

รายงานวิจัยฉบับสมบูรณ์

ความแปรปรวนทางพันธุกรรมของลักษณะการเป็นหมัน เนื่องจากขาดธาตุโบรอนในข้าวบาร์เลย์

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FINAL REPORT

GENOTYPIC VARIATION IN BORON DEFICIENCYINDUCED MALE STERILITY IN BARLEY

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กิตติกรรมประกาศ

ผู้วิจัยขอขอบคุณ สำนักงานกองทุนสนับสนุนการวิจัยเป็นอย่างสูง ที่ให้ทุนสนับสนุนงาน วิจัยหลังปริญญาเอกในครั้งนี้

ขอขอบคุณศูนย์วิจัยเพื่อเพิ่มผลผลิตทางเกษตรและภาควิชาพืชไร่ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ ที่ให้ความอนุเคราะห์ด้านสถานที่ แปลงทดลอง และเครื่องมือวิทยาศาสตร์ที่ ใช้ในการวิจัย

เมล็ดพันธุ์ข้าวบาร์เลย์ที่ใช้ในโครงการวิจัยนี้ได้รับการอนุเคราะห์จาก ศูนย์วิจัยการปรับปรุง ข้าวโพดและข้าวสาลีนานาชาติ (CIMMYT) คุณสุชีรา มูลศรี สถานีทดลองข้าวไร่และธัญพืชเมือง หนาว สะเมิง กรมวิชาการเกษตร รองศาสตราจารย์สุทัศน์ จุลศรีไกวัล ภาควิชาพืชไร่ คณะ เกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ และ ดร. จันทรวิภา ธนะโสภณ บริษัทที่ซีซีการเกษตร ขอ ขอบคุณ คุณสิทธิชัย ลอดแก้ว ศูนย์วิจัยเพื่อเพิ่มผลผลิตทางเกษตร มหาวิทยาลัยเชียงใหม่ ที่ให้ ความช่วยเหลือด้านการวิเคราะห์ธาตุโบรอน

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ความแปรปรวนทางพันธุกรรมของลักษณะการเป็นหมันเนื่องจากขาดธาตุ

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วัตถุประสงค์:

เพื่อบ่งชี้และประเมินความแตกต่างทางพันธุกรรมในลักษณะการเป็นหมัน

เนื่องจากขาดธาตุโบรอน

ขอบเขตการวิจัย: ศึกษาลักษณะการดอบสนองของพันธุกรรมข้าวบาร์เลย์ต่อระดับธาตุโบรอนใน สภาพ sand culture และแปลงทดลอง ประเมินขอบเขตของความหลากหลายทางพันธุกรรมในการ ตอบสนองต่อการขาดธาตุดังกล่าว คัดเลือกสายพันธุ์ที่มีประสิทธิภาพการใช้ธาตุโบรอนแตกต่างกัน มาศึกษาเปรียบเทียบและสร้างลูกผสมชั่วที่ 1 ในขั้นสุดท้าย เปรียบเทียบความสามารถของลูกผสมชั่วที่ 1 และสายพันธุ์พ่อแม่เมื่อปลูกในสภาพโบรอนระดับต่าง ๆ

ผลที่ได้รับ: การขาดธาตุโบรอนทำให้การติดเมล็ตและจำนวนช่อดอกของข้าวบาร์เลย์ลดลงและส่ง ผลให้ผลผลิตลดตามไปด้วย พบความแตกต่างทางพันธุกรรมในลักษณะประสิทธิภาพการใช้ธาตุโบรอนแต่ข้าวบาร์เลย์ส่วนใหญ่มักมีประสิทธิภาพการใช้ธาตุโบรอนด่ำ อย่างไรก็ตามพบข้าว บาร์เลย์จำนวน 2 สายพันธุ์แสดงประสิทธิภาพการใช้ธาตุโบรอนได้สูง และลักษณะประสิทธิภาพ การใช้ธาตุโบรอนสูงนี้ถูกควบคุมโดยพันธุกรรมสามารถถ่ายทอดไปยังรุ่นต่อไปได้

สรุปสาระสำคัญของผลที่ได้จากการวิจัย: พบแหล่งของความแปรปรวนทางพันธุกรรมในลักษณะ ประสิทธิภาพการใช้ธาตุโบรอนสูงในข้าวบาร์เลย์ สามารถหลีกเลี่ยงปัญหาผลผลิตลดลงเนื่องจาก การเป็นหมันเมื่อปลูกในสภาพธาตุโบรอนต่ำๆ ได้โดยการคัดเลือกและปรับปรุงพันธุ์เพื่อประสิทธิ ภาพการใช้ธาตุโบรอน สายพันธุ์ BRB 9604 และ BRB 9624 สามารถใช้แนะนำปลูกในพื้นที่ที่มี ธาตุโบรอนต่ำๆ หรือใช้เป็นสายพันธุ์พ่อแม่ในโครงการปรับปรุงพันธุ์ต่อไป

ข้อเสนอแนะสำหรับงานวิจัยในอนาคต: น่าจะได้มีการศึกษาจำนวนยืนที่ควบคุมประสิทธิภาพ การใช้ธาตุโบรอนสูงและตำแหน่งของยืนบนโครโมโซม ความเข้าใจในเรื่องจำนวนยืนจะเป็น ประโยชน์ในการตัดสินใจเลือกใช้ชนิดวิธีการคัดเลือกและปรับปรุงพันธุ์เพื่อประสิทธิภาพการใช้ โบรอน หากทราบตำแหน่งยืนจะเป็นประโยชน์ในการสร้างแผนที่ยืน ซึ่งจะช่วยเพิ่มประสิทธิภาพใน การคัดเลือกโดยใช้ลักษณะหรือยืนบ่งชื้อื่นๆ ที่อยู่ใกล้กันเข้ามาช่วยในการคัดเลือกได้แม่นยำยิ่งขึ้น

คำหลัก: ข้าวบาร์เลย์ ชาตุโบรอน ความแปรปรวนทางพันธุกรรม

Abstract

Project Code:

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Project Title:

Genotypic variation in boron deficiency-induced sterility in barley

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Project Period:

1 August 1997- 31 July 2000

Objectives:

To identify and evaluate genetic variation in boron response in barley.

Methodology: Boron (B) responses of barley genotypes were identified in sand culture and in the field. Genotypic variation in the response to B was determined. Genotypes with different levels of B efficiency were selected and evaluated. Selected genotypes were also used as parents to produce F₁ hybrids. F₁ hybrids were then evaluated for their response to B, compared to their respective parents.

Results: Grain yield of barley was severely depressed by B deficiency through reduction of grain set and spikelet number. Genotypic variation in B efficiency was identified, with most genotypes fell into the moderately B inefficient and inefficient classes. Two lines were identified as B efficient. Response of F₁ hybrids between B efficient and inefficient genotypes indicated that B efficiency in barley is a heritable trait.

Discussion & Conclusion: Sources of genotypic variation for B efficiency are available in barley. Grain yield reduction caused by sterility under B deficiency can be prevented by selection and breeding for B efficiency. Two advanced lines, BRB 9604 and BRB 9624, may be recommended for low B areas or used as parents in breeding programmes.

Suggestion/ Further Implication/ Implementation: The number of gene(s) controlling B efficiency and their chromosomal locations should be identified. This will assist in determining appropriate strategies for breeding and selecting for B efficiency. Identifying chromosomes controlling B efficiency would help in establishing linkage map, thus allowing a closely linked marker to be identified and used for selection.

Key words: Barley, boron, Hordeum vulgare, genotypic variation

1 EXECUTIVE SUMMARY

Project Code: PDF/74/ 2540

Project Title: Genotypic variation in boron deficiency-induced sterility in barley

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Project Period: 1 August 1997- 31 July 2000

Objectives: To identify and evaluate genetic variation in boron response in

barley.

