต้นหลักและก้านใบที่เหลือทั้งหมด) ของต้นถั่วเขียวในทุกกรรมวิธี เมื่อเทียบกับส่วนที่อ่อนกว่า (ยอดที่เจริญใหม่ ใบอ่อนที่สุดที่ขยายตัวเต็มที่ และลำต้นและก้านใบของใบอ่อนที่สุดที่ขยายตัวเต็มที่) อย่างไรก็ตามต้นที่ได้รับการเปลี่ยนแปลงโบรอน (B10/B0.5) มีปริมาณโบรอนในใบอ่อนที่สุดที่ขยายตัวเต็มที่ (YFEL) และเมล็ดสูงกว่าต้นที่ได้รับ B0.5 ตลอดการทดลอง (B0.5/B0.5)

วิจารณ์ผลการทดลอง

จากผลการทดลองจะเห็นได้ว่าโบรอนส่วนใหญ่ที่พืชได้รับในระยะแรกของการทดลอง (B10/B0.5) ถูกสะสมในใบแก่ ขณะที่พบว่ายังมีโบรอนจำนวนหนึ่งสามารถเคลื่อนย้ายไปยังใบอ่อนที่สุดที่ขยายตัวเต็มที่ และเมล็ดของถั่วเขียว เห็นได้จากปริมาณโบรอนในส่วนดังกล่าวสูงกว่าต้นที่ขาดโบรอนตลอดการทดลอง (Table 3.) ทั้งที่เนื้อเยื่อดังกล่าวพัฒนาในช่วงที่ขาดโบรอนเหมือนกัน ปริมาณโบรอนที่เพิ่มขึ้นนี้ยังไม่สามารถบอกได้ว่ามาจากส่วนไหนของพืช ซึ่งอาจมาจากส่วนที่เก็บสะสมไว้ในใบแก่ หรืออาจมาจากโบรอนที่ได้รับใน 25 วันแรกยังคงเหลืออยู่ในท่อลำเลียงของลำต้น รวมทั้งไม่สามารถบอกถึงเส้นทางการขนส่งโบรอนดังกล่าวได้ ซึ่งลักษณะการเคลื่อนย้ายทางโฟลเอ็มตามที่ได้มีรายงานจะพบการเคลื่อนย้ายในปริมาณมาก ดังเช่นการเคลื่อน

ย้ายโบรอนไปยังส่วนที่มีการสร้างผลผลิตของถั่วลิสง และ suterranean clover ที่มีการพัฒนาในวัสดุปลูกที่ปราศจากโบรอน (Campbell *et al.*, 1975) ซึ่งน้ำหนักแห้งในส่วนที่ให้ผลผลิต และปริมาณโบรอนมีค่าไม่ต่างจากต้นที่มีการพัฒนาผลผลิตในวัสดุปลูกที่มีโบรอน ในการทดลองนี้พบว่าปริมาณโบรอนที่เพิ่มขึ้นมีเพียง 20 μM แต่อย่างไรก็ตามโบรอนในปริมาณเล็กน้อยนี้สามารถทำให้ผลผลิตเมล็ดจากต้น B10/B0.5 น้อยกว่าต้น B10/B10 เพียง 1.2 เท่า ขณะที่มากกว่า B0.5/B0.5 ถึง 3.5 เท่า (Table 1.)

การที่พืชให้ผลผลิตเมล็ดลดลงเมื่อได้รับโบรอน 0.5 μM ในช่วง 30 วันหลัง (B10/B0.5 และ B0.5/B0.5) แต่ไม่พบผลต่อการเจริญทางต้นและใบในการทดลองนี้ เนื่องจากในระยะที่พืชมีการเจริญพันธุ์จะอ่อนแอต่อการขาดโบรอนมากกว่าการเจริญเติบโตทางต้นและใบ (Marschner, 1995) โดยเฉพาะอย่างยิ่งในระหว่างที่พืชมีการสร้างผลผลิต Bell *et al.* (1990) พบว่าการขาดโบรอนในระยะดังกล่าว ทำให้จำนวนฝักต่อต้น และจำนวนเมล็ดต่อฝักของถั่วเขียวลดลง

ความเข้มข้นโบรอนที่ลดลงในทุกส่วนของต้นถั่วเขียวจากทุกกรรมวิธีที่อายุ 55 วัน เป็นผลมาจากการที่ต้นถั่วเขียวมีการเจริญเติบโตมากขึ้นจากอายุ 25 วัน น้ำหนักแห้งในส่วนต่างๆ ที่เพิ่มมากขึ้น (dilution effect) ไม่สามารถนำมาใช้เป็นข้อพิจารณาถึงลักษณะการเคลื่อนย้ายของโบรอนได้ อย่างไรก็ตาม พบว่าในใบแก่ของต้นที่ได้รับโบรอนเพียงพอ (B10/B10) และต้นที่มีการเปลี่ยนแปลงระดับการให้โบรอน (B10/B0.5) ยังคงมีความเข้มข้นโบรอนที่สูงอยู่เมื่อเทียบกับส่วนอื่น เนื่องจากในใบแก่เป็นบริเวณที่มีการคายน้ำสูง ซึ่งแสดงถึงการขนส่งโบรอนเกิดขึ้นในไซเลม

จากข้อมูลที่ได้สามารถสรุปได้ว่ามีโบรอนจำนวนหนึ่งสามารถเคลื่อนย้ายไปยังส่วนที่มีการเจริญขึ้นมาใหม่หลังจากที่พืชขาดโบรอน แม้ว่าปริมาณโบรอนดังกล่าวมีเพียงเล็กน้อย แต่ก็สามารถทำให้ผลผลิตเมล็ดที่ได้จากต้น B10/B0.5 สูงกว่าต้นที่ได้จาก B0.5/B0.5 มาก แต่จากการทดลองนี้ยังไม่ทราบแน่ชัดว่าโบรอนดังกล่าวสามารถเคลื่อนย้ายมาจากส่วนที่ถูกเก็บสะสมไว้หรือไม่ ซึ่งจะได้ทำการทดลองต่อไปโดยใช้วิธีที่แม่นยำยิ่งขึ้น

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เอกสารอ้างอิง

เบญจวรรณ ฤกษ์เกษม. 2537. โบรอนในการผลิตถั่วภาคเหนือ. ว. ดินและปุ๋ย 16 (3): 130-154.

Bell, R.W., McLay, L., Plaskett, D., Dell, B., and Loneragan, J.F. 1990. Internal boron requirements of green gram (*Vigna radiata*). In: van Beusichem, M.L., ed., Plant nutrition- physiology and applications. Dordrecht, Kluwer Academic Publishers. 275-280.

Broughton, W.J. and Dilworth, M.J. 1971. Control of leghaemoglobin synthesis in snake bean. Biochem J. 125: 1075-1080.

Brown, P.H. and Hu, H. 1996. Phloem mobility of boron is species dependent. Evidence for phloem mobility in sorbitol rich species. Ann. Bot. 77: 497-505.

Brown, P.H. and Shelp, B.J. 1997. Boron mobility in plants. Plant and Soil. 193: 85-101.

Campbell, L. C., Miller, M. H. and Loneragan, J.F. 1975. Translocation of boron to plant fruits. Aust. J. Plant Physiol. 2: 481-487.

Delgado, A. Benlloch, M. and Fernández-Escobar, R. 1994. Mobilization of boron in olive trees during flowering and fruit development. HortScience. 29(6): 616-618.

Hanson, E.J. 1991. Movement of boron out of tree fruit leaves. HortScience. 26 (3): 271-273.

Hu, H. and Brown, P.H. 1994. Localisation of boron in cell walls of squash and tobacco and its association with pectin. Plant Physiol. 105: 681-689.

Hu, H., Penn, S.G., Lebrilla, C.B. and Brown, P.H. 1997. Isolation and characterization of soluble boron complexes in higher plants. Plant Physiol. 113: 649-655.

Loshse, G. 1982. Microanalytical azomethine-H method for boron determination in plant tissues. Commun. Soil. Sci. Plant Anal. 13: 127-134.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd edition. Academic Press. New York. 899 p.

Oertli, J.J. 1993. The mobility of boron in plants. Plant and Soil. 155/156: 301-304.

Smith, F.W. and Loneragan, J.F. 1997. Interpretation of plant analysis: Concepts and principles. In: Reuter, D.J. and Robinson, J.B., eds., Plant analysis and interpretation manual. 2nd edition. Collingwood Vic.: CSIRO. 3-33.

Table 1. Dry weight (g plant^{-1}) of green gram at day 25 (H1) and day 55 (H2). Values are means of three replicates (SE).

Plant parts[a]	B supply				
	H1		H2		
	B10	B0.5	B10/B10[b]	B10/B0.5	B0.5/B0.5
Growing point	0.161 (0.020)	0.133 (0.013)	0.486 (0.079)	0.535 (0.105)	0.382 (0.053)
YFEL, LB	0.228 (0.004)	0.181 (0.024)	0.557 (0.017)	0.389 (0.201)	0.581 (0.020)
YFEL, S+P	0.079 (0.003)	0.071 (0.006)	0.382 (0.062)	0.372 (0.043)	0.525 (0.054)
Old LB	0.305 (0.017)	0.324 (0.057)	3.014 (0.090)	3.592 (0.420)	4.094 (0.193)
Old S+P	0.190 (0.003)	0.174 (0.019)	2.621 (0.089)	2.933 (0.321)	3.282 (0.229)
Roots	0.300 (0.008)	0.314 (0.017)	1.697 (0.129)	1.593 (1.139)	1.707 (0.124)
Nodule	0.114 (0.002)	0.115 (0.011)	0.430 (0.080)	0.529 (0.149)	0.675 (0.057)
Seeds			5.537 (0.382)	3.532 (0.256)	1.056 (0.081)
Total	1.376 (0.045)	1.312 (0.066)	14.722 (0.342)	13.474 (1.203)	12.302 (0.597)

[a] Plant parts: Growing point – all parts above the youngest fully expanded leaf

YFEL – the youngest fully expanded leaf blades

YFEL, S+P – the stems + petioles below YFEL

Old LB – the rest of the leaf blades of the main stem (old leaf blades)

Old S+P – the rest of the main stem

[b] B10/B10 - B10 throughout the experiment

B10/B0.5 - B10 for the first 25 days followed by B0.5 for the following 30 days

B0.5/B0.5 – B0.5 throughout the experiment

Table 3. Boron contents (μg plant^{-1}) of green gram at day 25 (H1) and day 55 (H2). Values are means of three replicates (SE).

Plant parts[a]	B supply				
	H1		H2		
	B10	B0.5	B10/B10[b]	B10/B0.5	B0.5/B0.5
Growing point	7.31 (0.81)	2.16 (0.17)	15.55 (1.86)	2.77 (0.48)	2.02 (0.50)
YFEL, LB	9.14 (0.30)	2.49(0.28)	18.34 (1.44)	4.20 (0.60)	2.86 (0.04)
YFEL, S+P	2.99 (0.07)	1.27 (0.11)	10.12 (1.19)	2.65 (0.13)	2.98 (0.18)
Old LB	11.52 (0.76)	6.07 (0.98)	117.76 (2.25)	52.04 (0.83)	27.88 (0.95)
Old S+P	4.96 (0.09)	2.96 (0.37)	56.24 (0.85)	31.74 (1.78)	21.37 (1.40)
Roots	7.11 (0.28)	5.52 (0.26)	33.55 (2.32)	24.26 (1.90)	19.16 (0.83)
Seeds			118.26 (7.36)	15.80 (1.50)	4.44 (0.71)
Total	43.03 (1.82)	20.47 (1.20)	369.83 (9.93)	133.47 (2.56)	80.71 (2.76)

[a]Plant parts:

Growing point – all parts above the youngest fully expanded leaf

YFEL – the youngest fully expanded leaf blades

YFEL, S+P – the stems + petioles below YFEL

Old LB – the rest of the leaf blades of the main stem (old leaf blades)

Old S+P – the rest of the main stem

[b] B10/B10 - B10 throughout the experiment

B10/B0.5 - B10 for the first 25 days followed by B0.5 for the following 30 days

B0.5/B0.5 – B0.5 throughout the experiment

การตอบสนองต่อความเป็นพิษของโบรอนในข้าวสาลีสามพันธุ์ที่มีระดับความทนทานต่อการขาดโบรอนแตกต่างกัน

Response to Boron Toxicity in Three Wheat Genotypes with Varying Responses to Boron Deficiency

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Abstract

Significant wheat yield reductions have been attributed to boron (B) deficiency and toxicity all over the world. Because of a wide range of genotypic variation in response to both B deficiency and toxicity, there may be some differences in responses to B. It is as yet unclear how the two responses are related in wheat genotypes. To measure and examine responses to B toxicity in three wheat genotypes with known B deficiency responses, two experiments were set out at Multiple Cropping Center and Agronomy Department, Faculty of Agriculture, Chiang Mai University. Three genotypes used in this experiment were Fang 60 (Efficient; E) Bonza and Turkey 1473 (Inefficient; I) were grown for 23 days in sand culture with five levels of B added to nutrient solution (10, 50, 100, 150, 200 mg B L^{-1}). Plant growth was assessed by measuring root and shoot length, root and shoot dry weight, leaf and tiller number, toxicity symptoms (necrosis, chlorosis and symptom score). From the result, root and shoot length, root and shoot dry weight of all genotypes were depressed by B concentration higher than 10 mg B L^{-1}. Fang 60 exhibited the shortest root length whereas shoot length of all genotypes reduced equally. However, root dry weight of all genotypes were reduced equally by high B concentration whereas shoot dry weight of Fang 60 reduced the most at 50, 100 and 150 mg B L^{-1}. Besides, B toxicity depressed tiller number but not leaf number. At 50 mg B L^{-1}, all genotypes depressed tiller number except in Bonza. Boron toxicity also affected on symptom of toxicity such as necrosis and chlorosis by increasing in severe symptom in all genotypes. Boron toxicity symptom developed in YEB+1 more than in YEB. Bonza exhibited the least symptom of necrosis in YEB and YEB+1 at 50, 100 and 150 mg B L^{-1} and also exhibited the least symptom of chlorosis in YEB at 50, 100 and 150 mg B L^{-1} and in YEB+1 at 50 and 100 mg B L^{-1}. In case of symptom score, Fang 60 had more severe of B toxicity than Turkey 1473 and Bonza. So that the symptom score can be used to separate differences in B toxicity response between genotypes. It has also been demonstrated that genotype tolerant to B deficiency such as Fang 60 was not tolerant to B toxicity. In contrast,

Bonza and Turkey 1473 (inefficient to B deficiency) were tolerant to B toxicity resulted in less severe symptoms of B toxicity. Finally, the data in this experiment indicated that there may be some association between tolerant to B deficiency and to B toxicity.

บทคัดย่อ

การขาดและการเป็นพิษของโบรอน (boron, B) ทำให้ผลผลิตข้าวสาลีลดลงในหลายประเทศทั่วโลก เนื่องจากข้าวสาลีมีความแตกต่างทางพันธุกรรมในการตอบสนองต่อทั้งการขาดและการเป็นพิษของโบรอนและความแตกต่างนี้มีช่วงกว้างจึงอาจทำให้ข้าวสาลีมีการตอบสนองแตกต่างกัน จึงยังไม่เป็นที่แน่ชัดว่าการตอบสนองต่อทั้งการขาดและความเป็นพิษของโบรอนในพันธุ์ข้าวสาลีมีความเกี่ยวข้องหรือไม่และเป็นไปในลักษณะใด เพื่อวัดและตรวจสอบการตอบสนองต่อความเป็นพิษของข้าวสาลีสามพันธุ์ที่มีระดับความทนทานต่อการขาดโบรอนแตกต่างกัน จึงทำการทดลองที่ศูนย์วิจัยเพื่อเพิ่มผลผลิตทางการเกษตรและภาควิชาพืชไร่ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ ข้าวสาลีสามพันธุ์ที่ใช้ในการทดลองได้แก่ Fang 60 (ทนต่อการขาดโบรอน; E) Bonza และ Turkey 1473 (อ่อนแอต่อการขาดโบรอน; I) ให้โบรอน 5 ระดับในสารละลายธาตุอาหารพืช (10, 50, 100, 150, 200 mg B L^{-1}) ใช้รดข้าวสาลีที่ปลูกในทราย ทดสอบเป็นเวลา 23 วัน บันทึกข้อมูลความยาวรากและต้น น้ำหนักแห้งรากและต้น จำนวนใบ จำนวนหน่อ อาการเป็นพิษ (อาการ necrosis, chlorosis และการให้คะแนนความเป็นพิษ) จากผลการทดลองพบว่าความยาวรากและต้น น้ำหนักแห้งรากและต้นของข้าวสาลีทุกพันธุ์ลดลงเมื่อระดับโบรอนเพิ่มมากกว่า 10 mg B L^{-1} โดยพันธุ์ Fang 60 มีความยาวรากลดลงมากที่สุด ในขณะที่ความยาวต้นของทุกพันธุ์ลดลงเท่าๆ กัน อย่างไรก็ตามพบว่าน้ำหนักแห้งรากของข้าวสาลีทุกพันธุ์ลดลงเท่าๆ กัน ในขณะที่น้ำหนักแห้งต้นของพันธุ์ Fang 60 ลดลงมากที่สุดที่ 50, 100 และ 150 mg B L^{-1} ความเป็นพิษของโบรอนยังมีผลทำให้จำนวนหน่อของข้าวสาลีลดลงแต่ไม่มีผลต่อจำนวนใบ ที่ 50 mg B L^{-1} ข้าวสาลีทุกพันธุ์แตกกอลดลงยกเว้นพันธุ์ Bonza ความเป็นพิษของโบรอนมีผลทำให้เกิดอาการ necrosis และ chlorosis เพิ่มขึ้นในข้าวสาลีทุกพันธุ์ อาการจะพัฒนาที่ใบแก่ (YEB) มากกว่าใบที่อ่อนกว่า (YEB+1) พันธุ์ Bonza แสดงอาการ necrosis ในใบ YEB และ YEB+1 ที่ 50, 100 และ 150 mg B L^{-1} น้อยที่สุด และยังแสดงอาการ chlorosis น้อยที่สุดในใบ YEB ที่ 50, 100 และ 150 mg B L^{-1} และใน YEB+1 พันธุ์ Bonza ยังคงมีอาการน้อยที่สุดที่ 50 และ 100 mg B L^{-1} และเมื่อพิจารณาความรุนแรงของความเป็นพิษโดยใช้คะแนนเป็นตัววัดจะพบว่า Fang 60 แสดงอาการเป็นพิษมากที่สุด รองลงมาคือ Turkey 1473 และ Bonza ตามลำดับ ดังนั้นการให้คะแนนความเป็นพิษสามารถจำแนกความแตกต่างระหว่างพันธุ์ในการทนต่อความเป็นพิษได้ ดังนั้นจึงกล่าวได้ว่าพันธุ์ข้าวสาลีที่ทนต่อการขาดโบรอน Fang 60 ไม่ทนต่อความเป็นพิษของโบรอน เนื่องจากแสดงความรุนแรงของการเป็นพิษมากที่สุด ในขณะที่พันธุ์อ่อนแอต่อการขาดโบรอนเช่น Bonza และ Turkey 1473 จะทนต่อความเป็นพิษของโบรอน เนื่องจากแสดงความรุนแรงของอาการเป็นพิษน้อยกว่า จากการทดลองชี้ให้เห็นว่ามีความเกี่ยวข้องกันในการทนต่อการขาดและความเป็นพิษของโบรอนอีกด้วย

Index words: ความเป็นพิษของโบรอน, ข้าวสาลี, ความทนทานต่อการขาดโบรอน
boron toxicity, wheat, boron deficiency tolerance

คำนำ

โบรอนที่เป็นพิษเกิดขึ้นได้ในพื้นที่เพาะปลูกที่มีสภาพแห้งแล้งและกึ่งแห้งแล้ง เช่นที่พบในประเทศมาเลเซีย (Shorrocks, 1964) อินเดีย (Takkar, 1982) ออสเตรเลียตอนใต้ (Cartwright *et al.*, 1984) และเตอรกี (Kalayci *et al.*, 1998) เป็นต้น การปลูกข้าวสาลีในดินที่มีโบรอนสูง (มากกว่า 5 mg B L^{-1}) ทำให้ข้าวสาลีแสดงอาการเป็นพิษเช่น chlorosis และ necrosis (เนื้อเยื่อสูญเสียคลอโรฟิลล์และเนื้อเยื่อตาย) ทำให้มีสีซีดและไหม้ที่ปลายใบ ทำให้ผลผลิตลดลงในที่สุด เนื่องจากข้าวสาลีมีความแตกต่างทางพันธุกรรมที่มีช่วงกว้างในการทนต่อการขาด (Rerkasem and Jamjod, 1997) และทนต่อความเป็นพิษของโบรอน (Paull *et al.*, 1991) จึงมีโครงการปรับปรุงพันธุ์เพื่อคัดเลือกพันธุ์ทน เช่นโครงการปรับปรุงพันธุ์ข้าวสาลีให้ทนต่อความเป็นพิษของโบรอนในประเทศเตอรกีและออสเตรเลียและพบพันธุ์ทนพิษหลายพันธุ์ ซึ่งมีกลไกการทนต่อความเป็นพิษโดยการไม่สะสมโบรอนในต้น (Nable *et al.*, 1988) และในประเทศไทยพบว่า พันธุ์ Fang 60 เป็นพันธุ์ที่ทนต่อการขาดโบรอนดีที่สุด (Jamjod *et al.*, 1992) จนมีการนำพันธุ์ Fang 60 ไปเป็นแหล่งพันธุกรรมของความทนทานต่อการขาดโบรอนในโครงการปรับปรุงพันธุ์ข้าวสาลีนานาชาติของสถาบันวิจัยข้าวโพดข้าวสาลีนานาชาติ (CIMMYT) เนื่องจากดินที่ขาดโบรอนและมีโบรอนมากจนเป็นพิษอาจเกิดขึ้นในพื้นที่ใกล้กันโดยธรรมชาติ เช่นที่เตอรกีหรือออสเตรเลีย และเมื่อมีการใส่ปุ๋ยโบรอนลงในดินอย่างไม่ทั่วถึง ในขณะที่กลไกการทนทานต่อการขาดโบรอนยังไม่ชัดเจน (Rerkasem and Jamjod, 1997) จึงจำเป็นต้องทราบว่าพันธุ์ Fang 60 มีการตอบสนองต่อความเป็นพิษของโบรอนอย่างไรเทียบกับพันธุ์ที่อ่อนแอต่อการขาดโบรอน เช่นพันธุ์ Bonza (สุภาวดี, 2543) และ Turkey 1473 (Pers. Comm.) ดังนั้นในโครงการปรับปรุงพันธุ์จึงต้องมีการทดสอบและคัดเลือกพันธุ์ทนเพื่อนำไปใช้แก้ปัญหาดังกล่าว แต่เนื่องจากยังไม่เป็นที่แน่ชัดว่าข้าวสาลีพันธุ์มาตรฐานที่มีระดับการตอบสนองต่อการขาดโบรอนแตกต่างกัน จะสามารถเจริญเติบโตได้ดีหรือไม่ในสภาพที่โบรอนเป็นพิษ เพื่อวัดและตรวจสอบการตอบสนองต่อความเป็นพิษของข้าวสาลีสามพันธุ์ที่มีระดับความทนทานต่อการขาดโบรอนแตกต่างกัน

อุปกรณ์และวิธีการทดลอง

การทดลองนี้ดำเนินการที่ศูนย์วิจัยเพื่อเพิ่มผลผลิตทางเกษตรและภาควิชาพืชไร่คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ ใช้ข้าวสาลี 3 พันธุ์ที่ทราบระดับความทนทานต่อการขาดโบรอน ได้แก่ Fang 60 (ทนต่อการขาดโบรอน; E) Bonza และ Turkey 1473 (อ่อนแอต่อการขาดโบรอน; I) โดยเพาะเมล็ดข้าวสาลีบนกระดาษเพาะความงอกที่ชุ่มน้ำใน petri dish ประมาณ 2 วันก่อนนำไปปลูกทดสอบในกระถางบรรจุทราย (sand culture) ใช้กระถางขนาดเส้นผ่านศูนย์กลาง 30 ซม. ลึก 30 ซม.

รองก้นกระถางด้วยถุงพลาสติกที่เจาะรูระบายน้ำ ปลูกถั่วเขียวพันธุ์ Regur ก่อนปลูกข้าวสาลีประมาณ 1 สัปดาห์ เพื่อทดสอบปริมาณธาตุโบรอนที่ตกค้างในทราย (ถ้าทรายขาดโบรอนถั่วเขียวพันธุ์ Regur จะไม่มีใบประกอบชุดแรก (trifoliate)) และสารละลายธาตุอาหารพืชที่ใช้รดร่วมกับระดับโบรอนต่างๆ มีดังนี้ $CoSO_4$ (0.1 μM) Na_2MoO_2 (0.1 μM) $CuSO_4$ (0.2 μM) $ZnSO_4$ (0.5 μM) $MnSO_4$ (2 μM) FeEDTA (10 μM) K_2SO_4 (250 μM) $MgSO_4$ (250 μM) KH_2PO_4 (500 μM) $CaCl_2$ (1,000 μM) (Broughton and Dilworth, 1971) และ KNO_3 (5,000 μM) ทดสอบข้าวสาลีทั้ง 3 พันธุ์ในกระถางบรรจุทรายที่ให้สารละลายธาตุอาหารพืชและใส่โบรอน 5 ระดับ คือ 10, 50, 100, 150 และ 200 mg B L^{-1} (แสดงเป็น B10, B50, B100, B150, B200 ตามลำดับ) วางแผนการทดลองแบบ CRD 2 ปัจจัยมี 3 ซ้ำแต่ละกระถาง (แต่ละซ้ำ) ปลูกข้าวสาลี 3 พันธุ์ แต่ละพันธุ์ปลูก 5 ต้น ปลูกจำนวน 3 กระถางต่อหนึ่งระดับโบรอน (รวมเป็น 15 กระถาง) โดยข้าวสาลีแต่ละพันธุ์หยอด 10 หลุม หลุมละ 1 เมล็ด หลังจากนั้น 7 วัน ถอนให้เหลือ 5 ต้น รดสารละลายธาตุอาหารทุกวัน เช้า-เย็น กระถางละ 1 ลิตรต่อครั้ง

ข้อมูลที่บันทึก ได้แก่ ความยาวรากวัด seminal root ที่ยาวที่สุด (ซม.), ความยาวต้นวัดตั้งแต่โคนต้นถึงยอด (ซม.), น้ำหนักแห้งราก (กรัมต่อต้น) แสดงเป็น relative root dry weight ((น้ำหนักแห้งรากที่ B+/น้ำหนักแห้งรากที่ B10) x 100), น้ำหนักแห้งต้น (กรัมต่อต้น) แสดงเป็น relative shoot dry weight ((น้ำหนักแห้งต้นที่ B+/น้ำหนักแห้งต้นที่ B10) x 100), จำนวนใบและจำนวนหน่อ แสดงเป็น relative tiller number ((จำนวนหน่อที่ B+/จำนวนหน่อที่ B10) x 100), อาการเป็นพิษวัดเป็น necrosis และ chlorosis ในใบ YEB (the Youngest Expanded Blade) และ YEB+1 (the second Youngest Expanded Blade) (Paull *et al.*, 1990) โดยวัดเป็น necrosis (%) ((ความยาวของอาการ necrosis/ ความยาวใบนั้น) x 100) และวัดเป็น chlorosis (%) ((ความยาวของอาการ chlorosis / ความยาวใบนั้น) x 100) และวัดอาการเป็นพิษโดยการให้คะแนนความรุนแรงของอาการเป็นพิษโดยให้คะแนน 1-9 ดังนี้

คะแนน 1	ไม่แสดงอาการเป็นพิษ	คะแนน 7	½ ของใบเกิด necrosis
คะแนน 3	เกิด necrosis ที่ปลายใบขนาด 1 ซม.	คะแนน 9	ใบตาย
คะแนน 5	¼ ของใบเกิด chlorosis ร่วมกับเกิด necrosis ที่ปลายใบขนาด 1 ซม.		

วิเคราะห์ข้อมูลโดยวิเคราะห์ความแปรปรวน (Analysis Of Variance) ตามแผนการทดลองแบบ CRD และเปรียบเทียบความแตกต่างระหว่างสิ่งทดลองโดยใช้ LSD ที่ระดับความเชื่อมั่น 95%

ผลการทดลอง

ความยาวรากและความยาวต้น

พบว่ามีความแตกต่างระหว่างพันธุ์ในการตอบสนองต่อความเป็นพิษของโบรอนในลักษณะความยาวราก เมื่อปลูกข้าวสาลีทั้ง 3 พันธุ์ในทราย ที่ B10 ข้าวสาลีทั้ง 3 พันธุ์มีความยาวรากอยู่ระหว่าง

40-42 ซม. (ภาพที่ 1a) พันธุ์ Fang 60 มีความยาวรากสั้นลงเมื่อเพิ่มระดับโบรอนโดยเหลือ 29 ซม. ที่ B50 และ 10 ซม. ที่ B100 แต่ไม่ลดลงอีกที่ B150 และ B200 ในขณะที่การเพิ่มโบรอนไม่มีผลต่อความยาวรากใน Bonza และ Turkey 1473 จนกว่าจะถึง B150 ซึ่งทำให้ข้าวสาลี 2 พันธุ์ มีรากยาว 33 ซม. และลดลงเหลือ 23 ซม. ที่ B200 สำหรับความยาวต้นนั้นพบว่าเมื่อระดับโบรอนเพิ่มขึ้นจาก B10 ความยาวต้นของข้าวสาลีทั้ง 3 พันธุ์ลดลง (ภาพที่ 1b) เมื่อปลูกที่ B10 ข้าวสาลีมีความยาวต้นอยู่ระหว่าง 21-26 ซม. เมื่อเพิ่มระดับโบรอนเป็น B200 ความยาวต้นของ Turkey 1473, Bonza และ Fang 60 เหลือเพียง 12, 9 และ 8 ซม. ตามลำดับ

น้ำหนักแห้งรากและน้ำหนักแห้งต้น

จากการทดลองไม่พบว่ามีความแตกต่างระหว่างพันธุ์เมื่อระดับโบรอนเพิ่มขึ้นในลักษณะน้ำหนักแห้งรากของข้าวสาลีโดยพิจารณาจากค่า Relative root dry weight (ภาพที่ 2a) ระดับโบรอนที่เพิ่มขึ้นทำให้น้ำหนักแห้งรากของข้าวสาลีลดลง เมื่อระดับโบรอนเพิ่มขึ้นจาก B10 เป็น B50 มีผลทำให้ข้าวสาลีทั้งสามพันธุ์มีน้ำหนักแห้งรากลดลงเหลือ 40% ที่ B100 ลดลงเหลือ 19% ที่ B150 ลดลงเหลือ 13% และที่ B200 ลดลงเหลือ 9% และยังพบว่ามีความแตกต่างระหว่างพันธุ์ในลักษณะน้ำหนักแห้งต้นเมื่อระดับโบรอนเพิ่มขึ้น โดยพิจารณาจากค่า Relative shoot dry weight (ภาพที่ 2b) ระดับโบรอนที่เพิ่มขึ้นทำให้น้ำหนักแห้งต้นของข้าวสาลีลดลงยกเว้นพันธุ์ Bonza โดยพบว่าที่ B50 พันธุ์ Bonza มีน้ำหนักแห้งต้นลดลงน้อยที่สุดโดยลดลงเหลือเท่ากับ 63% รองลงมาคือ Turkey 1473 ลดลงเหลือ 49% และ Fang 60 ลดลงมากที่สุด เหลือเท่ากับ 32% แต่พบว่าที่ B100, B150 และ B200 การลดลงของน้ำหนักแห้งต้นนั้นไม่มีความแตกต่างระหว่างพันธุ์

จำนวนใบและจำนวนหน่อ

ไม่พบว่าโบรอนมีผลต่อจำนวนใบในข้าวสาลี ข้าวสาลีทั้งสามพันธุ์มีจำนวนใบต่อต้นเฉลี่ยประมาณ 3 ใบ (ภาพที่ 3a) สำหรับจำนวนหน่อนั้นมีความแตกต่างระหว่างพันธุ์ในการตอบสนองต่อความเป็นพิษของโบรอน (ภาพที่ 3b) เมื่อระดับโบรอนเพิ่มจาก B10 เป็น B200 ทุกพันธุ์มีการแตกกอลดลงโดยพิจารณาจากค่า Relative tiller number (% เทียบกับจำนวนหน่อที่ B10) โบรอนทำให้พันธุ์ข้าวสาลีแตกกอต่างกันเห็นได้ชัดเจนที่สุดที่ B50 โดยที่พันธุ์ Fang 60 มีจำนวนหน่อลดลงครึ่งหนึ่ง Turkey 173 ลดลง 20% และ Bonza ไม่ลดลงเลยเมื่อเทียบกับ B10 การแตกกอของข้าวสาลีทุกพันธุ์ลดลงอีกเมื่อเพิ่มโบรอนเป็น B100 แต่ยังคงรักษาความแตกต่างระหว่างพันธุ์ไว้เช่นเดิมคือ Fang 60 มีจำนวนหน่อลดลงมากที่สุด ตามด้วย Turkey 1473 และ Bonza ซึ่งลดลงน้อยที่สุด ที่ B150 และ B200 ข้าวสาลี 3 พันธุ์มี Relative tiller number ใกล้เคียงกันคือประมาณ 40% ของที่ B10

อาการเป็นพิษที่แสดงเป็น necrosis (%) และ chlorosis (%) ในใบ

จากภาพที่ 4 อาการ necrosis (%) ในใบพบตั้งแต่ B50 จนถึง B200 โดยจะไหม้จากปลายใบเข้าสู่โคนใบ และเกิดที่ใบแก่ (YEB+1) มากกว่าใบที่อ่อนกว่า (YEB) ข้าวสาลีทุกพันธุ์แสดงอาการเป็นพิษ

เพิ่มขึ้นเมื่อระดับโบรอนเพิ่มขึ้น โดยที่ B200 ข้าวสาลีทุกพันธุ์แสดงอาการรุนแรงเท่ากันโดยไม่สร้างใบ YEB พันธุ์ Bonza แสดงอาการ necrosis น้อยที่สุด และโบรอนที่เป็นพิษมีผลทำให้เกิด necrosis ในใบ YEB+1 ในพันธุ์ข้าวสาลีแตกต่างกัน ข้าวสาลีพันธุ์ Bonza ยังคงแสดงอาการ necrosis น้อยที่สุดที่ B50, B100 และ B150 ในขณะที่ Fang 60 แสดงอาการรุนแรงมากที่สุดที่ B50 และ B100 เมื่อความเข้มข้นโบรอนเพิ่มเป็น B200 ทุกพันธุ์แสดงอาการเป็นพิษรุนแรงมากขึ้นเท่าๆ กัน จากภาพที่ 5 พบว่าอาการ chlorosis (%) ในใบ YEB ของพันธุ์ Bonza แสดงอาการน้อยที่สุดที่ B50, B100 และ B150 ส่วนที่ B200 นั้นข้าวสาลีทุกพันธุ์ไม่สร้างใบ YEB ส่วนในใบ YEB+1 พบว่าพันธุ์ Bonza ยังคงแสดงอาการน้อยที่สุดที่ B50 และ B100 รองลงมาคือ Turkey 1473 และ Fang 60 ที่ทุกระดับโบรอน เมื่อความเข้มข้นโบรอนเพิ่มเป็น B150 และ B200 ข้าวสาลีทุกพันธุ์แสดงอาการ chlorosis ที่ YEB+1 เพิ่มขึ้นทุกพันธุ์และไม่ต่างกันระหว่างพันธุ์

อาการเป็นพิษที่วัดเป็นคะแนน 1-9

จากการวัดคะแนนความเป็นพิษ พบว่าข้าวสาลีทุกพันธุ์แสดงอาการรุนแรงที่ใบ YEB+1 มากกว่า YEB ในใบ YEB+1 พันธุ์ Bonza และ Turkey 1473 แสดงอาการรุนแรงน้อยกว่า Fang 60 ที่ B50 ส่วนที่ B100 พันธุ์ Bonza และ Turkey 1473 แสดงอาการรุนแรงใกล้เคียงกันในขณะที่ Fang 60 แสดงอาการมากกว่า อย่างไรก็ตามเมื่อปลูกที่ B100 และ B150 ข้าวสาลีทุกพันธุ์แสดงอาการเป็นพิษรุนแรงใกล้เคียงกัน (ตารางที่ 1)

วิจารณ์ผลการทดลอง

จากการทดลองพบว่าพันธุ์ข้าวสาลีที่ทนต่อการขาดโบรอน เช่น Fang 60 จะไม่ทนต่อความเป็นพิษของโบรอน และพันธุ์ที่ไม่ทนต่อการขาดโบรอน เช่น Bonza และ Turkey 1473 จะทนต่อความเป็นพิษของโบรอน ความเป็นพิษของโบรอนมีผลทำให้ความยาวรากและความยาวต้นข้าวสาลีลดลง โดยทำให้ความยาวรากลดลงแตกต่างกันระหว่างพันธุ์ แต่ไม่ทำให้ความยาวต้นลดลงแตกต่างกันระหว่างพันธุ์ Holloway and Alston (1992) กล่าวว่าความเข้มข้นโบรอนสูงๆ จะจำกัดการเจริญของรากข้าวสาลีและทำให้ผลผลิตลดลงในธัญพืช (Rathjen *et al.*, 1987) จากการทดลองพบว่าพันธุ์ที่ทนต่อการขาดโบรอน เช่น Fang 60 มีความยาวรากลดลงมากที่สุด ในขณะที่ Bonza และ Turkey 1473 มีความยาวรากลดลงน้อยกว่าเมื่อปลูกที่ระดับโบรอนสูงๆ การเป็นพิษของโบรอนยังทำให้ข้าวสาลีพันธุ์ Fang 60 แสดงอาการ necrosis และ chlorosis มากกว่า Bonza และ Turkey 1473 อาการเป็นพิษรุนแรงในใบแก่มากกว่าใบอ่อนกว่า Paull *et al.* (1990) กล่าวว่าเมื่อปลูกข้าวสาลีในทรายและเพิ่มระดับโบรอนภายนอกให้สูงขึ้น พบว่าพันธุ์ที่ทนต่อการเป็นพิษจะมีอาการ necrosis น้อยกว่าพันธุ์อ่อนแอและมีการแตกกอดีกว่า โดย Fang 60 มีการแตกกอลดลงมากกว่า Bonza และ Turkey 1473 เมื่อระดับโบรอนสูงขึ้น (ที่ B100

และ B150) อาการเป็นพิษที่วัดเป็นคะแนนยังสามารถจำแนกความแตกต่างระหว่างพันธุ์ในการทนต่อความเป็นพิษได้ชัดเจนในใบแก่ (YEB+1) มากกว่าใบที่อ่อน (YEB) ที่ B100 และ B150 ซึ่งให้ผลเช่นเดียวกับการใช้ necrosis (%), chlorosis (%) และความยาวราก โดย Fang 60 มีคะแนนความเป็นพิษมากที่สุด สอดคล้องกับการมีความยาวรากที่ลดลงมากที่สุดเมื่อระดับโบรอนสูงขึ้น อย่างไรก็ตามหากจะคัดเลือกพันธุ์ที่ทนต่อความเป็นพิษของโบรอนสามารถคัดเลือกได้โดยใช้ความยาวรากหรือการวัดโดยให้คะแนนและไม่สามารถคัดเลือกได้ที่ B150 และ B200 เนื่องจากเป็นระดับที่เป็นพิษมากเกินไปซึ่งทำให้ข้าวสาลีทั้ง 3 พันธุ์ได้รับผลกระทบจากการเป็นพิษเท่าๆ กัน ระดับ B50 และ B100 จึงเป็นระดับที่เหมาะสมเมื่อคัดเลือกพันธุ์ข้าวสาลีในทราย

ดังนั้นหากนำพันธุ์ที่ทนต่อการขาดโบรอนเช่น Fang 60 หรือสายพันธุ์ #145 ของ CIMMYT (มี Fang 60 และ SERI เป็นพันธุ์พ่อแม่) ที่พบว่าทนต่อการขาดโบรอนเช่นกัน (สุพรรณิการ์, 2546) ไปปลูกในพื้นที่มีโบรอนเป็นพิษจะทำให้เกิดความเสียหายได้ จากการศึกษาดังกล่าวจะเป็นแนวทางและเป็นประโยชน์ในการคัดเลือกและวัดการตอบสนองของพันธุ์ข้าวสาลีต่อการเป็นพิษของโบรอนโดยใช้วิธีการที่รวดเร็วและสะดวก และช่วยตัดสินก่อนนำพันธุ์ไปปลูกในพื้นที่ที่มีโบรอนเป็นพิษต่อไป

กิตติกรรมประกาศ

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เอกสารอ้างอิง

สุพรรณิการ์ พันชนะ. 2546. ความสัมพันธ์ระหว่างการตอบสนองต่อการขาดและการเป็นพิษของโบรอนในพันธุ์ข้าวสาลี. วิทยานิพนธ์วิทยาศาสตรมหาบัณฑิต บัณฑิตวิทยาลัย มหาวิทยาลัยเชียงใหม่. 154 หน้า.