Yield reduction due to sterility is a major obstacle to expansion of temperate cereals area in Thailand. Boron (B) deficiency has been identified as one of the major causes of sterility. This project set out to evaluate genotypic variation in responses to B. It was found that B deficiency depressed grain number and grain yield in most barley tested. In the most severe case, B deficiency also promoted late tillering but depressed number of spikes plant and spikelets spike. We have developed an index to measure grain set in barley genotypes, termed Barley Grain Set Index (BGSI). It has been used as a selection criteria for B efficiency. About 300 genotypes from various breeding stations were tested in low B soil and sand culture. A wide range of genotypic variation in responses to B, comparable to that found in wheat, has been found. Sources of B efficiency were identified from two advanced lines, BRB 9604 and BRB 9624, selected in Northern Thailand. At low B soils, grain set and grain yield of these lines were not affected while those of inefficient genotypes were severely depressed. This suggested that these two lines could be recommended in low B areas. BRB 9604 was used to study inheritance of B efficiency by crossing to the moderately inefficient and inefficient genotypes. Response of F₁ hybrids indicated that B efficiency was a heritable trait and non-maternal controlled. B efficiency in F₁ hybrids was expressed within a range from nearly complete dominance to nearly complete recessive depended on cross combination and severity of B deficiency. These findings provide a basis for screening segregating populations in genetic studies and breeding programmes serving low B soils such as Northern Thailand.

2. BACKGROUND AND OBJECTIVES

2.1 Background

Economic importance of barley (*Hordeum vulgare* L.) is indicated by imported value exceeding one billion baht a year. Expansion of barley production is an objective in the National Agricultural Development Plan. However, severe sterility has been observed in barley grown so far in the country. Boron (B) deficiency was identified as the cause of this problem in Chiang Mai, Thailand (Rerkasem and Jamjod, 1989), Finland (Simojoki, 1972) and Malaysia (Ambak and Tadano, 1991). For example, yield of two barley varieties BRB 1 and BRB 2 grown at low B soil in Chiang Mai were reduced more than 50% by B deficiency (Rerkasem and Jamjod, 1989).

A number of studies suggested that B plays a significant role in reproductive development, for example, flower development and fertilization processes. In general, B deficiency symptoms of small grain cereals such as wheat (Rerkasem et al., 1987), barley (Simojoki, 1972) and rice (Garg et al., 1979) were observed on reproductive organ, resulted in grain set failure. Boron deficiency symptom of wheat was described as sterility, which can be seen at anthesis. Florets remain open, resulting in 'gaping glume' in which spikes are transparent appearance when viewed with the sun behind them. The anthers and pollens of these sterile plants were poorly developed (Li et al., 1978; Sthapit, 1988; Rerkasem et al., 1989).

In barley, Simojoki (1972) reported that sterility in barley has been observed when grown in soil with water-soluble B 0.1-0.2 mgL⁻¹ and an application of B fertilizer was able to correct the problem. The symptoms of B deficiency in barley were described as an abnormal of pollen and stamen, swelling of ovary, openness of spikelets at early flowering stage. Symptom was more severe on tillers than main stem. The B deficient spikes were proned to be infected by ergot fungus as the ears remain open. Ambak and Tadano (1991) reported that when barley cultivar Hoshimasaii was grown in micronutrient-deficient peat soil, the highest percentage of sterility was caused by B deficiency in this soil and the depression on vegetative was not observed. Rerkasem and Jamjod (1989) reported that the symptoms of B deficiency in two barley cultivars, BRB 1 and BRB 2, were observed only as sterility of the cars but not on the vegetative part when grown in soils

with hot water soluble B at 0.13 mg kg⁻¹. However, when grown in nutrient solution, Nable et al. (1990) reported that B deficiency symptom of barley can be observed on leaf blades, described as wrinkled, mishapen, irregularly chlorotic and occasionally had split margins "saw-tooth edges" and longitudinal splits.

Genotypic variation for response to low B has been reported in several crops (Rerkasem and Jamjod, 1997) suggesting that the widespread B deficiency in barley could be managed by growing B efficient cultivars. In wheat, Jamjod et al. (1992a) screened germplasm originating from CIMMYT and adapted to northern Thailand, were among the most B efficient. Further screening has established the response to low B when tested in sand culture without add B compared to add B. Altogether, 252 genotypes, collected from Thailand, Bangladesh, Nepal, China, India, South America, Australia and CIMMYT, were screened and classified into five distinctive classes including very inefficient, inefficient, moderately inefficient, moderately efficient and efficient. In this screening only nine genotypes, seven from Thailand and two from China and India each were identified as B efficient (Rerkasem and Jamiod, 1997). At very low B the very inefficient genotypes were completely sterile while the B efficient genotypes set grain normally.

Genetic control of response to B deficiency has been studied for a number of species. Response to B deficiency in celery (Pope and Munger, 1953), tomato (Wall and Andrus, 1962) and red beet (Tehrani et al., 1971) was reported to be under monogenic control. However, Kelly and Gabelman (1960) found that the genetic control of B efficiency in table beets was complex. In sunflower, Blamey et al. (1984) found that general combining ability effects were significant for the concentration of B in leaf, deficiency symptom and grain yield. These three characters were highly heritable and additive or additive epistatic gene action predominated. In genetic studies with two crosses between B inefficient x B efficient wheat genotypes, broadsense and narrow sense heritability for B efficiency were 0.50-0.58 and 0.35-0.42, respectively (Jamjod et al., 1992b), suggested a high possibility of breeding and selection for B efficiency trait in this crop.

In contrast to wheat, there is no information of genetic variation in response to low B in barley. Extensive studies with the mechanism and genetics of response to B in barley have been done with B toxicity (Nable et al., 1997). As barley is becoming an important crop to agricultural and industrial production of Thailand, it is therefore important to study the incident of B deficiency-induced sterility in this crop. Understanding genotypic

variation in B efficiency and its inheritance will assist in breeding and selection for B efficient varieties to grow in problem areas

2.2 Objectives

The objective of this project was to increase yield of barley by preventing sterility through breeding and selection for B efficiency when grown in low B areas through:

- Identification of genetic variation and source of B efficiency;
- Development of screening procedures for large number of genotypes;
- Identification of inheritance for B efficiency.

3. MATERIALS AND METHODS

3.1 Sand culture experiments

In sand culture plants were grown in freely drained earthenware pots (30cm diameter and 30cm deep) or trays (45cm x 70cm x 35cm) contained washed river quartz sand with no detectable available B. The sand was watered twice daily with an otherwise complete nutrient solution (Table 3.1) with varying levels of boron supply (0, 0.1 or 1.0 μ M B, designated as B0, B0.1 and B1.0, respectively) in three replications. The pots/trays were flushed with water once every 4-5 weeks.

Table 3.1. Nutrient solution for sand culture.

Stock	Element	Salt	G/I	M	μ M
solution					
1	Ca	CaCl ₂ .2H ₂ O	294.1	2.0	1000
2	P	KH₂PO₄	136.1	1.0	500
3	Fe	Fe-Citrate	6.7	0.02	10
	Mg	MgSO ₄ .7H ₂ O	123.3	0.5	250
	K	K₂SO₄	87.0	0.5	250
	Mn	MinSO₄.H₂O	0.338	0.002	2
4	Zn	ZnSO ₄ .7H ₂ O	0.288	0.001	0.5
	Cu	CuSO₄.5H₂O	0.100	0.0004	0.2
	Co	CoSO ₄ .7H₂O	0.056	0.0002	0.1
	Мо	Na ₂ MoO ₂ .2H ₂ O	0.048	0.002	0.1
5	N	KNO₃	101.0	1.0	5000
6	В	H₃BO₃	0.247	0.004	2

Boron free:

For each 10 litres of full strength nutrient solution, take 5.0 ml each of solutions 1 to 4, 50 ml of solution 5, dilute to 10 litres with water. Adjust pH to 6.6-6.8 with NaOH or HCL With Boron:

Adding 5.0 ml of solution 6 as well as 5 ml of solution 1 to 4 and 50 ml of solution 5 to make 10 litres of nutrient solution will give boron concentration of 2 μ M or 0.02 ppm.

Adapted from Broughton and Dilworth (1971).

6

Response of genotypes were assessed at two harvesting times; at booting stage for flag

leaf B concentrations and at maturity stage for number of spikes plant⁻¹, spikelets spike⁻¹.

grains spikelet⁻¹. 100 seed weight (100SW), straw yield and grain yield.

3.2 Field experiments

Field experiments were carried out in the field on low B sandy loam (hot water soluble

boron at 0.10-0.14 mg B kg⁻¹) soil at Multiple Cropping Center, Chiang Mai University in

1997/98 and 1998/99 growing seasons. Boron treatments were varied into four levels by

applying either lime or B to the soil before sowing, designated as follow:

BL: plus 1.6 t of lime ha-1

B0: nil

B1: 1 kg Borax ha

B2: 10 kg Borax ha⁻¹

The experiments were arranged in split-plot design with four replications. Boron

treatments were arranged as mainplot and genotypes as subplot. In each plot, genotypes

were sown in three, 2-m row with 25 cm between rows. At early boot stage, twenty

spikes of each plot were bagged to prevent outcrossing. At maturity, the bagged spikes

were harvested separately and determined for number of spikelets spike 1, grains spike 1,

grains spikelet and grain set. Grain yield was measured from the remaining spikes in

the three-row plot.

3.3 The Barley Grain Set Index

The effect of B on grain set in wheat was measured as the Grain Set Index (GSI), which

is defined as the percentage of the twenty basal florets from ten central spikelets on a

spike with grain (Rerkasem and Loneragan, 1994). In the present study with barley, the

term Barley Grain Set Index (BGSI) was used in order to distinguish it from the GSI used

in wheat. BGSI was defined as:

percentage of the ten median spikelets on the central of a spike with grain for two-row

barley, and

· percentage of the twenty lateral spikelets on the central of a spike with grain for six-

row barley

3.4 Genotypes

- (1) Two barley and one wheat genotypes in sand culture with three B levels in 1997/98.
- (2) 21 entries from the Barley Thailand Yield Nurseries 97/98, in sand culture with B0.
- (3) 9 advanced barley lines/locally adapted varieties in the field with three B levels in 1997/98.
- (4) 244 barley lines from the International Barley Yield Trial (IBYT 98/99, 219 entries) and International Barley Observation Nursery (IBON 98/99, 25 entries), in sand culture with B0 in 1998/99.
- (5) Wheat genotypes with known levels of B response were included as checks in all experiments except (1).

3.5 F, production and evaluation

Parents with contrasting levels of response to B were chosen from 3.4 (2). Crossing plots were conducted in the field in 1998/99 with four sowing dates, to synchronize flowering time. In each plot, four 2-m rows of each parental line were grown with a spacing of 30 cm between row. Hand emasculation of spike form the female parent was performed at booting stage and then bagged to prevent cross-pollination. Three days after emasculation, the bagged spikes were pollinated with pollen from the male parent by swirling method (Starling, 1980). Ten to twenty spikes from each cross combination were made. At maturity, spikes were harvested separately and kept at 4 °C until used.

 F_1 hybrids were tested for response to B and compared to their parents in sand culture in 1999/2000 in two experiments. In Experiment 1, two crosses namely, BRB 9604 x BRB 9 and BRB 9 x BCMU 96-9, and their reciprocal crosses were evaluated in two levels of B, 0 μ M B (B0) and 10 μ M B (B10). Ten plants of F_1 hybrid or parent were grown in each pot without replication. Data were recorded for number of tillers plant every five days. At maturity, number of spikes plant, spikelets spike, grains spikelets and BGSI were measured from every tiller. In Experiment 2, crosses between the most B efficient barley, BRB 9604 and four lines, BRB 9, BCMU 96-9, Stirling and SMGBL 94003 were evaluated in four levels of B (0, 0.1, 1.0 and 10.0 μ M B) in sand culture. Parents and their F_1 hybrid from each combination were sown in the same pot with two replications. At maturity, BGSI, number of grains spikelet and spikelets spike were determined.

4. RESPONSE OF BARLEY TO BORON DEFICIENCY

4.1 Visual symptoms

Foliar symptom

Symptom of boron deficiency on morphological character was not observed when barley genotypes were grown in the field and in sand culture in 1997/98 and 1998/99. However, in 1999/2000 foliar symptom was observed on younger leaves of Stirling barley grown in sand culture at B0 that was similar to those described by Robson and Snowball (1986) and Nable et al. (1990). The symptom was observed as the splitting of the newer leaves along the leaves close to the midrib (i.e. saw-tooth edges). Leaf blades were wrinkled, mishapen and chlorotic. These symptoms were not observed in any wheat genotypes tested in this study.

Spike

An abnormal of spike development was observed in exotic, advanced lines of barley introduced from CIMMYT when grown in sand culture at B0 in1998/99. The spikes were emerged but deaths of spikelets at the tip were observed. In Cu-deficient wheat, rat-tail spikes were observed with full grain in the base of the spike, shriveled grains in the middle of the head and a withered necrotic tip (Robson and Snowball, 1986). The symptom found in barley was differed from that in the Cu-deficient wheat as the spikelets adjacent to those which die grow normally but no grain was set in any spikelets of the B-deficient spike. In the present study, six-row barley tended to have higher number of accessions exhibiting abnormal spikes than two-row barley (Table 4.1.1). This symptom was not observed in bread wheat.

Table 4.1.1 Number of barley lines (% of 244 lines) exhibited normal and abnormal spikes when tested in B0. Sand culture screening 1997/98.

Spike type	Normal spike	Abnormal spike
Two-row barley	89	11
Six-row barley	28	72

4.2 Yield and yield components

Boron deficiency has been reported to affect grain yield of wheat through grain set (Rerkasem et al., 1987). The present study in barley was consistent to that found in wheat. In sand culture experiment, it was found that under B deficiency (0 μ M B) grain yield of BRB 2 and Stirling were severely depressed (Table 4.2.1) at the rate similar to SW 41 wheat. Straw yields of BRB 2 and SW 41 were not effected by B deficiency but that of Stirling was reduced significantly. Grain yield of all genotypes was depressed by B deficiency through grain set i.e. grains spikelets and GSI (Figures 4.2.1 and 4.2.2). Moreover, in Stirling, B deficiency also depressed spikes plant and spikelets spike 1.

Table 4.2.1 Effects of B treatments on (a) grain yield and (b) straw yield of barley and wheat genotypes. Sand culture experiment 1997/98. (Jamjod and Rerkasem, 1999).

Genotype	B treatments ^b (μM)			
	0	0.1	1.0	
(a) Grain yield (g pot ⁻¹)	_		-	
Stirling	2.6 a	6.2 a	19.0 b	
BRB 2	7.3 a	9.5 a	25.6 b	
SW 41	24.8 a	29.2 a	36.9 b	
F-test B***, G***, B x G**				
b) Straw yield (g pot-1)				
Stirling	88.9 a	96.3 ab	115.9 b	
BRB 2	79.7 a	101.2 a	82.2 a	
SW 41	78.5 a	70.9 a	64.5 a	
F-test B'*, G***; B x G**				

^{*} and *** Significant at 0.01 and 0.001 probability levels, respectively; * not significant.

Means within a row with the same letter do not differ significantly at 5% level with Dancan's Multiple Range Test.