สุภาวดี ง้อเหรียญ. 2543. การตอบสนองของประชากรข้าวสาลีที่มีการกระจายตัวทางพันธุกรรมต่อการขาดโบรอน. วิทยานิพนธ์วิทยาศาสตรมหาบัณฑิต บัณฑิตวิทยาลัย มหาวิทยาลัยเชียงใหม่. 68 หน้า.

Broughton, W.J. and M.J., Dilworth, 1971. Control of leghaemoglobin synthesis in snake beans. *Biochem. J. 125*: 1075-1080.

Cartwright, B., B.A., Zarcinas and A.H., Mayfield, 1984. Toxic concentration of boron in red brown earth at Gladstone, South Australia. *Aust. J. Soil. Res. 22*: 261-272.

Holloway, R.E. and A.M., Alston. 1992. The effect of salt and boron on growth of wheat. *Aust. J. Agric. Res. 43*: 987-1001.

Jamjod, S., C.E., Mann and B., Rerkasem. 1992. Screening for boron deficiency in wheat. *In* Boron deficiency in wheat: Wheat special report No.11 February 17-19, 1992. (Eds. C.E. Mann and B. Rerkasem). CIMMYT, Mexico. pp 79-82.

Kalayci, M., A., Alkan, I., Çakmak, O., Bayramoğlu, A., Yilmaz, M., Aydin, V., Ozbek, H., Ekiz, and F., Ozberisoy. 1998. Studies on differential response of wheat cultivars to boron toxicity. *Euphytica 100*: 123-129.

Paull, J.G., A.J., Rathjen and B., Cartwright. 1991. Major gene control of tolerance of wheat (*Triticum aestivum* L.) to high concentrations of soil boron. *Euphytica 55*: 217-228.

Paull, J.G., A.J., Rathjen, B., Cartwright and R.O., Nable. 1990. Selection parameters for assessing the tolerance of wheat to high concentrations of boron. *In* Genetic aspects of plant mineral nutrition. Kluwer academic publishers, Netherlands. pp 361-369.

Rathjen, A.J., B., Cartwright, J.G., Paull, D.B., Moody, and J., Lewis. 1987. Breeding for tolerance of mineral toxicities in Australian cereals with special reference to boron. *In* Priority in soil/plant relation research for plant production. (Eds. P.G.E. Searle and B.G. Devy). School of crop sciences, The University of Sydney, Australia. pp 110-130.

Rerkasem, B. and S., Jamjod. 1997. Genotypic variation in plant response to low boron and implications for plant breeding. *Plant and Soil 193*: 169-180.

Shorrocks, V.M. 1964. Boron toxicity in *Hevea brasiliensis*. *Nature 204*: 599-600.

Takkar, P.N. 1982. Micronutrients: forms, content, distribution in profile, indices of availability and soil test methods. *In* 12th Int. Soil Sci. Cong., Part 1. New Delhi. 136 p.

Nable, R.O., B., Cartwright and R.C.M., Lance. 1988. Genotypic differences in boron accumulation in barley: Relative susceptabilities to boron deficiency and toxicity. *In* Genetic aspects of plant mineral nutrition. (Eds. N. El Bassam). Kluwer academic publisher, Netherlands. pp 243-251.

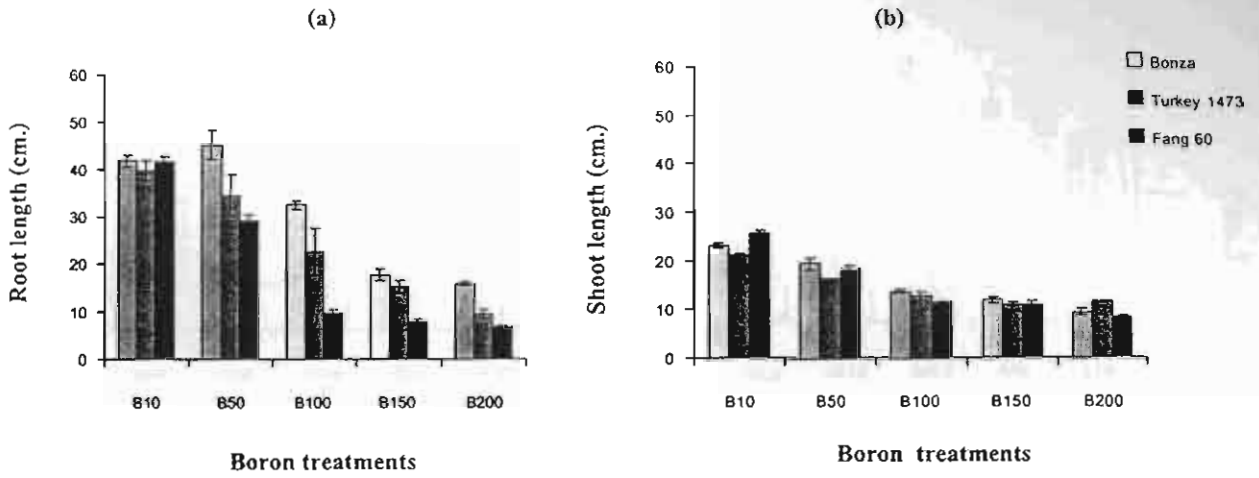

Figure 1 (a) Root length (cm.) and (b) shoot length (cm.) of three wheat genotypes grown in sand culture with five B treatments at 23 days after sowing. Vertical bars presented as standard error of 3 replications. (1a) BxG** (significant at $p < 0.01$), (1b) BxG* (significant at $p < 0.05$). B = boron, G = genotype.

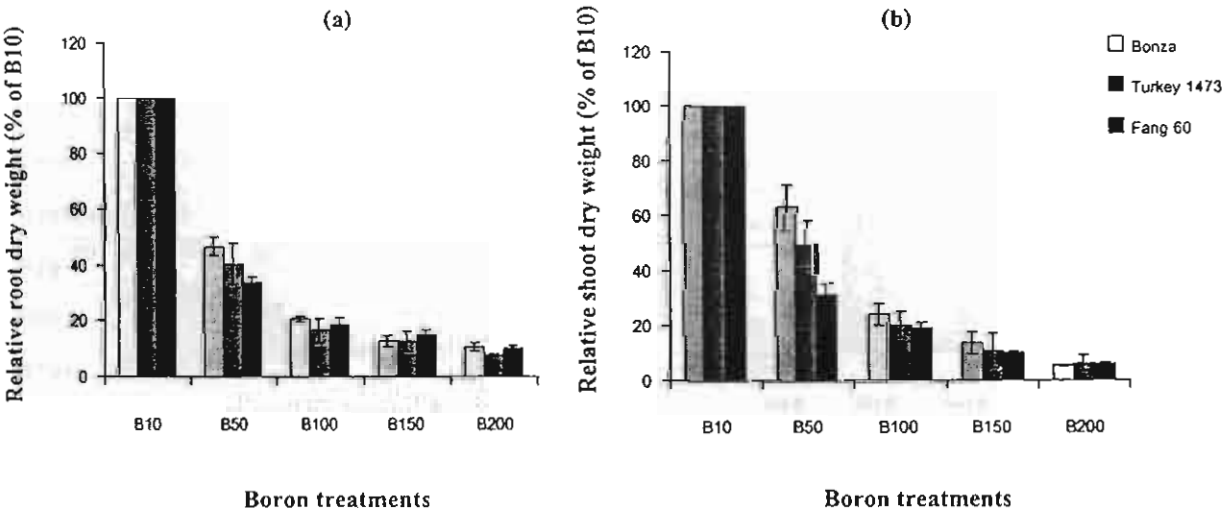

Figure 2 Effect of B treatments on (a) root dry weight (expressed as relative root dry weight (% of B10)) and (b) shoot dry weight (expressed as relative shoot dry weight (% of B10)) of three wheat genotypes grown in sand culture at 23 days after sowing. Vertical bars presented as standard error of 3 replications. (2a) BxG^{ns}, (2b) BxG^{ns} (^{ns} non significant at $p < 0.05$). B = boron, G = genotype.

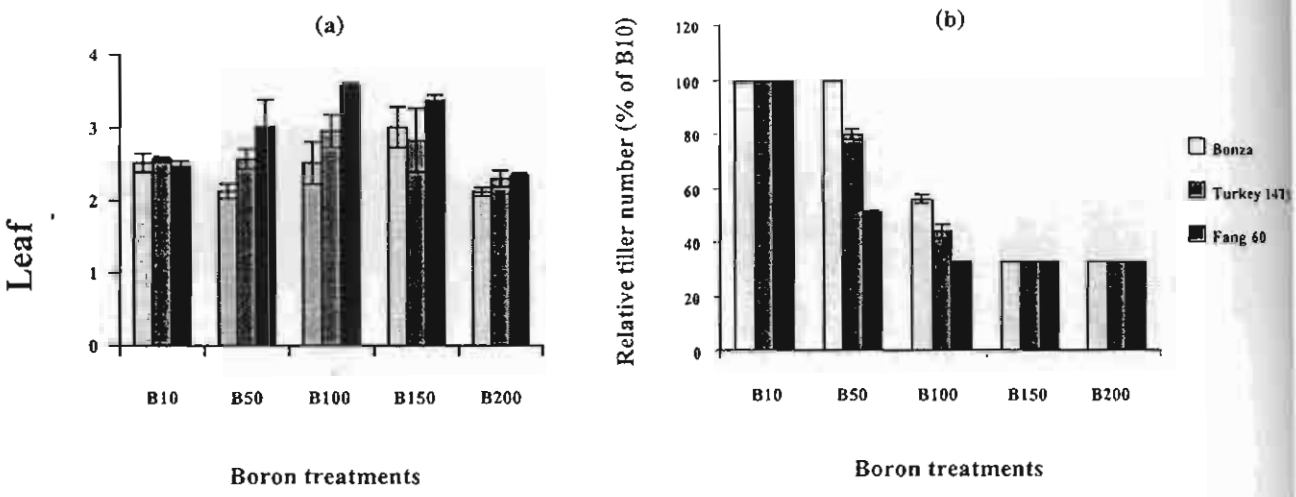

Figure 3 Effect of B treatments on (a) leaf number and (b) tiller number (expressed as relative tiller number (% of B10)) of three wheat genotypes grown in sand culture at 23 days after sowing. Vertical bars presented as standard error of 3 replications. (3a) BxG^{ns} (non significant at $p < 0.05$), (3b) BxG** (significant at $p < 0.01$). B = boron, G = genotype.

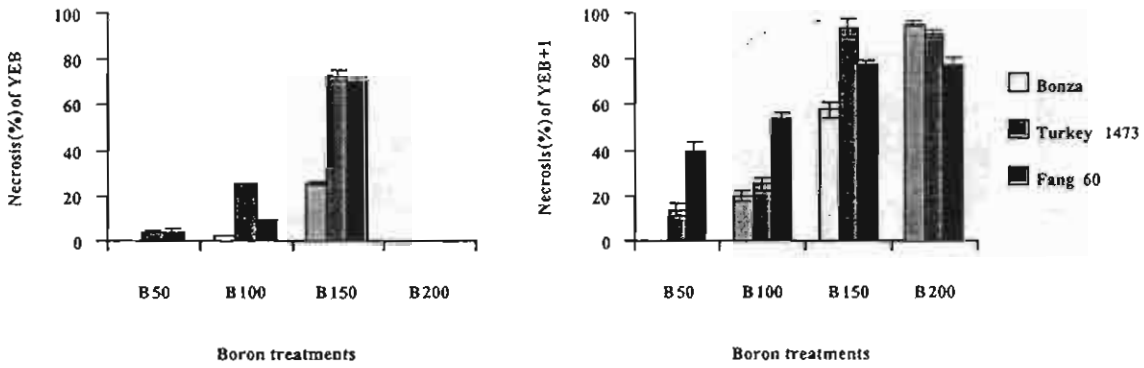

Figure 4 Effect of B treatments on necrosis (%) of YEB and YEB+1 of three wheat genotypes grown in sand culture at 23 days after sowing. Vertical bars presented as standard error of 3 replications. BxG^{ns} (YEB), BxG* (YEB+1) (^{ns} non significant at $p < 0.05$, * significant at $p < 0.05$). B = boron, G = genotype.

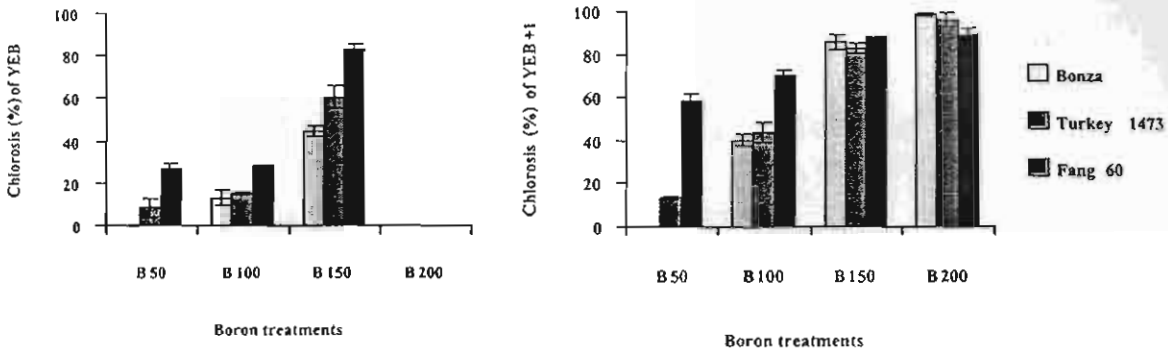

Figure 5 Effect of B treatments on chlorosis (%) of YEB and YEB+1 of three wheat genotypes grown in sand culture at 23 days after sowing. Vertical bars presented as standard error of 3 replications. BxG^{ns} (YEB), BxG* (YEB+1) (^{ns} non significant at $p < 0.05$, * significant at $p < 0.05$). B = boron, G = genotype.

Table 1 Boron toxicity symptom expression of YEB and YEB+1 of three wheat genotypes grown in sand culture with five B treatments at 23 days after sowing.

Boron	YEB			YEB+1		
treatments	Bonza (I)	Turkey 1473 (I)	Fang 60 (E)	Bonza (I)	Turkey 1473 (I)	Fang 60 (E)
B10	1	1	1	1	1	1
B50	1	1	2	2	2	5
B100	1	3	3	3	4	6
B150	4	6	7	7	8	8
B200	8	8	8	9	9	9

Visual rating	Description of B damage
1	no visual symptoms
3	tip necrosis (1 cm.)
5	¼ leaf blade severe chlorosis with > 1 cm. tip necrosis
7	½ leaf blade necrosis
9	leaf dead

อิทธิพลของโบรอนต่อคุณภาพเมล็ดในถั่วเขียวต่างพันธุ์

Genotypic Variation in the Effect of Boron on Seed Quality in Mungbeans (*Vigna mungo* (L.) Hepper and *V. Radiata* (L.) Wilczek)

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บทคัดย่อ

การขาดโบรอนนอกจากทำให้ผลผลิตถั่วเขียวลดลงยังอาจมีผลต่อคุณภาพเมล็ดด้วย แต่เนื่องจากถั่วเขียวเป็นพืชชนิดหนึ่งที่พบว่ามีความแตกต่างระหว่างพันธุ์ในการทนต่อการขาดโบรอน การศึกษานี้จึงมุ่งประเมินความแตกต่างระหว่างพันธุ์ถั่วเขียวในการตอบสนองต่อโบรอนในแง่ของคุณภาพเมล็ด โดยใช้ถั่วเขียวผิวดำ (*Vigna mungo* (L.) Hepper) พันธุ์ M1, Regur, CPI79563 และถั่วเขียวผิวมัน (*V. Radiata* (L.) Wilczek) พันธุ์ กำแพงแสน1, VC2755, VC1163 ปลูกใน sand culture รดด้วยสารละลายธาตุอาหารที่ให้โบรอนในระดับความเข้มข้น 4 ระดับคือ 0, 0.5, 3 และ 5 μM โดยทั่วไปพันธุ์ถั่วเขียวมีโบรอนในเมล็ดต่ำสุดใน B0 และมีความเข้มข้นโบรอนในเมล็ดเพิ่มขึ้นตามระดับโบรอนจนถึงสูงสุดที่ B3 ที่แปลกไปคือถั่วเขียวผิวดำสายพันธุ์ CPI79563 ซึ่งมีโบรอนในเมล็ดสูงสุด 24.3 mg B/kg ใน B0 และลดลงเป็น 4.6 mg B/kg ใน B0.5 แล้วจึงเพิ่มเป็น 18 mg B/kg ใน B3 และ B5 เมื่อนำเมล็ดไปเพาะในทรายที่ได้รับสารละลายอาหารที่มีการเติมโบรอนในอัตรา 0 และ 10 μM B (B0 และ B10) พบว่าปริมาณโบรอนในเมล็ดมีผลต่อคุณภาพเมล็ดต่างกันในถั่วเขียวต่างพันธุ์ โดยถั่วเขียว 6 สายพันธุ์ที่มีโบรอนในเมล็ดในช่วง 3.9-7.7 mg B/kg มีเพียงถั่วเขียวผิวดำสายพันธุ์ M1 และ CPI79563 เท่านั้นที่เปอร์เซ็นต์ความงอกถูกจำกัด แต่เปอร์เซ็นต์ความงอกจะเพิ่มขึ้นเมื่อความเข้มข้นโบรอนในเมล็ดสูงขึ้น การเพาะเมล็ดใน B10 มีความงอกสูงกว่าใน B0 นอกจากความงอกแล้วปริมาณโบรอนในเมล็ดยังมีผลต่อความสมบูรณ์ของต้นอ่อนด้วยแต่ความแตกต่างระหว่างพันธุ์เป็นไปตามความแตกต่างในระดับโบรอนในเมล็ด โดยเปอร์เซ็นต์ต้นอ่อนผิดปกติเมื่อเพาะใน B0 กับความเข้มข้นโบรอนในเมล็ดของถั่วเขียวทั้ง 6 พันธุ์ มีปฏิสัมพันธ์ทางลบร่วมกันที่มีนัยยะสำคัญยิ่ง ($p < 0.01$) และมีค่าวิกฤติของโบรอนในเมล็ดที่ 10 mg B/kg เมล็ดที่มีโบรอนต่ำกว่านี้มีต้นอ่อนผิดปกติมากกว่าร้อยละ 50 เมื่อเพาะใน B10 จำนวนต้นอ่อนผิดปกติของถั่วเขียวที่มีโบรอนในเมล็ดต่ำลดลงเกือบหมด ยกเว้นพันธุ์ CPI79563 และพันธุ์ VC2755 ซึ่งยังคงมีต้นอ่อนผิดปกติอยู่ประมาณร้อยละ 20 การศึกษานี้แสดงให้เห็นชัดว่าพันธุ์ถั่วเขียวมีความแตกต่างในการตอบสนองต่อโบรอนในเมล็ดในด้านคุณภาพ ทั้งในเปอร์เซ็นต์ความงอกและความสมบูรณ์ของต้นอ่อน ถั่วเขียวผิวดำสายพันธุ์ M1 และ CPI79563 นับว่ามีคุณภาพเมล็ดต่ำที่สุดระหว่างพันธุ์ที่มีโบรอนในเมล็ดในระดับเดียวกัน

Abstract

Boron (B) deficiency depresses seed quality in mungbeans. Large genotypic variations have been found in the response of mungbean species to low B in terms of growth and grain yield. This study aims to examine variation in seed quality response to seed B among genotypes of mungbeans. The objectives of this experiment were to evaluate B accumulating ability in seeds and B requirement for seedling growth in different genotypes. Black gram (*Vigna mungo* (L.) Hepper) genotypes included M1, Regur, CPI79563 and green gram (*Vigna radiata*(L.) Wilczek) genotypes included KPS1, VC2755, VC1163 were multiplied in sand culture with 4 levels of applied B (0, 0.5, 3 and 5 μM). Seed B of the most of mungbeens genotypes was lowest in B0 and increasing solution B from B0 to B3 increased it. But Seed B concentration of CPI79563 genotype was highest in B0 (24.3 mg B kg^{-1}) and it was decreased to 4.6 mg B kg^{-1} in B0.5 and it was increased to 18 mg B kg^{-1} by increasing solution B to B3 and B5. Genotypes in range of low seed B, 3.9-7.7 mg B/kg, germination of black gram M1 and CPI79563 was limited. Germination increased with seed B. In general applying B to the germinating medium also increased germination. In addition to germination percentage, low seed B also adversely affected seedling growth. Differences between genotypes, however, were associated with the levels of seed B. When germinated in sand without added B, the six genotypes of mungbeans all fitted into one significant negative correlation ($p < 0.01$) between seed B concentration and % abnormal seedlings. The critical seed B concentration for normal seedling growth was 10 mg B/kg, below this more than half of the seedlings developed abnormally. Applying 10 μM B to the nutrient solution overcame the effect of low seed B in all but two genotypes. The exceptions were black gram M1 and CPI79563, which still had 20% abnormal seedlings in B10. In conclusion, this study has clearly shown that mungbean genotypes differ in the response to seed B in terms of seed quality, as germination percentage and number of normal seedlings. The black gram genotype M1 may be considered to the most sensitive among genotypes with the same range of seed B concentrations.

บทนำ

การขาดโบรอนเป็นปัจจัยหนึ่งที่จำกัดการให้ผลผลิตของพืชตระกูลถั่ว สำหรับในถั่วเขียวแล้ว ถั่วเขียวผิวดำอ่อนแอต่อการขาดโบรอนมากกว่าถั่วเขียวผิวมัน (เบญจวรรณ, 2537) การขาดโบรอนในถั่วเขียวที่เห็นได้ชัดเจนคือการเกิดต้นอ่อนผิดปกติในระยะงอก Rerkasem (1990) พบว่าเมื่อนำเมล็ดของถั่วเขียวผิวดำพันธุ์ Regur และ ถั่วเขียวผิวมันพันธุ์ อู่ทอง 1 ที่มีโบรอนในเมล็ดต่ำ (7.7 และ 5.0 mg B kg^{-1} ตามลำดับ) ไปปลูกในดินที่มีโบรอนต่ำ (0.08 mg B kg^{-1}) จะพบต้นอ่อนผิดปกติถึง 70 % สำหรับพันธุ์ Regur และ 55 % สำหรับพันธุ์ อู่ทอง 1 แต่ต้นอ่อนผิดปกตินี้จะหมดไปเมื่อปลูกในดินที่มีโบรอนสูง

(0.36 mg B kg^{-1}) หรือเมื่อใช้เมล็ดที่มีความเข้มข้นของโบรอนในเมล็ดสูง (14 mg B kg^{-1} สำหรับ Regur และ 10 mg B kg^{-1} สำหรับ อู่ทอง 1) ดังนั้นปริมาณโบรอนในเมล็ดจึงเป็นปัจจัยสำคัญที่กำหนดคุณภาพเมล็ดเมื่อปลูกถั่วเขียวในดินที่มีโบรอนต่ำ

ความเข้มข้นของโบรอนในเมล็ดถูกกำหนดโดยปริมาณโบรอนในดินที่ใช้ผลิตเมล็ดในฤดูปลูกที่ผ่านมา Predisripipat (1988) พบว่าเมล็ดถั่วเขียวที่ผลิตจากดินที่ให้ปุ๋ยโบรอนมีความเข้มข้นของโบรอนในเมล็ดสูงกว่าเมล็ดที่ผลิตจากดินที่ไม่ให้ปุ๋ยโบรอน

ในขณะเดียวกันมีการศึกษามากมาย ที่รายงานว่ามีความหลากหลายทางพันธุกรรมในการทนทานต่อการขาดโบรอนในดิน ในด้านการเจริญเติบโตและการสร้างผลผลิตเมล็ดในพืชหลายชนิด (Rerkasem and Jamjod, 1997) ถั่วเขียวเป็นพืชหนึ่งที่ได้มีรายงานว่ามีสายพันธุ์ที่สามารถเจริญเติบโตและให้ผลผลิตได้ดี ในดินที่มีโบรอนต่ำจนพันธุ์อื่นๆมีการเจริญเติบโตและผลผลิตเมล็ดลดลงอย่างเห็นได้ชัด (Rerkasem 1990) องค์ความรู้เรื่องความแตกต่างระหว่างพันธุ์ในการตอบสนองต่อโบรอนในเมล็ดของคุณภาพเมล็ดน่าจะเป็นประโยชน์ต่อการปรับปรุงและคัดเลือกพันธุ์ถั่วเขียวเพื่อปลูกในดินโบรอนต่ำที่มีแพร่หลายในประเทศไทย ทั้งในภาคเหนือ (เบญจวรรณ 2532) และตะวันออกเฉียงเหนือ (เพิ่มพูน 3538) อีกทั้งอาจมีส่วนช่วยอธิบายกลไกการทนทานต่อการขาดโบรอนในพันธุ์ถั่วเขียวด้วย

วัตถุประสงค์

เพื่อประเมินความแตกต่างระหว่างพันธุของถั่วเขียวในการตอบสนองต่อโบรอนในเมล็ดในด้านคุณภาพเมล็ด

อุปกรณ์และวิธีการทดลอง

ใช้ถั่วเขียวผิวดำและถั่วเขียวผิวมันชนิดละ 3 สายพันธุ์ ถั่วเขียวผิวดำประกอบด้วยพันธุ์ M1 พันธุ์ Regur และสายพันธุ์ CPI79563 ถั่วเขียวผิวมันประกอบด้วยพันธุ์ กำแพงแสน1 (กำแพงแสน 1) สายพันธุ์ VC2755 และ VC1163 ปลูกถั่วเขียวแต่ละสายพันธุ์ สายพันธุ์ละหกเมล็ดในกระถางดินเผาเส้นผ่าศูนย์กลาง 30 เซนติเมตร ลึก 30 เซนติเมตร รองกระถางด้วยถุงพลาสติกเจาะรูและบรรจุทรายแม่น้ำที่ล้างแล้ว ปลูกโดยวิธีเจาะหลุมหยอดเมล็ดแล้วปลูกเชื้อไรโซเบียมด้วยวิธีโรยผงเชื้อลงในหลุมปลูกตามหลังเมล็ด (ใช้เชื้อไรโซเบียมสำหรับถั่วเขียวจากกองปฐพีวิทยา กรมวิชาการเกษตร) หลังงอกรดด้วยสารละลายที่ให้ธาตุอาหารที่ดัดแปลงจากสูตรของ Broughton and Dilworth (1971) ซึ่งไม่ให้ ไนโตรเจน โดยให้โบรอนในรูป H_3BO_3 ในระดับที่แตกต่างกัน 4 ระดับคือ 0, 0.5, 3 และ 5 μM (ให้สัญลักษณ์เป็น B0, B0.5, B3 และ B5 ตามลำดับ) ให้หนึ่งกระถางเป็นหนึ่งหน่วยการทดลองปลูก 3 ซ้ำ ถอนแยกให้

เหลือ 3 ต้นต่อกระถางเมื่ออายุได้ 15 วันหลังงอก เมื่อสุกแก่เก็บเมล็ดแต่ละสายพันธุ์จากแต่ละระดับโบรอนแยกกันแล้วนำเมล็ดที่ผลิตได้ไปหาความเข้มข้นของโบรอนในเนื้อเยื่อโดยวิธี Azomathine-H (Lohse, 1982) จากนั้นเลือกเมล็ดของแต่ละสายพันธุ์ที่ผลิตจากระดับโบรอนที่ต่างกันมารับละ 1 ซ้ำ จากทั้งหมด 3 ซ้ำ ไปเพาะในตะกร้าพลาสติกขนาด กว้าง 30 เซนติเมตร ยาว 40 เซนติเมตร ลึก 12 เซนติเมตร รองก้นตะกร้าด้วยถุงพลาสติกเจาะรูบรรจุด้วยทรายแม่น้ำที่ล้างแล้ว ก่อนปลูกคลุกเมล็ดด้วยเชื้อไรโซเบียม ใช้ระยะปลูก 3 x 3 ซ.ม. ปลูก 1 เมล็ดต่อหลุม หนึ่งหน่วยการทดลองมี 4 แถวแถวละ 5 หลุม หลังปลูกรดด้วยสารละลายที่มีธาตุอาหารที่ให้โบรอนในระดับที่แตกต่างกัน 2 ระดับคือ ไม่ให้โบรอนในสารละลาย (B0) และให้โบรอนในสารละลายในรูป H_3BO_3 10 μM (B10) เมื่ออายุได้ 11 วันหลังปลูก ซึ่งต้นอ่อนมีใบจริงคู่แรกกางเต็มที่และใบประกอบชุดแรกกำลังคลี่กาง ตรวจนับเปอร์เซ็นต์ความงอกและเปอร์เซ็นต์ต้นอ่อนผิดปกติ ที่มีลักษณะดังต่อไปนี้

(ก) ใบจริงคู่แรกขาดหาย มีใบเหลือไม่ถึง 50 % ของใบปกติ

(ข) หยุดชะงักการเจริญเติบโตหลังจากใบจริงคู่แรกคลี่กางเต็มที่

(ค) ใบประกอบชุดแรกบิดงอผิดรูปร่าง

ผลการทดลอง

ความเข้มข้นของโบรอนในเมล็ด

เมื่อปลูกขยายเมล็ดในสภาพที่ไม่ให้โบรอน (B0) ถั่วเขียว 5 พันธุ์มีความเข้มข้นโบรอนในเมล็ดใกล้เคียงกันในช่วง 4.3-6.9 mg B/kg ที่แปลกไปคือ ถั่วเขียวผิวดำสายพันธุ์ CPI79563 มีความเข้มข้นของโบรอนในเมล็ดสูงถึง 24.3 mg B kg^{-1} เมื่อผลิตเมล็ดในสภาพที่ให้โบรอน 0.5 μM (B0.5) ความเข้มข้นของโบรอนในเมล็ดของสายพันธุ์ส่วนใหญ่เพิ่มขึ้นยกเว้นสายพันธุ์ CPI79563 ที่โบรอนในเมล็ดลดลงเหลือ 4.6 mg B kg^{-1} โดยถั่วเขียวผิวดำพันธุ์ M1 มีโบรอนในเมล็ดสูงกว่าพันธุ์อื่นๆ (13.8 mg B kg^{-1}) ส่วนสายพันธุ์ที่เหลือมีโบรอนในเมล็ดพอๆ กันในช่วง 9.4-11.1 mg B kg^{-1} และเมื่อผลิตเมล็ดในสภาพที่ให้โบรอน 3 μM (B3) พบว่าโบรอนในเมล็ดของทุกสายพันธุ์เพิ่มขึ้น โดยสายพันธุ์ VC2755 มีโบรอนในเมล็ดสูงที่สุด (22.0 mg B kg^{-1}) ส่วนสายพันธุ์อื่นๆ มีโบรอนในเมล็ดพอๆ กันในช่วง 17.2-18.1 mg B kg^{-1} และเมื่อเพิ่มระดับโบรอนที่ใช้ผลิตเมล็ดเป็น 5 μM (B5) มีพียงพันธุ์ กำแพงแสน 1 เท่านั้นที่มีโบรอนในเมล็ดเพิ่มขึ้นเป็น 20.7 mg B kg^{-1} ส่วนโบรอนในเมล็ดของสายพันธุ์อื่นๆ ยังคงที่ (Table 1)

เปอร์เซ็นต์ความงอก

การเพาะเมล็ดถั่วเขียวผิวดำพันธุ์ M1 และ CPI79563 ที่มีความเข้มข้นโบรอนในเมล็ดต่ำ (4.4 และ 4.7 mg B kg^{-1}ตามลำดับ) ไม่ว่าจะใน B0 หรือ B10 เปอร์เซ็นต์ความงอกจะถูกจำกัด แต่จะสามารถ

งอกได้ปกติเมื่อใช้เมล็ดมีโบรอนในเมล็ดไม่น้อยกว่า 13.5 mg B kg^{-1} สำหรับพันธุ์ M1 และ 18.6 mg B kg^{-1} สำหรับสายพันธุ์ CPI79563 ในขณะที่เมล็ดของถั่วเขียวพันธุ์อื่นๆ แม้จะมีโบรอนในเมล็ดต่ำ (3.9-7.7 mg B kg^{-1}) ก็สามารถงอกได้ปกติได้ปกติ การเพาะเมล็ดใน B10 ทำให้เปอร์เซ็นต์ความงอกโดยเฉลี่ยจากทุกสายพันธุ์สูงกว่าการเพาะเมล็ดใน B0 2.5% (Table 3)

เปอร์เซ็นต์ต้นอ่อนผิดปกติ

เมื่อเพาะเมล็ดใน B0 ความเข้มข้นของโบรอนในเมล็ดกับเปอร์เซ็นต์ต้นอ่อนผิดปกติของถั่วเขียวทั้ง 6 พันธุ์ มีปฏิสัมพันธ์ทางลบร่วมกันอย่างมีนัยสำคัญยิ่ง ($p < 0.01$) มีค่าสัมประสิทธิ์สหสัมพันธ์ (r) -0.857 เมื่อโบรอนในเมล็ดต่ำทำให้จำนวนต้นอ่อนผิดปกติเพิ่มขึ้น และด้วยวิธีการของ Cate and Nelson (1971) ทำให้ทราบว่าค่าวิกฤติของความเข้มข้นโบรอนในเมล็ดเท่ากับ 10 mg B kg^{-1} หากเมล็ดมีความเข้มข้นโบรอนต่ำกว่านี้จะทำให้เกิดต้นอ่อนผิดปกติมากกว่า 50 % เมล็ดถั่วเขียวผิวดำพันธุ์ M1 ที่มีโบรอนในเมล็ด 4.4 mg B kg^{-1} ทำให้เกิดต้นอ่อนผิดปกติ 54 % ขณะที่เมล็ดพันธุ์ Regur และ CPI79563 ที่มีโบรอนในเมล็ด 4.8 และ 4.7 mg B kg^{-1} ทำให้เกิดต้นอ่อนผิดปกติถึง 100 และ 94.1 % ตามลำดับ ส่วนเมล็ดถั่วเขียวผิวมันสายพันธุ์ VC2755 ที่มีโบรอนในเมล็ด 6.2 mg B kg^{-1} ทำให้เกิดต้นอ่อนผิดปกติ 66.2 % ในขณะที่พันธุ์ กำแพงแสน 1 และ VC1163 ที่มีโบรอน 3.9 และ 7.7 mg B kg^{-1} ทำให้เกิดต้นอ่อนผิดปติ 92.5 และ 81.1 % ตามลำดับ

เมื่อเพาะเมล็ดใน B10 ความเข้มข้นของโบรอนในเมล็ดกับเปอร์เซ็นต์ต้นอ่อนผิดปกติไม่มีความสัมพันธุ์กัน ($r = -0.485$) จำนวนต้นอ่อนผิดปกติของถั่วเขียวที่มีโบรอนในเมล็ดต่ำลดลงจนเกิบหมดไปแต่ในถั่วเขียวผิวดำสายพันธุ์ CPI79563 และ ถั่วเขียวผิวมันสายพันธุ์ VC2755 ยังพบต้นอ่อนผิปกติอยู่ 19.4 และ 22.5 % ตามลำดับ

วิจารณ์และสรุปผลการทดลอง

ดังที่มีรายงานว่าถั่วเขียวมีความแตกต่างระหว่างพันธุ์ในการทนทานต่อการขาดโบรอนในดิน (Rerkasem,1990; อยุธย์ 2545) ซึ่งนำเสนอว่า ถั่วเขียวบางพันธุ์อาจมีการเจริญเติบโตและสร้างผลผลิตเมล็ดได้ดี ในดินที่บางพันธุ์มีการเจริญเติบโตและให้ผลผลิตต่ำเนื่องจากขาดโบรอน การศึกษานี้ได้พบว่าพันธุ์ถั่วเขียวมีความแตกต่างกันในการตอบสนองต่อโบรอนในด้านคุณภาพเมล็ดด้วย

ความเข้มข้นของโบรอนในเมล็ดขึ้นอยู่กับพันธุ์และโบรอนในดินที่ใช้ผลิตเมล็ดนั้น การผลิตเมล็ดในสภาพที่ให้โบรอนสูงก็จะทำให้โบรอนในเมล็ดสูงตามไปด้วย แต่สำหรับสายพันธุ์ CPI79563 การผลิตเมล็ดในสภาพที่โบรอนต่ำมากๆ กลับทำให้โบรอนในเมล็ดสูงผิดปกติ (Table 2) ซึ่งอาจเป็นผลมาจาก Piper-Steenbjerg effect (Marschner, 1995) เพราะในสภาพที่ไม่ให้โบรอน สายพันธุ์ CPI79563 แทบไม่ให้ผลผลิตเลย (Table 1) แต่เมื่อผลิตเมล็ดในสภาพที่โบรอนไม่ต่ำจนเกินไป (B0.5) พันธุ์ M1 มีความสามารถในการดึงโบรอนไปสะสมในเมล็ดได้สูงที่สุด

โบรอนในเมล็ดมีผลต่อคุณภาพเมล็ดทั้งต่อเปอร์เซ็นต์ความงอกและ การเจริญของต้นอ่อน เมื่อพิจารณาคุณภาพเมล็ดพันธุ์ในแง่เปอร์เซ็นต์ความงอกแล้ว ความงอกของพันธุ์ M1 และ CPI79563 อ่อนไหวต่อโบรอนในเมล็ดมากกว่าพันธุ์อื่นๆ ดังจะเห็นว่าเมื่อโบรอนในเมล็ดต่ำทำให้ทั้งสองพันธุ์มีเปอร์เซ็นต์ความงอกลดลง ในขณะที่ความงอกของพันธุ์อื่นๆ ไม่ได้รับผลกระทบแม้จะมีโบรอนในเมล็ดต่ำพอๆ กัน (Table 3) การให้โบรอนจากภายนอกสามารถชดเชยความต้องการโบรอนในการงอกได้โดยเปอร์เซ็นต์ความงอกจะเพิ่มขึ้นเมื่อเพาะเมล็ดในสภาพที่ให้โบรอน

ต้นอ่อนต้องการโบรอนในเมล็ดเพื่อการเจริญ การขาดโบรอนในระยะต้นอ่อนแสดงออกโดยการเกิดต้นอ่อนผิดปกติค่าวิกฤติของโบรอนในเมล็ดต่อการเจริญของต้นอ่อนเท่ากับ 10 mg B kg^{-1} แต่ถั่วเขียวต่างพันธุ์ที่มีโบรอนในเมล็ดต่ำพอๆ กันแต่กลับมีเปอร์เซ็นต์ต้นอ่อนผิดปกติต่างกันโดยพันธุ์ M1 ที่มีโบรอนในเมล็ดต่ำ (4.4 mg B kg^{-1}) แต่กลับมีเปอร์เซ็นต์ต้นผิดปกติน้อยกว่า พันธุ์ Regur, CPI79563 และ กำแพงแสน 1 ที่มีโบรอนในเมล็ดต่ำพอๆ กัน (3.9-4.8 mg B kg^{-1}) (Figure 1) แสดงให้เห็นว่าการเจริญของต้นอ่อนของถั่วเขียวสามพันธุ์นี้อ่อนไหวต่อระดับโบรอนในเมล็ดมากกว่าพันธุ์ M1 การให้โบรอนจากภายนอก (ปลูกในสภาพโบรอนสูง) ก็สามารถชดเชยความต้องการโบรอนในเมล็ดได้โดยต้นอ่อนผิดปกติลดลงจนแทบจะหมดไปปลูกในสภาพที่ให้โบรอน (Figure 2)

จากการทดลองนี้จะเห็นว่าความแตกต่างระหว่างพันธุ์ในด้านการเจริญเติบโตและให้ผลผลิตอาจไม่สอดคล้องกับด้านของคุณภาพเมล็ดดังจะเห็นจากถั่วเขียวผิวดำพันธุ์ M1 ซึ่งจัดเป็นพันธุ์ทนทานสามารถเจริญเติบโตและให้ผลผลิตได้ดีในสภาพโบรอนต่ำแต่กลับมีคุณภาพเมล็ดในด้านความงอกต่ำกว่าพันธุ์อื่นที่มีโบรอนในเมล็ดพอๆ กัน

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เอกสารอ้างอิง

เบญจวรรณ ฤกษ์เกษม. 2537. โบรอนในการผลิตถั่วในภาคเหนือ วารสารดินและปุ๋ย. 16 :130 – 154

เพิ่มพูน กีรติกสิกร. ผลงานวิจัยธาตุอาหารเสริมสำหรับพืชตระกูลถั่วที่เป็นอาหารในภาคตะวันออกเฉียงเหนือ วารสารดินและปุ๋ย. 16 : 155-167

Bell, R.W., McLay, L., Plaskett, D., Dell, B., and Lonerragan, J.F. 1989. Germination and vigor of black gram (*Vigna mungo* (l.) Hepper) seed from plant grown with and without boron. Aus. J. Agric. Res. 40, 237-279.

Cate, R.B. and Nelson, L.A. 1971. A simple statistical procedure for partitioning soil test correlation data in two classes. Soil Sci. Soc. Ame. Proc., 35: 658-660

Marschner, H. 1995. Mineral Nutrition of Higher Plant. 2nd ed. Academic Press. London. pp. 899.

Predisripipat, S. 1988. Response to boron applications in *Vigna.* Master thesis, Chiangmai University.

Rerkasem, B. 1990. Comparison of green gram(*Vigna radiata)* and black gram (*Vigna mungo)* in boron deficiency. In Proceedings of Mung bean Meeting 90. Eds. C.Thavarasook, P. Srinives, N. Bookerd, H. Imai, A. Pookpakdi, P. Laosuwan and U. Pupipat. pp. 167-174. Bangkok Office of Tropical Agricultural Research Center , Japan.

Table 1 Effect of solution B on seed yield of six mungbeans genotypes.

genotypes	Solution B				mean
	B0	B0.5	B3	B5	
Black gram					
M1	15.7 ± 2.0	17.4 ± 2.1	7.7 ± 0.6	9.1 ± 2.6	71.8
Regur	2.2 ± 2.1	14.1 ± 3.3	24.0 ± 8.0	22.0 ± 2.6	64.9
CPI79563	0.1 (0.1)	18.4 ± 3.1	41.5 ± 7.1	47.8 ± 4.2	56.4
Green gram					
KPS1	18.7 ± 1.4	19.6 ± 2.1	21.0 ± 3.6	7.0 ± 1	79.0
VC2755	16.8 ± 1.8	22.2 ± 2.3	27.4 ± 11.2	13.3 ± 3	72.8
VC1163	5.5 ± 0.9	14.3 ± 5.9	10.8 ± 2.4	13.2 ± 3.1	75.0
mean	47.1	78.7	84.5	69.6	70.0

Significant differences in the same low are designated by different lower case letter and in the same column by different upper case letters. B = Boron level, G = genotype, BxG = interaction between B and G: * significant at $p < 0.05$, ** $p < 0.01$

Table 2 Effect of solution B on seed B concentration (mg B kg^{-1}) of six mungbeans genotypes

Genotypes	Solution B			
	B0	B0.5	B3	B5
Black gram				
M1	6.2 a AB	13.8 b C	18.0 c A	17.4 c A
Regur	4.9 a AB	9.4 b B	17.6 c A	17.1 c A
CPI79563	24.3 c C	4.6 a A	18.1 b A	18.5 b AB
Green gram				
KPS1	4.3 a A	11.0 b B	17.7 c A	20.7 d BC
VC2755	5.3 a AB	11.0 b B	22.0 c B	22.4 c C
VC1163	6.9 a B	11.1 b B	17.2 c A	18.2 c A
F test	B**	G**	GxB**	
$LSD_{0.05}$	0.96	1.17	2.34	

Significant differences in the same low are designated by different lower case letter and in the same column by different upper case letters. B = Boron level, G = genotype, BxG = interaction between B and G, SB = source of seed : * significant at $p < 0.05$, ** $p < 0.01$

Table 3 Effect of Seed B concentration on % germination of six mungbeans genotypes in two levels of B application

Treatment	Genotype	Seed B	% Germination		
		Mg B kg^{-1}	Solution B levels		Mean
			B0	B10	
1	M1	4.4	70.0	90.0	80.0 A
2	M1	13.5	100.0	100.0	100.0 B
3	M1	17.7	95.0	97.5	96.3 B
4	M1	18.0	94.9	97.5	96.2 B
5	Regur	4.8	90.0	97.5	93.8 B
6	Regur	8.6	100.0	97.5	98.8 B
7	Regur	17.3	100.0	100.0	100.0 B
8	Regur	17.2	100.0	95.0	97.5 B
9	CPI79563	4.7	77.5	90.0	83.8 A
10	CPI79563	18.6	100.0	100.0	100.0 B
11	CPI79563	18.7	92.5	100.0	96.3 B
12	KPS1	3.9	95.0	92.5	93.8 B
13	KPS1	11.4	100.0	100.0	100.0 B
14	KPS1	19.8	97.5	100.0	98.8 B
15	KPS1	21.2	100.0	100.0	100.0 B
16	VC2755	6.2	97.5	100.0	98.8 B
17	VC2755	11.4	92.5	100.0	96.3 B
18	VC2755	22.0	97.5	100.0	98.8 B
19	VC2755	22.4	100.0	100.0	100.0 B
20	VC1163	7.7	95.0	100.0	97.5 B
21	VC1163	12.2	97.5	97.5	97.5 B
22	VC1163	16.7	97.5	97.5	97.5 B
23	VC1163	16.9	97.5	92.5	95.0 B
24		mean	95.1 a	97.6 b	
F test	Trt**	B*	TrtxB^{NS}		
LSD	6.5	1.9			

Significant differences in the same low are designated by different lower case letter and in the same column by different upper case letters. Trt = treatment combination between genotype and seed B, B = solution B, TrtxB = interaction between treatment combination and solution B

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Arbuscular mycorrhizal fungi from the rhizosphere of a fallow enriching tree, *Macaranga denticulata* Muell. Arg., and their effect on the host plant

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Abstract

A fallow enriching tree, *Macaranga denticulata* Muell. Arg., has been shown to increase rice yield in a rotational shifting cultivation system in northern Thailand through increased accumulation of mineral nutrients. As arbuscular mycorrhizal (AM) fungi may play an important role in nutrient accumulation, AM fungi in the rhizosphere of *M. denticulata* and the effects of the indigenous soil inoculum on the host plant were investigated. The diversity and abundance of AM fungi were documented for the rhizosphere of *M. denticulata* in the field for two years. Based on morphology, 29 species of AM fungi were found in the rhizosphere of *M. denticulata* growing in farmers' fields. Root colonization ranged from 63.5 to 81.5% in the first year and 68.7 to 79.9% in the second year of study. The highest spore density was observed at the end of the wet season. The effects of indigenous soil inoculum, and N and P fertilizers on the host plant were investigated in pots for four months. Inoculation with soil-containing AM fungi strongly increased plant growth and nutrient contents when P was limiting but N was applied. Application of N and P together strongly depressed root colonization and spore density of AM fungi, whereas applying them separately had much less effect. AM fungi may play an important role in nutrient accumulation in *M. denticulata*-rich fallow and thus in nutrient cycling that is beneficial to the maintenance of upland rice yield and sustainability of the rotational shifting cultivation system.

Key words: Arbuscular mycorrhizal fungi, fertilizer, *Macaranga denticulata* rotation, shifting cultivation, Thailand, upland rice

Introduction

Shifting cultivation is a form of land use that can be productive and sustainable if the fallow vegetation has an opportunity to grow and accumulate sufficient amount of nutrients between the cropping phases (Zinke et al., 1978). *Macaranga denticulata* Muell. Arg. (*Euphorbiaceae*) is a small to medium-sized, evergreen tree and is a common pioneer species in moist open areas and secondary forests (Kerby et al., 2000). In the mountains of Northern Thailand, *M. denticulata* is used as a fallow enriching species (Rerkasem et al., 2002). Rice is grown after a mature fallow, in the seventh year, has been slashed and burned. Rice is cultivated for one crop season and then the forest is allowed to regenerate for seven years before the next cropping. Karen farmers in the village of Haui Tee Cha, Sob Moei District of Mae Hong Son Province have been reported to manage this fallow-enriching species successfully and achieved much higher upland rice yield with dense stands of *M. denticulata* compared with fallow plots in which the trees had been poorly established (Yimyam et al., in press). The effect of *M. denticulata* was shown to be associated with the species ability to accumulate nutrients, especially P, K, Ca and Mg compared with other species of the natural fallow vegetation.

In recent years, there is increasing evidence that microbial communities of soils and plants have an important role in the development of sustainable agriculture (Duponnois et al., 2001). Arbuscular mycorrhizal associations are of widespread occurrence and represent the natural status of most plant species growing in a normal soil (Siqueira et al., 1998). AM fungi are known to improve the nutritional status of plants (e.g. N, P, K, Ca, Mg, Mn, Cu and Zn) resulting in increased growth (Marschner and Dell,

1994; Taylor and Harrier, 2001), to protect plants against root pathogens, to confer resistance to drought and salinity conditions (Bagyaraj and Varma, 1995), and to increase the formation of soil aggregates (Douds and Millner, 1999).

This study has two aims. Firstly we set out to examine AM fungi associated with *M. denticulata* in a highland rotational shifting cultivation system where the tree is in use as a fallow enriching species. Secondly, we evaluated the effect of the indigenous soil inoculum on growth and nutrient accumulation of the host plant as a potential option for other rotational shifting cultivation farmers.

Materials and methods

The study site

The village of Haui Tee Cha, Sob Moei District, Mae Hong Son Province is located at 19° 78′ N, 93° 84′ E, altitude 800 m. This village was established more than 200 years ago. Good forest cover dominates the village landscape. *M. denticulata* is a local fallow-enriching species in this village (Rerkasem et al., 2002). The soil at the study site is an acidic (pH 4.83-4.93, 1:5 soil: distilled water) clay loam containing sand at 33.7%, silt 40.2% and clay 26.1%. The soil contained 0.21-0.29% total nitrogen (Kjeldahl method), 3.00-4.29 mg kg^{-1} available P (Bray II method), 156-196 mg kg^{-1} extractable K (1 *N* NH_4OAc, pH 7) and 3.43-3.84% organic matter (Walkley-Black method). The climate of the study site is tropical monsoonal. Mean seasonal rainfall and maximum-minimum temperatures for the two sample years (Year one: June 2000-May 2001; Year two: June 2001- May 2002) are given in Table 1.

Soil and root sampling

In first year, soil and fine root samples (0-20 cm depth) under *M. denticulata* were collected from four locations each in dense (4200) and sparse (1000 plants ha^{-1}) stands of *M. denticulata* in their first year of regeneration from seed. Samples were collected at the end of the wet (October 2000), cool (February 2001) and hot (May 2001) seasons. In the second year, comparable soil and root samples were collected seasonally (October 2001 to May 2002) from four locations each in stands of *M. denticulata* aged five months, three and six years.

Evaluation of root colonization

Roots of *M. denticulate* were washed over a 2 mm sieve under running water. The root samples were cut into pieces 1-2 cm in length, cleared in 10% KOH at 121 °C for 15 minutes and rinsed with water on a 90 μm sieve. Cleared roots were stained with 0.05% trypan blue in lactoglycerol at 121 °C for 15 minutes (Brundrett et al., 1996). Thirty pieces of fine roots were taken at random from each sample and mounted on microscopic slides to assess root colonization (McGonigle et al., 1990).

Determination of AM spores

AM spores were separated from 2 × 25 g of each soil sample in the rhizosphere of *M. denticulata* by wet sieving through 750, 250, 90, and 53 μm mesh sieves. The fractions

from the 250, 90 and 53 μm sieves were processed separately. Each was centrifuged for five minutes at 2000 rpm to remove floating debris. The pellet was resuspended in 50% sucrose with vigorous shaking. The samples were centrifuged for one minute at 2000 rpm to separate spores from dense soil compartments. After centrifugation, spores in the supernatant were poured over the finest sieve and washed with water to remove the sucrose before vacuum filtration on filter paper with gridlines. Spores on filter paper were kept in Petri dishes. Spores were counted under a stereomicroscope. Different types of spores were selected to observe under a compound microscope. Identification of AM fungi was according to morphological characteristics of published AM spore descriptions (e.g. Schenck and Perez, 1988; INVAM webpages).

In the first year of study, soil and root samples from the rhizosphere of some other trees (e.g. *Cratoxylum formosum* Dyer, *Dipterocarpus obtusifolius* Teijsm, *Gluta usitata* Hou, *Lithocarpus elegansi* Hatus ex Soepadmo and *Xylia xylacarpa* Taub.) in the same area of study were also collected to evaluate spore density.

Preliminary experiment for the effect of indigenous soil inoculum on the host plant

To assess the potential of soil inoculum to improve the growth of *M. denticulata*, a pot trial was undertaken consisting of three treatments (soil inoculum from the rhizosphere at the village of Haui Tee Cha collected when?, soil pasteurized with steam for four hours, non-inoculated) each with three replicate pots. The soil inoculum contained blab la spores per g soil The pot soil was an upland soil from Chiang Mai in which no *M. denticulata* was growing and was steamed for four hours. It had a pH of 5.9 (in water) and sandy loam texture (sand 57.5%, silt 24.0% and clay 18.5%). The soil contained 0.09% total N

(Kjedahl method), 4.10 mg kg^{-1} available P (Bray II method), 53 mg kg^{-1} extractable K (1 *N* NH_4OAc, pH 7) and 1.87% organic matter (Walkley-Black method). Seeds of *M. denticulata* from the village of Haui Tee Cha were surface sterilized in 1.2% sodium hypochloride for five minutes, washed with sterile water twice and sown in a seedling tray containing the pasteurized soil. After one month (2 cm tall, with three leaves), one seedling was transplanted into each pot. The pots (20 cm top diameter, 12 cm bottom diameter and 18 cm depth containing 4 kg air dry soil) were placed in the open at Chiang Mai University, Chiang Mai, Thailand. Thirty grams of soil inoculum was applied to the bottom of the planting hole before transplanting. Two weeks after transplanting, nitrogen fertilizer (urea) at 50 mg N kg^{-1} soil was split applied in three equal weekly doses to every pot for three weeks and, similarly, potassium chloride at 50 mg K kg^{-1} soil in five equal weekly doses. The experiment was harvested three months after transplanting. Prior to harvest, shoot height was recorded for each plant. Shoots were separated from roots at the soil surface and dried at 70 °C for three days to constant weight. Roots were sampled by coring (3 cm diameter) twice in each pot from the soil surface to the bottom of the pot, mid-way between the stem and the pot wall. Roots in one of the soil cores were washed and dried at 70 °C for three days to calculate root dry weight. Soil from the second soil core was assessed for spore density and AM fungal colonization of roots.

Pot experiment for the effect of indigenous soil inoculum and fertilizer on the host plant

The experiment was a full factorial with soil inoculation (soil inoculum from rhizosphere of *M. denticulata* from a shifting cultivation plot at the village of Haui Tee Cha, and non-inoculation), two levels of N (urea at 0 and 50 mg N kg^{-1} soil), and two levels of P

(superphosphate at 0 and 50 mg P kg^{-1} soil) with four replications. Give source of inoculum – was it same as in first experiment and give The soil inoculum contained blab la spores per g soil The same soil as in the preliminary study was used, but was acidified with $Al_3(SO_4)_2.18H_2O$ at the rate of 1 g kg^{-1} soil before steam pasteurization. After pasteurization, the soil pH was 4.9 (1:5 in water), thus similar to the field soil in Huai Tee Cha. Three month-old seedlings (4-5 cm tall, with 4 leaves) of *M. denticulate,* that were prepared in the same way as in the preliminary experiment, were transplanted into well drained clay pots (30 cm top diameter, 23 cm bottom diameter and 27 cm depth) containing 17 kg pasteurized soil with 50 g of indigenous soil inoculum in the bottom of the planting hole (inoculated treatment). The N and P treatments were applied weekly in five weekly doses, beginning two weeks from transplanting. Each pot received a basal treatment of potassium chloride at the rate of 50 mg K kg^{-1} in ten equal weekly doses. The pots were placed in a greenhouse at Chiang Mai University. At four months, plant height was measured in all pots and the experiment was harvested with shoot and root samples treated in the same way as in the preliminary experiment. An exception was that there were four soil cores instead of two, from which two were used to estimate root dry weight and two for determination of root colonization and spore density. After drying, shoots were ground and analyzed for N (Kjeldahl method), P (Bray II method) and K (1 *N* NH_4OAc, pH 7).These methods can not be right. Presumably they were wet or dry ashed and analysed colourimetrically or flame or ICP or? Ask Sittichai and give a reference if needed.

Statistical analysis

Statistical tests were performed with SPSS software version 10.0. The data were analyzed by analysis of variance (ANOVA) to test the effect of the factors or treatments. Duncan's Multiple Range Test ($P < 0.05$) was used to compare means. Was any transformation of the data required?

Results and discussion

AM fungi from the rhizosphere of M. denticulata *at the village of Haui Tee Cha in Northern Thailand*

AM colonization of *M. denticulata* fine roots or spore density of the fungi in the rhizosphere of young *M. denticulata* plants did not differ with host stand density. However, root colonization and spore density of AM fungi varied greatly with the season ($P < 0.001$), with the highest root colonization and spore density at the end of the wet season and the lowest at the end of the hot season (Figure 1). The density of AM spores in the rhizosphere was higher in *M. denticulata* than in other tree species in the same area (Table 2). In the second year of study, percent root colonization was slightly less at the end of the wet season in five month-old trees, but the difference disappeared in older trees (Figure 2a). Age of *M. denticulata* did not significantly affect spore density of AM fungi. This is similar to observations by Jayapatne check spelling as different from the references and Waidyanatha (1982) on rubber (*Hevea*). Spore densities at the end of the wet season were about twice as high as at the end of the cool or hot seasons (Figure 2b). Soil moisture and temperature in the wet season may be more suitable for spore production of AM fungi in the rhizosphere of *M. denticulata*. Schenck and Smith (1982) determined the efficacy of

AM fungi at soil temperature ranging from 18 to 41 °C and found that maximum colonization, sporulation and growth enhancement in soybean was at 30 °C.

Root colonization of young *M. denticulata* plants in the first year of the study was only slightly different from the young plants in the second year of the study. Means of root colonization in the first year were 81.5, 74.6 and 63.5%, and those in the second year were 79.9, 76.2 and 68.7% at the end of the wet, cool and hot seasons, respectively. Results from both years showed a similar trend in decreasing from the wet to the hot seasons.

Twenty-nine species of AM fungi were recorded from the rhizosphere of *M. denticulata.* Based on morphology, they were placed in six genera: *Acaulospora*, *Archaeospora*, *Gigaspora*, *Glomus*, *Paraglomus* and *Scutellospora* (Table 3). *Acaulospora elegans* Trappe & Gerdemann and *Glomus multicaule* Gerdemann & Bakshi were the most frequent species, occurring in most samples. The diversity of AM fungi associated with *M. denticulata* in the area of study was high compared with some other trees elsewhere. For example, 11 AM fungi were found in the rhizosphere of rubber (*Hevea*), Sri Lanka (Jayaratne and Waidyanatha, 1982); 14 AM fungi in bamboo forest, Taiwan (Wu and Chen, 1986); and 28 AM fungi in a *Eucalyptus* plantation, South China (Chen et al., 1998).

Effect of soil inoculation on the host plant

In the preliminary experiment, inoculation with fresh soil markedly ($P < 0.01$) increased height and dry weight of *M. denticulata*, root colonization and spore density of AM fungi. Pasteurized soil and non-inoculation treatments were similar and stunted (Table 4).

Because of the confirmed soil microbial response, the main experiment was undertaken without a pasteurized soil control.

In the main pot experiment, there were interactions between the effect of soil inoculation, and N and P fertilization on plant height, shoot and root dry weights. The largest response to inoculation occurred when N was applied without P. Without AM inoculation, N and P fertilizer application, plant height and dry weight of *M. denticulata* were clearly depressed by N and P deficiency (Table 5). Applying N or P alone did not increase plant growth, nor did application of the soil inoculum in the absence of the fertilizers. Plant height and dry weight were increased significantly with N application, in combination with either P fertilizer or AM inoculation. The effect of AM inoculation was very small when the N and P fertilizers were applied together.

Shoot N, P and K contents of the host plant were significantly affected by soil inoculation, and N and P applications (Table 6). Soil inoculum increased the nutrient content of all treatments. In uninoculated treatments, the N contents of the host plants were depressed to a similar extent in all treatments except where both N and P were applied. Further, the shoot P contents were very low in uninoculated plants not supplied with P fertilizer. By contrast, in inoculated soil treatments, applying only N fertilizer significantly increased shoot N, P and K contents. Thus, though the soil was deficient in both P and N for growth of nonmycorrhizal plants, it appears that the AM fungi were more efficient in accessing and supplying P from soil reserves than N to the host plant.

Root colonization and spore density were significantly affected by soil inoculation, and N and P fertilizer ($P < 0.05$). The highest root colonization and spore density occurred in plants without N and P (Figure 3). Application of both N and P reduced root colonization and spore density but not when they were applied separately. Many workers have shown that application of high rates of P can suppress root colonization by AM

fungi (e.g. Kucey and Paul, 1983; Khaliq and Sanders, 2000 and Valentine et al., 2001). However, N additions have been reported to both stimulate and suppress root colonization (Sylvia and Neal, 1990). Further, Chamber check correct spelling et al. (1980) reported that both NH_4^+ and NO_3^- depressed AM colonization of *Trifolium subterraneum* roots.

Relevance of findings for hill farmers

In the shifting cultivation area at the village of Haui Tee Cha in Northern Thailand, Yimyam et al. (in press) reported that, at the end of seven years of fallow regeneration, above ground biomass of dense stands of *M. denticulata* was about 22.2 t ha^{-1} and sparse stands of *M. denticulata* was about 9.4 t ha^{-1}. The grain yield of upland rice in plots after dense stands of *M. denticulata* was about three times higher than in plots after sparse stands of *M. denticulata.* They reported that the amount of N, P and K taken up by the rice crop much smaller fraction of the nutrients accumulated above ground in seven-year old-fallow in the sparse than in the dense stands of *M. denticulata.*Unclear Although much of the above-ground N in the fallow would have been lost with burning by Karen farmers, most of the P, K and some other nutrients could be assumed to remain in the ash. In this study, we found that root colonization and spore density of AM fungi in the rhizosphere of *M. denticulata* in dense and sparse stands of *M. denticulata* were not significantly different. However, regardless of stand density, the AM fungi in the rhizosphere of *M. denticulata* could improve growth and nutrient contents of the host plant. Therefore, dense stands of *M. denticulata* would accumulate more biomass and nutrient content per area than sparse stands of *M. denticulata*. The pot trial suggests that, even though *M.*

denticulata is a colonizer species, it is highly dependent on AM fungi for rapid growth on soils that are low in available P.

This study has shown that *M. denticulate*, in the shifting cultivation area of the village of Haui Tee Cha in Northern Thailand, had a high diversity and extensive root colonization of AM fungi. Further, the AM fungi in soil inoculum improved the growth and nutrient accumulation of *M. denticulata*. Since AM fungi may have a broad host range, some of the species that were associated with *M. denticulata* are likely to form effective associations with upland rice. Work is continuing to determine the contribution of these fungi to the sustainability of nutrient cycling in rotational shifting cultivation in the region.

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References

Bagyaraj DJ and Varma A (1995) Interactions between arbuscular mycorrhizal fungi and plants: their importance in sustainable agriculture in arid and semiarid tropics. Adv Microbiol Ecol 14: 119-142

Brundrett M, Bougher N, Dell B, Grove T and Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. ACIAR Monograph, Canberra, Australia, 374 pp

Chamber CS, Smith SE and Smith FA (1980) Effects of ammonium and nitrate ions on mycorrhizal infection, nodulation and growth of *Trifolium subterraneum*. New Phytol 85: 47-62

Chen Y, Gong M, Wang F, Chen Y, Zhang M, Dell B and Malajczuk N (1998) Diversity of putative ectomycorrhizal fungi and arbuscular mycorrhizal fungi in *Eucalyptus* plantations in southern China. In: Gong M, Xu D, Chonglu Z, Chen Y, Dell B and Brundrett M (eds) Mycorrhizal Fungi Biodiversity and Applications of Inoculation Technology, pp 21-28. Proceedings of the Guangzhou ACIAR international workshop, Beijing, China

Duponnois R, Plenchette C, Thioulouse J and Cadet P (2001) The mycorrhizal soil infectivity and arbuscular mycorrhizal fungal spore communities in soils of different aged fallows in Senegal. Appl Soil Ecol 17: 239-251

Douds DD, and Millner PD (1999) Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. Agric Ecosyst Environ 74: 119-142

Jayaratne AHR and Swaidyanatha UPS (1982) Endomycorrhizas of rubber growing soils of Sri Lanka and their effect on water uptake. In: Proceeding of Training Course on Mycorrhizal Research Techniques, pp 328-359. Serdang, Malaysia

Kerby J, Elliot S, Maxwell JF, Blakesley D Anusarnsunthorn V (2000) Tree Seeds and

Seedlings for Restoring Forests in Northern Thailand. Biology Department, Science Faculty, Chiang Mai University, Chiang Mai, Thailand, 151 pp

Khaliq A and Sanders FE (2000) Effects of vesicular-arbuscular mycorrhizal inoculation on the yield and phosphorus uptake of field-grown barley. Soil Biol Biochem 32: 1691-1696

Kucey RMN and Paul EA (1983) Vesicular arbuscular mycorrhizal spore populations in various Saskatchewan soils and the effect of inoculation with *Glomus mosseae* on faba bean growth in greenhouse and field trials. Can J Soil Sci 63: 87-95

Marschner, H and Dell, B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159: 89-102

McGonigle TP, Miller MH, Evans DG, Fairchild GL and Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115: 495-501

Rerkasem K, Yimyam N, Kosamphan C, Thong-Ngam C and Rerkasem B (2002) Agrodiversity lessons in mountain land management. Mt Res Dev 22: 4-9

Schenck NC and Perez Y (1988) Manual for identification of VA mycorrhizal fungi 2nd ed. INVAM Gainesville, Florida, USA, 241 pp

Schenck NC and Smith GS (1982) Response of six species of vesicular-arbuscular mycorrhizal fungi and their effects on soybean at four soil temperatures. New Phytol 92: 193-201

Siqueira JO, Carneiro MAC, Curi N, Rosado SCS and Davide C (1998) Mycorrhizal colonization and mycotrophic growth of native woody species as related to successional groups in Southeastern Brazil. For Ecol Manage 107: 241-252

Sylvia DM and Neal LH (1990) Nitrogen affects the phosphorus response of VA mycorrhiza. New Phytol 115: 303-310

Taylor J and Harrier LA (2001) A comparison of development and mineral nutrition of micropropagated *Fragaria* x *ananassa* cv. Elvira (strawberry) when colonised by nine species of arbuscular mycorrhizal fungi. Appl Soil Ecol 18: 205-215

Valentine AJ, Osborne BA and Mitchell DT (2001) Interaction between phosphorus supply and total nutrient availability on mycorrhizal colonization, growth and photosynthesis of cucumber. Sci Hort 88: 177-189

Wu CG and Chen ZC (1986) The endogonaceae of Taiwan I, a preliminary investigation on *Endogonaceae* of bamboo vegetation at Chi-Tou areas, central Taiwan. Taiwania 31: 65-88

Yimyam N, Rerkasem K and Rerkasem B Fallow enrichment with pada (*Macaranga denticulata* (Bl.) Muell. Arg.) trees in rotational shifting cultivation in Northern Thailand. Agrofor Syst. In press

Zinke P, Sabhasri S and Kunstadter P (1978) Soil fertility aspects of the Lua forest fallow system of shifting cultivation. In: Kunstader P, Chapman EC and Sabhasri S (eds) Farmers in the Forest, pp 134-159. University Press of Hawaii, Honolulu

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Table 1. Means of rainfall and maximum-minimum temperatures for the wet (June-October), cool (November-February) and hot seasons (March-May) in 2000 to 2002.

Seasons	Rainfall (mm)		Temperature (°C)	
	1st year	2nd year	1st year	2nd year
Wet	172.6	188.4	31.7-23.3	31.8-23.2
Cool	0.4	16.2	32.8-15.0	32.3-14.8
Hot	73.7	86.9	35.6-20.8	36.7-19.0

Data from Mae Sariang Station of the Northern Meteorological Center about 15 km from the study area

Table 2. Spore density of AM fungi associated with common tree species in the area of study at the end of the cool season.

Tree species	Family	Spore density (spores g^{-1} soil)
Cratoxylum formosum Dyer	*Hypericarpaceae*	2
Dipterocarpus obtusifolius Teijsm	*Dipterocarpaceae*	1
Gluta usitata (Wall.) Hou	*Anacardiaceae*	2
Lithocarpus elegans Hatus. Ex Soepadmo	*Fagaceae*	3
Macaranga denticulata Muell. Arg.	*Euphorbiaceae*	19
Xylia xylocarpa Taub.	*Leguminosae*	3

Table 3. AM fungi associated with *M. denticulata* in the area of study.

Genus	Number of species	Relative frequency of each genus found
Acaulospora	6	+ + + + +
Archaeospora	1	+
Gigaspora	2	+ + +
Glomus	17	+ + + + +
Paraglomus	1	+
Scutellospora	2	+ + +

+, rarely; + + +, moderately; and + + + + +, frequently found

Table 4. Effect of soil inoculation of the preliminary study on growth of *M. denticulata* and AM fungi.

Align the colums as a table

Treatment	Height (cm)	Plant dry wt (g)	AM colonization (%)	Spore density (spores g^{-1} soil)
Live soil inoculum	9.0 a	5.36 a	53.87 a	21.31 a
Pasteurized soil inoculum	4.2 b	0.12 b	1.59 b	0.45 b
Non-inoculated	3.2 b	0.12 b	2.78 b	0.3 b

Numbers in the same column followed by different letters are significantly different by Duncan's Multiple Range Test ($P < 0.05$).

Table 5. Effect of inoculation and fertilization on height, shoot and root dry weight of seven month-old *M. denticulata* seedlings.

Treatment	Height (cm)	Shoot dry weight (g)	Root dry weight (g)
M0N0P0	15.38d	3.93d	3.55c
M0N0P50	25.25cd	9.47cd	8.67c
M0N50P0	16.00d	5.37d	3.65c
M0N50P50	42.38b	26.96b	20.66b
M1N0P0	21.75cd	11.43cd	7.05c
M1N0P50	26.50c	16.52c	9.44c
M1N50P0	53.50a	46.26a	30.59a
M1N50P50	46.88ab	40.64a	27.57ab
Analysis of variance			
M	***	***	***
N	***	***	***
P	**	**	*
M x N	**	***	**
M x P	***	**	**
N x P	ns	ns	ns
M x N x P	**	**	*

M0, non-inoculated; M1, inoculated with soil containing AM fungi; N0, no added N; N50, 50 mg N kg^{-1} soil; P0, no added P; P50, 50 mg P kg^{-1} soil. Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test. *, **, ***, significant at $P < 0.05$, 0.01, 0.001 respectively; ns, not significant.

Table 6. Effect of inoculation and fertilization on shoot N, P and K contents of seven month-old *M. denticulata* seedlings.

Treatment	Shoot content (mg plant^{-1})		
	N	P	K
M0N0P0	42.97e	6.87d	44.06d
M0N0P50	92.92de	19.60cd	110.46cd
M0N50P0	59.96e	8.86d	59.31d
M0N50P50	258.50b	35.90ab	244.66b
M1N0P0	114.33cd	18.84cd	107.22cd
M1N0P50	151.33c	31.98bc	161.00c
M1N50P0	375.47a	46.34ab	357.98a
M1N50P50	394.44a	49.65a	271.32b
Analysis of variance			
M	***	***	***
N	***	***	***
P	***	***	**
M x N	***	ns	**
M x P	**	ns	***
N x P	*	ns	ns
M x N x P	**	ns	*

M0, non-inoculated; M1, inoculated with soil containing AM fungi; N0, no added N; N50, 50 mg N kg^{-1} soil; P0, no added P; P50, 50 mg P kg^{-1} soil. Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test. *, **, ***, significant at P<0.05, 0.01, 0.001 respectively; ns, not significant.

Distribution and structure of protein and phytin bodies relating to mineral nutrient contents in seed of four rice genotypes

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As the nutrient intake of many people is dependent on rice as their staple food, we investigated the histochemistry, ultrastructure and elemental composition of storage protein bodies in four rice genotypes, selected because of differences in their seed Fe content. Grain was harvested from paddy rice grown in a uniform environment in Chiang Mai, northern Thailand. In thin sections, endosperm cells of all genotypes had about a quarter of the number of protein bodies than cells in the embryo and aleurone layer. Protein bodies were spherical, rod-shaped or irregular in outline and diameters ranged from <1 to 4 μm. The relative distribution and density of protein bodies were similar for the four genotypes. Some of the protein bodies in the non-endospermic part of the seed contained crystalline inclusions. From their histochemistry and FEGTEM-EDX-analysis, showing enrichment in P, Mg, K, Mn, Fe, and Zn, it was concluded that the inclusions were phytin bodies. The phytin bodies ranged from <1 to 2 μm in diameter, and were round, oval or irregular in shape. Phytin bodies were more abundant in the embryo and aleurone layer of the high Fe (IR68144, CMU122) than the low Fe genotypes (KDML105, UBON2). Seed with more abundant phytin bodies also contained higher concentrations of Cu, Mn, Mg, K and P.

Keywords: ***Oryza sativa*****, rice, iron, tissue distribution, protein bodies, phytin**

Abbreviation: FEGTEM-EDX?????

INTRODUCTION

Rice is the staple food of most people in Asia, providing organic and inorganic nutrients that are essential for the human diet. Protein bodies in rice are synthesized during seed development (Tanaka et al., 1973; Ogawa et al., 1975; Zakaria et al., 2000). At seed maturity, the cereal grain contains two main types of protein bodies, those with and those without crystalline inclusions. The distribution of protein bodies is generally not uniform across the whole grain. For example, protein bodies with crystalline inclusions are common in aleurone cells of rice (Harris and Juliano, 1977; Tanaka et al., 1980; Ogawa et al., 1987) and sorghum (Balz, 1966; Spichiger, 1969) but are usually absent from much of the endosperm of rice (Harris and Juliano, 1977; Bechtel and Pomeranz, 1978; Bechtel and Juliano, 1980). The crystalline inclusions are called phytin bodies by many researcher because they contain phytin, a salt of myo-inositol hexaphosphoric acid (Ashton and Williams, 1958; Ogawa et al., 1975). Phytin is usually associated with a wide range of minerals including Ca, Zn, Fe and Mg (Lolas and Markakis, 1975; Bassiri and Nahapetian, 1977). Most studies on protein and phytin bodies in rice have focussed on either the aleurone layer (Ogawa et al., 1975) or the endosperm (Harris and Juliano, 1977; Bechtel and Juliano, 1980; Tanaka et al., 1980). However, the embryo is an important source of protein and minerals for human nutrition and for the growth of rice seedlings. Furthermore, there is no comparative information on protein bodies across rice genotypes. Hence, this paper documents the type and distribution of storage protein bodies in seed from four rice genotypes which differ in their seed Fe content (Prom-u-thai and Rerkasem, 2001). As protein content may vary with environmental conditions during plant growth

(Prida and Mitra, 1989), the seeds used in this experiment were harvested from plants grown concurrently in one uniform field.

MATERIALS AND METHODS

Plant materials

Seed of rice (*Oryza sativa*); with high Fe (Table 1) content (IR68144, an improved genotype from IRRI in the Philippines; and CMU122, an upland Thai genotype) and low Fe content (KDML105, a popular Thai genotype; and Ubon2, a newly released Thai genotype) were harvested from paddy plants grown in the same season on a sandy loam (San Sai soil) at Chiang Mai University, Thailand (18° 48' N; 98° 59' E). Nitrogen concentration of brown rice, separated into embryo and endosperm segments, was determined colourimetricaly after Kjeldahl digestion (Yoshida et al., 1976). Other elements were measured by ICP (VISTA Simultaneous ICP-AES Spectrometer, Varian) after microwave digestion (Huang et al., 2003) using nitric acid (Zarcinas et al, 1987).