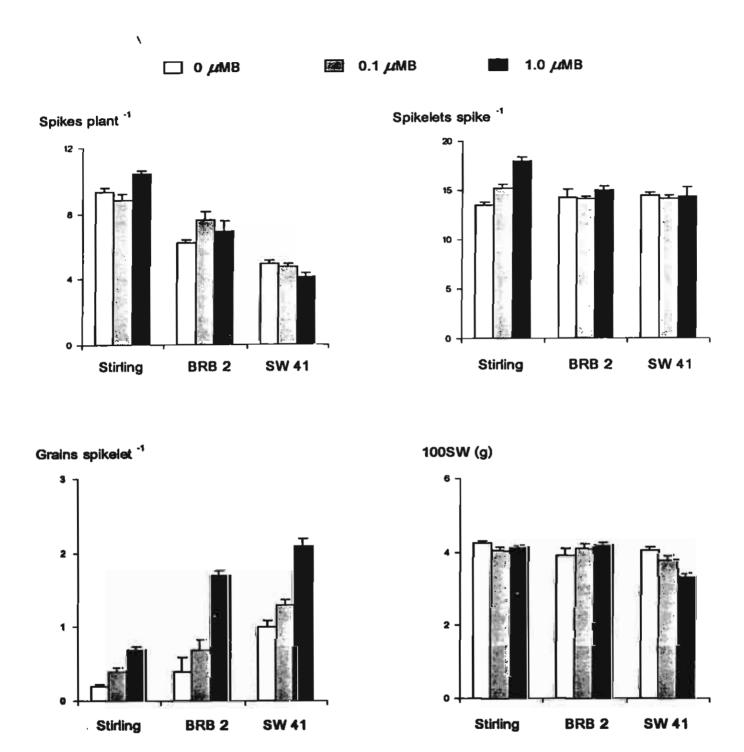


Figure 4.2.1 Effects of B treatment on yield components of two barley genotypes, Stirling and BRB 2, and SW 41 wheat grown in sand culture with 0, 0.1 and 1.0 μM B. Sand culture experiment 1997/98. (Jamjod and Rerkasem, 1999).

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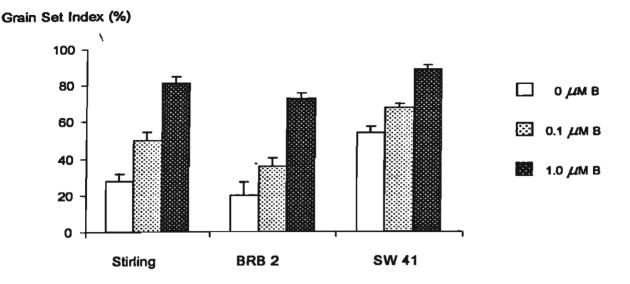


Figure 4.2.2 Effects of B treatment on Grain Set Index (GSI, %) of two barley genotypes, Stirling and BRB 2, and SW 41 wheat grown in sand culture with 0, 0.1 and 1.0 MB. Sand culture experiment 1997/98. (Jamjod and Rerkasem, 1999).

Grain Set Index (GSI) has been used successfully in wheat to compare the effect of B to grain set, as this criteria discriminates effects of B on grain set from those on grain filling (Rerkasem and Loneragan, 1994; Anantawiroon et al., 1997). As found in wheat, GSI of barley responded positively to external B levels and closely correlate to grain yield (Table 4.2.2). Highly significant correlation of GSI with grains spike 1, grains spikelet 1 and grain yield was shown under low B levels. At high B level, GSI did not correlated with grains spike 1 and grain spikelet 1, which indicates the dependence of GSI on B levels. The term GSI, generally, refers to the fertility percentage of ten central spikelets of barley or wheat. Since the structure of barley spikes is different from that of wheat, and two-row barley is also different from six-row barley. It is recommended that the term "Barley Grain Set Index" or BGSI, defined as percentage of the ten central spikelets (ten median spikelets for two-row barley and twenty lateral spikelets for six-row barley) on a spike with grains, should be used in order to distinguish it from the GSI used in wheat.

Table 4.2.2 Correlation coefficient (r) between Grain Set Index (GSI) or grain yield (italics) and yield components. Sand culture experiment 1997/98. (Jamjod and Rerkasem, 1999).

B treatment	1	Spikelets spike ⁻¹	Grains spike ⁻¹	Grains spikelet ⁻¹	100SW	GSI
0 <i>μ</i> Μ	GSI	0.11	0.89**	0.89**	0.38	-
•	Grain yield	0.27	0.88**	0.87**	0.09	0.81**
0.1 <i>μ</i> Μ	GSI	0.01	0.72**	0.70**	-0.49*	-
•	Grain yield	-0.23	0.80**	0.89**	-0.48*	0.75**
1.0 <i>µ</i> M	GSI	0.13	0.37	0.29	-0.64**	-
•	Grain yield	-0.08	0.79**	0.68**	-0.49*	0.65**

^{*} and ** Significant at the 0.05 and 0.01 probability levels, respectively.

4.3 Boron in flag leaf

At boot stage, flag leaf B concentrations were increased with increasing B levels (Figure 4.3.1). At low B, both barley and wheat contained >2-4 mg B kg⁻¹ and increased to >4-8 mg B kg⁻¹ at B 1.0 M. The present results are consistent with previous studies (Rerkasem and Loneragan, 1994; Simojoki, 1972). Flag leaf B concentration can be used as a general indicator for B status in barley and concentrations <4 mg B kg⁻¹ appear to predict a decline in grain set from B deficiency.

[B] (mg kg⁻¹)

10

8

6

4

2

Stirling BRB 2 SW 41

Figure 4.3.1 Effects of B treatment on flag leaf B concentration (mg B kg $^{-1}$) of two barley genotypes, Stirling and BRB 2, and SW 41 wheat grown in sand culture with 0, 0.1 and 1.0 μ M B. Sand culture experiment 1997/98. (Jamjod and Rerkasem, 1999).

5 GENOTYPIC VARIATION IN BORON EFFICIENCY IN BARLEY

5.1 Evaluation of advanced lines

Field experiment was carried out in 1997/98 to evaluate B responses of 9 locally adapted, advanced lines or cultivars of barley. The objectives are to identify the genotypic range of B responses within these 9 genotypes and to identify specific response which may be used in further study or breeding programmes. Barley genotypes were grouped into two-row and six-row classes. Boron efficient (Fang 60) and moderately inefficient (SW 41) wheat genotypes were included as checks. Genotypes differed significantly in response to B in spikelets spike 1, grains spike 1, grains spikelets 1 and BGSI (Table 5.1.1-5.1.4).

Boron had no effect on number of spikelets of all six-row barley genotypes, as well as wheat checks, Fang 60 and SW 41 (Table 5.1.1). However, the number of spikelets spike⁻¹ of three two-row barleys, namely, Stirling, SMGBL 91002 and SMGBLS 94003, showed clear positive responses to increasing B.

Very significant interaction between B treatments and genotypes was observed on grains spike⁻¹ (Table 5.1.2), grains spikelet⁻¹ (Table 5.1.3) and GSI (Table 5.1.4). Grains spike⁻¹ and grains spikelet⁻¹ of SW 41, the B inefficient check and most barley genotypes were lowest in the BL treatment and increased with increasing B, whereas those of Fang 60 remained constant.

When all genotypes were compared using GSI (Table 5.1.4), they can be classified into four different groups. Fang 60 wheat was the most efficient genotype and had more than 90% GSI in all B treatments. CMBL 92029, BRB 9, SMGBLS 91002, SMGBLS 94003, BCMU 96-5 and BRB 2 had low GSI similar to SW 41 and were classed as inefficient to B. The GSI of BCMU 96-9 and BCMU 96-1 at BL and B0 were intermediate between those of SW 41 and Fang 60. Stirling was the most inefficient genotypes, its GSI was 19, 23 and 79% in BL, B0 and B+ respectively.

Table 5.1.1 Effect of B treatments on spikelets spike⁻¹ of barley and wheat genotypes. Field experiment 1997/98. (Jamjod and Rerkasem, 1999).