Microscopy

For histochemistry, seeds were imbibed for 4-5 hours in distilled water, the embryo third removed with a teflon blade and fixed in 3% gluteraldehyde in 0.025 M phosphate buffer, pH 7, at 4 °C overnight. The samples were processed into glycol methacrylate (Pro Sci Tech, Australia) resin (Feder and O'Brien 1968), and sections (2 μ m, Fig.1 – plane of sectioning) were stained for phytin (toluidine blue O - C.I. 52040), protein (amido black 10B - C.I. 20470), and starch and carbohydrate (Periodic Acid/Schiff's reaction) (Feder and O'Brien, 1968).

For ultrastructural studies, seeds were imbibed as for light microscopy, and tissues of the embryo/scutellum region were fixed in 3% gluteraldehyde in 0.1 M HEPES buffer,

pH 7, at 4 °C overnight. The specimens were dehydrated in acetone and embedded in Spurr's resin. Sections (90-150 nm) were stained with 0.1% aqueous lead citrate (Venable and Coggeshall, 1965) and saturated aqueous uranyl acetate (Millonig, 1976) and examined in a Philips CM100 transmission electron microscope at 80 kV. For EDX analysis, the specimens were processed as for normal TEM. Thick sections (150-190 nm) were placed on formvar-coated titanium grids (Polaron). Grids were mounted on a Gatan double-tilt low-background (Beryllium cup) specimen holder to minimise interference from the holder. EDX analysis was carried out by an Oxford Instruments INCA Energy 200 EDX system (ATW window, take-off angle 23°) on a JEOL-3000F Field Emission Gun Transmission Electron Microscope (FEGTEM) at 100 kV. In each case, the electron probe was defocussed to cover an area approximately half the size of the protein body (over the crystalline inclusions when present) in cells of the embryo. All spectra of macro and micro-elements were collected for 100 s live time at an acquisition rate of 2-4,000 cps. EDX data were also obtained from control areas without protein bodies in the resin, cell wall and cytoplasm.

RESULTS

Histochemistry

Protein bodies were abundant in the embryo (coleorhiza, coleoptile, embryonic axis, scutellum) and aleurone layer, but were scarce in the endosperm (Fig. 2 A, C, E). As for seed N concentration, the pattern of distribution of protein bodies and their relative abundance did not differ with rice genotype (Tables 1 & 2).

Toluidine blue-positive phytin bodies were observed in the embryo (mostly in the embryonic axis and scutellum) and aleurone layer but were absent from the endosperm

(Fig. 2 B, D, F). The low Fe genotypes, KDML105 and UBON2, had visibly less phytin bodies than the high Fe genotypes, IR68144 and CMU122 (Table 1, Fig. 3).

Comparison of the distribution of protein and phytin bodies in adjacent sections of the same cells showed that not all protein bodies contained phytin bodies as inclusions. Protein bodies without inclusions were present in the coleoptile and coleorhiza but they also occurred at low frequency in other tissues including the endosperm.

Ultrastructure and EDX analysis

Under the transmission electron microscope, protein bodies varied in shape from amorphous to spheres and rods (Fig. 4). They occurred in small groups (1-3 units) or in larger clusters (Fig. 4A). The protein bodies ranged in diameter from < 1 to 4 μm in diameter, depending on cell type. The larger protein bodies occurred in the embryo axis (Fig. 4A, B, C) and the smaller protein bodies were in the aleurone layer (Fig. 4E) and scutellum (Fig. 4D). Crystalline inclusions were present in some protein bodies (Fig. 4F). The crystalline inclusions ranged from <1 to 2 μm in diameter and varied in shape (round, oval and irregular).

From EDX analysis, protein bodies with crystalline inclusions were rich in macronutrients, especially P, Mg and K, and some also contained Mn, Fe, and Zn (Fig. 5). There was considerable variation in the micronutrient composition of protein bodies within the same cell and across cell types. For example, some protein bodies had high levels of Mn whereas others had high levels of Fe (data not presented). Also, large protein bodies appeared to contain less macro- and micronutrient reserves than small protein bodies. Areas in the same cells, away from protein bodies, lacked signals for these elements as did the resin without cells.

DISCUSSION

The protein bodies of the four rice genotypes were more concentrated in the embryo and aleurone regions than in the endosperm. Although the density of protein bodies differed with seed part, it was not genotype dependent. Seed of the four genotypes had similar N contents and similar relative abundance of protein bodies in thin sections. Previous investigations on protein bodies in rice have emphasised the endosperm and aleurone layer (Harris and Juliano, 1977; Bechtel and Juliano, 1980; Tanaka et al., 1980; Ogawa et al., 1987). In the endosperm, differences in seed protein content were related to the number of protein bodies (del Rosario et al., 1968; Juliano et al., 1973), and protein bodies were more concentrated (del Rosario et al., 1968) and more diverse in their morphology (Harris and Juliano, 1977; Bechtel and Pomeranz, 1975) in the peripheral than the central endosperm cells. The protein bodies in our study were similar in size to the protein bodies in the endosperm of several other rice genotypes (Harris and Juliano, 1977).

For other cereals, protein bodies have been studied in greater detail. For example, Campbell et al. (1981) showed that protein bodies of wheat endosperm are largely spherical and occurr in a wide range of sizes up to 30 μm in diameter, though small and numerous protein bodies may occur in the epithelial tissue (Fulcher et al., 1972). In other parts of the seed, protein bodies are uniform in size in the aleurone layer whereas in the scutellum they decrease in size towards the scutellar node (Swift and O'Brien, 1971).

Using histochemistry (toluidine blue O), most protein bodies in the embryo and aleurone layer contained phytin. Phytin was confirmed by EDX analysis of the associated macro and micronutrients in the crystalline inclusions. In polished rice (embryo removed), phytin particles have been isolated from the aleurone layer (Ogawa et al., 1975) with dextran-polyethylene glycol and observed by both scanning and transmission

electron microscopy (Tanaka et al., 1973). Over 90% of the isolated phytin particles consisted of phytic acid, K and Mg (Ogawa et al., 1975). Other minerals, including Ca, Mn, Fe and Cu, were present in rather small amounts. The same laboratory, using TEM and EDX-analysis, detected phytin in protein bodies in the scutellum and aleurone layer of rice seed (Tanaka et al., 1977). Phytin has been observed previously in some protein bodies in rice endosperm (Tanaka et al., 1980, Ogawa et al., 1987) but not in all studies (Tanaka et al., 1977). Phytin has been detected in the aleurone layer of barley (Jacobsen et al., 1971) and sorghum (Adams and Novellie, 1975), and is probably universally present in this tissue in all members of the food Gramineae (O' Dell et al., 1972). Furthermore, phytin has been reported in the scutellum of a number of cereals, including wheat (Swift and O'Brien, 1971).

The light microscope study showed that the relative distribution of phytin bodies differed with rice genotype. The high Fe genotypes (IR68144 and CMU122) had more phytin bodies than the low Fe genotypes (KDML105 and UBON2), especially in the embryo where the Fe is more concentrated. This suggests an association between Fe storage and the distribution of phytin bodies in the seed that is independent of the number of protein bodies. However, the high Fe genotypes also had higher concentrations of some other elements, notably Cu, Mn, Mg, K and P in the embryo portion (Table 1). This is to be expected if the majority of the Fe is associated with phytin and does not accumulate as a metalloprotein such as ferritin.

During polishing into white rice, most of the embryo and aleurone layer is lost. Hence much of the phytin and mineral nutrient content, including Fe, is lost in milling. From a human nutrition point of view, the association of minerals with phytin is unfortunate as phytin inhibits the bioavailability of some micronutrients in the digestive

tract (Lolas and Markakis, 1975; Bassiri and Nahapetian, 1977). Work is in progress to further localize the distribution of Fe and other minerals in these rice genotypes. We are particularly interested in those genotypes where mineral accumulation is not phytin dependent. This information is needed to enable breeders to select for traits that may help to increase the mineral intake of people who depend on rice as their staple food.

ACKNOWLEDGEMENTS

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LITERATURE CITED

Adams CA, Novellie L. 1975. Composition and structure of protein bodies and spherosomes isolated from ungerminated seed *Sorghum bicolor* (Linn.) Moench. *Plant Physiology* 55: 1-6.

Ashton WM, Williams PC. 1958. The phosphorus compounds of oats. I. The content of phytate phosphorus. *Journal of the Science of Food and Agriculture* 9: 505-511.

Balz HP. 1966. Intrazellulare lokalisation and funktion von hydrolytischen enzymen bei Tabak. *Planta* 70: 207-236.

Bassiri A, Nahapetian A. 1977. Differences in concentrations and interrelationships of phytate, phosphorus, magnesium, calcium, zinc, and iron in wheat varieties grown under dryland and irrigated conditions. *Journal of Agricultural and Food Chemistry* 25:1118-1122.

Bechtel DB, Juliano BO. 1980. Formation of protein bodies in the starchy endosperm of rice (*Oryza sativa* L.): A reinvestigation. *Annals of Botany* 45: 503-509.

Bechtel DB, Pomeranz Y. 1978. Ultrastructure of the mature ungerminated rice (*Oryza sativa*) cariopsis. The starchy endosperm. *American Journal of Botany* 65: 684-691.

Campbell WP, Lee JW, O'Brien TP and Smart MG. 1981. Endosperm morphology and protein body formation in developing wheat grain. *Australian Journal of Plant Physiology* 8: 5-19.

Del Rosario AR, Briones VP, Vidal AJ and Juliano BO. 1968. Composition and endosperm structure of developing and mature rice kernel. *Cereal Chemistry* 45: 225-235.

Feder N, O'Brien TP. 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* 55: 123-142.

Fulcher RG, O'Brien TP, Simonds DH. 1972. Localization of arginine-rich proteins in mature seed of some members of the Gramineae. *Australian Journal of Biological Science* 25: 487-97.

Harris N, Juliano BO. 1977. Ultrastructure of endosperm protein bodies in developing rice grains differing in protein content. *Annals of Botany* 41:1-5.

Huang, L, Bell RW, Dell B, Woodward J. 2003. Rapid nitric acid digestion of plant material with an open-vessel microwave system. *Communications in Soil Science and Plant Analysis* (in press, C2230).

Jacobsen JV, Knox RB, Pyliotis NA. 1971. The structure and composition of aleurone grains in the barley aleurone layer. *Planta* **101**: 189-209.

Juliano BO, Antonio AA, Esmama BV. 1973. Effects of protein content on the distribution and properties of rice protein. *Journal of the Science of food and Agriculture* **24**: 295-306.

Lolas GM, Markakis P. 1975. Phytic acid and other phosphorus compounds of beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry* **23**:13-15.

Millonig G. 1976. Laboratory Manual of Biological Electron Microscopy. Vercilli, Italy. 53.

O'Dell LB, de Boland RA, Koirtyohann RS. 1972. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. *Journal of Agricultural and Food Chemistry* **20**: 718-721.

Ogawa M, Kumamaru T, Satoh H, Iwata N, Omura T, Kasai Z, Tanaka K. 1987. Purification of protein body-I of rice seed and its polypeptide composition. *Plant Cell Physiology* **28**: 1517-1527.

Ogawa M, Tanaka K, Kasai Z. 1975. Isolation of high phytin containing particles from rice grains using an aqueous polymer two phase system. *Agricultural and Biological Chemistry* **39**: 695-700.

Prida RC, Mitra GN. 1989. Effect of genotype and environment on grain quality in rice. *Orissa Journal of Agricultural Research* **2**: 74-77.

Prom-u-thai C, Rerkasem B. 2001. Grain iron concentration in Thai rice germplasm. Development in plant and soil science **92**: 351-352.

Spichiger JU. 1969. Isolation and charaktersierung sphare glyoxisomen aus tabakendosperm. *Planta* **89**: 56-75.

Swift JG, O'Brien TP. 1971. Vascular differentiation in the wheat embryo. *Australian Journal of Botany* 19: 63-71.

Tanaka K, Ogawa M, Kasai Z. 1977. The rice scutellum. II. A composition of scutellar and aleurone electron-dense particles by transmission electron microscopy including energy-dispersive X-ray analysis. *Cereal Chemistry* 54: 684-689.

Tanaka K, Sugimoto T, Ogawa M, Kasai Z. 1980. Isolation and characterization of two types of protein bodies in the rice endosperm. *Agricultural and Biological Chemistry* 44: 1633-1639.

Tanaka K, Yoshida T, Asuda K, Kasai Z. 1973. Subcellular particles isolated from aleurone layer of rice seeds. *Archives of Biochemsirty and Biophysics* 155: 136-143.

Venable JM, Coggeshall R. 1965. A simplified lead citrate for use in electron microscopy. *Journal of Cell Biology* 25: 407.

Yoshida S, Forno D, Cock J, Gomez K. 1976. Laboratory Manual for Physiological Studies of Rice, 3rd eds. The International Rice Research Institute. Los Banos, Philippines. 14-16.

Zakaria S, Matsuda T, Nitta Y. 2000. Effects of nitrogen application on the development and accumulation of protein bodies in developing rice seed. *Plant Production Science* 3: 84-93.

Zarcinas BA, Cartwright B, Spouncer LR. 1987. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Communication in Soil Science and Plant Analysis* 18: 131-146.

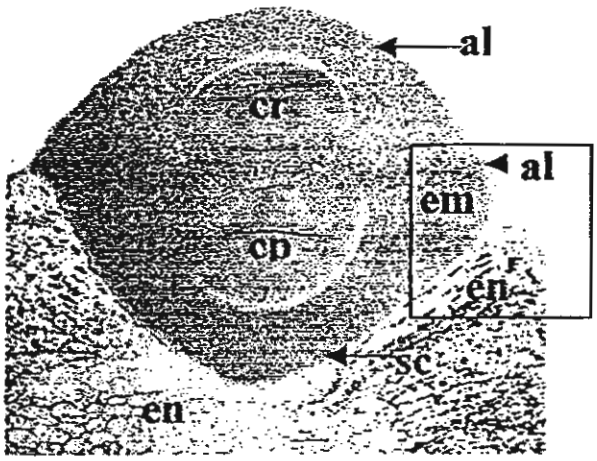


Fig. 1 Light micrograph of median longitudinal section of a rice seed showing location of tissue where the phytin and protein bodies were investigated, em; embryonic axis, sc; scutellum, cp; coleoptile, cr; coleorhiza, en; endosperm, al; aleurone layer.

Table 1 The iron andnitrogen concentration in embryo and endosperm in brown rice of four genotypes

Genotype	Embryo (including aleurone layer and scutellum)		Endosperm (including aleurone layer)	
	Fe (mg Fe/kg)	N (%)	Fe (μ Fe/kg)	N (%)
IR68144	42.2 ± 0.8	2.6 ± 0.05	17.6 ± 0.5	1.8 ± 0
CMU122	44.4 ± 3.6	3.0 ± 0.04	12.7 ± 0.7	1.4 ± 0.14
KDML105	21.7 ± 0.6	2.7 ± 0.05	8.3 ± 0.2	1.4 ± 0.06
UBON2	19.9 ± 1.8	2.9 ± 0.02	8.2 ± 0.6	1.5 ± 0.04

Mean % nitrogen and Fe concentration ± SE

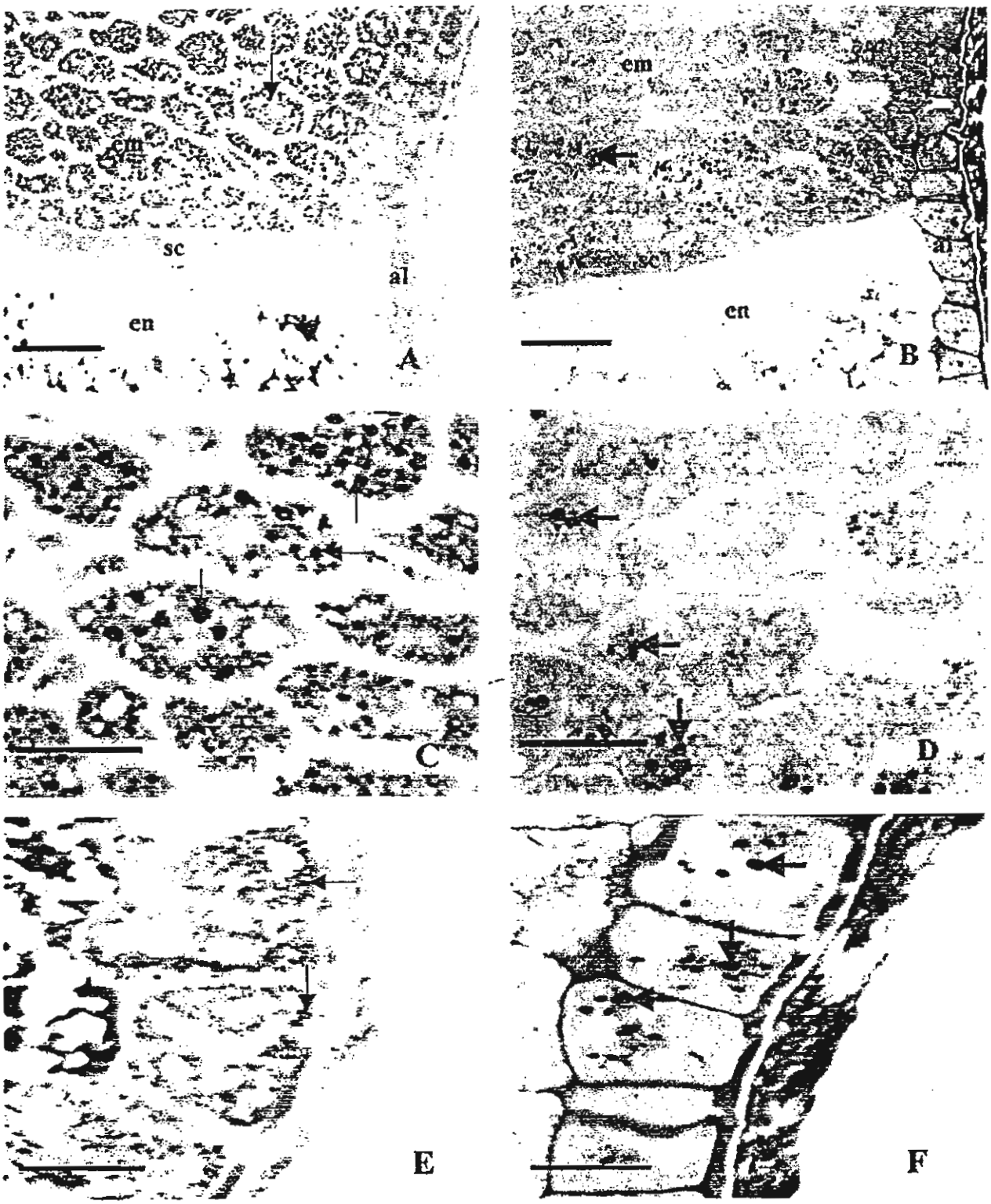


Fig. 2 Light micrograph of KDML105 seed, showing the distribution and abundance of storage protein (A, C, E – thin arrows) and phytin bodies (B, D, F – thick arrows). A, B region shown in Fig. 1; C, D – detail of embryonic cell; E, F – detail of aleurone layer. A, C, E stained with amido black 10B, B, D, F stained with toluidine blue.

Scale bar A, B, = 50 μm, C, D, E, F = 20 μm.

Table 2 Relative distribution of storage protein and phytin bodies in three regions of the seed of four rice genotypes

Cultivar	Protein bodies			Phytin-like bodies		
	Endosperm	Embryo[2]	Aleurone	Endosperm	Embryo	Aleurone
IR68144	+[1]	++++	++++	0	+++	+++
CMU122	+	++++	++++	0	+++	+++
KDML105	+	++++	++++	0	++	++
UBON2	+	++++	++++	0	++	++

1) 0 = 0, + = 1 – 10, + + = 11 – 20, + + + = 21 – 30, + + + + = > 30 bodies per section of the cells

2) Embryonic axis cells

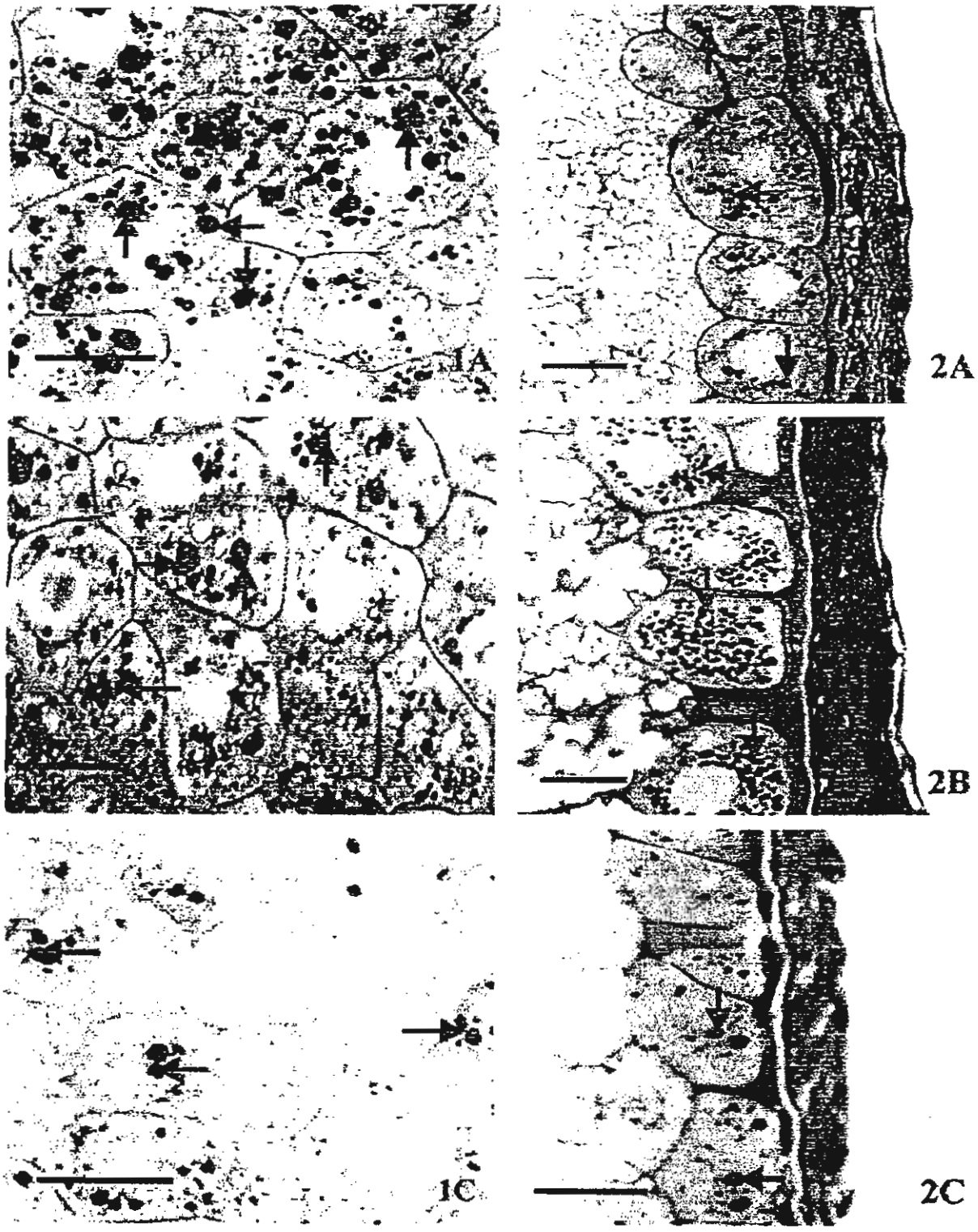


Fig. 3 Light micrographs of rice seed showing the distribution and density of phytin bodies (arrows) detected by toluidine blue in embryonic cells (1A, 1B, 1C) and aleurone cells (2A, 2B, 2C) of three genotypes; IR68144 (A), CMU122 (B): high distribution and UBON2 (C): the low density of phytin bodies.
Scale bar = 20 μm.

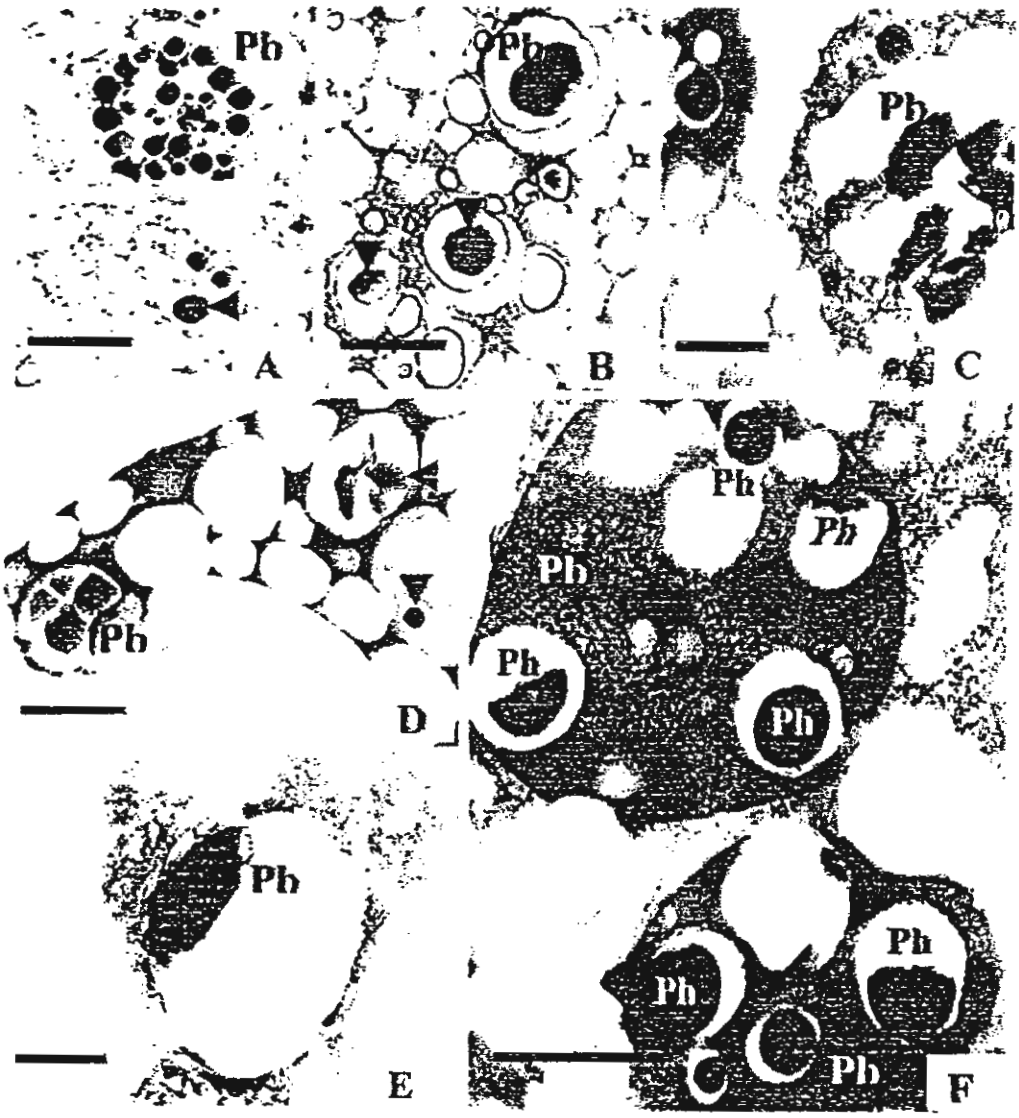
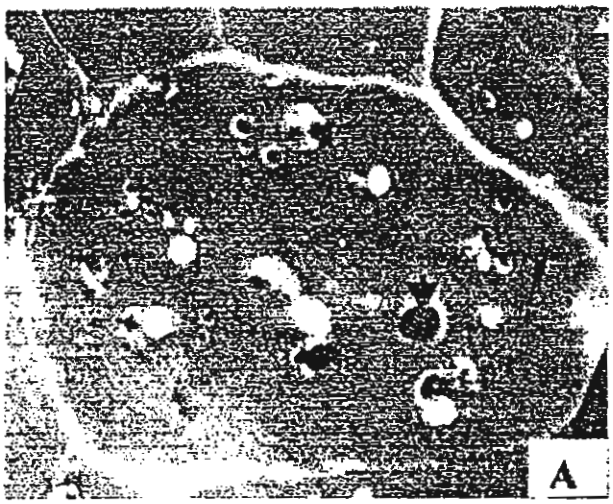


Fig 4.Electron micrographs of rice seed, showing the ultra structure of protein bodies (Pb; arrow) and phytin bodies (Ph). In embryonic cell, protein bodies without obvious inclusion (A – C) occur in larger or small cluster (A) and are spherical (A, B) or amorphous (C). Small protein bodies occur in the scutellum (D) and aleurone layer (E). Protein bodies with phytin-like inclusion in embryonic cell (E).
Genotype; A, C, F = IR68144, B = UBON2, D, E = CMU122
Scale bar; A= 5 μm, B, C, D = 2 μm, E= 200 nm, F = 1 μm

ภาคผนวก 2.2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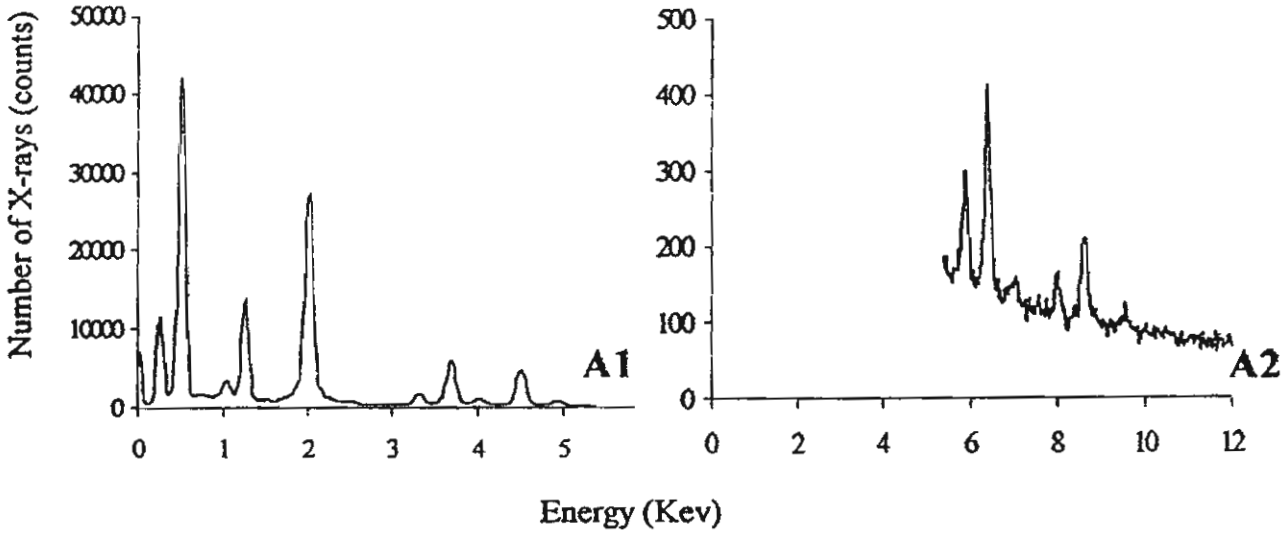
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30000
20000
10000
0
0
1
2
3
4
5
A1
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300
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100
0
0
2
4
6
8
10
12
A2
Energy (Kev)

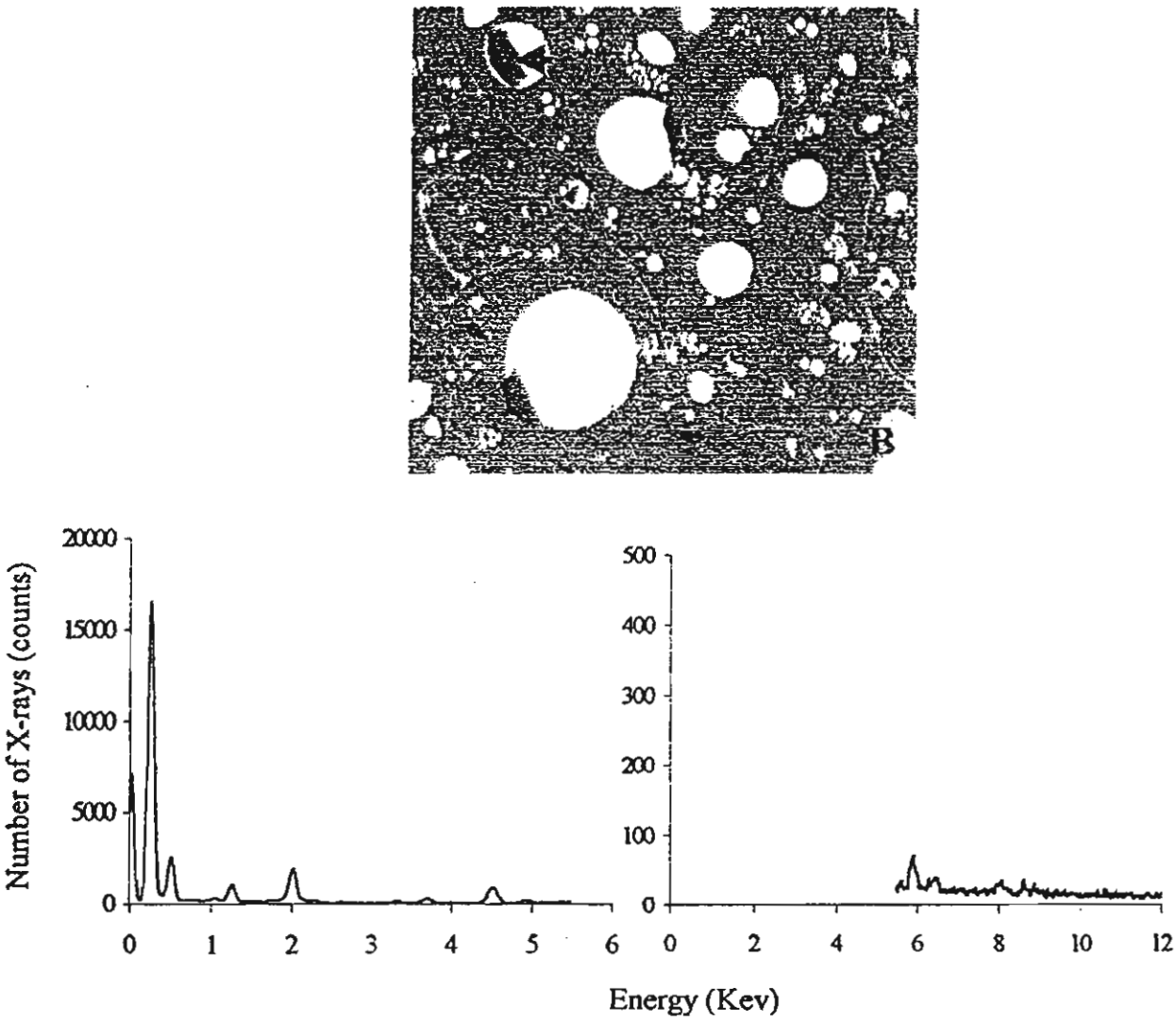

Fig. 5 Electron micrographs of protein bodies (A, B; arrow) in embryonic cell with phytin inclusion of IR68144 (A) and EDX-analysis spectrum from A (macronutrient; A1 and micronutrient; A2). Protein bodies without phytin inclusion of KDML105 (B) and EDX-analysis spectrum from B have no minerals showing in the spectrum (macronutrient; B1 a micronutrient; B2).

Boron efficient germplasm identified in *Vigna mungo* (L.) Hepper and *Vigna radiata* (L.) Wilczek

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Abstract

Boron (B) deficiency affect to seedling vigor of mungbean, black gram (*Vigna mungo* (L.) Hepper) and green gram (*Vigna radiata* (L.) Wilczek) and it affect to seed yield of black gram. Using B efficient genotypes can alleviate boron deficiency in mungbean. The aims of this study were to evaluate responses to B in black gram (*Vigna mungo* (L.) Hepper) and green gram (*Vigna radiata* (L.) Wilczek) genotypes, to determine the range of B efficiency in the two species and to identify germplasm for a breeding program to improve B efficiency. Sixteen genotypes of black gram (BG) and 26 genotypes of green gram (GG) were screened for B efficiency in sand culture without added B by scoring symptoms in 3 weeks old seedlings. In black gram, 81.3% of the genotypes were rated as inefficient, 12.5% as moderately inefficient and one genotype (M1) was identified as efficient. In green gram, no genotypes were rated as inefficient, 58% were rated as moderately inefficient and 42% as moderately efficient. Six mungbean genotypes: M1 (BG: efficient), Regur (BG: inefficient), CPI79563 (BG: inefficient), KPS1 (GG: moderately efficient), VC2755 (GG: moderately efficient), VC1163 (GG: moderately inefficient) were

selected from the first screening and evaluated in sand culture with 4 levels of applied B: 0, 0.5, 3 and 5 μM (designated as B0, B0.5, B3, and B5, respectively). In B0, seed yield of Regur, CPI79563 and VC1163 were depressed by B deficiency but M1, KPS1 and VC2755 were unaffected. Seed yield of M1, KPS1 and VC275, however, were limited when grown in high B (≥3 μM B for M1; 5 μM B for KPS1 and VC2755). The results suggest that B-efficient mungbean genotypes can be readily identified by screening growth of three weeks old seedlings in sand culture without added B. The M1 (black gram), KPS1 and VC2755 (green gram) genotypes were apparently B-efficient genotypes and could be used on low B soil and in breeding for increasing the B efficiency of mungbean.

Key words: Boron deficiency, Boron efficiency, Black gram, Genotypic variation, Green gram, Mung bean

Introduction

Soils with low available-B are widespread (Sillanpaa, 1982) and extensive areas of low B soil have been identified in northern Thailand (Rerkasem et al., 1989). Boron deficiency enhanced abnormal seedlings (Rerkasem et al.,1990) and depressed seed yield of black gram and green gram in northern Thailand (Predisripipat, 1988). Boron deficiency could be alleviated by applying B fertilizer but the availability of B fertilizer depends on many factors such as soil pH, soil texture (Goldberg, 1997), soil and air temperature (Forno et al., 1979). Alternatively, B-efficient genotypes could be used on these problem soils.

Some genetic variation in B efficiency was found in green gram and black gram genotypes, although most green gram lines were not as sensitive to B deficiency as black gram (Rerkasem, 1990). However, some genotypes of green gram were moderately sensitive to B deficiency and some genotypes of black gram were moderately tolerant to low soil B. A suitable and rapid screening method is needed to identify B-efficient germplasm for a breeding program for mungbean in northern Thailand. If the prevalence of abnormal seedling growth in low B is related to seed yield in low B, than seedlings could be used to screen for B efficiency. The objectives of this study were to evaluate responses to B deficiency in green gram and black gram germplasm and determine the range of B efficiency in the two species at both the seedling stage and at maturity. The aim was to evaluate the effectiveness of a potential screening method that could identify B-efficient genotypes early in seedling growth.

Materials and methods

Experiment 1: Sand culture screening of seedlings

Sixteen genotypes of black gram and twenty-six genotypes of green gram were multiplied at Agronomy Department Faculty of Agriculture Chiang Mai University then all genotypes were screened for B efficiency in sand culture. The screening was conducted in trays (0.45 m wide, 0.7 m long and 0.35 m deep) containing washed river quartz sand. Before sowing, the B concentration of each genotype was determined using the Azomethine – H procedure (Lohse, 1982) after

dry ashing . Twenty-four seeds of each genotype were sown separately in 0.4 m rows. There are were 12 rows per tray. Check varieties with known B efficiency, Regur (black gram: inefficient Predisripipat, 1988) and KPS1 (green gram: moderately efficient Rerkasem ,1990) were sown in every tray. Two replication trays were tested. Trays were supplied twice daily with complete nutrient solution without added B. The nutrient solution, adapted from Broughton and Dilworth (1971), consisted of (μM): KNO_3, 5000; $CaCl_2$ 1000; $MgSO_4$ 250; KH_2PO_4 500; $C_6H_5O_7Fe$ 10; K_2SO_4 250; $MnSO_4$ 2; $ZnSO_4$ 0.5; $CuSO_4$ 0.1; Na_2MoO_2 0.1. At three weeks after sowing, seedlings were classified into 8 classes (Table 1) according to symptoms of abnormal apical and leaf growth (after Rekasem at al., 1990).

Experiment 2: The response of six mungbean genotypes to boron

Three black gram (M1, Regur and CPI79563) and three green gram genotypes (KPS1, VC2755 and VC1163) were chosen from experiment 1 to represent a range of B efficiency. The seeds of these genotypes were multiplied again at Agronomy Department. Than seven seeds of each genotype were sown in freely drained, earthenware pots (0.3 m diameter and 0.3 m deep) containing washed river quartz sand. Seeds were inoculated with Rhizobium. Pots were supplied twice daily with the nutrient solution described in experiment 1, but without KNO_3, amended with four levels of B: 0 (B0), 0.5 (B0.5), 3 (B3) and 5 (B5) μM. Boron treatments and genotypes were arranged in a factorial combination with 3 replications for each of two harvest times (H1 and H2). At 15 days after sowing seedlings were thinned to 3 plants per pot. At the R3 stage (H1), total dry matter, root dry matter, nodule dry matter and B concentration in the youngest fully expanded leaf (YFEL) of each pot were determined. At maturity (H2), total dry matter, root dry matter, nodule dry matter,

seed yield, pods per plant, seed per pod, 1000 seed weight and seed B concentration were determined. Boron concentration in plant tissue was determined as in experiment 1. Data were analysed statistically by analysis of variance. Mean of treatment was compared by Least Significant Difference (LSD). Correlation coefficient between characteristics was computed from the mean of two replications in experiment 1 and three replication in experiment 2.

Results

Experiment 1

At three weeks after germination, seedling scores of check varieties, Regur and KPS1, were 1-2 and 5-6, respectively. Genotypes were classified into 4 categories, namely, inefficient (I: seedling score 1-2), moderately inefficient (MI: seedling score 3-4), moderately efficient (ME: seedling score 5-6) and efficient (E: seedling score 7-8). In the black gram, 81.3% of the genotypes (13 genotypes) were rated as inefficient, 12.5% (2 genotypes) as moderately inefficient and only one genotype, M1, as efficient. For the green gram, no genotype was rated as inefficient, 57.7% of genotypes (15 genotypes) were rated as moderately in efficient and 42.3% (11 genotypes) as moderately efficient (Figure 1).

In the black gram population, there was no relationship between seedling score and seed B concentration but a significant correlation ($p<0.01$) between seed B concentration and seedling score was found when the M1 genotype was omitted ($r = 0.666$: Figure 2 a). In the green gram population, seed B concentration, which ranged

from 8.06 to 18.02 mg B kg^{-1}, did not correlate with seedling score (r=0.353: Figure 2 b).

Experiment 2

Boron concentrations in the YFEL of all genotypes increased with B supply. At the R3 stage, the B concentration in the YFEL of M1 was higher than in all other genotypes in B0, but at high B levels (B3 and B5), the B concentration in the YFEL of M1 was similar to most of the other genotypes (Table 2). Shoot dry matter of all genotypes, at the R3 stage, was not affected by B supply. However, at maturity, shoot dry matter of M1 and the three green gram genotypes was not affected by B deficiency but that of M1 and KPS1 was depressed in high B (B3 and B5 for M1; B5 for KPS1). Shoot dry matter at maturity of Regur and CPI79563 genotypes was depressed at B0 (Table 3).

Grain yield and pod number of Regur, CPI79563 and VC1163 genotypes were depressed by B deficiency at low B supply (B0 for Regur and VC1163: B0 and B0.5 for CPI79563) (Table 4). Grain yield and pod number of M1, KPS1 and VC2755 genotypes were not affected by B deficiency but grain yield of these genotypes was depressed in high B (Table 4). Most of the variation in seed yield in B0 could be explained by changes in pod number per plant (Figure 3).

Seed weight of Regur, CPI79563 and VC1163 genotypes was increased by B deficiency but seed weight of other genotypes was not affected by B levels (Table 5). In all genotypes except CPI79563 seed B concentration was increased by increasing B levels (Table 5). Seed B concentration of CPI79563 was very high in B0 although minimal seed was actually produced.

Discussion

As in previous studies (Rerkasem, 1990), we have found evidence in genetic variation of B efficiency in black gram and green gram genotypes. However, whereas previous reports emphasized the relative B efficiency of green gram and the inefficiency of black gram, the most efficient genotype in the two experiments was the black gram, M1. This suggest highly B efficient germplasm exists in both species and that selection for B efficiency could readily identify superior germplasm for the low B soils in North and North East Thailand (Rattanarat et al., 1994; Rerkasem, 1994). In addition to M1, two green gram genotypes (KPS1, VC2755) were rated as B efficient. The efficient genotypes might be sensitive to B toxicity because grain yield of these genotypes was depressed in high B. Therefore, these B-efficient genotypes should not be planted in areas with high soil B. Boron deficiency particularly limited processes related to pod setting in inefficient genotypes. It was shown previously that B deficiency reduces grain yield by reduces number of pod per plant.

In this study the seedling score from experiment 1 and seed yield in low B solution in experiment 2 were closely correlated (Figure 4). The genotypes which produced normal seedlings in low B also produced high grain yield in low B. The efficient and moderately efficient genotypes from experiment 1 were not affected by B deficiency in experiment 2. Therefore, screening for B efficiency based on visual symptoms in seedlings, 3 weeks after germination in low B sand culture appears to be effective in identifying B-efficient mungbeans. However, the screening method has to consider in seed B concentration because low B concentration in black gram seed depresses vigor of seedling (Rerkasem et al., 1990).

References

Bell, R.W., Rerkasem, B., Keerati-Kasikorn, P., Phetchawee, S., Hiranburana, N., Ratanarat, S., Pongsakul, P., Loneragan, J.F., 1990. Mineral Nutrition of Food Legumes in Thailand with Particular Reference to Micronutrients. ACIAR Technical Reports No.16, 52p. ACIAR, Canberra.

Broughton, W.J., Dilworth, M.J., 1971. Control of leghaemoglobin synthesis in snake bean. Biochem J. 125, 1075-1080.

Forno, D.A., Asher, C.J., Edwards, D.G., 1979. Boron nutrition of cassava, and boron x temperature interaction. Field Crops Res. 2, 265-279.

Lohse, G., 1982. Microanalytical azomethine-H method of boron determination in plant tissues. Commun. Soil Sci. Plant Anal. 13,127-134.

Predisripipat, S., 1988. Response to boron applications in *Vigna.* M.Sc. (Agronomy) Thesis, Chiangmai University, Thailand.

Rattanarat, S., Prachawe, S., Bell R.W., Gilmour, R., Sumbatsiri, T., Plaskett, D., Loneragan, J.F., 1994. Effect of boron application on peanut growth on farmers' fields in Northern, Northeastern, Eastern and Western Thailand. Thai J. Soil Fert. 16, 187-203. *(In Thai with English abstract)*

Rerkasem, B., 1986. Boron deficiency in sunflower and green gram at Chiang Mai. J. Agric. Res. J3, 171-175. *(In Thai with English abstract)*

Rerkasem, B., Netsangtip, R., Bell, R.W., Loneragan, J.F., Hiranburana, N., 1988. Comparative species response to boron on a typic Tropaqualf in northern Thailand. Plant Soil. 106, 15-21

Rerkasem, B., Netsangtip, R., Lodkaew, S., Tongrod, P., Predisripipat, S., Meejui, S., Polwong, N. 1989. A survey of hollow heart in groundnuts as an indicator of boron deficiency in Northern Thailand. *In* Proceedings of the 7th Thailand Nation Groundnut Meeting. pp 377-383. Publisher and place

Rerkasem, B., 1990. Comparision of green gram(*Vigna radiata)* and black gram (*Vigna mungo)* in boron deficiency. *In* Proceedings of Mung bean Meeting 90. Eds. C.Thavarasook, P. Srinives, N Boonkerd, H. Imai, A. Pookpakdi, P. Laosuwan and U. Pupipat. pp 167-174. Bangkok Office of Tropical Agricultural Research Center, Japan City not country.

Rerkasem, B., Bell, R.W., Loneragan, J.F., 1990. Effect of seed and soil boron early ? in seedling growth of black gram and green gram (*Vigna mungo* and *V. radiata). In* Plant Nutrition – Physiology and Application. Ed. M.L. Van Beusichem. pp281-285. Kluwer Academic Publishers, Dordrecht.

Rerkasem, B., 1994. Boron deficiency in food legumes in Northern Thailand. Thai J. Soil Fert. 16, 130-154. *(In Thai with English abstract)*

Sillanpaa, M., 1982 Micronutrients and nutrient status of soils. FAO Soil Bull.48.

Table 1. Scoring criteria for mungbean seedlings at 3 weeks after germination

Symptom	Score
Apical necrosis and plant mortality within 3 weeks from germination	1
Apical necrosis and main shoot missing above the unifoliate leaves	2
Main shoot missing above the first trifoliate leaf. Trifoliate leaf fails to extend, misshapen	3
Main shoot missing above the first trifoliate leaf. Trifoliate leaf fully extended but misshapen	4
Rosette of leaves above the unifoliate leaves	5
Second trifoliate leaf misshapen	6
Third trifoliate leaf misshapen	7
Normal seedling: 2-3 sets of trifoliate leaves and active apical growth	8

Table 2. Effect of B supply on B concentration in the YFEL (mg Bkg^{-1} dry wt) at the R3 growth stage

Genotype	Solution B concentration (μM)			
	0	0.5	3	5
Black gram				
M1	16.7 a B	26.9 b B	42.9 c B	43.0 c BC
Regur	10.0 a A	21.1 b B	38.9 c B	42.0 c ABC
CPI79563	8.3 a A	14.5 b A	38.3 c B	45.0 d BC
Green gram				
KPS1	6.4 a A	16.2 b A	41.5 c B	47.8 d C
VC2755	8.7 a A	12.7 a A	39.6 b B	41.4 b AB
VC1163	8.8 a A	16.5 b A	28.8 c A	36.1 d A
F test	G**	B**	GxB**	
$LSD_{0.05}$	3.0	2.4	6.0	

Values within a row are significantly different ($P<0.05$) unless followed by the same lower case letter. Values within a column are significantly different ($P<0.05$) unless followed by the same capital letter

B=Boron level, G=genotype, BxG=interaction between B and G

Table 3. Effect of B supply on shoot dry matter (g pot^{-1}) in six mungbean genotypes at two growth stages

Genotype	Solution B concentration (μM)				Mean
	0	0.5	3	5	
			R3 stage		
Black gram					
M1	5.8	6.2	4.2	3.8	5.0 A
Regur	5.4	11.1	10.4	17.1	11.0 B
CPI79563	10.8	16.8	20.4	14.3	15.6 C
Green gram					
KPS1	12.9	10.0	13.7	9.4	11.5 B
VC2755	11.8	13.5	15.7	13.0	13.5 BC
VC1163	5.2	9.0	13.0	13.0	10.1 B
F test	B^{NS}	G**	GxB^{NS}		
$LSD_{0.05}$	-	4	-		
			Seed maturity		
Black gram					
M1	60.1 b	29.2 a	12.5 a	16.4 a	29.5
Regur	30.9 a	41.2 ab	38.0 ab	61.5 b	42.9
CPI79563	47.4 a	89.2 b	84.6 b	100.4 b	80.4
Green gram					
KPS1	46.2 b	40.2 ab	39.9 ab	18.1 a	36.1
VC2755	42.6 a	45.3 a	54.0 a	33.2 a	43.8
VC1163	16.9 a	40.1 a	21.2 a	28.1 a	26.6
F test	B^{NS}	G**	BxG**		
$LSD_{0.05}$	-	13.0	25.9		

Values within a row are significantly different ($P<0.05$) unless followed by the same lower case letter. Values within a column are significantly different ($P<0.05$) unless followed by the same capital letter

B=Boron level, G=genotype, BxG=interaction between B and G

Table 4. Effect of B supply on relative yield (yield as % of yield in sufficient B) and relative pod number (pod number as % of pod number in sufficient B) in six mungbean genotypes

Genotype	Solution B concentration (μM)			
	0	0.5	3	5
		Relative yield		
Black gram				
M1	90.2 ab C	100.0 b B	44.6 a A	52.3 a ABC
Regur	9.0 a A	58.7 b AB	100.0 b B	91.7 b BC
CPI79563	0.3 a A	38.5 a A	86.9 b AB	100.0 b C
Green gram				
KPS1	89.2 b C	93.6 b B	100.0 b B	33.3 a A
VC2755	61.6 ab BC	81.3 ab AB	100.0 b B	48.5 a AB
VC1163	32.2 a AB	100.0 b B	75.6 ab AB	92.0 b BC
F test	B**	G^{NS}	BxG**	
LSD0.05	21.9	-	46.6	
		Relative pod number		
Black gram				
M1	100.0 b D	95.2 b B	48.3 a A	44.4 a A
Regur	10.0 a AB	63.7 b AB	88.6 b B	100.0 b B
CPI79563	0.3 a A	51.9 b A	78.5 bc AB	100.0 c B
Green gram				
KPS1	100.0 b D	72.1 b AB	74.3 b AB	30.2 a A
VC2755	68.6 ab CD	73.3 ab AB	100.0 b B	49.5 a A
VC1163	42.5 a BC	100.0 b B	69.8 ab AB	87.7 b B
F test	B*	G^{NS}	BxG**	
$LSD_{0.05}$	15.1	-	37.0	

Values within a row are significantly different (P<0.05) unless followed by the same lower case letter. Values within a column are significantly different (P<0.05) unless followed by the same capital letter.

B=Boron level, G=genotype, BxG=interaction between B and G

Table 5. Effect of B supply on 1000 seed weight and seed B concentration in six mungbean genotypes

Genotype	Solution B concentration (μM)			
	0	0.5	3	5
	1000 seed weight (g)			
Black gram				
M1	34.48 a	30.65 a	33.15 a	35.94 a
Regur	55.89 c	40.61 b	45.70 b	36.62 a
CPI79563	37.79 c	32.76 bc	27.52 ab	22.25 a
Green gram				
KPS1	46.50 a	49.49 a	49.12 a	53.71 a
VC2755	54.24 a	52.52 a	51.65 a	56.90 a
VC1163	62.56 b	54.25 a	59.24 ab	59.23 ab
F test	B*	G**	BxG**	
$LSD_{0.05}$	3.34	4.09	8.16	
	Seed B concentration (mg B kg^{-1})			
Black gram				
M1	6.2 a AB	13.8 b C	18.0 c A	17.4 c A
Regur	4.9 a AB	9.4 b B	17.6 c A	17.1 c A
CPI79563	24.3 d C	4.6 a A	18.1 b A	18.5 b AB
Green gram				
KPS1	4.3 a A	11.0 b B	17.7 c A	20.7 d BC
VC2755	5.3 a AB	11.0 b B	22.0 c B	22.4 c C
VC1163	6.9 a B	11.1 b B	17.2 c A	18.2 c A
F test	B**	G**	GxB**	
$LSD_{0.05}$	0.96	1.17	2.34	

Values within a row are significantly different ($P<0.05$) unless followed by the same lower case letter. Values within a column are significantly different ($P<0.05$) unless followed by the same capital letter.

B=Boron level, G=genotype, BxG=interaction between B and G

Figure 1. Seedling scores in green gram and black gram genotypes after three weeks growth in sand culture without added B.

Seedling score 1-2 = inefficient

Seedling score 3-4 = moderately inefficient

Seedling score 5-6 = moderately efficient

Seedling score 7-8 = efficient

(For detailed criteria for scores see Table 1)

Figure 2. The relationship between seed B concentration and seedling score in black gram (a) and green gram genotypes (b).

Figure 3. The relationship between relative pod number (pod number in B0 as % of pod number insufficient B) and relative yield (seed yield in B0 as % of seed yield in sufficient B).

Figure 4. The relationship between seedling score form in experiment 1 and relative yield (seed yield in B0 as % of seed yield in sufficient B) from experiment 2

(For detailed criteria for seedling scores see Table 1)

Figure 1

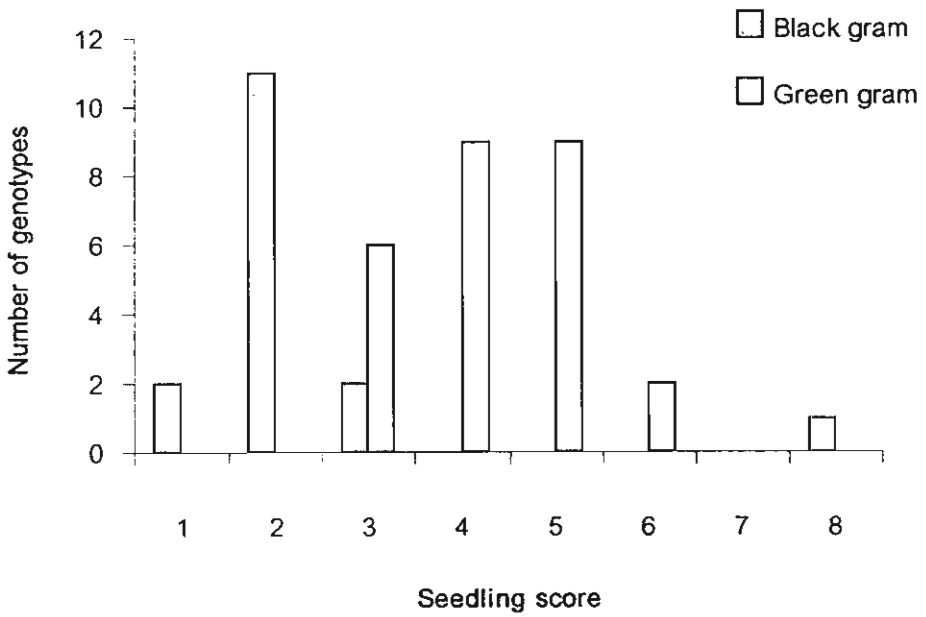
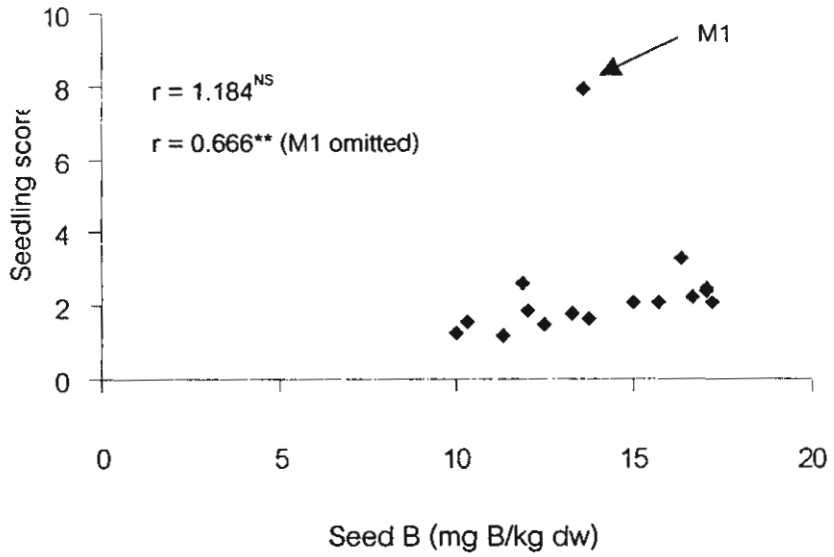

Black gram
Green gram
Number of genotypes
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
Seedling score

Figure 2

(a)


10
8
6
4
2
0
Seedling score
r = 1.184NS
r = 0.666** (M1 omitted)
M1
0
5
10
15
20
Seed B (mg B/kg dw)

(b)

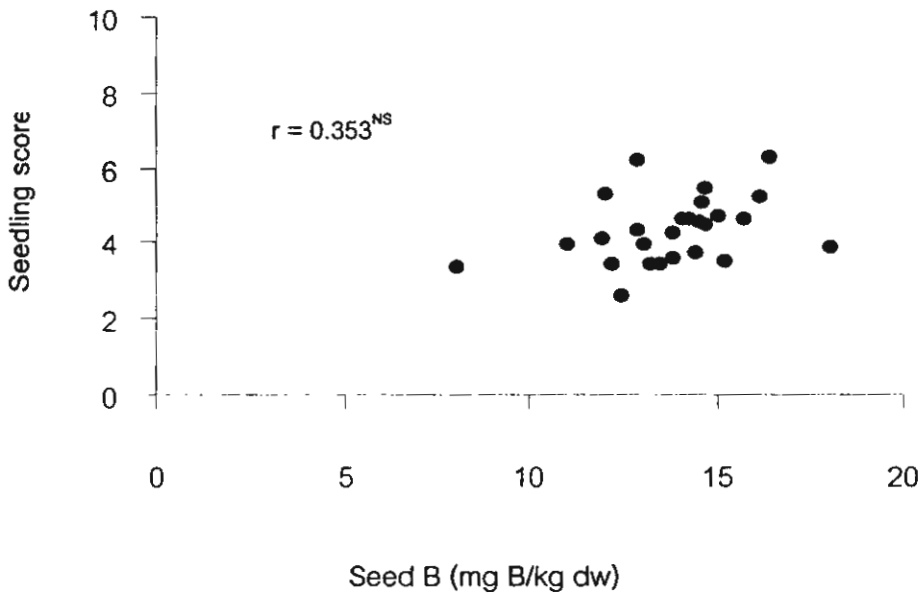

10
8
6
4
2
0
Seedling score
r = 0.353NS
0
5
10
15
20
Seed B (mg B/kg dw)

Figure 3

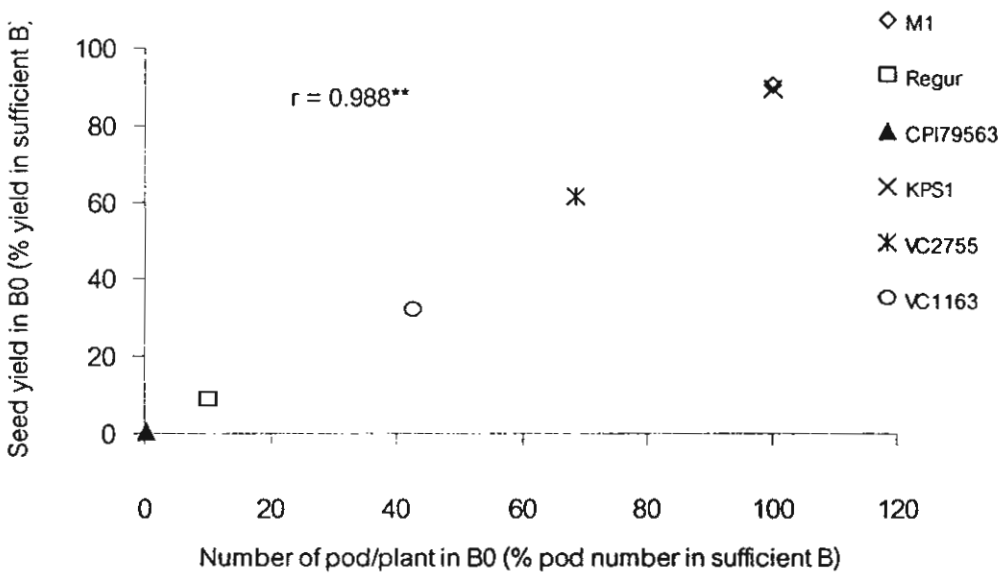
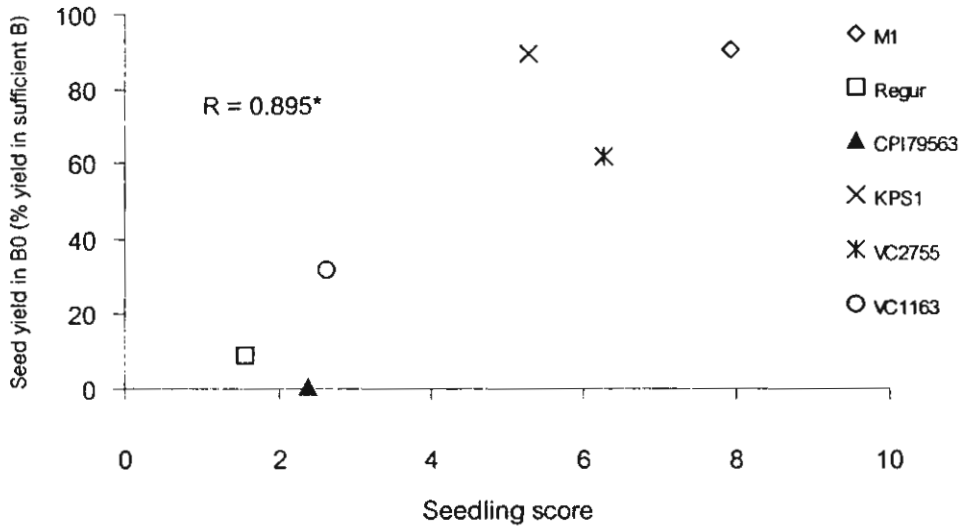

Seed yield in B0 (% yield in sufficient B)
100
80
60
40
20
0
r = 0.988**
0
20
40
60
80
100
120
Number of pod/plant in B0 (% pod number in sufficient B)
M1
Regur
CPI79563
KPS1
VC2755
VC1163

Figure 4


Seed yield in B0 (% yield in sufficient B)
100
80
60
40
20
0
R = 0.895*
0
2
4
6
8
10
Seedling score
M1
Regur
CPI79563
KPS1
VC2755
VC1163

Do Boron Deficiency and Toxicity Responses Correlate in Wheat Genotypes?

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Abstract

There is a wide range of genotypic variation in responses to boron (B) deficiency and toxicity in wheat. Yield reductions in cultivated area occur because of either B deficiency or toxicity. The relationship between B deficiency and toxicity responses in wheat is not understood. The objectives of this study were to measure the responses between B deficiency and toxicity in wheat genotypes and examine relationship between them. Two experiments, with nine genotypes known to differ in response to B deficiency (Fang 60, CMU 88-9, SW 41, Bonza) and toxicity (Turkey 1473, BT-Schomburgk, Schomburgk, Tatiara, Kenya Farmer), were undertaken. The response to B deficiency was evaluated in sand culture with two levels of applied B (0 and 10 μM B) in the nutrient solution. After maturity, grain set index (GSI; percentage of the 20 basal florets from 10 spikelets with grain), grain weight spike^{-1}, tiller plant^{-1} and straw yield were measured. B toxicity response was assessed in solution culture with three levels of B added (0, 100, 150 mg B L^{-1}). After 12 days, root and shoot length were measured. Tissue B analysis was conducted to compare B utilization in Fang 60, Bonza and Turkey 1473 by growing in sand culture with three B added (0, 10, 50 mg B L^{-1}) for 21 days.

In low B sand, GSI and grain weight spike^{-1} of all genotypes were depressed, except in Fang 60 (B-efficient), but tiller plant^{-1} and straw yield were not affected. At toxic levels B, root and shoot length reduction occurred in all genotypes. The tolerant genotypes, Turkey 1473 and Bonza, maintained the longest root length. However shoot length did not differ between genotypes. When grown without added B, deficiency tolerant Fang 60 did not differ significantly in B concentration in the whole plant and specific parts including YEB and YEB+1 from deficiency sensitive Bonza

and Turkey 1473. When grown at 50 mg B L^{-1}, efficient genotype, Fang 60 had higher B concentration in all parts than inefficient genotype, Bonza and Turkey 1473. Tissue B in the three genotypes was closely related to their toxicity response but not their deficiency response. These results suggested that sensitivity to B toxicity in Fang 60 be due to its tendency ability to accumulate more B in its plant part resulting in more severity symptom and root length reduction. In contrast, Bonza and Turkey1473 tolerant to B toxicity because they accumulated less B in their tissue.

We are postulating that there may be four types of the relationship between B deficiency and toxicity responses in wheat genotypes, on the basis of GSI and relative root length comparisons. The genotypes studied fell into three groups: (1) efficient (E) genotypes sensitive to high B i.e. Fang 60 (high GSI with short root length), (2) tolerant genotypes (T) sensitive to low B such as Turkey 1473 and Bonza (long root length with low GSI), and (3) inefficient genotypes sensitive to high B and low B such as BT-Schomburgk, Shomburgk, Tatiara, Kenya Farmer, SW 41 and CMU 88-9 (low GSI and short root length). The fourth possible type may be those genotypes that are tolerant to both B deficiency and toxicity, although none has yet been found so far.

Keywords: Boron deficiency, Boron toxicity, Wheat

Introduction

Boron (B) is an essential micronutrient for crop growth and there is a narrow range of deficiency and toxicity for growth (Reisenauer et al., 1973) and the sufficient range is narrow (Mahalakshmi et al., 1995).

Yield reductions in cultivated area occur because of either boron (B) deficiency or toxicity. Low B in soils contributes to male sterility in wheat and can depress grain yield. This problem has been reported in many countries in Asia including Nepal (Subedi, 1992), India (Tandon and Naqvi, 1992), Bangaladesh (Ahmed and Hossain, 1997), China (Yang, 1992) and Thailand (Rerkasem et al., 1989). The application of B fertilizer can ameliorate yield loss on low B soils (Rerkasem and Jamjod, 1997b), but the use of B-efficient genotypes is recommended (Jamjod et al., 2000). High B in soils causes necrosis and chlorosis of vegetative organs resulting in reduced grain

yield in wheat and also affects the growth at all stages of development as found in South Australia (Cartwright et al., 1984) and Turkey (Kalayci et al., 1998).

There is a wide range of genotypic variation in responses to B deficiency (Rerkasem and Jamjod, 1997b) and toxicity (Paull et al. 1991) in wheat. Boron efficiency is an ability of plant to grow well in soil, which is deficient for standard genotype. Boron efficiency mechanisms might be due to the ability to acquire B from soil, the way that B is distributed, and its utilisation within plant, while B tolerance mechanism is the ability to maintain lower B by B restriction in plant. It is as yet unclear how mechanisms governing tolerance to B deficiency and toxicity are related in wheat. This study set out to relate responses to B deficiency and toxicity in wheat genotypes covering a wide range of tolerance to measure the responses between B deficiency and toxicity in wheat genotypes and examine relationship between them.

Materials and methods

Genetic material

Nine standard wheat genotypes were evaluated in sand and solution culture.

Group 1 Genotypes known the range of response to B deficiency (Rerkasem and Jamjod, 1997b).

(1) Fang 60 (E; Efficient)

(2) CMU 88-9 (ME; Moderately efficient)

(3) SW 41 (MI; Moderately inefficient)

(4) Bonza (I; Inefficient)

Group 2 Genotypes known the range of response to B toxicity (Chantachume et al., 1995).

(1) Turkey 1473 (T; Tolerant)

(2) BT-Schomburgk (MT; Moderately tolerant)

(3) Schomburgk and Tatiara (MS; Moderately sensitive)

(4) Kenya Farmer (VS; Very sensitive)

Experiment 1: Sand culture experiment.

Nine wheat genotypes were grown in 0.3 m diameter earthenware pots containing washed river quartz sand with ten seeds of each genotype. The pots were watered twice daily with complete nutrient solution at two levels of B added (0 and 10 μM B),

referred to B0 and B10, respectively. The complete nutrient solution, adapted from Broughton and Dilworth (1971), consisted of: $CoSO_4$ (0.1 µM) Na_2MoO_2 (0.1 µM) $CuSO_4$ (0.2 µM) $ZnSO_4$ (0.5 µM) $MnSO_4$ (2 µM) FeEDTA (10 µM) K_2SO_4 (250 µM) $MgSO_4$ (250 µM) KH_2PO_4 (500 µM) $CaCl_2$ (1,000 µM) and KNO_3 (5,000 µM). Genotypes and B treatments were arranged randomly in blocks with four replications. After maturity, tiller plant^{-1}, straw yield, Grain Set Index (GSI) (measured as percentage of the 20 basal florets from 10 spikelets with grain) (Rerkasem and Loneragan, 1994) and grain weight spike^{-1} were measured.

Experiment 2: Solution culture experiment.

Nine wheat genotypes were assessed by the aerated solution culture method adapted from Campbell et al. (1998) with three levels of B concentrations (0, 100 and 150 mg BL^{-1}) assigned as B0, B100 and B150, respectively, prepared from boric acid (H_3BO_3), $ZnSO_4 \cdot 7H_2O$ (2.5 µM) (Webb and Loneragan, 1990) and $Ca(NO_3)_2 \cdot 4H_2O$ (500 µM) (Haynes and Robbins, 1948). The B treatments and genotypes were arranged factorially with two replications. Plastic mesh stuck to the underside of a foam sheet which had a row of holes punched was used in this experimental apparatus. Foam sheet was placed on plastic box that had the same size of length and width (0.37x0.23 m) and used the hose of an air pump to pass the air through into the solution (it was necessary to maintain growth of seedlings).

Seeds of the nine wheat genotypes were germinated in Petridishes for two days at 18 °C. The germinants were transferred to small plastic mesh containers inserted into foam sheet floating on the surface of a solution in plastic boxes on a laboratory bench at room temperature. Root and shoot length were measured after 12 days and the severity of symptoms of B toxicity was by evaluating the percentage of necrosis (necrosis (%) = (necrosis length/ length of that leaf) x 100). Root length was measured as the longest seminal root at B100 and B150, relative to B0 (relative root length; RRL). Likewise, shoot length was measured as the coleoptile length at B100 and B150, relative to B0 (relative shoot length; RSL).

Experiment 3: Tissue boron analysis.

Fang 60, Bonza and Turkey 1473 were grown in sand culture to compare B utilization with three levels of B (0, 10, 50 mg B L^{-1}) referred to B0, B10, B50 respectively added to nutrient solution adapted from Broughton and Dilworth (1971).

Genotypes and B treatments were arranged factorially with three replications. After 21 days, plants were collected to analyze tissue B concentration by azomethine-H method (Lohse, 1982) in YEB, YEB+1 and whole plant. Root and shoot length, root and shoot dry weight, symptom of B toxicity in YEB and YEB+1 were measured.

Statistical analysis

Data were analyzed by analysis of variance (AOV). Significant differences between means were calculated by the LSD test at 95% probabilities. Few data were transformed by square root arcsine transformation and square root transformation.

Results

Response to boron deficiency

There were significant differences between genotypes and boron treatments for GSI (Figure 1) and grain weight spike^{-1}, but not for tiller plant^{-1} and straw yield (Table 1), in experiment 1. Boron deficiency in wheat (B0) depressed GSI of all genotype compared with B10 except in Fang 60 (92.2 % GSI at B0). At B10, GSI of Fang 60 was not different from B0 (94.9 % GSI). Further, the GSI of Turkey 1473 (T), BT-Schomburgk (MT) and Schomburgk (MS) was more severely decreased at B0 than in B-efficient genotypes (GSI<40%). Genotypes that were more susceptible to B toxicity were also more sensitive to B deficiency (Figure 1). Tatiara (MS) and Kenya Farmer (VS) had very low GSI<20% at B0) but Tatiara set grain less than Kenya Farmer.

Grain weight spike^{-1} was affected by B0 in the same way as GSI in all genotypes (Table 1). Fang 60 had more grain weight spike^{-1} than sensitive ones at both B levels. For tillering and straw yield, all genotypes were not affected in these characters by B levels. Tiller plant^{-1} and straw yield were genotype dependent.

Response to boron toxicity

In experiment 2, higher B concentration affected root and shoot length of wheat genotypes differently (Figure 2). High B affected root length more than shoot length. The clearest differences in response to B toxicity occurred at B100 and B150 (Table 2). At B100, Bonza showed the least root length reduction (RRL = 70.7%) followed by Turkey 1473 (RRL = 68.9%) and BT-Schomburgk (RRL = 51.2%), whereas Kenya Farmer, Tatiara and Fang 60 showed the most reduction in root length (RRL

range 39.4-50.3%). At B150, Turkey 1473 and Bonza still had less root length reduction than other genotypes, whereas Fang 60 and Tatiara had the most root length reduction.

For shoot length, high B supply reduced shoot length (range 75.2-94.0%) but there was no significant interaction between genotypes and B treatments when assessed by RSL (% of B0) (Table 2). Leaf necrosis (%) at B100 and B150, was severe in sensitive genotypes such as Fang 60, Kenya Farmer and SW 41 (necrosis (%) = 47.6, 46.4, 45.7, respectively) (Table 3). Bonza and Turkey 1473 showed less severe symptoms (26.6 and 30.7%, respectively).

Tissue boron analysis

Boron levels did not affect on root and shoot length, root and shoot dry weight of wheat genotypes at 21 days after sowing but affected on necrosis (%) significantly between genotypes (Figure 3). Bonza had the least toxicity symptoms in both YEB and YEB+1 whereas Fang 60 had the most.

When grown without added B, Fang 60 did not differ significantly in B concentration in the whole plant and specific parts including YEB and YEB+1 from Bonza and Turkey 1473 (Table 4). At 50 mg B L^{-1}, Fang 60 had higher B concentration in all parts of its plant than Bonza and Turkey 1473. Whole plant B concentration of Fang 60 was 806.55 mg B kg^{-1} and 678.19, 655.97 mg B kg^{-1} in Bonza and Turkey 1473 respectively. Furthermore, B concentration in YEB and YEB+1 of Fang 60 was higher than Bonza and Turkey 1473 that was 180.08, 131.82, 142.28 mg B kg^{-1} respectively in YEB and 273.66, 226.85, 226.17 mg B kg^{-1} respectively in YEB+1.

Discussion

From these studies, there had the relationship between B deficiency and toxicity responses categorized into four groups by assessing GSI (for B deficiency response) and relative root length (for B toxicity response) (Figure 4):

(1) Genotypes are efficient to B deficiency but sensitive to B toxicity i.e. Fang 60.