Genotype		B treatments ^b	
	BL	В0	B+
Two-row barley			
BCMU 96-9	22.2 a	23.8 a	22.9 a
BRB 9	14.6 a	14.0 a	13.3 a
CMBL 92029	17.9 a	19.7 a	20.5 a
SMGBLS 91002	21.4 a	23.3 ab	25.0 b
SMGBLS 94003	21.5 a	24.4 b	23.8 ab
Stirling	15.6 a	18.5 b	19.4 b
Six-row barley			
BCMU 96-1	18.9 a	18.5 a	16.8 a
BCMU 96-5	15.7 a	18.5 a	16.9 a
BRB 2	17.0 a	18.4 a	16.5 a
Wheat			
Fang 60	13.2 a	13.9 a	12.8 a
SW 41	14.6 a	15.4 a	15.6 a

F-test B", G**, BxG*

and ** Significant at 0.05 and 0.01 probability levels, respectively; and not significant.

Mean within a row with the same letter do not differ significantly at 5% level with Dancan's Multiple Range Test.

Table 5.1.2 Effect of B treatments on grains spike of barley and wheat genotypes. Field experiment 1997/98. (Jamjod and Rerkasem, 1999).

Genotype		B treatments ^b	
	BL	В0	B+
Two-row barley			
BCMU 96-9	12.1 a	18.3 a	19.1 a
BRB 9	6.6 a	7.9 a	11.7 a
CMBL 92029	8.1 a	6.0 a	12.2 a
SMGBLS 91002	3.6 a	10.1 ab	18.5 b
SMGBLS 94003	5.8 a	10.7 ab	16.4 b
Stirling	2.6 a	3.8 a	11.3 b
Six-row barley			
BCMU 96-1	30.8 a	38.6 ab	41.0 b
BCMU 96-5	18.0 a	30.0 b	40.3 c
BRB 2	23.5 a	34.0 b	36.6 b
Wheat			
Fang 60	36.3 a	39.0 a	33.0
SW 41	14.8 a	19.0 a	34.2 b

^{**} Significant at 0.01 probability level.

Mean within a row with the same letter do not differ significantly at 5% level with Dancan's Multiple Range Test.

Table 4.1.3 Effect of B treatments on grains spikelet⁻¹ of barley and wheat genotypes. Field experiment 1997/98. (Jamjod and Rerkasem, 1999).

Genotype		B treatments ^b	
	BL	ВО	B+
Two-row barley			
BCMU 96-9	0.5 a	0.8 a	0.8 a
BRB 9	0.5 a	0.6 a	0.9 a
CMBL 92029	0.4 a	0.3 a	0.6 a
SMGBLS 91002	0.2 a	0.4 ab	0.7 b
SMGBLS 94003	0.3 a	0.4 a	0.7 a
Stirling	0.2 a	0.2 a	0.6 b
Six-row barley			
BCMU 96-1	1.6 a	2.1 ab	2.4 b
BCMU 96-5	1.1 a	1.6 b	2.4 c
BRB 2	1.4 a	1.8 ab	2.2 b
Wheat			
Fang 60	2.7 a	2.8 a	2.6 a
	1.0 a	1.2 ab	2.2 b

^{* **} Significant at 0.01 probability level.

Mean within a row with the same letter do not differ significantly at 5% level with Dancan's Multiple Range Test.

Table 4.1.4 Effect of B treatments on Grain Set Index (%) of barley and wheat genotypes. Field experiment 1997/98. (Jamjod and Rerkasem, 1999).

Genotype		B treatments	s ⁶
	BL	В0	B+
Two-row barley	-		
BCMU 96-9	65.6 e	84.2 de	91.2 ab
BRB 9	48.1 cde	61.5 bc	90.5 ab
CMBL 92029	42.3 bcd	33.0 a	67.3 a
SMGBLS 91002	27.0 ab	61.5 bc	92.8 b
SMGBLS 94003	36.4 abc	55.8 bc	85.4 ab
Stirling	19.0 a	23.1 a	79.1 ab
Six-row barley			
BCMU 96-1	60.1 de	74.4 cd	89.1 ab
BCMU 96-5	43.9 bcd	63.8 bcd	86.6 ab
BRB 2	40.5 bcd	71.9 bcd	83.4 ab
Wheat			
Fang 60	94.4 f	97.0 e	96.6 b
SW 41	42.7 bcd	52.5 b	93.0 Ь

F-test B**, G**, BxG**

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Significant at 0.01 probability level.

Mean within a row with the same letter do not differ significantly at 5% level with Dancan's Multiple Range Test.

5.2 Evaluating germplasm for B efficiency

Very limited genotypic variation for B efficiency was found within germplasm introduced from oversea. Without B added to the nutrient solution, none of advanced lines of barley from the International Barley Nurseries (IBON + IBYT) was B efficient. More than 90% of these germplasms were classified as B inefficient (GSI <20) and 6% were similar to or less than the moderately in check efficient (Table 5.2.1). In contrast, genotypic variation for B response within advanced lines selected in Thailand was demonstrated with 10% of barley lines from the National nurseries (BTYN97/98) exhibited GSI in the range of 81-100%.

Table 5.2.1 Grain Set Index in advanced lines from Barley Thailand Yield Nurseries (BTYN) and International Barley Nurseries (IBON + IBYT) in sand culture without added B. Sand culture screening 1997/98 and 1998/99.

Nursery	Total tested	GSI				
	_	0-20	21-40	41-60	61-80	81-100
			Num	ber of lin	es (%)	
BTYN97/98	21	5	24	29	33	10
IBON 98/99 +	244	94	4	2	0	0
IBYT 98/99						

Note: GSI of Fang 60 (B efficient) and SW 41 (moderately B inefficient) wheat checks were 95-98% and 65-71%, respectively.

Within the BTYN 97/98, the GSI of two genotypes were in the range of 81-100% (Table 5.1.2). However, only BRB 9604 set grain better than SW 41 and comparable to Fang 60, and was classified as B efficient. The remaining lines set grain either similar to or less than the moderately inefficient check, with the exception of BRB 9624 and BRB 9. These two lines were intermediate between the two wheat checks.

Considerable genotypic variation in B efficiency was observed within advanced lines of barley screened in soil and sand culture. BGSI of five lines were higher than the

^a BTYN 1997/98 = Barley Thailand Yield Nurseries 97/98, IBON = International Barley Observation Nursery 1998/99 and IBYT = International Barley Yield Trail 1998/99

Table 5.2.2 Response of advanced lines from Barley Thailand Yield Nurseries 1997/98 tested in sand culture with B0. Sand culture screening 1997/98. (Jamjod and Rerkasem, 1999).

Line	Origin [#]	GSI (%)	% of	checks
			Fang 60	SW 41
Two-row barley			_	
BRB 9604	BRB	90.0	91	138 *
BRB 9624	BRB	85.3	87	131
BRB 9	BRB	79.9	81	123
BRB 9609	BRB	75.2	76 *	115
BRB 10	BRB	74.5	76 *	114
BRB 11	BRB	72.5	74 *	111
LARTC-BL9101 (4)	LMP	64.1	65 **	98
BRB 9621	BRB	61.5	62 **	94
LARTC-BL9119 (11)	LMP	60.7	62 **	93
LARTC-BL9408	LMP	47.9	49 ***	73
SMGBL 94026	SMG	42.3	43 ***	66 *
LARTC-BL9410	LMP	40.4	41 ***	62 *
SMGBL 94003	SMG	4.8	5 ***	8 ***
Six-row barley				
FNBL 8309-34-SMG-1-1	SMG	55.2	56 ***	85
FNBL 8306-BC-SMG-1-1	SMG	46.5	47 ***	71
FNBL #140	SMG	45.9	47 ***	70
FNBL 8404-4-SMG-1-1-1	SMG	38.9	40 ***	60 **
FNBL 8403-6-SMG-1-2-1	SMG	37.0	38 ***	57 **
FNBL 8403-17-SMG-1-1-1	SMG	34.5	35 ***	53 **
SMG 1	SMG	31.9	32 ***	49 **
BRB 2	BRB	24.7	25 ***	38 ***
Wheat (checks)				
Fang 60		98.3	100	151 ***
SW 41		65.1	66 **	100

BRB = Boon Rawd Brewery Co. Ltd; LMP = Lampang Agricultural Research Training Center; SMG

⁼ Samoeng Upland Rice and Temperate Cereals Experiment Station.

^{*, **} and *** Significantly different from check cultivars at p< 0.05, 0.01 and 0.001, respectively.

moderately B inefficient wheat, SW 41 (Tables 5.1.4 and 5.2.2). All lines were selected in Northern Thailand, where B deficiency has been reported in wheat and some barley lines (Rerkasem and Jamjod, 1989). The majority of segregating populations and advanced lines used in barley breeding programmes in Thailand were obtained through CIMMYT. Substantial numbers of B efficient wheat lines have been identified from CYMMYT materials (Jamjod et al., 1992a) and the same may be true for barley. It is likely that B efficiency in barley is already available in these germplasms. However, the present results have screened only a small selection of the total variation for all barley. Further screening of germplasms from other sources is required to define the full range of B response for barley.

5.3 Evaluation of genotypes difference in B efficiency

Four selections of two-row, six-row barley and wheat each were tested in the soil with four levels of B. The wheat genotypes represented the whole range of response to B, including, Fang 60 (efficient, E), CMU 88-9 (moderately efficient, ME), SW 41 (moderately inefficient, MI) and Bonza (inefficient, I).

When tested in the soil, B levels had no effect on number of spikelets of all barley and wheat genotypes tested (Table 5.3.1). Genotypes displayed differential number of spikelet spike⁻¹. BCMU 96-9 exhibited the highest number of spikelets spike⁻¹.

Very significant interaction between B treatments and genotypes were observed for grains spike⁻¹ (Figure 5.3.1), grains spikelet⁻¹ (Figure 5.3.2), GSI (Figure 5.3.3) and grain yield (Figure 5.3.4). Grain set and grain yield of CMU 88-9, SW 41, Bonza, SMGBL 91002, BCMU 96-9, SMG 1, BCMU 96-1 and BRB 2 were reduced by B deficiency while those of Fang 60, BRB 9624, 9604 and FNBL 8309 were not adversely affected.

Grain Set Index was used to classify response to B of the tested barley compared to the wheat checks. Within the checks, Fang 60 was rated as the most B efficient, CMU 88-9 as moderately efficient, SW 41 as moderately inefficient and Bonza as inefficient (Figure 5.3.3). Compared to checks, barley genotypes were classified into three groups as follow: a) BRB 9624, BRB 9604 (two row) and FNBL 8309 (six-row) barley genotypes expressed

similar responses to B as Fang 60 wheat, and being classified as B efficient; b) SMGBL 91002 (two row, BCMU 96-1 and BRB 2 (six-row) showed the same responses as SW 41 wheat and were classified as moderately B inefficient; and c) BCMU 96-9 (two-row) and SMG 1 (six-row) showed the same responses Bonza wheat and were classified as B inefficient.

Table 5.3.1 Effect of B treatments on spikelets spike⁻¹ of selected two-row and six-row barley, and wheat genotypes. Field experiment 1998/99. (Jamjod et al., 2000).

Genotype*		B to	reatments		Mean
	BL	В0	B1	B2	
Two-row barley					
BRB 9624	14.2	14.9	15.6	15.5	15.0 b
BRB 9604	16.5	15.9	15.6	15.9	16.0 cd
SMGBL 91002	21.1	24.1	25.5	24.6	21.2 f
BCMU 96-9	23.8	24.1	25.5	24.6	24.5 g
Six-row barley					
FNBL 8309	13.6	13.0	13.6	13.2	13.3 a
SMG 1	14.4	14.9	15.4	15.0	14.9 b
BCMU 96-1	17.7	17.1	18.2	17.5	17.6 e
BRB 2	16.2	16.9	17.1	16.5	16.7 d
Bread wheat					
Fang 60	15.0	15.8	16.6	15.8	15.0 bcd
CMU 88-9	16.4	16.1	17.2	15.7	16.4 d
SW 41	17.3	17.2	18.8	17.6	17.7 e
Bonza	15.1	14.7	16.5	15.4	15.4 bc

F-test: B^{ns}, G***, BxG^{ns}; *** Significant at p< 0.001

Mean within a column with different letters are differ significantly at p = 0.05 with LSD. Note: B treatments are BL = 1.6 t lime ha⁻¹, B0 = nil, B1 = 1.0 kg Borax ha⁻¹ and B2 = 10 kg Borax ha⁻¹.

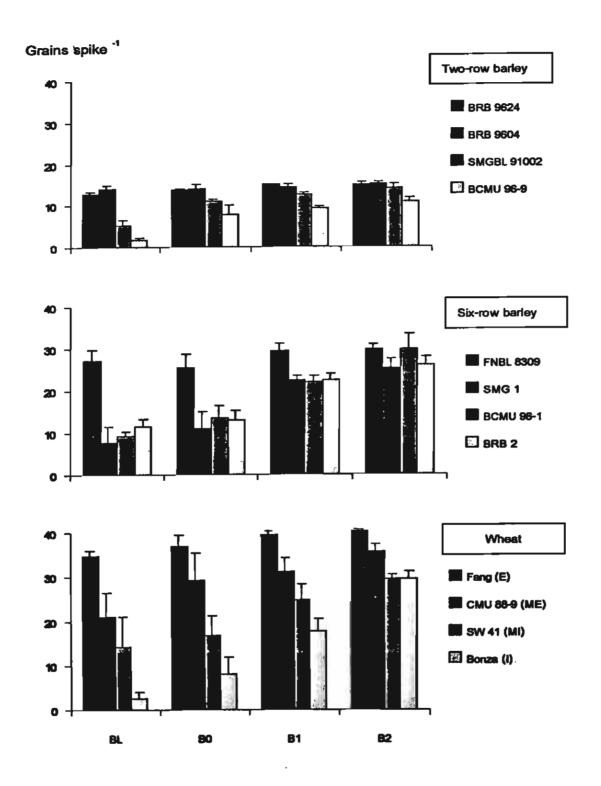


Figure 5.3.1 Effect of B treatment (B) on number of grains spike⁻¹ of barley and bread wheat genotypes (G). B x G significant at p<0.001, LSD (0.05) = 6.5.

B treatments are BL = 1.6 t lime ha⁻¹, B0 = nii, B1 = 1.0 kg Borax ha⁻¹ and B2 = 10 kg Borax ha⁻¹. Field experiment 1998/99. (Jamjod et al., 2000).

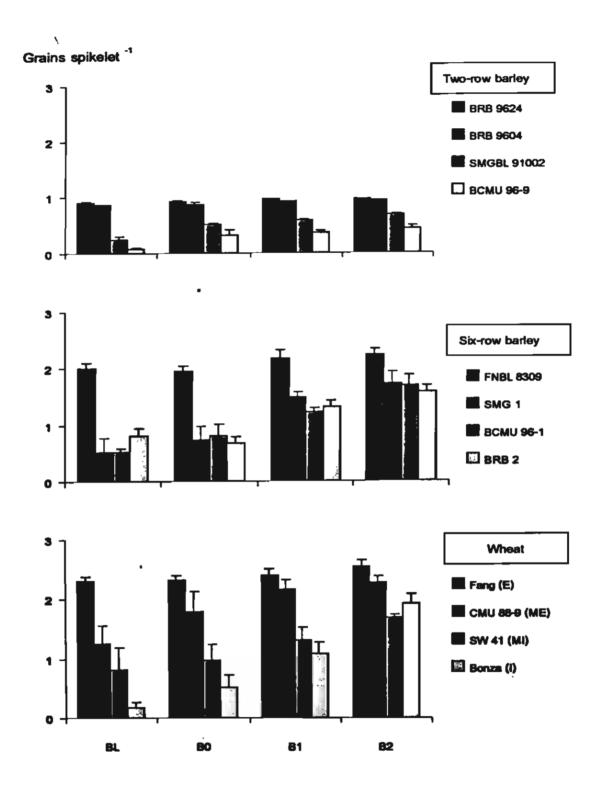


Figure 5.3.2 Effect of B treatment (B) on number of grains spikelet⁻¹ of barley and bread wheat genotypes (G). B x G significant at p<0.001, LSD (0.05) = 0.4.