(2) Genotypes are inefficient to B deficiency and sensitive to B toxicity i.e. CMU 88-9, SW 41, BT-Schomburgk, Schomburgk, Tatiara, Kenya Farmer.

(3) Genotypes are inefficient to B deficiency but tolerant to B toxicity i.e. Bonza and Turkey 1473.

(4) Genotypes are efficient to B deficiency and tolerant to B toxicity, have not been found yet. However, such genotypes have so far not been identified.

Relationship between B deficiency and toxicity in wheat in this study was resemblance with the response in barley. In case of efficient genotype, it has one or more efficient mechanisms such as the ability to acquire B, the way that B is distributed, and its utilization within the plant (Rerkasem and Jamjod, 1997a). Besides, phloem mobility is one of efficient mechanism in efficient genotype whereas *genotype that is unable to translocate B in phloem is found in genotype that tolerate to* B toxicity (Brown and Shelp, 1997). So, genotype that had ability to translocate B in phloem will susceptible to B toxicity (Brown and Hu, 1996). Nable (1992) reported that there has a general trend for species more tolerance of high B concentrations to be more prone to B deficiency such as tobacco, *Eucalyptus* and barley.

In the first experiment, the significant interaction between genotype and B treatment for GSI and grain weight spike^{-1} confirms that genetic variation for B efficiency occurs in wheat and B deficiency impairs reproductive growth. So that GSI is an appropriate index to compare the effect of B on grain set (Anantawiroon et al., 1997) by avoiding effects from spike type, spike size and it was used for B efficiency screening by growing wheat in B deficient condition without comparing with B sufficient condition. Fang 60 still efficient to B deficiency because of high GSI but not in CMU 88-9. SW 41 and Bonza that were classed in the same range as the study from Rerkasem and Jamjod (1997a). Besides this, Turkey 1473, BT-Schombirgk, Schomburgk, Tatiara and Kenya Farmer were sensitive to B deficiency. In addition to this, B deficiency has no effect on tiller plant^{-1} and straw yield since B deficiency affects on reproductive growth more than on vegetative growth in wheat (Rerkasem and Loneragan, 1994).

From the results in experiment two, higher B concentrations affected both root and shoot length of all genotypes, but B toxicity reduced root length more than shoot length. Bonza had long root length as same as Turkey 1473 when grown at higher B levels which ought to classified as B toxicity tolerance as Chantachume et al. (1995) have mentioned. By contrast, Fang 60, CMU 88-9, SW 41, BT-Schomburgk, Schomburgk, Tatiara and Kenya Farmer had short root length which could be suggested that they were not tolerant to B toxicity. In addition to this, Holloway and Alston (1992) found that high B concentration will restrict root growth of wheat and decrease yield (Cartwright et al., 1984) and tolerant genotype had longer root length

than sensitive genotype. Furthermore, susceptible genotypes had more symptoms of necrosis than tolerant genotypes when grown at higher B concentrations. Therefore, root length is an appropriate index for selecting or screening tolerance genotype because of quick and easy method (Campbell et al., 1998; Chantachume et al., 1995).

For tissue B analysis, deficiency tolerant Fang 60 did not differ in B concentration in the whole plant, YEB and YEB+1 from deficiency sensitive Bonza and Turkey 1473 when grown at 0 mg B L^{-1}. Therefore genotypic variation for B efficiency did not measure by tissue B analysis. At 50 mg B L^{-1}, Fang 60 had higher B concentration in any parts of its plant than Bonza and Turkey 1473. Tissue B in the three genotypes was closely related to their toxicity response (necrosis and chlorosis) but not their deficiency response. It also demonstrated that Fang 60 was not tolerant to B toxicity due to maintain more B in any parts at high B with resulted in more severity of B toxicity symptom. In contrast, Bonza and Turkey 1473 remained tolerant to B toxicity due to ability to maintain less B in any parts at high B. Nable (1988) stated that barley and wheat cultivars examined displayed a large range of resistance to B toxicity that was governed in both species by the ability of cultivars to restrict B accumulation in the plant. This mechanism is called exclusion. In addition, Nable et al. (1988) found that tolerant barley genotype was susceptible to B deficiency. Tolerant genotype accumulated less B concentration than sensitive genotype in both root and shoot. He concluded that resistance to B toxicity was governed by the ability of cultivars to restrict B accumulation in the plant. Fang 60 exemplifies the highest tolerance to B deficiency and Bonza and Turkey 1473 the highest tolerance to B toxicity. Tissue B data indicated that their may be some association between responses to the two extremes of B supply.

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References

Ahmed S and Hossain M B 1997 The problem of boron deficiency in crop production in Bangladesh. *In* Boron in Soil and Plants. Eds R W Bell and B Rerkasem. pp 1. Kluwer academic publishers, The Netherlands.

Anantawiroon P, Subedi K D and Rerkasem B 1997 Screening wheat for boron efficiency. *In* Boron in Soils and Plants. Eds R W Bell and B Rerkasem. pp 101-104. Kluwer Academic Publishers, The Netherlands.

Broughton W J and Dilworth M J 1971 Control of leghaemoglobin synthesis in snake beans. Biochem. J. 125, 1075-1080.

Brown P H and Hu H 1996 Phloem mobility of boron in species dependent: evidence for phloem mobility in sorbital-rich species. Ann. Bot. 77, 497-505.

Brown P H and Shelp B J 1997 Boron mobility in plants. *In* Boron in Soils and Plants. Eds B Dell P H Brown and R W Bell. pp 85-101. Kluwer Academic Publishers, The Netherlands.

Campbell T A, Rathjen A J, Paull J G and Islam A K M R 1998 Method for screening bread wheat for tolerance to boron. Euphytica 100, 131-135.

Cartwright B, Zarcinas B A and Mayfield A H 1984 Toxic concentration of boron in red brown earth at Gladstone, South Australia. Aust. J. Soil. Res. 22, 261-272.

Chantachume Y, Smith D, Hollamby G J, Paull J G and Rathjen A J 1995 Screening for boron toxicity in wheat (*T. aestivum*) by solution culture in filter paper. Plant and Soil 177, 249-254.

Haynes J L and Robbins W R 1948 Calcium and boron as essential factors in the root environment. J. Am. Soc. Agron. 40, 795-803.

Holloway R E and Alston A M 1992 The effect of salt and boron on growth of wheat. Aust. J. Agric. Res. 43, 987-1001.

Jamjod S, Boonsit P and Rerkasem B 2000 The genetic source for boron tolerance in barley. J. Agric. (CMU) 16, 53-64.

Kalayci M, Alkan A, Çakmak I, Bayramoğlu O, Yilmaz A, Aydin M, Ozbek V, Ekiz H and Ozberisoy F 1998 Studies on differential response of wheat cultivars to boron toxicity. Euphytica 100, 123-129.

Lohse G 1982 Microanalytical azomethine-H method for boron determination in plant tissue. Commun. Soil Sci. Plant Anal. 13, 127-134.

Mahalaksmi V, Yau S K, Ryan J and Peacock J M 1995 Boron toxicity in barley (*Hordeum vulgare* L.) seedlings in relation to soil temperature. Plant and Soil 177, 151-156.

Nable R O 1988 Resistance to boron toxicity amongst several barley and wheat cultivars: a preliminary examination of the resistance mechanism. Plant and Soil 112, 45-57.

Nable R O 1992 Mechanism of tolerance to boron toxicity in plants. *In* Boron deficiency in wheat. Eds C E Mann and B Rerkasem. pp.98-109. CIMMYT Wheat Special Report No.11. CIMMYT, Mexico.

Nable R O, Cartwright B and Lance R C M 1988. Genotypic differences in boron accumulation in barley: Relative susceptibilities to boron deficiency and toxicity. *In* Genetic aspects of plant mineral nutrition. Eds N El Bassam. pp 243-251. Kluwer academic publishers, The Netherlands.

Paull J G , Rathjen A J and Cartwright B 1991 Major gene control of tolerance of wheat (*Triticum aestivum* L.) to high concentrations of soil boron. Euphytica 55, 217-228.

Reisenauer H M, Walsh L M and Hoeft R G 1973 Testing soils for sulphur, boron, molybdenum and chlorine. *In* Soil Testing and Plant Analysis. Eds L M Walsh and J D Beaton. pp 173-200. Soil Sci. Soc. of America Inc. Madison/ Wisconsin.

Rerkasem B and Jamjod S 1997b Genotypic variation in plant response to low boron and implications for plant breeding. Plant and Soil 193, 169-180.

Rerkasem B, and Jamjod S 1997a Boron deficiency induced male sterility in wheat *(Triticum aestivum* L.) and implications for plant breeding. Euphytica 96, 257-262.

Rerkasem B, and Loneragan J F 1994 Boron deficiency in two wheat genotypes in a warm, subtropical region. Agron. J. 86, 887-890.

Rerkasem B, Saunders D A and Dell B 1989 Grain set failure and boron deficiency in wheat in Thailand. J. Agric. (CMU) 5, 1-10.

Subedi K 1992 Wheat sterility in Napal: A Review. *In* Boron deficiency in wheat. Eds C E Mann and B Rerkasem. pp 57. CIMMYT Wheat Special Report No.11. CIMMYT, Mexico.

Tandon J P and Naqvi S M A 1992 Wheat varietal screening for boron deficiency in India. *In* Boron deficiency in wheat. Eds C E Mann and B Rerkasem. pp 76-78. CIMMYT Wheat Special Report No.11. CIMMYT, Mexico.

Webb M J and Loneragan J F 1990 Zinc translocation to wheat roots and its implications for a phosphorus/zinc interaction in wheat plants. J. Plant. Nutr. 13, 1499-1512.

Yang Y 1992 Wheat boron deficiency in Yunnan, China. *In* Boron deficiency in wheat. Eds C E Mann and B Rerkasem. pp 72. CIMMYT Wheat Special Report No.11. CIMMYT, Mexico.

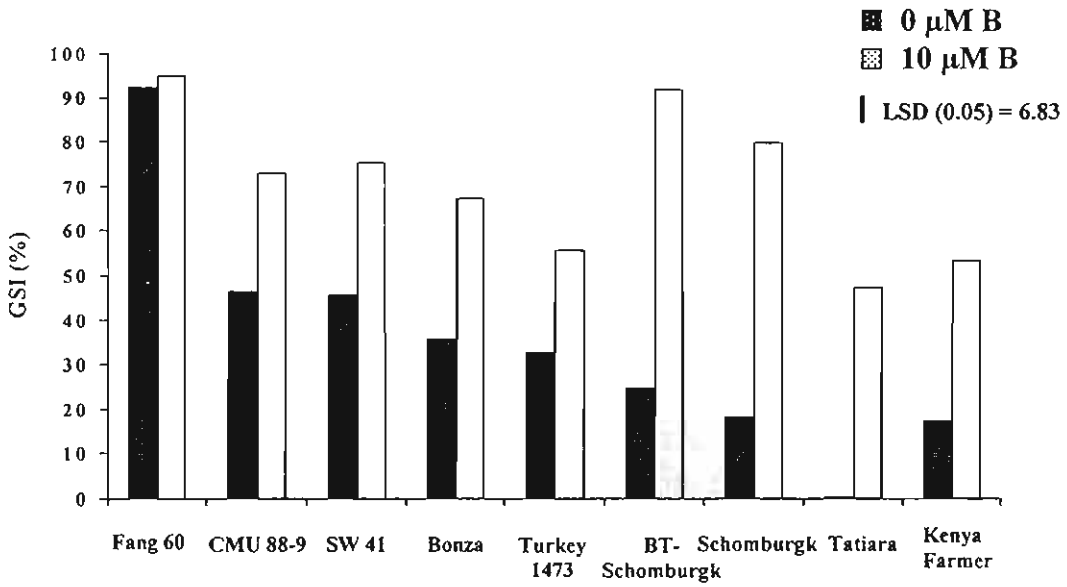

Figure 1 Effect of B supply on grain set index (GSI; %) of nine wheat genotypes grown in sand culture. (Experiment 1)

Table 1 Effects of B supply on grain weight spike^{-1} (g), tiller plant^{-1} and straw yield (g plant^{-1}) of nine wheat genotypes grown in sand culture. (Experiment 1)

Genotype	Grain weight spike^{-1}			Tiller plant^{-1}			Straw yield		
	B supply		*Mean*	B supply		*Mean*	B supply		*Mean*
	B0	B10		B0	B10		B0	B10	
Fang 60	0.87 aA	1.01 aA	*0.94*	7.3	7.3	*7.3 D*	9.38	9.36	*9.37 D*
CMU 88-9	0.52 bBC	0.97 aA	*0.74*	6.7	6.3	*6.5 DE*	10.38	10.26	*10.32 CD*
SW 41	0.57 bB	0.73 aB	*0.65*	6.9	6.7	*6.8 DE*	10.86	8.88	*9.87 D*
Bonza	0.37 bC	0.59 aBC	*0.48*	8.7	9.0	*8.9 BC*	15.27	14.11	*14.69 A*
Turkey 1473	0.39 aC	0.49 aCD	*0.44*	5.9	6.4	*6.1 E*	11.80	14.03	*12.92 ABC*
BT-Schomburgk	0.19 bD	0.62 aBC	*0.41*	10.9	10.8	*10.8 A*	11.07	9.81	*10.44 CD*
Schomburgk	0.14 bDE	0.48 aCD	*0.31*	10.3	9.0	*9.6 B*	13.29	13.16	*13.22 AB*
Tatiara	0.00 bE	0.35 aD	*0.18*	8.1	8.8	*8.5 C*	11.97	11.69	*11.83 BCD*
Kenya Farmer	0.19 bD	0.37 aD	*0.28*	7.4	6.3	*6.8 DE*	13.50	9.13	*11.32 BCD*
Mean	*0.36*	*0.62*	*0.49*	*8.0*	*7.8*	*7.9*	*11.95*	*11.16*	*11.55*
F-test	B**	G**	BxG**	B^{ns}	G**	BxG^{ns}	B^{ns}	G**	BxG^{ns}
$LSD_{0.05}$	0.05	0.11	0.15	-	1.03	-	-	2.62	-
CV (%)	21.83			12.92			22.57		

^{ns} not significant ($p<0.05$), ** significant at $p<0.01$.

Means within a row with the same lowercase letter and within a column with the same uppercase letter do not differ significantly at 5% with LSD.

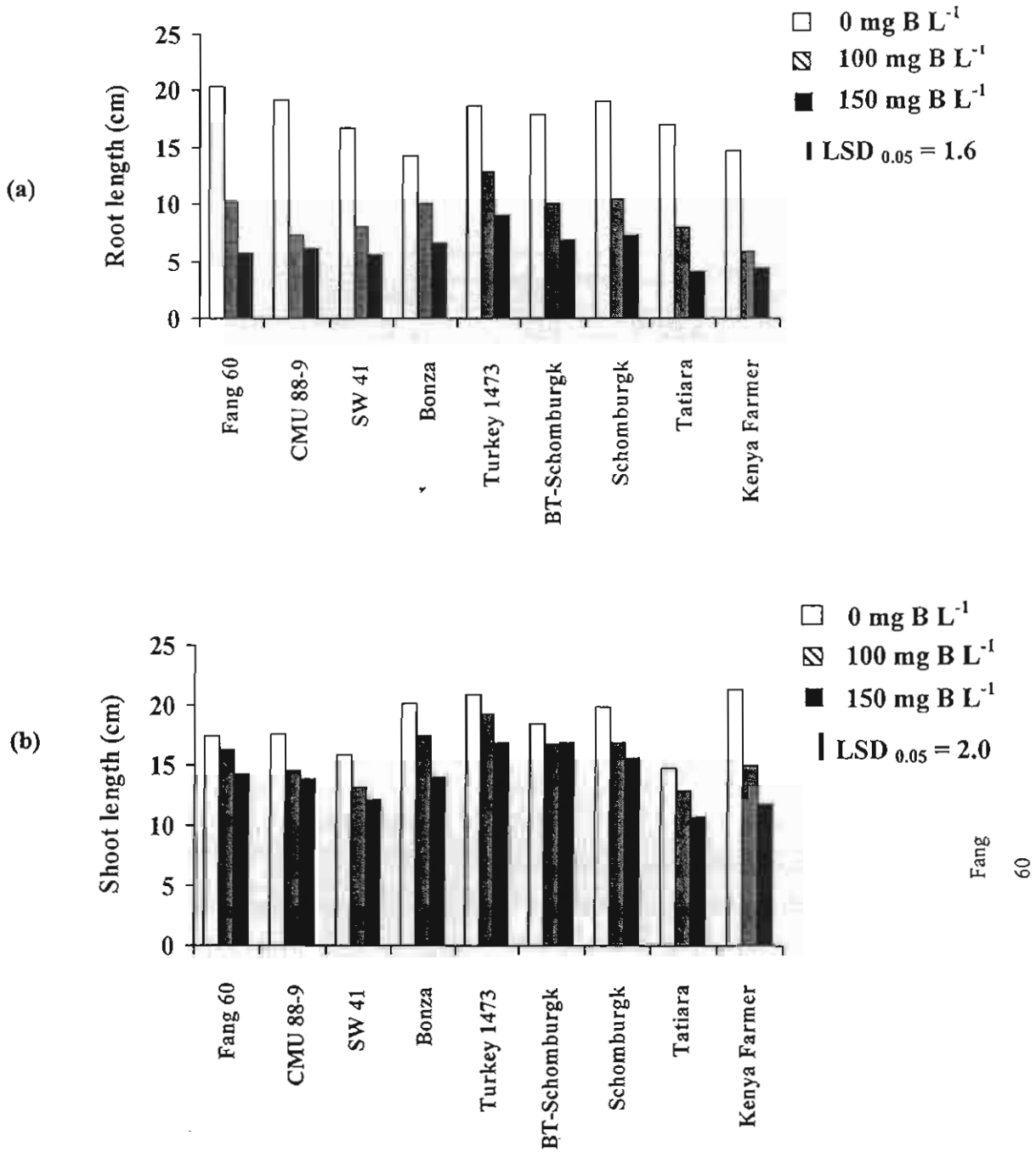

Figure 2 Root and shoot length of nine wheat genotypes grown in solution culture with three levels of B concentrations. (a) root length (cm.) (b) shoot length (cm.). (Experimet 2)

Table 2 Relative root length (RRL; % of B0) and relative shoot length (RSL; % of B0) of nine standard genotypes grown in solution culture with three B supplies. (Statistical value was transformed by square root arcsine transformation based on the data) (Experiment 2)

Genotype	B supply			*Mean*	B supply			*Mean*
	RRL				**RSL**			
	B0	B100	B150		B0	B100	B150	
Fang 60	100 aA	50.3 bCD	28.1 cDE	*59.5*	100	93.1	81.6	*91.6 AB*
CMU 88-9	100 aA	38.5 bF	32.5 bCD	*57.0*	100	83.1	78.8	*87.3 AB*
SW 41	100 aA	48.1 bD	33.2 cCD	*60.4*	100	82.1	75.9	*86.0 B*
Bonza	100 aA	70.7 bA	46.3 cAB	*72.3*	100	86.8	69.2	*85.3 B*
Turkey 1473	100 aA	68.9 bAB	48.7 cA	*72.6*	100	93.0	81.1	*91.4 AB*
BT-Schomburgk	100 aA	57.2 bBC	38.3 cBC	*65.2*	100	90.7	91.3	*94.0 A*
Schomburgk	100 aA	55.1 bCD	38.4 cBC	*64.5*	100	85.8	79.4	*88.4 AB*
Tatiara	100 aA	47.1 bDE	24.6 cE	*57.2*	100	87.5	73.3	*87.0 AB*
Kenya Farmer	100 aA	39.4 bEF	30.1 bDE	*56.5*	100	70.8	54.9	*75.2 C*
Mean	*100*	*52.8*	*35.6*	*62.8*	*100*	*85.9*	*76.2*	*87.4*
F-test	B**	G**	BxG**		B**	G**	BxG^{ns}	
$LSD_{0.05}$	0.023	0.039	0.068		0.039	0.068	-	
CV (%)	3.81				5.39			

RRL = (root length at B+/root length at B0) x 100

RSL = (shoot length at B+/shoot length at B0) x 100

^{ns} not significant ($p<0.05$), * significant at $p<0.05$, ** significant at $p<0.01$

Means within a column with the same letter do not differ significantly at 5% with LSD.

Table 3 Symptom of B toxicity (% of necrosis) of the oldest leaf of nine standard genotypes grown in solution culture with two levels of B supplies. (Statistical value was transformed by square root transformation based on the data in this table) (Experiment 2)

Genotype	B treatment		*Mean*	*Rank*
	B100	B150		
Fang 60	40.9	54.4	*47.6 A*	*9*
CMU 88-9	36.5	41.2	*38.9 ABC*	*6*
SW 41	37.5	54.0	*45.7 AB*	*7*
Bonza	20.3	32.9	*26.6 E*	*1*
Turkey 1473	27.0	34.4	*30.7 DE*	*2*
BT-Schomburgk	31.5	40.4	*35.9 CD*	*4*
Schomburgk	37.1	40.4	*38.7 BC*	*5*
Tatiara	32.1	37.9	*35.0 CD*	*3*
Kenya Farmer	38.6	54.1	*46.4 AB*	*8*
Mean	*33.5 b*	*43.3 a*	*38.39*	
F-test	B**	G**	BxG^{ns}	
$LSD_{0.05}$	0.033	0.071	-	
CV (%)	7.61			

%necrosis = (length of necrosis/length of that leaf) x 100

^{ns} not significant ($p<0.05$), ** significant at $p<0.01$

Means within a row with the same lowercase letter and within a column with the same uppercase letter do not differ significantly at 5% with LSD.

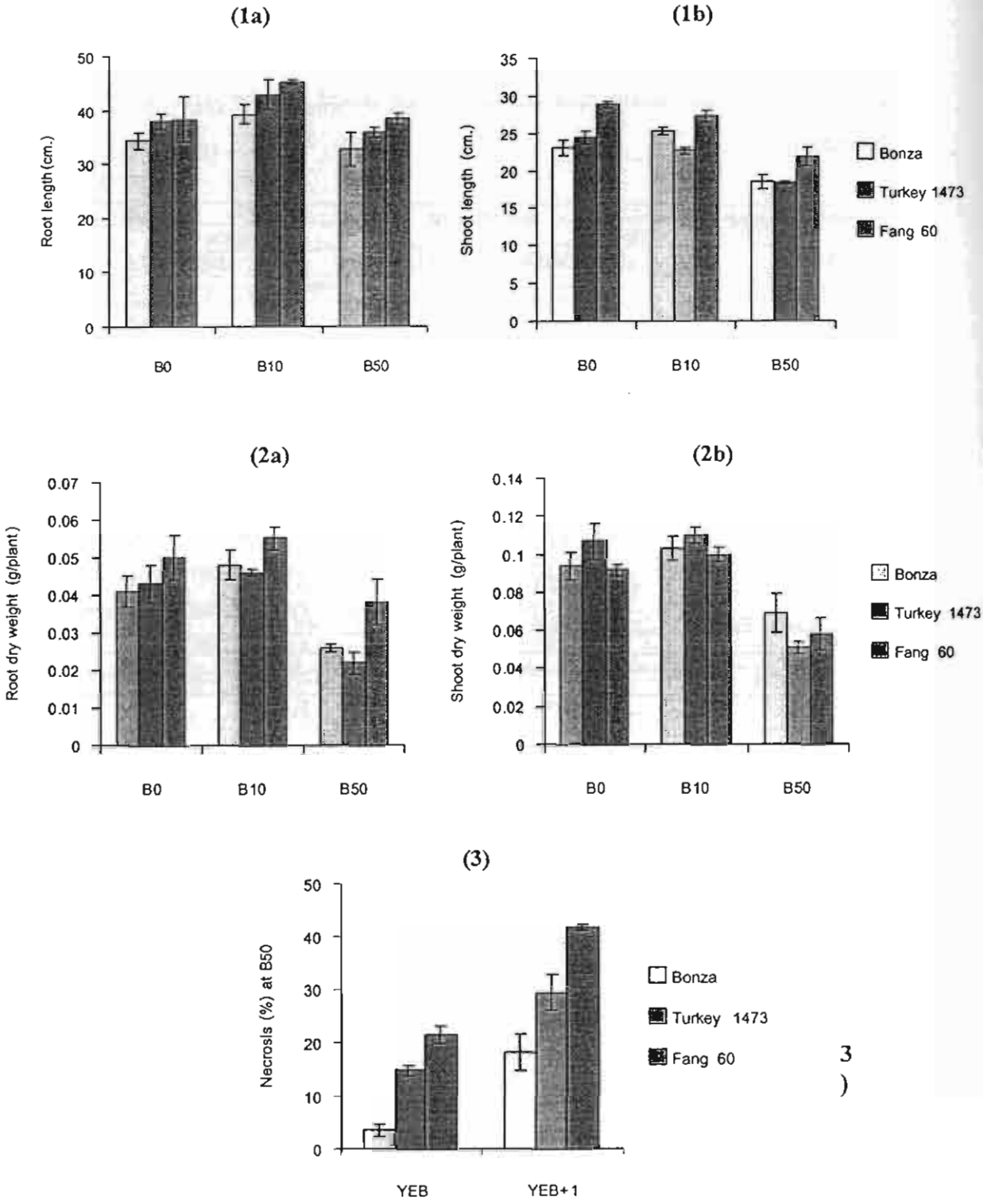

Figure 3 Root and shoot length (1a, 1b), root and shoot dry weight (2a, 2b) and necrosis (%) of YEB and YEB+1 (3) of three wheat genotypes grown in sand culture with three B supplies at 21 days after sowing. (Vertical bars presented as standard error of three replications) (Experiment 3)

Table 4 Effects of B supply on B concentration (mg B kg^{-1}) in YEB, YEB+1 and whole plant of three wheat genotypes grown in sand culture 21 days after sowing. (Statistical value was transformed by square root transformation based on the data in this table) (Experiment 3)

Genotypes	B supply			*Mean*
	B0	B10	B50	
YEB				
Fang 60 (E)	1.60 (1.26) bA	2.05 (1.43) bA	180.08 (13.41) aA	*61.24 (5.37)*
Bonza (I)	2.41 (1.55) bA	2.34 (1.53) bA	131.82 (11.48) aB	*45.53 (4.85)*
Turkey 1473 (T)	2.01 (1.40) bA	3.44 (1.85) bA	142.28 (11.87) aB	*49.24 (5.04)*
Mean	*2.01 (1.41)*	*2.61 (1.60)*	*151.40 (12.25)*	*52.00 (2.00)*
F-test	B**	G^{ns}	BxG**	
$LSD_{0.05}$	0.53	-	0.92	
CV (%)	10.58			
YEB+1				
Fang 60 (E)	7.92 (2.81) bA	8.04 (2.84) bA	273.66 (16.53) aA	*96.54 (7.39)*
Bonza (I)	7.65 (2.76) bA	7.80 (2.79) bA	226.85 (15.06) aB	*80.77 (6.87)*
Turkey 1473 (T)	1.65 (1.29) cB	7.69 (2.77) bA	226.17 (15.02) aB	*78.50 (6.36)*
Mean	*5.74 (2.29)*	*7.84 (2.80)*	*242.23 (15.54)*	*85.31 (6.87)*
F-test	B**	G**	BxG**	
$LSD_{0.05}$	0.44	0.44	0.77	
CV (%)	6.49			
Whole plant				
Fang 60 (E)	22.18 (4.71) cA	28.38 (5.33) bA	806.55 (28.40) aA	*285.70 (12.81)*
Bonza (I)	22.06 (4.69) bA	25.18 (5.02) bA	678.19 (26.04) aB	*241.81 (11.92)*
Turkey 1473 (T)	14.86 (3.85) cB	28.84 (5.37) bA	655.97 (25.60) aB	*233.22 (11.61)*
Mean	*19.70 (4.42)*	*27.47 (5.24)*	*713.57 (26.68)*	*253.58 (12.11)*
F-test	B**	G**	BxG**	
$LSD_{0.05}$	0.33	0.33	0.58	
CV (%)	2.78			

^{ns} not significant ($p<0.05$), ** significant at $p<0.01$

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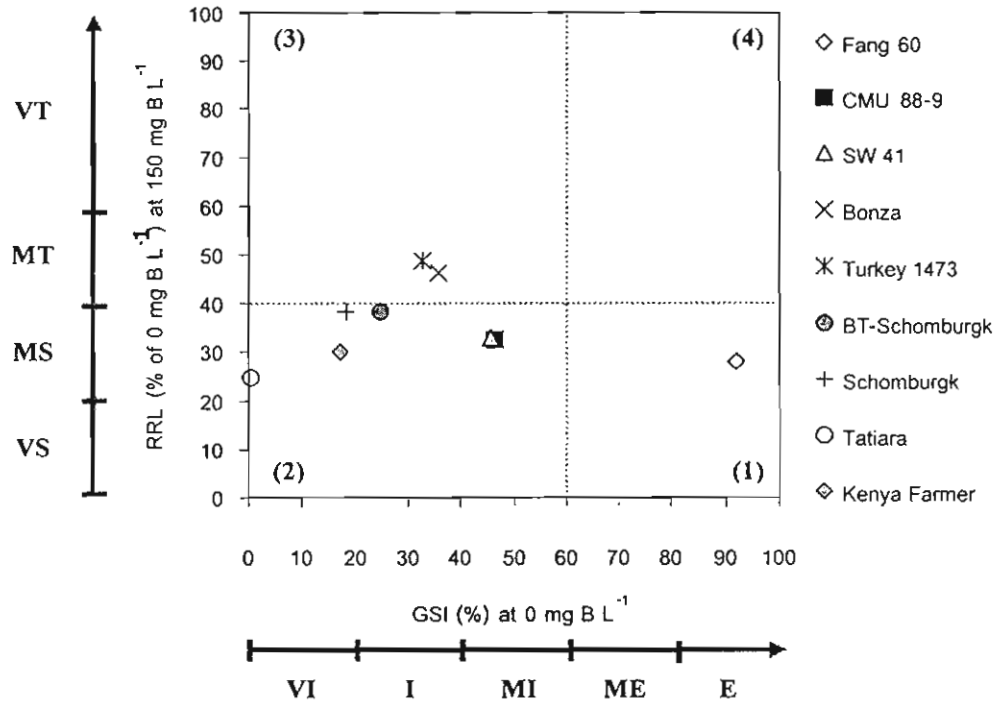

Figure 4 Relationship between RRL (at 150 mg BL^{-1}) and GSI (at 0 mg BL^{-1}) of nine standard wheat genotypes that could be categorized into three groups:

(1) GSI>60%, RRL<40% Tolerant to B deficiency but sensitive to B toxicity.

(2) GSI<60%, RRL<40% Sensitive to B deficiency and toxicity.

(3) GSI<60%, RRL>40% Sensitive to B deficiency but tolerant to B toxicity.

and the fourth possible group were:

(4) GSI>60%, RRL>40% Tolerant to B deficiency and toxicity that have not been found yet.

Iron in the Grain of High and Low Iron Density Rice Grown in Different Water Regimes

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Abstract

Throughout the rice growing areas of the world, water supply may range from intermittent rains, irrigation with relatively constant soil saturation, or to flooding several meters deep. This study sets out to measure the effect of water supply on Fe accumulation in the rice grain. Two rice genotypes, one with high (IR68144) and one with low (KDML105) grain Fe content, were grown in 4 water regimes (well-drained, W00; early drainage, from transplanting to anthesis, W0+; waterlogged, W++; late drainage, from anthesis to harvesting, W+0). Grain Fe concentration and content in IR68144 were generally higher than in KDML105. The water regimes affected both grain yield and grain Fe in the two genotypes differently. . In IR68144, grain Fe concentration in W00 and W++ were lower than in W0+ and W+0 whereas in KDML105, grain Fe concentrarion in W+0 was lower than in W00, W0+ and W++. By contrast, in IR68144, grain Fe content in W++ was lower than in W0+, but there was not differed from in W00 and in W+0. In KDML105, grain Fe content in W++ was higher than in W00 and W0+, but there was not differed from W+0. However, a very small fraction of the Fe was uptake from the shoot into the grain. In IR68144, grain Fe content in W00, W0+, W++ and W+0 were 2.9, 2.8, 1.8, and 2.3% respectively of Fe in the whole shoot, whereas, in KDML105, 0.7, 0.4, 1.0 and 0.4 respectively, it was lower Fe in shoots allocated to the

grain than in IR68144. In IR68144, the grain yield was not affected by water regimes whereas, in KDML105, the grain yield in W++ and W+0 were higher than in W00 and W0+. The grain Fe concentration and content was depended on rice genotype and water supply to the root.

Keyword: *Orysa sativa*, rice, water supply, grain Fe, transportation

Introduction

It is now generally agreed that increasing grain Fe concentration can help to solve problems associated with Fe-deficiency anemia (Senadhira et al., 1998; Welch and Graham, 1999). Previous studies found that grain Fe varied widely among rice genotypes and environmental conditions (Senadhira et al., 1998; Prom-u-thai and Rerkasem, 2001; Lauguet et al., 2001). Throughout the rice growing areas of the world, water supply may range from intermittent rains, irrigation with relatively constant soil saturation, or to flooding several meters deep (Naklang et al., 1996; Lauguet et al., 2001; Dingkuhn and Le Gal, 1996). Rainfed rice crops are commonly grown in either lowland paddies or upland fields in tropical Asia. Under rainfed, lowland conditions the use of the bunds and puddling of soils helps retain standing water, whereas aerobic soil conditions are usually maintained for upland crops (Naklang et al., 1996). Rice yield is highly responsive to water deficit during reproductive development and grain filling. The most sensitive stage is flowering, followed by gametogenesis (booting stage) and grain filling (O' Toole, 1982). Dingkhun and Le Gal (1996) found that early drainage, 3 to 4 days after flowering, reduced grain yield when compared to late drainage. Rice plants growing in flooded soil contain much higher levels of Fe then those growing in dry-land soil condition (Beyrouty et al., 1994). It is not known how grain Fe is influenced by such difference in Fe uptake

into the rice plant. The literature also lacks data on the distribution of Fe between the shoot and grain, as well as comparative information across genotypes. This paper documents how grain Fe is influenced by variation in Fe supply to the root by varying the water regimes in rice genotypes with high (IR68144) and low (KDML105) grain Fe content.

Materials and Methods

Two rice genotypes with high (IR68144 a improved rice genotype from IRRI) and low grain Fe content (KDML105 a popular genotype from Thailand) were grown under four soil conditions, in four replications. Five rice plants were grown in each black plastic bag containing 12 kg of soil (San Sai Series). The soil conditions were W00 (well-drained soil through out), W0+ (early drainage, the black plastic bag was completely drained 10 days after sowing and then submerged in a 10 L container of water after the anthesis stage), W++ (waterlogging through out) and W+0 (late drainage, the black plastic bag was submerged in a 10 L container of water and completely drained after anthesis). Basal fertilizer was applied at the rate of 0.707 g N/pot, 0.497 g P_2O_5/pot and 0.497 g K_2O/pot and 0.707 g N/pot, 0.497 g P_2O_5/pot and 0.497 g K_2O/pot four weeks later. Iron concentration was determined in mature grain (unhusked and brown rice), straw by dry-ashing and atomic absorption spectrometry (Emmanuel *et al.*, 1984).

Results

The Water regimes affected grain yield and both grain Fe concentration and content differently in two genotypes. In IR68144, grain Fe concentration in W00 and W++ were lower than in W0+ and W+0 whereas in KDML105, grain Fe concentrarion in

W+0 was lower than in W00, W0+ and W++ (Fig. 1). By contrast, in IR68144, grain Fe content in W++ was lower than in W0+, but there was not differed from in W00 and in W+0. In KDML105, grain Fe content in W++ was higher than in W00 and W0+, but there was not differed from W+0 (Table 1). Grain Fe concentration and content in IR68144 was higher than in KDML105 in 4 water regimes (Fig. 1 and Table 1). The effect of water regimes to grain Fe concentration and content were different from the percent empty grain and grain yield of two genotypes. In IR68144, the percent of empty grain in W00 and W+0 were higher than in W0+ and W++ , but in KDML105, the percent of empty grain in W0+ and W00 were higher than in W++ and W+0 (Table 2). These was affected to the grain yield of two genotypes grown in four water regimes. In IR68144, the grain yield was not affected by water regimes whereas, in KDML105, the grain yield in W++ and W+0 were higher than in W00 and W0+ (Table 2).

There was no effect of water regimes to Fe concentration in the whole shoot of two genotypes. The genotype was affected to Fe concentration in the whole shoot. These were differed from the effect of water regimes to grain Fe concentration in two genotypes. In IR68144, the Fe concentration in the whole shoot was 389.7 mg Fe/kg whereas in KDML105 the Fe concentration in the whole shoot was 555.6 mg Fe/kg. However, the Fe concentration in the grain was only a fraction of that in the whole shoot (Fig. 1). The Fe content of the whole shoot was affected by genotype and water regime (Table 2). But, there was no interaction between genotype and water regimes to shoot Fe content. The Fe content in the whole shoot of KDML105 was higher than IR68144 (Table 2). The Fe content of the whole shoot in W0+ and W+0 were higher than in W00, but there was not differed from W++ (Table 2). However, the grain accounted for a very small fraction of the whole shoot Fe in both genotypes. There was very little percent of Fe from the shoot was transported into the grain of two genotypes. In IR68144, grain Fe content in

W00, W0+, W++ and W+0 were 2.9, 2.8, 1.8, and 2.3% respectively of Fe in the whole shoot, whereas, in KDML105, 0.7, 0.4, 1.0 and 0.4% respectively, it was lower Fe in shoots allocated to the grain than in IR68144. There was no relationship between the grain and shoot and Fe concentration and content in two rice genotypes grown in four water regimes.

Discussion

Previous study found that Fe in soil solution increases to concentrations as high as 600 ppm within 1-3 weeks of submergence and later show a steep, roughly exponential, decrease to levels of 50-100 ppm, which persist of several months (Cho and Ponnamperuma, 1971) and in calcareous soil the concentration of water-soluble Fe rarely exceeds 20 ppm (Ponnamperuma, 1972).

The Fe uptake from the root into the whole shoot was affected by genotype and water regimes. There was not interacted between genotype and water regime. The Fe uptake into the whole shoot of two genotypes in W++ was higher than in W00 in this study. These were supproted the previous studies that when soils are flooded, the concentration of Fe^{+2} in the soil solution increases to several times that in aerated soil (Ponnamperuma, 1972). Furthermore, rice plants growing in flooded soil have been reported to contain Fe at many times those growing in well-drained soil (Beyrouty et al., 1994). However, The Fe uptake in the whole shoot in W++ was not differed from in W0+ and W+0. Trust, the Fe uptake into the whole shoot was affected by the stage of rice growing in different water regimes. The water can be supply to the rice plant before or after anthesis stage instead of waterlogged throughout. There was no previous result supported the Fe uptake of rice in different water regimes. However, Fe is an important

minerals for chlorophyll and enzyme synthesis process in plant function (Marschner, 1995). Therefore, the Fe uptake in rice plant was related to the growth stage of rice in different water regime may depending on the function of plant on that time. Moreover, Fe uptake was also affected by rice genotype in four water regimes. The Fe uptake in KDML105 was higher than in IR68144 in four water regimes. These were related to the grain Fe concentration and grain yield of two genotypes. The grain Fe concentration in IR68144 was low in W00 and W++ but the grain yield was not affected by water regimes. By contrast, the grain Fe concentration in KDML105 was low in W+0 but there was high grain yield in W++ and W+0.

Rice grain is the edible part of rice plant. Therefore, Fe uptake from the plant into the grain need to be discuss. In IR68144, the Fe transportation from the shoot into the grain much higher than in KDML105 in 4 water regimes. There was differed effect from the uptake of Fe in the whole shoot. In IR68144, Fe transportation from the shoot into the grain in W0+ was higher than in W++ but there was not differed in W00 and W+0 Whereas, in KDML105 in W++ was higher than in W00 and W0+ but there was not differed in W+0. The effect of water regimes to Fe uptake into the grain of two genotypes was not related to the previous studies about Fe in the soil solution ((Ponnamperuma, 1972). Clearly, The effect of water regimes to Fe uptake from the shoot into rice grain of two genotypes was differed from the uptake of Fe from the soil solution into the whole plant. However, there was a very small fraction of Fe from the shoot was transported into the grain in two genotypes. In IR68144, the distribution of Fe in the grain as percent of total Fe in W00, W0+, W++ and W+0 were 2.9, 2.7, 1.8 and 2.2% respectively, whereas in KDML105 were 0.9, 0.3, 0.9 and 0.4% respectively.

The Fe distribution in the grain is clearly depending on the rice genotype and water regimes. Previous study found that grain Fe in rice was varied widely among genotype

and water condition (Prom-u-thai and Rerkasem, 2001). Grain Fe was increased among the Fe supply into the soil, e.g. the suppling of nitrogen fertilizer (Senadhira et al., 1998; Prom-u-thai and Rerkasem, unpublish datas). Therefore, to solve the Fe-deficiency anemia problem by consuming the Fe from rice grain. High grain Fe content can be managed by genotype and supplying of Fe into the soil. The supplying of Fe into the soil was also correlated with the growth stage of rice.

Acknowledgement

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References

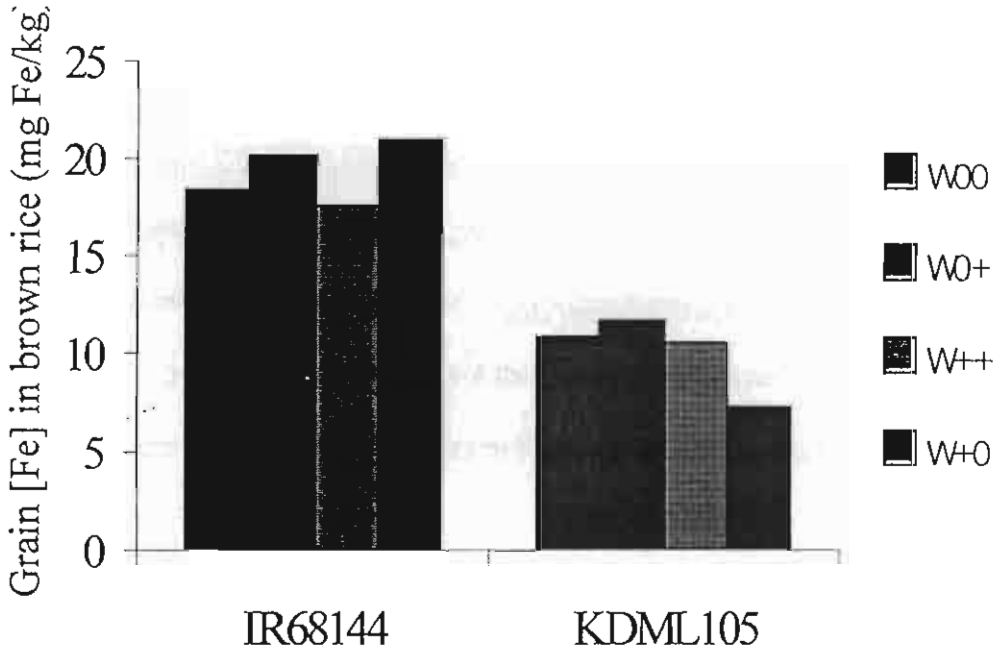

Fig. 1 Grain Fe concentration in brown rice of two rice genotypes grown under four water conditions (LSD=1.8)

Table 1 The Fe content in shoot and grain of two genotypes grown under four water regimes

Genotype	Water condition	Fe content (mg Fe/pot)	
		Shoot	Grain
IR68144	W00	20.4	0.6 cd
	W0+	25.5	0.7 d
	W++	28.2	0.5 bc
	W+0	26.9	0.6 cd
KDML5	W00	23.1	0.2 a
	W0+	66.8	0.2 a
	W++	44.1	0.4 b
	W+0	72.0	0.3 ab
Analysis of variance			
p (Genotypes)		**	***
p (Water)		*	ns
p (G X W)		ns	**

Table 1 The dry weight of grain and straw and percent of empty grain of two rice genotypes grown in different water regimes

Genotype	Water condition	Dry weight (g/pot)		% Empty grain
		Grain	Straw	
IR68144	W00	31.6 b	40.1 ab	25 b
	W0+	35.1 bc	44.4 b	10 a
	W++	29.9 b	32.1a	9 a
	W+0	28.5 b	29.6a	16 ab
KDML105	W00	14.5 a	58.1c	62 d
	W0+	20.9 a	67.1d	52 d
	W++	40.7 c	63.8 cd	18 ab
	W+0	37.2 c	63.4 c	33 c
Analysis of variance				
p (Genotypes)		*	**	**
p (Water)		**	*	**
p (G X W)		**	*	**

Lower case used for comparison between each columns

Response of F_2 population derived from boron efficient (Fang 60) x boron inefficient (Bonza) wheat (*Triticum aestivum* L.) genotypes to boron levels

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Abstract

Responses of a F_2 population between boron (B) efficient (Fang 60) and B inefficient (Bonza) wheat (*Triticum aestivum* L.) parents to levels of B were studied in two experiments. The three B levels; nil (B0), limed at the rate 1 t ha^{-1} (BL) and boron at the rate 10 kg borax ha^{-1} (B+), were applied to three populations, the two parents and their F_2. Response to B, i.e. number of grains $spikelet^{-1}$, grain set index (GSI) and grain yield, were found to differ among populations when grown in low B (BL and B0). Mean grain sets of F_2 in low B were intermediate between the two parents but closer to the more efficient parent, Fang 60. This suggests that B efficiency was expressed as a dominant trait. Segregation of the F_2 fit 15 B efficient plus intermediate: 1 B inefficient ratio, indicating that B efficiency was controlled by two genes. From each B level, 24 F_2 plants with the highest grain set and grain yield were selected and their F_3 progenies were evaluated in sand culture without B in the second experiment. Segregation for response to B was found within F_2-derived F_3 families selected from all B treatments. Populations selected from low B (BL and B0) displayed a higher proportion of B efficient genotypes than those from sufficient B (B+). F_2 populations selected from BL and B0 comprised B efficient, homozygous and heterozygous genotypes but no B inefficient genotype was found. Both B efficient and inefficient genotypes were found within populations selected under B+ condition, indicating that selecting for B efficiency in BL and B0 is more effective than in B+. In conclusion, B efficiency was qualitatively inherited in wheat. This character can be improved in breeding programme by backcrossing. As dominant gene action was involved, progeny testing method should be employed in selection for B efficient genotype.

Introduction

Low boron (B) soils are widespread in many wheat growing, subtropical areas (Sillanpaa, 1982). These include the northern and northeastern regions of Thailand, where wheat is being promoted (Rerkasem et al., 1988; Keerati-Kasikorn et al., 1987). Boron deficiency causes yield reduction by inducing male sterility, resulting in grain set failure (Cheng and Rerkasem, 1993). Wide range of genotypic variation for response to low B has been identified and genotypes were classified into four distinct groups, namely, efficient, moderately efficient, moderately inefficient and inefficient (Rerkasem and Jamjod, 1997). At low B, the inefficient genotypes were completely sterile and set only a few or no grain, while the efficient genotypes set grain normally. Evidence of genotypic variation offers a solution to B deficiency through selection and breeding for B efficient cultivars. In this study, the B efficient (Fang 60) and inefficient (Bonza) genotypes were used as parents to produce F_2 generation. Response of F_2 population to B levels and their genetic control are described. Understanding response of segregating population and genetic control will facilitate selection and breeding for B efficiency.

Materials and methods

Genetic materials

Fang 60 (B efficient, Jamjod et al., 1992) and Bonza (B inefficient, Rerkasem and Jamjod, 1997) were used as parents. F_1 plants were sown in B sufficient soil to produce the F_2 generation. The F_2 population was tested at 3 levels of B in the soil in

Experiment 1. Seventy-two F_2 plants were selected and multiplied. Selected F_3 families were tested in sand culture in Experiment 2.

Experiment 1: *Evaluation of F_2 response to B levels*

Parents and F_2 were sown in a low B soil at Chiang Mai University. The experiment was arranged as a split plot design with 3 replications. Three B levels assigned to main plots were nil (B0), limed at the rate 1 t ha^{-1} (BL) and B at the rate 10 kg borax ha^{-1} (B+), applied to the soil and the three populations, two parents (Fang 60 and Bonza) and their F_2, were sown in sub plots. In each plot, 10 plants each of Fang 60 and Bonza, and 120 F_2 plants were sown. At booting, the first two emerged ears from each plant were bagged to prevent outcrossing. At maturity, the bagged ears were harvested and analysed for number of spikelets ear^{-1}, grains $spikelet^{-1}$ and Grain Set Index (GSI, percentage of the 20 basal lorets from 10 central spikelets with grain; Rerkasem and Loneragan, 1994). All ears from each plant were counted, pooled, threshed and determined for grain yield. Responses to B of parents and the F_2 population were compared by LSD. Chi-square tests were used to examine the segregation ratio of F_2 population for each B treatment.

Experiment 2: *Evaluation of selected F_2-derived F_3 families*

Twenty-four F_2 plants which had the highest grain yield and gain set were selected from each B level, and seeds harvested from these plants represented F_2-derived F_3 families selected from BL, B0 and B+. All families were grown in trays containing washed river quartz sand with no detectable available B. Families were grown in rows, 12 plant row^{-1}, with a spacing of 6 cm between plants and 6 cm between rows. Grid rows of Fang 60 and SW 41 parents were included every 6 rows. Trays were watered twice daily with a

complete nutrient solution without added B. At booting, two ears from each plant were bagged and at maturity the bagged ears were harvested and GSI determined. For each F_3 family, mean and within family variance were calculated and compared to both parents.

Results and discussion

Parents used in this study displayed genotypic differences for response to B (Table 1). Severe reduction in number of grains spikelet^{-1}, grain yield and GSI were found in Bonza while those of Fang 60 parent were not affected, compared to B+. However, B had no effect on number of ears plant^{-1} and spikelets ear^{-1} of both parents and their F_2. These findings were consistent to those reported by Jamjod et al. (1992) and Rerkasem and Jamjod (1997) for Fang 60 which is B efficient and Bonza which is B inefficient.

At B+, mean grain set and grain yield of the F_2 population and both parents were similar while in low B the F_2 population means were intermediate between the two parents but closer to Fang 60 (Table 1). F_2 distribution for GSI were used to study the segregation pattern of F_2 compared to parents in response to low B (Figure 1). No segregation of individual F_2 plants was found when grown in B+. However, when grown in low B, most of F_2 plants fell into the range of the efficient parent, Fang 60. This type of segregation suggested that B efficiency was controlled by dominant gene action. In addition, low B treatments in this study allow genetic variability to be expressed and could be used to screen other segregating populations.

As B efficiency was controlled by dominant gene action, the F_2 segregation ratio was calculated by the following criteria. If the cross was segregating at a single gene, the F_2 population was expected to segregate in a ratio of 3 efficient : 1 inefficient. If the cross was segregating at two genes, the population was expected to segregate in a ratio of 15 (efficient + intermediate) : 1 inefficient. In Figure 1, the GSI of Bonza and Fang 60 grown in BL were 2.1 and 96.8 % and in B0 were 8.6 and 97.0 % respectively. The mean GSI of each parent plus one standard deviation was used to classified the response of F_2 plants. For example, F_2 plants grown in BL having GSI less than 10.9 % were classified as B inefficient. According to this classification, it was found that the segregation ratio of the F_2 population grown in BL and B0 fit with 15 efficient plus intermediate : 1 inefficient ratio (Table 2). This indicates that Fang 60 and Bonza differed by two genes for B efficiency.

If B efficiency was dominant to inefficiency and controlled by two independent loci, *A* and *B*, then it is expected that genotype of Fang 60 in term of response to B should be *AABB* and Bonza should be *aabb*. Genotypes of F_2 plants that fell into the range of Fang 60 when grown in BL and B0 were likely to be *A-B-* (i.e. *AABB*, *AABb*, *AaBB* and *AaBb*) and those in the range of Bonza were all *aabb*. Genotypes of F_2 plants classified as intermediate were likely to be *A-bb* (*AAbb* and *Aabb*) and *aaB-* (*aaBB* and *aaBb*). Selection for B efficiency base on phenotype will not effective because it is not possible to distinguish between heterozygous (e.g. *AaBb*) and homozygous efficient genotypes.

The value of each F_2 individual was the expression of its phenotype, which included both genotypic and environmental effects. The measurement of B response of

F_2 derived F_3 families allowed the genotype of the F_2 to be identified. Twenty-four F_2 plants with the highest grain yield and grain set were selected from each B treatments. Their F_3 progenies were tested in low B and assigned into homozygous efficient, segregating or homozygous inefficient to B by comparing the family's mean and variance to the parents. Bonza parental check set no grain while mean GSI of Fang 60 were between 80-90% and variances were <1000 (Figure 2). Although all selected F_2 plants had GSI >95%, most of their progenies were segregating when grown in low B. The segregation within the F_3 families selected from BL and B0 confirmed the dominant gene action of B efficiency while segregation of families selected from B+ resulted from random selection for B efficient genes. It is suggested that progeny testing should be employed in selection for B efficiency.

High proportions of B efficient alleles were found from populations selected under low B, compared to those selected from B sufficient (Table 3). Four families or 8 % of selected populations from BL and B0 were homozygous efficient but none were homozygous inefficient. In contrast, in B+ treatment where B efficient genes were randomly selected, no homozygous efficient and 8 % homozygous inefficient were found. This indicates that low B in this study exerted selection pressure for B efficiency and high yield. Moreover, populations selected from non B stress condition were likely to contain a high proportion of inefficient genotypes (for example, 46 % compared to 29 % of those selected from BL, Table 3). In breeding programmes, genotypes or breeding materials should be screened for response to B prior to release or promote in low B areas.

Most cereal breeding programmes devote a considerable proportion of their resources to selection for grain yield *per se*. This involves sowing yield trials at several contrasting locations within the one target region and retaining those selections with a comparatively high mean yield. Advanced lines from CIMMYT germplasm, #144, #1510 and #1015, which are all sister lines, outyielded National trials during the early 1980s. Line #1015 was released as Fang 60 from Fang Rice and Winter Cereals Research Station, Chiang Mai, low B site, in 1987, and has shown outstanding yield performance until now. High correlation ($r = 0.81$) between GSI and grain yield was found when wheat and barley genotypes were grown in low B but no correlation was found when plants were grown in B sufficient conditions (Jamjod and Rerkasem, 1999). B efficient genes in Fang 60 might having been unconsciously selected by selecting for grain yield.

The results of this study demonstrated that B efficiency is qualitatively inherited. This character can be improved in breeding programmes by backcrossing. As complete dominant gene action was involved, progeny testing method should be employed in selection for B efficient genotype.

References

Cheng, C. and Rerkasem, B. 1993. Effects of boron on pollen viability in wheat. Plant Soil 155/156: 313-315.

Jamjod, S., Mann, C.E. and Rerkasem, B. 1992. Screening for boron deficeincy in wheat. *In* "Boron Deficiency in Wheat" (*Eds*. C.E. Mann and B. Rerkasem). pp. 79-82. CIMMYT What Special Report No. 11. CIMMYT, Mexico.

Keerati-Kasikorn P., Bell, R.W. and Loneragan, J.F. 1987. Nutrient deficiencies affecting peanut production in soils of northeastern Thailand. *In* "Food Legume Improvement for Asian Farming Systems". (*Eds*. E.S. Wallis and D.E. Byth) p 261, Ramsay are Printing, Melbourne.

Rerkasem, B. and Loneragan, J. F. 1994. Boron deficiency in two wheat genotypes in a warm subtropical region. Agron. J. 85: 887-890.

Rerkasem, B. and Jamjod, 1997. Boron deficiency induced male sterility in wheat (*Triticum aestivum* L.) and implications for plant breeding. Euphytica 96: 257-262.

Rerkasem, B., Netsangtip, R., Bell, R.W., Hiranburana, N. and Loneragan, J.F. 1988. Comparative species response to boron deficiency on a Typic Tropaqualf in northern Thailand. Plant Soil 106: 15-21.

Sillanpaa, M. 1982. Micronutrients and nutrient status of soils. FAO Bull. 48, FAO, Rome.

Table 1. Response of Fang 60 (E, efficient), Bonza (I, inefficient) and F_2 grown at nil boron (B) plus lime (BL), nil B (B0) and 10 kg B ha^{-1} (B+) (Experiment 1).

	B treatment		
Characters/population	BL	B0	B+
Ears plant^{-1}			
Bonza (I)	9.1	11.7	9.4
F_2	10.0	7.5	9.3
Fang 60 (E)	5.4	8.1	8.5
LSD(0.05) B x G ns			
Spikelets ear^{-1}			
Bonza (I)	15.3	16.1	17.2
F_2	17.2	16.8	17.4
Fang 60 (E)	16.0	16.7	17.0
LSD(0.05) B x G ns			
Grains spikelet^{-1}			
Bonza (I)	0.1	0.4	1.9
F_2	1.6	1.7	2.2
Fang 60 (E)	2.8	2.9	3.0
LSD(0.05) B x G 0.3			
Grain yield (g plant^{-1})			
Bonza (I)	0.2	0.8	6.6
F_2	6.3	4.6	6.0
Fang 60 (E)	5.6	8.8	8.1
LSD(0.05) B x G 3.2			
GSI (%)			
Bonza (I)	2.1	8.6	89.9
F_2	69.9	79.1	87.6
Fang 60 (E)	96.8	97.0	99.3
LSD(0.05) B x G 8.3			

Table 2. Response of F_2 population from Fang 60 (B efficient) x Bonza (B inefficient) grown in 3 levels of B (Experiment 1).

B		Number of F_2 plants				
		Model[a]	Efficient + Intermediate	Inefficient	χ^2	P
BL	Expected	3:1	130.5	43.5	26.67	<0.01
	Expected	15:1	163.1	10.9	0.96	0.33
	Observed		160.0	14.0		
B0	Expected	3:1	146.2	48.7	33.03	<0.01
	Expected	15:1	182.9	12.1	0.29	0.61
	Observed		181.0	14.0		
B+	Expected	3:1	127.5	42.5	-	-
	Expected	15:1	159.4	10.6	-	-
	Observed		No segregation observed			

[a] Expected ratio for single gene was efficient : inefficient = 3:1 and for two genes was efficient + intermediate : inefficient = 15:1.

Table 3. Variance of F_2-derived F_3 families selected from BL (limed soil), B0 (no added B) and B+ (10 kg B ha^{-1}) then grown in sand culture without added B(Experiment 2). Selections were categorized by GSI.

	Number of family (%)					
	Variance					
Mean	0	1-500	>500-1000	>1000-1500	>1500	*Total*
			BL selection			
0-25			17	13		*29*
>25-50				17	21	*38*
>50-75				13	12	*25*
>75-100		8				*8*
Total	*0*	*8*	*17*	*43*	*33*	
			B0 selection			
0-25		4	13	17		*33*
>25-50				8	25	*33*
>50-75				8	17	*25*
>75-100		4	4			*8*
Total	*0*	*8*	*21*	*33*	*42*	
			B+ selection			
0-25	8	4	25	8		*46*
>25-50				17	21	*38*
>50-75				4	13	*17*
>75-100						*0*
Total	*8*	*4*	*25*	*29*	*44*	

Figure 1

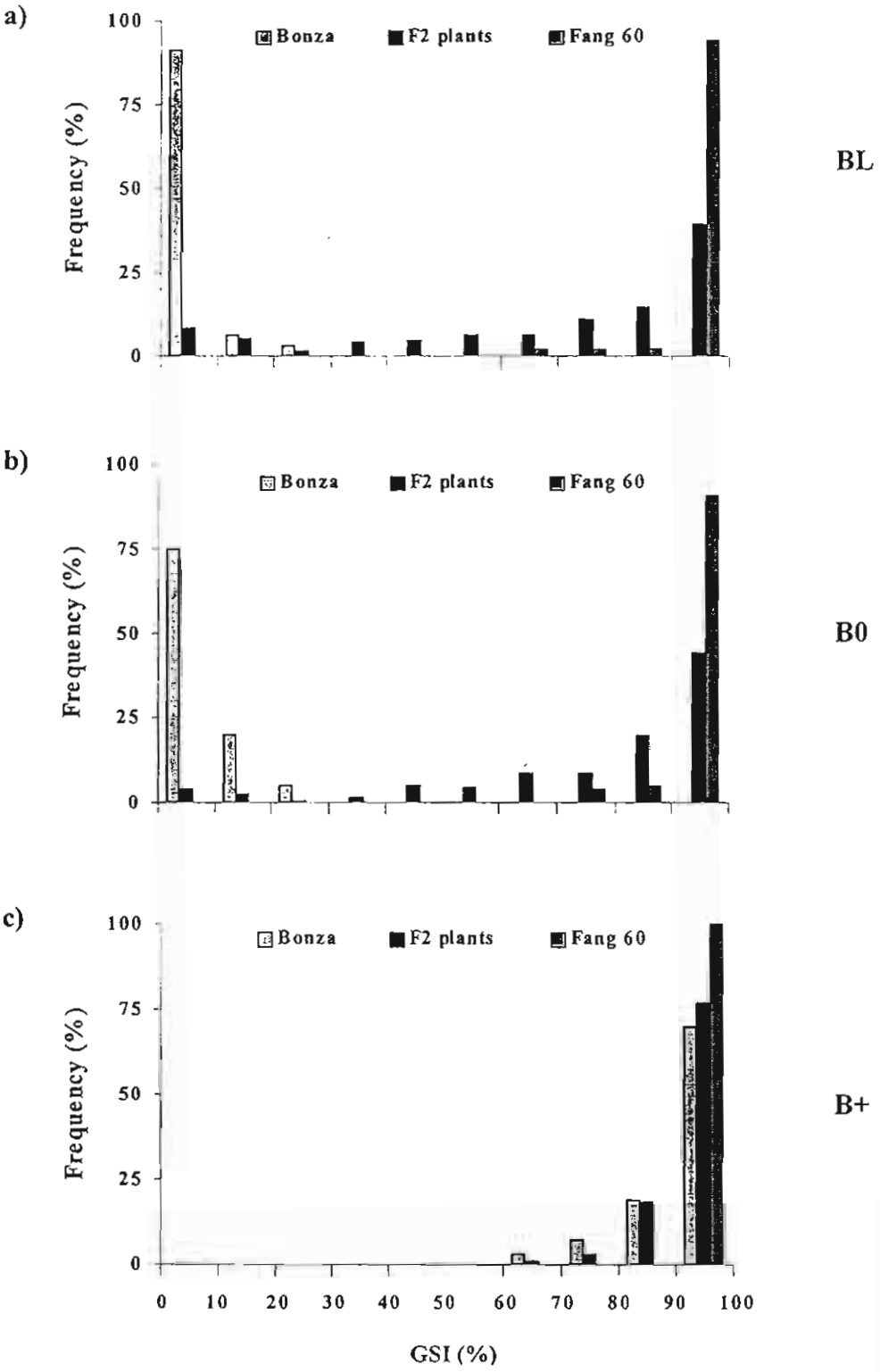
a)
b)
c)
Bonza
F2 plants
Fang 60
Frequency (%)
100
75
50
25
0
BL
B0
B+
0
10
20
30
40
50
60
70
80
90
100
GSI (%)

Figure 2

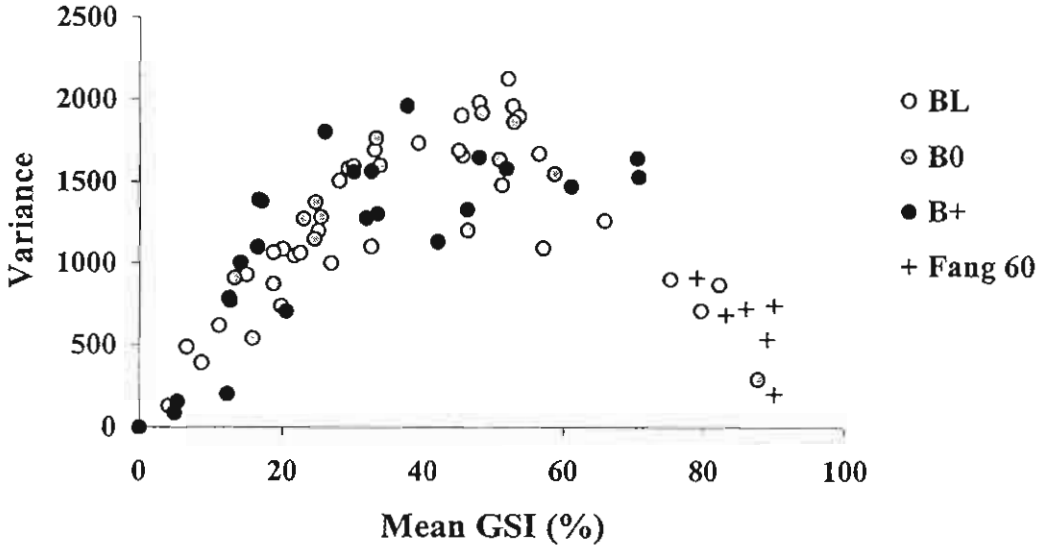
2500
2000
1500
1000
500
0
Variance
0
20
40
60
80
100
Mean GSI (%)
BL
B0
B+
Fang 60

Figure 1. Grain Set Index (GSI, %) of parents and F_2 plants of (Fang 60 x Bonza) grown in:

BL: mean (sd) of Bonza, F_2 population and Fang 60 were 2.1 (8.8), 69.9 (32.8) and 96.8 (9.8), respectively.

B0: mean (sd) of Bonza, F_2 population and Fang 60 were 5.6 (9.0), 79.1 (25.3) and 97.0 (5.1), respectively.

B+: mean (sd) of Bonza, F_2 population and Fang 60 were 89.9 (12.9), 87.6 (7.8) and 99.3 (1.4), respectively.

Figure 2. Mean and variance of F_2-derived F_3 families selected from BL (limed soil), B0 (no added B) and B+ (10 kg B ha^{-1}) and of Fang 60 parent grown in sand culture without B (Experiment 2). Note: Mean GSI and variance of Bonza parent were both 0 and obscured by F_3 families' data. GSI of all selected F_2 plants when grown in Experiment 1 were 95-100%.

Boron Deficiency in Wheat: a Review

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ABSTRACT

Although cereals are generally considered to be insensitive to boron (B) deficiency, incidences of B deficiency have been reported from many of the world's wheat growing countries since the 1960's. The most extensive region of B deficiency in wheat so far reported is the adjoining area of eastern Nepal, northeastern India and northwestern Bangladesh, through to southwestern China. Wheat is more prone to B deficiency than rice and maize, and some dicotyledons including soybean and mungbean. Although it has been reported to adversely affect many processes of wheat growth and development, B deficiency depresses commercial wheat yield primarily through grain set failure, which is in turn governed by male fertility. The function of wheat anthers has been found to be impaired when their B concentration per unit dry weight was 8-10 times that found limiting for vegetative growth. Wheat genotypes vary greatly in their adaptation to low B soils, B efficient genotypes may grow and yield normally on low B soils in which inefficient genotypes are so adversely affected by B deficiency that they set no grain at all. International germplasm from the International Maize and Wheat Improvement Centre (CIMMYT) on which most developing countries depend on for their new wheat cultivars is largely B inefficient. Increasing B efficiency in such internationally important germplasm should boost production in the world's growing areas on low B soils.

1. Incidences of boron deficiency in wheat

Boron (B) was shown to be essential to plant growth in 1923 (Warington, 1923). Among the micronutrients, deficiency of B is the most frequently encountered in the field (Gupta, 1979). However, along with other cereals, wheat has generally been considered to have a low requirement for B (Marten and Westermann, 1991). Lack of reports of deficiency in wheat and other small grains from areas where B deficiency in other crop species is otherwise widespread such as the USA (Lamb, 1967) further reinforced the perception of wheat being relatively free of B deficiency problem. Boron deficiency in field grown wheat was first observed almost concurrently on

different sides of the world following the spread of semi-dwarf, 'green revolution' wheat in the 1960's. Grain set failure associated with male sterility was observed in wheat in Brazil in 1962 (da Silva and de Andrade, 1980). Diagnosis of B deficiency was confirmed by positive responses to B application. The same was observed in Nepal in 1964, among introduced high-yielding Mexican and Indian wheat germplasm (Misra et al., 1992). Boron deficiency was found to be the cause of almost complete crop failure in some 40,000 ha of wheat in Heilongjian Province in the north of China in 1972 and 1973 (Li et al., 1978).

Countries where B deficiency, based on responses to B application, in wheat has been reported included Bangladesh, Brazil, Bulgaria, China, Finland, India, Madagascar, Nepal, Pakistan, South Africa, Sweden, Tanzania, Thailand, USA, USSR, Yugoslavia, Zambia (Shorrocks, 1997). Reports of B deficiency in wheat continue to come out of India (Singh et al., 1976; Ganguly, 1979; Sarkar and Chakraborty, 1980; Mandal and Das, 1988; Dwivedi et al, 1990.). Most of these were from the northeastern states of Bihar, West Bengal, Orissa, Meghalaya and Assam. It was common local knowledge in the 1980's that the problem does not stop at the international borders, but extends into neighboring areas of Bangladesh and Nepal (Reuter, 1987). "Wheat sterility", depressed percentage of florets with grain, has been reported from many parts of Nepal, with B deficiency, low temperature stress during reproductive development (Subedi, 1992), waterlogging at flowering, low soil nitrogen and hot dry wind (Misra et al, 1992) suggested as possible causes. An imprecise way in which the term "sterility" is used may sometimes lead to inaccurate diagnosis for the causal factor (see below). Except at higher altitudes, responses to B application have identified B deficiency as the cause of grain set failure in wheat in Nepal (Sthapit, 1988; Subedi et al, 1992). Boron deficiency was also found to be the cause of massive sterility observed in the northern wheat zone of Bangladesh. Application of B increased wheat yield in farmers' field by 8.5% to 14% (an equivalent of approximately 60% of the yield gains due to plant breeding in the past 20 years (D.A. Saunders, pers. comm.). Recent studies have confirmed widespread problem of B deficiency in wheat in this part of Asia (Kataki et al., 2001).

The adjoining areas of India, Nepal and Bangladesh is probably the world's most extensive area of B deficient wheat so far reported, covering at least several hundred thousand hectares. The problem of B deficiency affects wheat farmers of area, who are some the world's poorest, in two ways. Firstly, they continue to suffer grain set failure and yield loss each year. Secondly, they are prevented from realizing benefits from new improved wheat varieties with greater yield potential and better resistance to important diseases, which turned out to be poorly adapted to low B soils. Boron deficient wheat has also been found in northern Thailand (Rerkasem et al., 1989) and southwestern China to the Myanmar border (Yang, 1992; Rerkasem, 1996). Any attempt to improve wheat production in Myanmar should keep a close watch on B deficiency especially with introduced germplasm of semi-dwarf wheat from CIMMYT (Rerkasem et al., 2003 and see also section on adaptation to low B soils below).

2. Responses to low boron

2.1 Vegetative growth

The most rapid response to B deficiency in higher plants is the cessation of root elongation (Dugger, 1983; Marschner, 1995; Shelp, 1993; Dell and Huang, 1997), but this has rarely been seen in wheat. Boron deficiency in early vegetative growth is much less readily inducible in wheat than in dicots. For example, Snowball and Robson (1983) found that after a transfer to a solution culture to which no B had been added wheat root continued growing normally for a considerable time, while root growth in subterranean clover (*Trifolium subterraneum* L.) stopped immediately. In earlier studies, B deficiency was induced in vegetative growth in wheat only after B in the nutrient solution had been depleted by plant uptake. Characteristic symptoms in young wheat plants made B deficient in this way include a longitudinal splitting of the newer leaves close to the midrib and the development of a saw tooth effect on young leaves reflecting abnormal cellular development (Snowball and Robson, 1983).

Other evidence of lower requirement for B and lower sensitivity to B deficiency in wheat has all been based on early vegetative growth. Wheat has been shown to grow almost normally in a nutrient solution without added B in which various legumes were adversely affected by B deficiency in a comparison made on 30 days of early vegetative growth (Chapman et al., 1997). Similarly, the lower external and internal requirement for B in wheat than sunflower and marri (*Corymbia calophylla*) were judged on the basis of an early vegetative growth of 10-20 days (Asad et al., 2001). The young wheat plants grew well and were free of B deficiency symptoms in a nutrient solution with ≤ 0.13 μM B in which vegetative growth of the two dicots, marri and sunflower, was severely depressed by B deficiency. Maximum dry weight was attained in wheat with ≥ 0.6 μM B in the nutrient solution, compared with ≥ 1.2 μM B for Marri and sunflower. The difference was even greater in the B concentration in the young open leaf associated with maximum growth, referred by the authors as internal requirement. Ten days old wheat achieved maximum dry weight with ≥ 1.2 mg B kg^{-1} DW, whereas marri and sunflower did not until they had exceeded 18-20 mg B kg^{-1} DW. The lower B requirement in graminaceous species than dicotyledonous species is said to be related to their different cell wall composition (Marschner, 1995). Their shoots have been shown to contain 4-7 mg B kg^{-1} cell wall (CW) compared with 20-40 mg B kg^{-1} CW in dicotyledonous plants (Matoh, 1997).

These early vegetative responses to B in wheat, however, may or may not correlate with whole plant responses. None of the effects of low B on early vegetative growth cited above has ever been observed in the field. An exception is the longitudinal split along the mid-rib observed in solution culture (Snowball and Robson, 1983) which is common the field in Thailand, Bangladesh and Nepal (Rerkasem unpublished). In the field, however, the symptom is extremely ubiquitous and not restricted only to fields or plants that are otherwise proved to be B deficient.

2.2 Reproductive development

The first symptom of B deficiency in field grown wheat is seen during anthesis, when florets remain open longer than normal. When viewed from a distance of 3-4 m against the light, the ears have a translucent appearance, like paper lamps. Examination of the florets just before or during anthesis show poorly developed pollen and sometimes anthers, leading to the association between male sterility and B deficiency (Li et al., 1978; da Silva and de Andrade, 1983; Rerkasem et al., 1989).

A simple staining with iodine (in a KI/I_2 solution) may be used for pollen examination (Cheng et al., 1992). Dead pollen shows up misshapened, shrivelled and un-stained by iodine. However, pollen with starch deposits that stain blue black with iodine is not always viable. A more precise assessment of pollen viability may be made with a fluorochromatic (FCR) test or staining with 4'-6-Diamidino-2-phenylindole2HCl (DAPI). Pollen that exhibited an adverse effect of B deficiency in a 30-70% depression in viability by the FCR test was indistinguishable from B sufficient pollen by the iodine stain (Nachiangmai et al., 2002). The adverse effect of B deficiency on pollen viability was also shown in the absence of one or more of the nuclei by the DAPI test. In addition to male fertility, B deficiency has also been shown to depress the fertilization process (Cheng et al., 1993). Grain set was partially restored when B deficient female wheat flowers were fertilized with B sufficient pollen (Rerkasem et al., 1993) and further enhanced by an application of B to the stigma (Rerkasem and Jamjod, 1997).

That male sterility is the primary effect of B deficiency on grain set in wheat has been demonstrated by an increase in grain set in B deficient plants by hand pollinating with fertile pollen (Rerkasem et al., 1993). Anthers and carpel account for a much larger proportion of B content of the ear of wheat relative to their dry weight (Rerkasem and Lordkaew, 1996). Wheat anthers and carpel contain several times the B concentration of the whole ear (Table 2). Grain set failure was associated with less than 10 mg B kg^{-1} in the anthers and 8 mg B kg^{-1} in the carpel (Rerkasem et al., 1997). In contrast, the critical B deficiency concentration for vegetative growth in wheat has been estimated at 1 mg B kg^{-1} dry weight in the youngest open leaf (Huang et al., 1996; Asad et al., 2001) and 3 mg B kg^{-1} dry weight in the whole shoot (Reuter et al., 1997; Asad et al., 2001).

At present it is still unclear what function B is required for in the development of anthers, pollen and carpel that is different from that for somatic tissues including the secondary sexual part of the ear such as rachis, glumes and lemmas. Anthers with lower B contents appeared to have normal tapetum and lignified endothecium (Rerkasem et al., 1997). These were different from Cu deficient anthers, with their amoeboid tapetal cells (Jewell et al., 1988) and absent lignification of the endothecium (Dell, 1981). Pollen abortion has been found to take place some time after uninucleate vacuolar stage, microsporogenesis in B deficient wheat having proceeded normally prior to this (Rerkasem et al., 1997). This indicates the B requirement for the later stage of pollen development after meiotic and at least the earlier stage of mitotic cell division had occurred.

2.3 Whole plant response

To understand whole plant response to a nutrient deficiency, and thus to apply the understanding to field grown crops, it is necessary to consider responses of different growth processes in relation to one another as well as the response of individual processes. The relationship may be in the chronological order in which the processes occur in the life cycle of the plant, and in their relative sensitivity to the deficiency. Irreversible adverse effects that occur earlier may over-ride effects on more sensitive processes or organs that occur later. Boron deficiency causes flower buds to shed in some species, e.g. in apple (*Malus domestica*) (Dong et al., 1997); black gram

(Noppakoonwong et al., 1997) and sunflower (Blamey, 1987). In such cases it may be irrelevant if the development of the male and female gametes are more sensitive to B deficiency if the flower buds had been lost even before meiosis. On the other hand, a greater sensitivity of the development the stamen or carpel or the fertilization process may make one of them the limiting-step if the B deficiency is not severe enough cause prior irreversible damages. A key to understanding B deficiency in wheat appears to be the relative sensitivity of its reproductive process. Published accounts of responses to low B in field grown wheat invariably reported on the effect of B deficiency on male fertility, grain set and grain yield (Table 1). Evidence of adverse effects of low B on vegetative parameters such as straw yield, tiller number, and secondary reproductive organs such as number of spikelets ear^{-1} is rare. In contrast to the effect on male fertility and grain set, B deficiency tended to increase the weight of individual grains. Barley and triticale (x*Triticosecale* Wittmack) are different from wheat in one respect. As well as the grain set failure, B deficient barley and triticale have been reported to have shorter ears (fewer spikelets ear^{-1}), and also delayed ear emergence in the case of barley (Jamjod and Rerkasem, 1999; Pasook, 2000; Wongmo, 2001).