B treatments are BL = 1.6 t lime ha⁻¹, B0 = nil, B1 = 1.0 kg Borax ha⁻¹ and B2 = 10 kg Borax ha⁻¹. Field experiment 1998/99. (Jamjod et al., 2000).

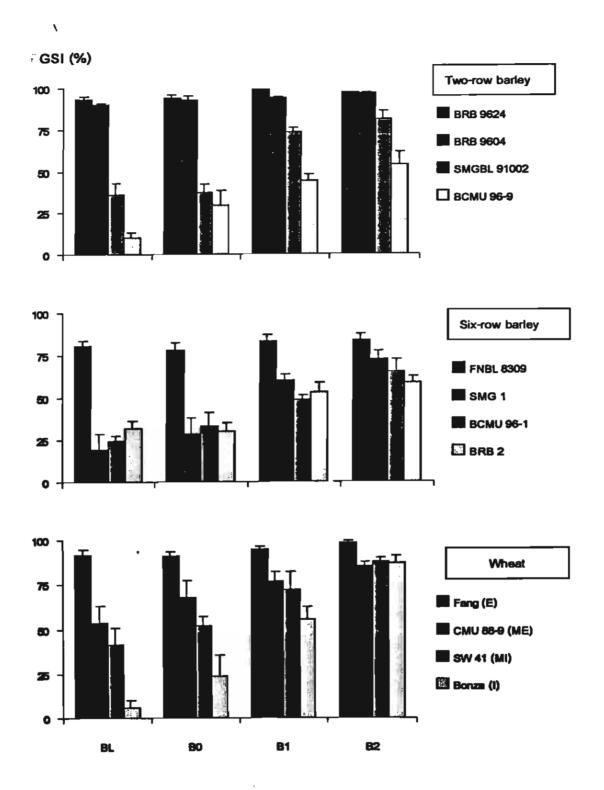


Figure 5.3.3 Effect of B treatment (B) on number of Grain Set Index, GSI (%) of barley and bread wheat genotypes (G). B x G significant at p<0.001, LSD (0.05) = 16.9. B treatments are BL = 1.6 t lime ha⁻¹, B0 = nil, B1 = 1.0 kg Borax ha⁻¹ and B2 = 10 kg Borax ha⁻¹. Field experiment 1998/99. (Jamjod et al., 2000).

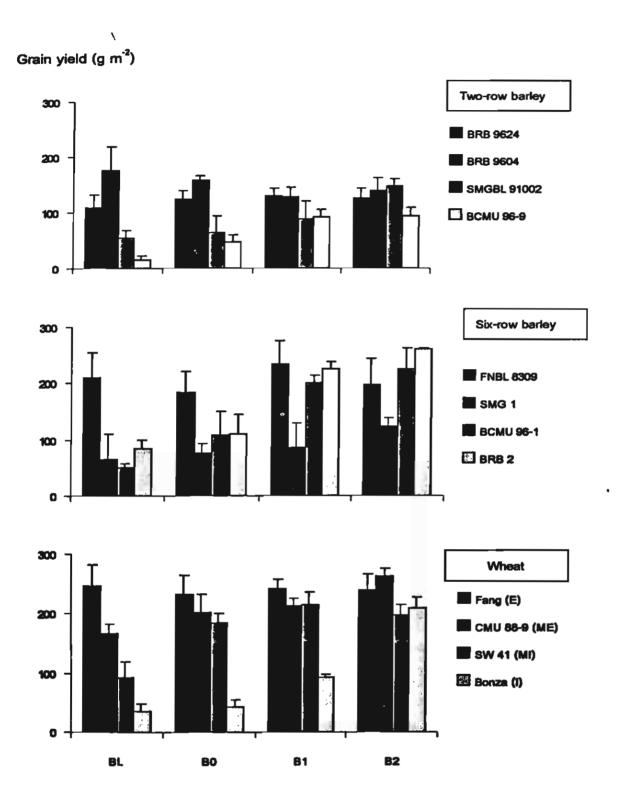


Figure 5.3.4 Effect of B treatment (B) on number of grain yield (g m⁻²) of barley and bread wheat genotypes (G). B x G significant at p<0.001, LSD (0.05) = 6.5.

B treatments are BL = 1.6 t lime ha⁻¹, B0 = nil, B1 = 1.0 kg Borax ha⁻¹ and B2 = 10 kg Borax ha⁻¹. Field experiment 1998/99. (Jamjod et al., 2000).

Genotypic variation for response to B within advanced lines of barley selected in Thailand was confirmed by these results. Two advanced lines, namely, BRB 9624 and BRB 9604, selected from the previous screening (Table 5.2.2) exhibited normal grain set and grain yield under B deficiency conditions. These lines can be recommended for barley production in low B areas. Moreover, they may be used to introduce B efficiency to the commercial cultivars by breeding.

Response to B of FNBL 8309 in this study contrasted with previous screening. In 1997/98 screening, this line displayed the highest GSI with the six-row barley tested but was rated as moderately B inefficient as SW 41 when tested in sand culture (Table 5.2.2). However, when tested in the soil, FNBL 8309 revealed the same level of B efficiency as Fang 60. Mechanism of B uptake when plants were grown in soils may be different from in sand culture or nutrient solution (Marschner, 1995). Boron efficiency of FNBL 8309 should be confirm before being recommended to farmers or used in breeding programmes.

Boron efficiency barley genotypes were identified among advanced lines derived from CIMMYT germplasm, selected and evaluated in Northern Thailand. This was consistent to that found in bread wheat that B efficient genotypes were identified from advanced lines originating from CIMMYT materials and selected in Thailand such as Fang 60 and #144 (Rerkasem and Jamjod, 1997). This suggests that low B in this region may have acted as a selection pressure upon the adaptation of these genotypes. Although sources of B efficiency are available in this germplasm, it was shown that very low frequency of advanced lines in International and National Yield Trials were B efficient. For example, 86% of BTYN 97/98 (Table 4.4.2) and all lines in IBYT 98/99 and IBON 98/99 were rated as moderately B efficient or B inefficient. As barley production is being promoted in areas where B deficiency is likely to be predominated. Results from this study suggest that yield loss due to B deficiency can be prevented by the use of B efficient genotypes. Boron efficiency should be included as selection criteria in breeding programmes.

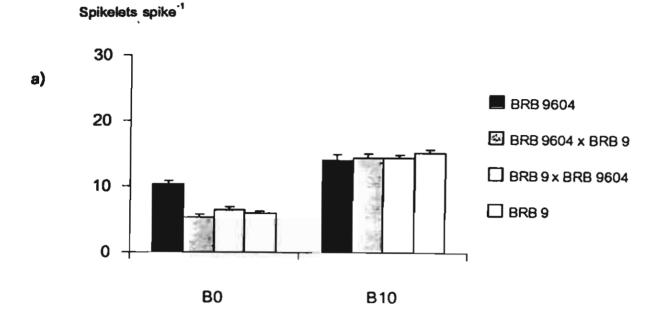
6. INHERITANCE OF OF BORON EFFICIENCY IN BARLEY

6.1 Comparison of reciprocal crosses

Boron deficiency reduced number of spikelets spike⁻¹ (Figure 6.1.1) grains spikelet⁻¹ (Figure 6.1.2) and BGSI (Figure 6.1.3) but increased number of tillers plant⁻¹ (Table 6.1.1 and Figure 6.1.4) of all parents and F₁ hybrids used in this study. The reductions in the first three characters of F₁ hybrids from both crosses were close to the more inefficient parents. For example, spikelets spike⁻¹ of F₁ from BRB 9604 x BRB 9 crosses at 80 were the same as the more inefficient barley, BRB 9 (Figure 6.1.1a). In BRB 9 x BCMU 96-9 crosses (Figure 6.1.1b), spikelets spike⁻¹ of F₁ at B0 were the same as both parents. At B10, spikelets spike⁻¹ of BCMU 96-9 was higher than BRB 9 and those of F₁ were not differed from BCMU 96-9. This indicates the more sensitive of BCMU 96-9 to B deficiency and responses of F₁'s were similar to BCMU 96-9. No different between reciprocal crosses were found.