In wheat (Subedi et al., 1997; Pant et al., 1998), along with barley (Ambak and Tandao, 1991; Jamjod and Rerkasem, 1999), the greater requirement for B for reproductive growth than vegetative growth may be indicated by the effect of B deficiency that actually increased tillering while at the same time depressing male fertility and grain set. In addition to the greater functional requirement for B in the anthers and carpel as discussed above, sensitivity to B deficiency of reproductive development in wheat, and the other Triticeae cereals, may be related to B supply to these organs during critical time. Boron is transported from plant roots via the xylem, driven by water potential gradient created by transpiration. The site of the reproductive process most sensitive to B deficiency in wheat along with barley, durum (*Triticum durum* Dest.) and triticale is in the non-transpiring ear, while it develops inside the leaf sheath. The effect of B deficiency on male fertility in wheat may therefore be expected to operate through interrupted supply of B for anthers and pollen development. On the other hand, it is as yet unclear how wheat avoids the effect of B deficiency on terminal spikelets development that causes depression in the number of spikelets ear^{-1} and the rat-tail symptom barley and triticale.

A greater requirement for B in reproductive than vegetative growth has been shown in canola and sunflower (Asad et al., 2002). In these species, however, B deficiency depressed dry weight of vegetative parts along with the flowers, and stamens that were determined in canola. Similarly in maize, adverse effects of B deficiency on vegetative growth were observed along with those on the pollen and anthers (Agarwala et al., 1981). Wheat is different in that male sterility and the associated grain set failure may be the only observable effect of B deficiency. It offers a unique model through which the effect of B deficiency on the primary sexual process may be studied without the confounding effects on other organs and processes.

2.4 Wheat in comparison to other species

Wheat may be judged to be relatively insensitive to B deficiency on the evidence of its early vegetative growth (see above). In farmers' fields, however, wheat is more prone to B deficiency than rice, maize and even some dicotyledons such as soybean

and mungbean. Cases of B deficiency in wheat in south Asia reviewed above were generally reported from areas growing rice-wheat, the region's most common cropping system. Yet B deficiency has never been observed in the associated rice crop. One of the earliest reports of B deficiency in wheat was made on rice-wheat and soybean-wheat cropping systems, in which neither rice nor soybean were deficient (da Silva and de Andrade, 1983). The wheat crop in a rice-wheat cropping system recorded grain set failure and yield losses of 30% to 70% over 3 consecutive years (Rerkasem and Loneragan, 1994). No adverse effect of B deficiency was ever observed in the rice crops in between the wheat seasons.

This difference between wheat and other crops may be based on relative tolerance to low B of the species. It may also reflect seasonal effects on plant response to soil B, since wheat in the subtropics is grown in the coolest months normally to be avoided for rice, soybean, maize and green gram. In non-alkaline soils wheat grain set and grain yield were depressed by the same level of hot water soluble (HWS) B that affected black gram (*Vigna mungo* (L). Hepper), peanut (*Arachis hypogaea* L.) and sunflower (*Helianthus annuus*) (Bell, 1997). This level of soil B, < 0.12 mg HWS B kg^{-1}, had no adverse effect on rice, soybean, green gram (Bell et al., 1990), maize and sorghum (Rerkasem, unpublished). However, the other Triticeae cereals, including barley (*Hordeum vulgare* L.), have shown responses to B in the same order as wheat (Jamjod and Rerkasem, 1999; Rerkasem and Jamjod, 2001).

2.5 Variation due to the environment

The variable nature of plant responses to B is well known and evidence of B deficiency being accentuated by suboptimal environmental conditions such temperature (too low as well as too high), water stress and high light intensity has been reviewed by Shorrocks (1997), adding to management difficulties. Variation in the response to low B among wheat genotypes is well established. On the same soil, some wheat genotypes may set no grain at all because of B deficiency while others set grain normally (see section on genotypic variation below). Waterlogging (Pintasen et al., 1997) and drought (Pant et al., 1998) have both been shown to accentuate B deficiency in wheat. Researchers in Bangladesh have induced "sterility" by shading, waterlogging or fogging (decreasing water vapour deficit), but the symptoms appeared to be very different from those observed in farmers' fields (Saifuzzaman and Meisner, 1996). No evidence was presented to show if these treatments had caused "sterility" by inducing B deficiency. An experiment in controlled environment showed that the effect of B deficiency in wheat was amplified by low light intensity but not high humidity (Rawson and Noppakoonwong, 1996). Many observations indicating an involvement of B in frost resistance have been reviewed, but it is still unclear if these are simply the effect of B deficiency being accentuated by low temperature stress (Shorrocks, 1997). On the other hand, the wheat sterility problem that occurs regularly at high altitudes in Nepal has not responded to B application (Sthapit, 1988; Subedi, 1992).

3. Diagnosis and measuring effects of B deficiency in wheat

A combination of methods including plant symptoms, soil and plant analysis and plant response to applied B has been employed for diagnosis of B deficiency (Bell, 1997). Vegetative symptoms are not very useful in wheat as they are hardly ever seen

in the field. Male sterility, with florets that stay open for many days at anthesis, followed by grain set failure, are the most common symptoms seen in field grown B deficient wheat (Li et al., 1978; da Silva and de Andrade, 1980; Sthapit 1988; Rerkasem et al., 1989; Rerkasem and Loneragan, 1994). However, similar symptoms associated with male sterility are also caused by Cu deficiency (Graham, 1975). In wheat Cu deficiency also causes the rat-tail symptom of the ear which B deficiency does not. It is however necessary to keep in mind that B deficiency does cause the rat-tail symptom in barley and triticale. Further evidence may be found in local knowledge of the history of B deficiency in the area. As it is now clear that wheat can be as prone to B deficiency as some legumes and other dicots, the history of B deficiency in other crops is also useful. In many crop species, however, genotypes have been observed to vary widely in their sensitivity to B deficiency, making the choice genotypes for indicator plants critical. The problem of B deficiency would never have been suspected in Nepal by the appearance and yield of two local lentil varieties 'Simal' and 'Simrik', although it almost completely destroyed an introduced germplasm of some 500 entries (Srivastava et al., 2000). Locally adapted wheat varieties such as Sonora 64 and Fang 60 are not very good indicators because they are extremely well adapted to low B soils (Anantawiroon et al., 1997; Rerkasem et al., 2003).

Available soil B levels found associated with B deficiency in wheat ranged from about 0.1 mg HWS B kg^{-1} in Thailand (Rerkasem and Loneragan, 1994) to 0.3 – 0.4 mg HWS B kg^{-1} in China (Li et al., 1978). As in other crops, the critical level of available soil B is likely to be highly variable. It is influenced by soil characteristics such as pH, moisture and texture (Shorrocks, 1992; Sims and Johnson, 1991), above ground conditions of light, humidity and temperature (Shorrocks, 1997) and genotypes (see below). Incidences of B deficiency in wheat in Bangladesh have been reported to be completely unrelated to the level of HWS B in the soil (Kataki et al., 2001). Wheat has been found to respond to B in areas dominated by soils with toxic levels B such as Pakistan (Rashid et al., 2001). Response to B has been observed in durum wheat in Turkey (Topal et al., 2001), where pockets of low B soils have also be found amongst toxicity levels B (Gezgin et al., 2001).

The critical B concentration for early vegetative growth in wheat is reported to be about 1 mg B kg^{-1} DW (Asad et al., 2001). In contrast, grain set failure in wheat has been found associated with less than 2 mg B kg^{-1} DW in the ear (Rerkasem and Lordkaew, 1992) and 3 mg B kg^{-1} in the flag leaf at boot stage (Rerkasem and Loneragan, 1994). At these concentrations the flag leaf and ear themselves appeared normal. The leaf and ear B concentration are all somewhat imprecise for diagnosis for B deficiency in wheat, for two reasons. The reproductive organs affected by deficiency, the anthers and carpel, account for only 4% of the whole ear dry weight at anthesis, and 10% of its B content (Rerkasem, 1996). Furthermore, the critical B deficiency concentration for the carpel and anthers, at 10-12 mg B kg^{-1} (Rerkasem et al., 1997), are several times the values for these vegetative and secondary reproductive tissues such as the lemma, palae and rachis.

Diagnosis for B deficiency with tissue analysis may be made with the most precision by analysing for B in the anthers. However, sampling for anthers is logistically impossible for use in farmers' fields. It is extremely time consuming, especially with the samples (larger amounts of B) needed for analysis by the colorimetric method

with Azomethine-H of Loshe (1982). The final verification of B deficiency is plant response to apply B. Thus after an observation of massive sterility in wheat in Bangladesh in 1986/87, widespread B deficiency was verified by the average 14% on-farm yield increase by B application in 1987/88 and 10% in 1988/89 (D.A. Saunders, pers. comm.). Although not as effective as B applied to the soil, foliar B may increase grain set significantly (Rerkasem and Jamjod 1988) and may be sufficient for the purpose of confirming B deficiency. A source of error in B response trials is "contamination". Large amounts of B have been found in common N, P and K fertilizers in Asia (Bell et al., 1990; Lordkaew, 1995). Thus B deficiency may be overcome even in the nil B treatment.

The occurrence of wheat sterility in farmer's field in Nepal (Sthapit, 1988; Misra et al., 1992; Tiwari, 1996) and Bangladesh (Saifuzzaman and Meisner, 1996) has often been reported to be highly variable. Confirmation of B deficiency as the cause, however, has sometimes been hindered by imprecise definition of "sterility". Percentage of florets without grain measures the combined success of two different processes, florets development (photosynthetic capacity) and fertilization (grain set). Boron deficiency, on the other hand, specifically affects grain set only (see above). Unless there is a B sufficiency control, grain set failure is more precisely measured with the grain set index (GSI). The GSI is defined as the percentage grain bearing in two basal florets of 10 central spikelets (Rerkasem and Loneragan, 1994). Among florets of a wheat spikelet, the two basal florets are the first to develop, while development of terminal florets is dependent on the supply of photosynthate. The absence of a grain in either or both of the basal florets of a spikelet is therefore indicative of grain set failure. Focusing on central spikelets removes the possibility of a confounding effect of incompletely developed basal and terminal spikelets. Without the need for a B sufficiency control, the GSI is very useful to evaluate responses to low B in nurseries with large entry numbers (Rerkasem and Jamjod, 1997a; Anantawiroon et al., 1997). The extent of B deficiency in the screening environment may be indicated by inclusion of a set of check genotypes covering a range of responses to low B. The GSI is also useful to quantify the problem of grain set failure in wheat, triticale and barley under a wide range of on-farm environmental conditions. The presence of common varieties with known susceptibility to B deficiency allows verification of B deficiency as the likely limiting factor.

4. Adaptation to low B soils

4.1 Genotypic variation

The ability of a genotype to grow and yield well in soils too deficient in a particular nutrient for a standard genotype has been defined as 'nutrient efficiency' (Graham, 1984). This simple definition enables performances of genotypes to be compared experimentally even when the mechanism behind their differences is yet to be explained. Without inferring a mechanism, the ability of a genotype to perform well in soils too deficient in B for other genotypes has been termed B efficiency (Rerkasem and Jamjod, 1997b). Germplasms of oilseed rape have successfully been evaluated for B efficiency based on their performance in low B relative to sufficient B (Xue et al, 1998; Stangoulis et al, 2000).).

Reports of B deficiency in wheat, from Brazil (da Silva and de Andrade, 1983), China (Li et al., 1978), to India (Ganguly, 1979), Nepal (Subedi et al., 1997) and Thailand (Rerkasem et al., 1989), have invariably noted large genotypic variations in the response to B. Variation in B efficiency in wheat is probably the widest possible of any species in response to a deficiency in any nutrient element. Wheat genotypes growing in the same low B condition may range from the most inefficient that set no grain at all to the most efficient that set grain normally (Rerkasem and Jamjod, 1997a; Rerkasem et al., 2003). Boron efficient and inefficient genotypes have been identified in different countries (Table 3). Some of these, which have shown consistent responses to low B, have been used as standard B efficiency checks.

Wheat genotypes have been ranked according to their adaptation to low B soils in into 5 different B efficiency classes (Rerkasem and Jamjod, 1997). The most efficient and most inefficient classes appear to be readily distinguishable by their tissue B concentrations. Growing in the same low B conditions, B concentration in the flag leaf and ear of the very inefficient Tatiara were only about half of those of B efficient Fang 60 (Wongmo et al., 2003). Those belonging to closer B efficiency classes, however, are less readily distinguishable by the concentration of B in their flag leaf (Rerkasem and Loneragan, 1994; Subedi et al., 1999) or ear (Rerkasem and Lordkaew, 1992). Male fertility and grain set are more closely correlated with B concentration in the anthers than in the leaf or secondary reproductive tissues such as the palea or lemmas (Rerkasem et al., 1997). Boron efficient genotypes appear to have escaped male sterility by maintaining anther B above the critical deficiency concentration (Figure 1).

The ability to recycle B from old tissues is a mechanism that enables some plant species to escape deficiency when external supply is low. Unique among the essential plant nutrients, B is freely mobile in the phloem of some species while having restricted mobility in other species, (Brown and Shelp, 1997). Some have suggested that certain wheat genotypes may be more tolerant to B deficiency than others because they are able to meet reproductive demand by their ability to recycle B into growing ears (Rawson, 1996; Subedi et al., 1999; Huang et al., 2001). No evidence of B remobilization has been found in the B efficient Fang 60 which had escaped B deficiency caused by withholding B for five days between premeiotic interphase and young microspore stage (Nachiangmai et al., 2003). The authors were able to trace B supply to the ear by first providing adequate B supply with ^{11}B at 10 μM and then labeling the much lowered supply at 0.1 μM with ^{10}B. They clearly demonstrated that with the lowered external supply to 0.1 μM, inefficient SW41 was unable to maintain adequate B supply to the ear, and so its pollen viability was depressed by 40-70% by B deficiency. In contrast, Fang 60 was unaffected by B deficiency because it was able to meet the demand for pollen development with the 0.1 μM ^{10}B.

4.2 Genetics of B efficiency

Genetic control of B efficiency has been reported for many species (Rerkasem and Jamjod, 1997). For wheat, analysis of B efficiency in F1s from seven crosses of genotypes with different adaptation to low B soils led to a suggestion of additive and dominant gene actions in the control of B efficiency (Jamjod et al., 1992). The F1s by B efficient Fang 60 as male parent and very inefficient female parents showed adaptation to low B soil that was almost identical to Fang 60 (Figure 2). The F2

populations of a Fang 60 x Bonza cross were found to fit the 1:15 ratio of B inefficient (similar to Bonza) to efficient (more efficient than Bonza to similar to Fang 60), suggesting two dominant genes controlling B efficiency (Ngorian, 2000).

For triticale, durum wheat and barley, a transfer of relevant genes from B efficient bread wheat such as Fang 60 will probably be essential. As it is present in the more B efficient bread wheat but not in the less B efficient durum wheat, triticale and barley, the D genome is of great interest as a possible source of genes for increasing B efficiency.

4.3 Boosting wheat production on low boron soils with B efficiency

The world's largest single source of wheat germplasm is the International Maize and Wheat Improvement Centre (CIMMYT). Thousands of advanced breeding lines are introduced into developing countries each year in various yield trials, screening and observation nurseries. Evidence of B inefficiency in the germplasm relative to B efficient Fang 60 (PI/FD//PI/MZ/3/MXP) or Sonora 64 was seen in lines after lines of male sterile genotypes in the various screening nurseries (e.g. the International Bread Wheat Screening Nursery, Semi-Arid Areas Wheat Screening Nursery) and yield trials (e.g. High Temperature Wheat Yield Trial, Elite Selection Wheat Yield Trial) in stations on low B soils of the Asian subtropics such as Nashipur in Bangladesh, Chiang Mai in Thailand and Yunnan in China (Rerkasem unpublished, C.E. Mann and D.A. Saunders, Pers. Comm.). This has been confirmed experimentally (Rerkasem and Jamjod, 2001; Rerkasem et al., 2003.). Some 30-40% of the germplasm set only 1-2 grains ear^{-1} or none at all in the same low B condition in which Fang 60 set grain normally. Most developing countries, including Nepal and Bangladesh, depend on CIMMYT for their new improved wheat cultivars. It is therefore not surprising that wheat varieties and advanced breeding lines from Nepal (Subedi et al., 1997) and Bangladesh (Ahmed et al., 2002) are mostly B inefficient. Similarly, promising lines selected in Thailand that turned out to be susceptible to B deficiency-induced male sterility when they reached on farm trials included ARTC87001 (Junco 'S'/Buc 'S', CM64478, from the 4th Hot Climate Wheat Screening Nursery) and SMGBW88001 (Rerkasem, 1996).

By screening the F2s in low B the author demonstrated the ease of transfer of the B efficiency genes. There is real scope for genetic improvement. The source of genes for B efficiency already exists in bread wheat, especially in advanced breeding lines already incorporating high yielding, disease resistance and other desirable characteristics. Screening can be done in the field on soil with low B or in a simple sand culture (Anatawiroon et al., 1997; Rerkasem et al., 2003). Many of the B efficient genotypes identified were advanced breeding lines, i.e. ready to be released as cultivars, that were included in CIMMYT international yield nurseries such as the 4HTWYT and 17ESWYT.

REFERENCES

Ambak, K. and Tadano, T. 1991 Effect of micronutrient application on the growth and occurrence of sterility in barley and rice in a Malaysian deep peat soil. Soil Sci. Plant Nutr. 37, 715-724.

Asad, A., Bell, R.W. and Dell, B. 2001. A critical comparison of the external and internal boron requirements for contrasting species in boron-buffered solution culture. Plant Soil 233, 31-45.

Asad, A., Blamey, F.P.C. and Edwards, D.G.. 2002. Dry matter production and boron concentrations of vegetative and reproductive tissues of canola and sunflower plants grown in nutrient solution. Plant Soil 243, 243-252.

Bell, R.W., Rerkasem, B., Keerati-Kasikorn, P., Phetchawee, S., Hiranburana, N., Ratanarat, S., Pongsakul, P. and Loneragan, J.F. 1990. Mineral Nutrition of Food Legumes in Thailand, with particular reference to micronutrients. ACIAR Technical Reports No. 16, Australian Centre for International Agricultural Research, Canberra, 52 p.

Bell, R.W. 1997. Diagnosis and prediction of boron deficiency for plant production. Plant and Soil 193,149-168.

Blamey, F.P.C., Edwards, D.G. and Asher, C.J. 1987. Nutritional Disorder of Sunflower. Department of Agriculture, University of Queensland, St. Lucia, Queensland. 72 p.

Brown, P.H. and Shelp, B.J. 1997. Boron mobility in plants. Plant Soil 193, 85-101

Chapman, F.P.C., Edwards, D.G., Blamey, F.P.C. and Asher, C.J. 1997. Challenging the dogma of a narrow supply range between deficiency and toxicity of boron. In: Bell, R.W. and Rerkasem, B. (Eds.), Boron in Soils and Plants, Kluwers Academic Publishers, Dordrecht, pp. 151-155.

Cheng, C., McComb, J.A. and Rerkasem, B. 1992. Techniques to study the anther in wheat. In Mann, C.E. and Rerkasem, B. (Eds.), Boron Deficiency in Wheat, Wheat Special Report No. 11, CIMMYT. Mexico, D.F., pp. 32-33.

Cheng, C. and Rerkasem, B. 1993. Effects of boron on pollen viability in wheat. Plant and Soil 155/156, 313-315.

da Silva, A.R. and de Andrade, J.M.V. 1980. A Cultura do Trigo nas Varzeas de Minas Gerais - possibilidades e dificuldades. Embrapa, Centro de Pesquisa Agropecuária dos Cerrados - CPAC. Circular Técnica no. 2. 69 p. (In Portuguese)

da Silva, A.R. and de Andrade, J.M.V. 1983. Efeito micronutrientes no chochamento do trigo de seqqueiro e nas culturas de soja e arroz, em latossolo vermelho-amarelo. [Influence of micronutrients on the male steriltiy, on upland wheat and on rice and soybean yields, in red-yellow latosol] Pesq. agropec. bras., Brasilia 18:593-601. (In Portuguese with English Abstract)

Dell, B. 1981 Male sterility and anther wall structure in copper-deficient plants. Ann. Bot. 48, 599-608.

Dell, B. and Huang, L. 1997. Physiological response of plants to boron. Plant and Soil 193, 103-120.

Dong, R.H., Noppakoonwong, R.N., Song, X.M. and Rerkasem, B. 1997. Boron and fruit quality of apple. In: Bell, R.W. and Rerkasem, B. (Eds.), Boron in Soils and Plants, Dordrecht, The Netherlands: Kluwer Academic Publishers, pp. 125-130.

Dugger, W.M. 1983. Boron in plant metabolism. In: A. Läuchli and R.L. Bieleski (Eds.), Encyclopedia of Plant Physiology, New Series vol. 15B,. Springer-Verlag, Berlin, pp. 626-650.

Dwivedi, G.K., Dwivedi, M. and Pal, S.S. 1990. Modes of application of micronutrients in acid soil in soybean-wheat crop sequence. J. Indian Soc. Soil Sci. 38, 458-463.

Ganguly, B. 1979. Note on seedlessness in some wheat varieties caused by boron deficiency. Indian J Agric. Sci. 43, 384-386.

Gezgin, S., Dursun, N., Hamurcu, M., Harmankaya, M., Önder, M., Sadea, B., Topal, A., Soylu, S., Akgün, N., Yorgancilar, M., Ceyhan, E., Çiftçi, N., Acar, B., Gültekin, I., Işik, Y., Şeker, C. and Babaoglu, M. 2002. Boron content of cultivated soils in central-southern Anatolia and its relationship with soil properties and irrigation water quality. In: Goldbach, H. E., Rerkasem, B., Wimmer, M. A., Brown, P. H., Thellier, M., and Bell, R.W. (Eds.), All Aspects of Plant and Animal Boron Nutrition. Kluwer and Plenum Academic Publishers, Dordrecht, The Netherlands, pp. 391-400.

Gupta, U.C. 1979. Boron nutrition of crops. Adv. Agron. 31, 273-307.

Huang L.B., Pant J., Bell R.W., Dell B. and Deane K. 1996. Effects of boron deficiency and low temperature on whet sterility. Sterility in Wheat in Subtropical Asia ACIAR Proceedings 72, 90-101.

Huang, L.B, Bel,l R.W., Dell, B. 2001. Boron supply into wheat (*Triticum aestivum* L. cv. Wilgoyne) ears whilst still enclosed within leaf sheath. J. Exp.Bot. 52, 1731-1738.

Jamjod, S. and Rerkasem B. 1999 Genotypic variation in responses of barley to boron deficiency. Plant Soil 215, 65-72.

Jewell, A.W., Murray, B.G. abd Alloway, B.J. 1988. Light and electron microscope studies on pollen development in barley (*Hordeum vulgare* L.) grown under copper-sufficient and deficient conditions. Plant Cell Env. 11, 273-281.

Kataki, P.K., Srivastava, S.P., Saifuzzaman, M. and Upreti, H.K. 2002. Sterility of wheat and response of field crops to applied boron in the Indo-Gangetic Plains. J. Crop Prod. 4, 133-165.

Lamb, C.A. 1967. Physiology. In: Quisenberry, K.S. and Reitz, L.P. (Eds.) Wheat and Wheat Improvement. Agronomy Monograph 13. ASA, Madison, WI., pp. 181-223.

Li, B. Li, H., Kui, W.H., Chao, M.C., Jern, W.S., Li, H.P., C R, Chu W J and Wang C L

Li, B.H., Li, W.H., Kui, M.C., Chao, W.S., Jern, H.P., Li, C.R., Chu, W.J. and Wang, C.L. 1978. Studies on cause of sterility of wheat. Journal of Northeastern Agricultural College 3, 1-19. (In Chinese, with English translation)

Lordkaew, S. 1995. Boron contamination in fertilizers. In: Bell, R. W. and Rerkasem, B. (eds.), Causes of sterility in wheat, Environmental Science Report 94/6. Murdoch University, Perth, Australia, pp. 150-153.

Rashid, A., S. Muhammad, and E. Rafique. 2002. Gynotypic variation in boron uptake and utilization in rice and wheat. In: Goldbach, H.E., Rerkasem, B., Wimmer, M.A., Brown, P.H., Thellier, M. and Bell, R.W., All Aspects of Plant and Animal Boron Nutrition, Kluwer and Plenum Academic Publishers, pp. 305-310.

Mandal, A.B. and Das, A.D. 1988. Response of wheat (Triticum aestivum) to boron application. Indian J Agric. Sci. 58, 681-683.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Marten, J.M. and Westermann, D.T. 1991. Fertilizer applications for correcting micronutrient deficiencies. In: Mordtvedt, J.J. et al. (Eds.) Micronutrients in Agriculture. SSSA Book Series no. 4. SSSA, Madison, WI, pp. 549-592.

Misra, R., Munankarmi, R.C., Pandey, S.P. and Hobbs, P.R. 1992. Sterility work in wheat at Tarahara in the eastern Terai of Nepal. In: Mann, C.E. and Rerkasem, B. (Eds.), Boron Deficiency in Wheat, Wheat Special Report No. 11, CIMMYT, Mexico, D.F., pp. 67-71.

NaChiangmai, D., Dell, B., Huang, L., Bell. R.W. and Rerkasem B. 2002. The effect of boron on pollen development in two wheat cultivars (*Triticum aestivum* L.). In: Goldbach, H.E., Rerkasem, B., Wimmer, M.A., Brown, P.H., Thellier, M. and Bell, R.W. All Aspects of Plant and Animal Boron Nutrition, Kluwer and Plenum Academic Publishers, pp. 181-185.

Noppakoonwong, R.N., Rerkasem, B. , Bell, R.W., Dell, B. and Loneragan, J.F.1997. Diagnosis and prognosis of boron deficiency in black gram (*Vigna mungo* L. Hepper) in the field by using plant analysis. In: Bell, R.W. and Rerkasem, B. (Eds.), Boron in Soils and Plants, Dordrecht, The Netherlands: Kluwer Academic Publishers, pp. 89-94

Pant, J., Rerkasem, B. and Noppakoonwong, R. 1998. Effect of water stress on the boron response of wheat genotypes under low boron field conditions. Plant Soil 202, 193-200.

Phasook, T. 2001. Boron Efficiency in Two Barley Genotypes. M.Sc. Thesis (Agronomy). Graduate School, Chiang Mai University.

Pintasen, S., Rerkasem, B. and Jamjod, S. 1997. Effects of waterlogging and boron on sterility in wheat. In: Julsrigaival, S. (Ed.), Proceedings of the Thailand International Temperate Cereals Conference, December 11-13, 1997, Chiang Mai University, pp. 217-225.

Rashid, A., Muhammad, S. and Rafique, E. 2000. Genotypic variation in rice susceptibility of boron deficiency. International Rice Research Notes 25, 29-30.

Rawson, H.M. 1996. The developmental stage during which boron limitation causes sterility in wheat genotypes and the recovery of fertility. Aust. J. Plant Phys. 23, 709-717.

Rawson, H.M. and Noppakoonwong, R.N. 1996. Effects of boron limitation in combination with changes in temperature, light and humidity on floret fertility in wheat. In: Rawson, H.M. and Subedi, K.D. (Eds.), Sterility in Wheat in Subtropical Asia: extent, causes and solution, ACIAR Proceedings No. 72, 85-89.

Rerkasem, B. 1996. Boron and plant reproductive development. In: Rawson, H.M. and Subedi, K.D. (Eds.), Sterility in Wheat in Subtropical Asia: extent, causes and solution, ACIAR Proceedings No. 72, 32-35.

Rerkasem, B. and Jamjod, S. 1989. Correcting boron deficiency induced ear sterility in wheat and barley. Thai J. Soils and Fert. 11, 200-209.

Rerkasem, B. and Jamjod, S. 1997. Boron deficiency induced male sterility in wheat (*Triticum aestivum* L.) and implications for plant breeding. Euphytica 96, 257-262.

Rerkasem, B. and Jamjod, S. 2001. Overcoming wheat sterility problem with boron efficiency. In: Horst, W. et al. (Eds.), Dev. Plant Soil Sci. 92, 82-83.

Rerkasem, B. and Lordkaew, S. 1992. Predicting grain set failure with tissue boron analysis. In: Mann, C.E. and Rerkasem, B. (Eds.), Boron Deficiency in Wheat, Wheat Special Report No. 11, CIMMYT, Mexico, D.F., pp. 9-14.

Rerkasem, B. and Loneragan, J.F. 1994. Boron deficiency in two wheat genotypes in a warm, subtropical region. Agron. J. 86, 887-890.

Rerkasem, B. and Lordkaew, S. 1996. Tissue boron. In: Rawson, H.M. and Subedi, K.D. (Eds.), Sterility in Wheat in Subtropical Asia: extent, causes and solution, ACIAR Proceedings No. 72, 36-38.

Rerkasem, B., Lordkaew, S. and Dell, B. 1997 Boron requirement for reproductive development in wheat. Soil Sci. Plant Nutr. 43, 953-957.

Rerkasem, B., Saunders, D.A. and Dell, B. 1989. Grain set failure and boron deficiency in wheat in Thailand. J. Agric. (CMU) 5, 1-10.

Rerkasem, B., Netsangtip, R., Lordkaew, S. and Cheng, C. 1993. Grain set failure in boron deficient wheat. Plant and Soil 155/156, 309-312.

Reuter, D.J. 1987. An Assessment of Wheat Nutrition Research in Bangladesh. A consultancy report for CIMMYT and CIDA. SAGRIC International. 45 p.

Reuter, D.J., Edwards, D.G. and Wilhelm, N.S. 1997. Temperate and sub-tropical crops. In: Reuter, D.J. and Robinson, J.B. (Eds.), Plant Analysis – An Interpretation Manual, CSIRO Publishing, Australia, pp. 81-284.

Saifuzzaman, M. and Meisner, C.A. 1996. Wheat sterility in Bangladesh: an overview of the problem, research and possible solutions. In: Rawson, H.M. and Subedi, K.D. (Eds.), Sterility in Wheat in Subtropical Asia: extent, causes and solution, ACIAR Proceedings No. 72, pp. 104-108.

Sarkar, A.K. and Chakraborty, S. 1980. Effect of micronutrient on yield of major field crops grown in the Gangetic delta region. Indian Agric 24, 259-266.

Shelp, B.J. 1993. Physiology and biochemistry of boron in plants. In: Gupta, U.C. (Ed.), Boron and Its Role in Crop Production, CRC Press, Boca Raton, FL., USA, pp. 53-85.

Shorrocks, V.M. 1997. The occurrence and correction of boron deficiency. Plant and Soil 193, 121-148.

Singh, R.P., Sinha, S.D. and Prasad, R.B. 1976. Effect of boron on seed setting in wheat under North Bihar conditions. Indian J. Agron. 21, 100-101.

Snowball, K. and Robson, A.D. 1983. Symptoms of Nutrient Deficiencies: Subterranean Clover and Wheat. Department of Soil Science and Plant Nutrition, University of Western Australia.

Srivastava, S. P., Bhandari, T. M. S., Joshi, M. and Erskine, W. 2000. Boron deficiency in lentil: yield loss and geographic distribution in a germplasm collection. Plant Soil 219, 147-151.

Sthapit B.R. 1988. Studies on Wheat Sterility Problem in the Hills, Tar and Tarai of Nepal. Technical Report No. 16/88. Lumle Agricultural Research Centre, Pokhara, Kaski, Nepal.

Subedi K.D. 1992. Wheat sterility in Nepal - a review. In: Mann, C.E. and Rerkasem, B. (Eds.), Boron Deficiency in Wheat, Wheat Special Report No. 11, CIMMYT, Mexico, D.F., pp. 57-64.

Subedi, K.D., Budhathoki, C.B. and Subedi, M. 1997. Variation in sterility among wheat (*Triticum aestivum*) cultivars in response to boron deficiency in Nepal. Euphytica 95, 21-26.

Subedi, K.D., Gregory, P.J. and Gooding, M.J. 1999. Boron accumulation and partitioning in wheat cultivars with contrast tolerance to boron deficiency. Plant Soil 214, 141-152.

Tiwari, T.P. 1996. Review of research into wheat sterility: the experience of Pakhribas Agricultural Centre. In: Rawson, H.M. and Subedi, K.D. (Eds.),

Sterility in Wheat in Subtropical Asia: extent, causes and solution, ACIAR Proceedings No. 72, 112-118.

Topal, A., S. Gezgin, N. Akgün, N. Dursun and M. Babaoglu. 2002. Yield and yield attributes of durum wheat (*Triticum durum* Desf.) as affected by boron application. In: Goldbach, H.E., Rerkasem, B., Wimmer, M.A., Brown, P.H., Thellier, M. and Bell, R.W., All Aspects of Plant and Animal Boron Nutrition, Kluwer and Plenum Academic Publishers, pp. 401-406.

Warington, K. 1923. The effect of boric acid and borax on the broadbean and certain other plants. Ann. Bot. 37, 457-466.

Wongmo, J. 2001. Comparative Response to Boron in Wheat and Barley. M.Sc. Thesis (Agronomy). Graduate School, Chiang Mai University.

Yang, Y.H. 1992. Wheat boron deficiency in Yunnan, China. In: Mann, C.E. and Rerkasem, B. (Eds.), Boron Deficiency in Wheat, Wheat Special Report No. 11, CIMMYT, Mexico, D.F., pp. 72-75.

Table 1. Responses to boron in wheat.

Responsive Parameters	% max†	Non-responsive parameters	References
Seed yield	51-61	Straw yield	Rerkasem et al (1989)
Grains spikelet^{-1}	25-73	Ears m^{-2}	
mg seed^{-1}	106-121‡	Spikelets ear^{-1}	
Seed yield	25-65	Straw yield	Rerkasem and
Grains spikelet^{-1}	21-57	Ears m^{-2}	Loneragan (1994)
Grain set index	31-73	Spikelets ear^{-1}	
mg seed^{-1}	123-141‡		
Seed yield	3-75	-	Subedi et al. (1997)
Grains ear^{-1}	9-79		
mg seed^{-1}	121-164‡		
Ears m^{-2}	95-161‡		
Seed yield	62-88	Above ground	Pant et al. (1998)
% ears with grain	63-79	biomass	
Grains ear^{-1}	40-85	Plant height	
Grain set index	66-75	Spikelets ear^{-1}	
mg seed^{-1}	120-130‡		
Ears m^{-2}	99-116‡		
Grains ear^{-1}	9-67	Florets ear^{-1}	Subedi et al. (1999)
Pollen viability	15-78	Dry weight of roots, stems, leaves	

† Relative to performance in B sufficiency

‡ Increased in low B

Table 2. Boron concentration in different parts of the wheat ear at ear emergence and anthesis in five wheat cultivars.

Genotype	Just emerged		Anthesis	
	Whole ear	Anthers	Whole ear	Carpel
	mg B kg^{-1}			
SW41	2.6	14.0	2.6	6.8
Glenaro 81	3.7	14.3	2.7	9.2
Glennson	2.9	18.0	2.5	8.9
Nesser	3.4	16.7	3.8	10.9
Seri 82	3.5	15.3	2.7	6.7
SE	0.6	5.2	0.7	2.0

Each number is mean of samples from three replicated plots of field grown plants.
Source: Rerkasem (1995).

Table 3. Boron efficiency of selected Asian wheat varieties and genotypes.

Country of origin	Efficiency	Reference
Bangladesh	Efficient: **(Fang 60)**[1]	Ahmed et al., 2002
	Inefficient: Kanchan, Gourab, E12, Sourav, **(SW41)**[2]	
	Inefficient: Ananda, Aghrani, Balaka, Inia 66, Kanchan, Kalyasona, Sonalika, Veery	Rerkasem unpublished
China	Inefficient: Saric F70, Tanori F71, Chapingo, Spring 980[3]	Yang, 1992
	Efficient: **Sonora 64**	
India	Inefficient: Janak, UP262, BW5, BW11, BW43	Reuter, 1987
	Inefficient: HP1102, HP1209, HD2285, HUW206	Tandon and Naqvi, 1992
	Efficient: HD2307, HDR77, C 306	
Nepal	Inefficient: **(SW41)**, BL1022, SW23	Subedi et al., 1993
	Efficient: **(Fang 60, Sonora 64)**	
	Inefficient: BL1022, Annapurna 4, Annapurna 3, BL1135, RR21, **(SW41)**	Subedi et al., 1999
	Efficient: BL1249, Nepal 297, **(Fang 60)**	
Thailand	Inefficient: Seri, Kauz, **SW41**, SW23	Jamjod et al., 1992
	Efficient: **Fang 60**, #144[4]	
	Inefficient: **SW41**	Rerkasem and Loneragan, 1994
	Efficient: **Sonora 64**	
	Inefficient: **SW41**, Bonza[5]	Wongmo, 2001
	Efficient: **Fang 60**	

[1]Boron efficicent check (PI/FD//PI/MZ/3/MXP), locally known as 1015 before release as Fang 60 in 1987 in Thailand.
[2] Boron inefficient check (Baya/Emu)
[3]Ald/Pci
[4]Another selection from PI/FD//PI/MZ/3/MXP
[5] Australian variety

Table 4. Effects of boron fertilizer on wheat line SW41

Boron Treatment†	Seed yield (kg ha^{-1})	Ears m^{-2}	Spikelets ear^{-1}	Grains spikelet^{-1}	Grain weight (mg)	Harvest Index (%)
Nil	531	270	15.5	0.49	42	10.4
Soil	2913	290	15.1	2.36	35	44.8
Foliar(31)‡	906	272	15.1	1.26	41	19.2
Foliar(39)	1144	290	14.3	1.23	43	22.3
Foliar(49)	1325	312	14.8	1.47	40	22.1
Foliar(52)	838	304	14.8	0.97	41	17.1
Foliar(54)	863	314	15.5	10.8	41	16.8
Foliar(57)	1238	316	14.6	1.18	41	20.2
LSD ($p < 0.05$)	594	NS	NS	0.4	3	9.0

† Soil application before sowing, with borax at 1 kg B ha^{-1}. Foliar application of at 50 g B ha^{-1} (as borax in 0.05% solution, w/v)

‡ Time of foliar application in brackets, in days from sowing: 31, tillering; 39, stem elongation; 49, flag leaf visible; 52, flag leaf emerged; 54, boot stage; 57, anthesis.

Source: Rerkasem and Jamjod, 1989.

Table 5. B efficiency in F3 populations from Fang 60 x Bonza crosses selected under B deficiency and sufficiency.

GSI in B0†	Selected in		
	BL	B0	B+
= Bonza	25.0	20.8	41.7
> Bonza	75.0	79.2	58.3
= Fang	29.2	29.2	16.7
Fang 60	85.9	78.9	81.9
Bonza	0	0	0

† $P < 0.05$

Source Ngorian (2000)

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