Grain set of F₁ hybrids at B0 were within the range of parents but were closer to the more inefficient parents. No different between reciprocal crosses were found. (Figures 6.1.2 and 6.1.3). Grains spikelets⁻¹ of BRB 9 x BCMU 96-9 at B10 were slightly lower than both parents. At B0, only BRB 9 parent set grain about 0.2 grains spike⁻¹ and its BGSI was 12%. F₁ hybrids and BCMU 96-9 set no grain under B0 (Figures 6.1.2b and 6.1.3b).

B deficiency resulted in an increasing in tiller number of parents and F₁ in the present study (Table 6.1.1 and Figure 6.1.4). Compared to B10, tillers plant⁻¹ of BRB 9604, BRB 9 and BCMU 96-9 at B0 were increased at 4, 12 and 26 tillers, respectively. F₁ hybrids tended to respond similar to the more responsive parents. In contrast to effect on tiller number, B deficiency had less effect on number of spikes (Table 6.1.1). No different between reciprocal crosses were found.



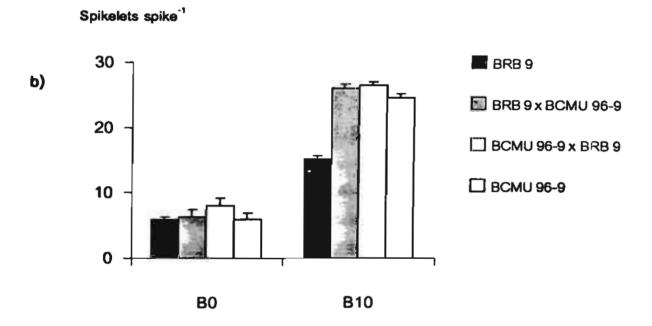
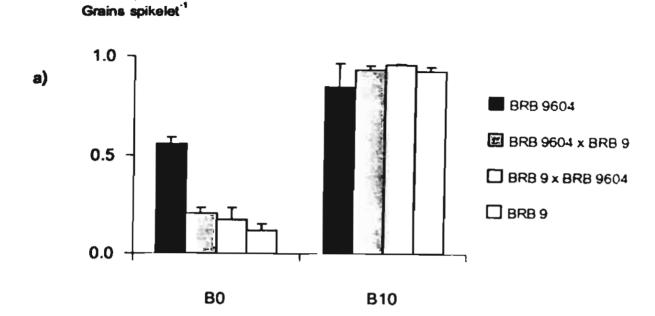


Figure 6.1.1 Number of spikelets spike⁻¹ of parents and F_1 hybrids from crosses a) BRB 9604 x BRB 9 and b) BRB 9 x BCMU 96-9, grown in sand culture with 0 μ MB (B0) and 10 μ MB (B10). Data are means with standard error bars. Sand culture experiment 1, 1999/2000.



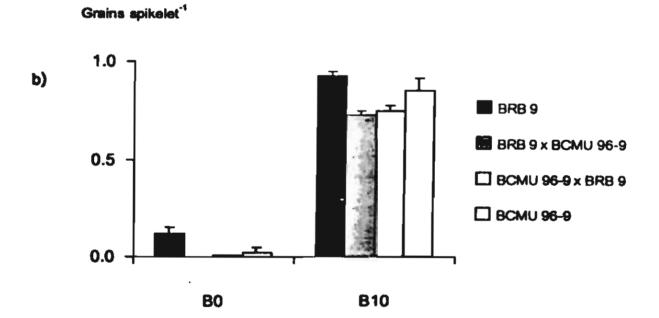
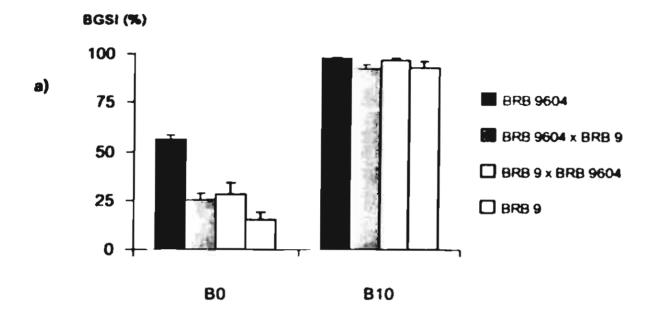


Figure 6.1.2 Number of grains spikelet⁻¹ of parents and F₁ hybrids from crosses a) BRB 9604 x BRB 9 and b) BRB 9 x BCMU 96-9, grown in sand culture with 0 μMB (B0) and 10 μMB (B10). Data are means with standard error bars. Sand culture experiment 1, 1999/2000.



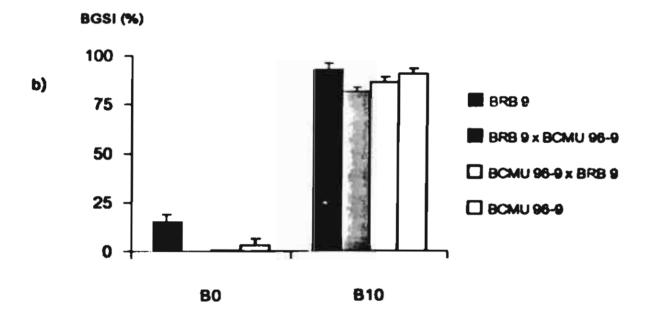


Figure 6.1.3 Barley Grain Set Index, BGSI (%) of parents and F₁ hybrids from crosses a) BRB 9604 x BRB 9 and b) BRB 9 x BCMU 96-9, grown in sand culture with 0 μMB (B0) and 10 μMB (B10). Data are means with standard error bars.

Table 6.1.1 Effect of B treatments (B0 and B10) on number of tillers plant and spikes plant at harvesting of parents and F₁ hybrids. Data are mean with standard error in parentheses. Sand culture experiment 1, 1999/2000.

Genotype	Tillers plant ¹		Spikes plant ⁻¹	
	В0	B10	B0	B10
BRB 9604	17 (1)	13 (1)	7 (1)	9 (1)
BRB 9604 x BRB 9	26 (2)	12 (1)	10 (1)	11 (1)
BRB 9 x BRB 9604	29 (2)	11 (1)	10 (1)	9 (1)
BRB 9	36 (3)	14 (1)	12 (2)	11 (1)
BRB 9 x BCMU 96-9	31 (5)	8 (1)	5 (1)	7 (1)
BCMU 96-9 x BRB 9	33 (4)	9 (1)	4 (1)	6 (1)
BCMU 96-9	33 (2)	6 (1)	4 (1)	5 (1)

As reciprocal crosses were not different, reciprocal F₁ hybrids from each cross were pooled (Figure 6.1.4). It was shown that F₁ from BRB 9604 x BRB 9 were intermediate between parents at only close to maturity stage. At early stage, 20-50 days after sowing, tillers number of all barley genotypes at B0 and B10 were not differed significantly. At 50-70 days after sowing, tillers plants⁻¹ of all parents and F₁ hybrids increased rapidly. This response may be a secondary effect of B deficiency induced by sterility. Differences between genotypes in spike number at B0 (Table 6.1.1) was not likely to be primarily induced by B deficiency, but it was likely caused by an ability of genotypes to develop each tiller into a spike at the later stage.

Parents and F₁ exhibited maximum number for spikelets spike⁻¹, grains spikelet⁻¹ and BGSI when grown at B10. The absence of reciprocal differences in the F₁ hybrids for all measured characters demonstrate that response to B in barley is not cytoplasmically controlled.