รายงานวิจัยฉบับสมบูรณ์

การเพิ่มประสิทธิภาพการผลิตเมล็ดพันธุ์ข้าวลูกผสมด้วยการสร้างความเข้าใจในความหลากหลายของกระบวนการสืบพันธุ์ด้วยเมล็ดและการใช้ธาตุอาหารโบรอน

(Improving Hybrid Rice Seed Production by Better Understanding of Diversity in the Breeding System and Boron Nutrition)

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(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

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Improving Hybrid Rice Seed Production by Better Understanding of Diversity in the Breeding System and Boron Nutrition

Executive summary

Hybrid seed technology has been successfully deployed to increase rice yield in many Asian countries e.g. China, India and Vietnam. The primary target in the development of hybrid seed technology is high yield of the F_1 hybrid seed. The higher yield will translate into lower cost per kg of the seed, which will in turn help to lower farmers' input cost. However, the key process in hybrid seed production in self pollinated crop such as rice is the pollination, fertilization and environmental factors. Common wild rice, the progenitor of cultivated rice is predominately outcrossing. In addition, a major constraint to successful pollination and seed set in cereals is boron deficiency. This project has contributed to knowledge in genotypic variation in breeding system and boron response to improve outcrossing in hybrid seed production, in four sets of objectives as follows.

1. **To identify genotypic variation in traits promote outcrossing in cultivated rice**

Rice is a self-pollinated crop. Up to 7% of outcrossing has been reported in specific varieties or conditions. Genotypic variation for floral morphology relation to outcrossing was evaluated in modern, popular, high yielding rice varieties released in Thailand. For traits require for the female parents, Pathumtani 1 (PTT1) and Neaw Phrae 1 (NP1) were the varieties with the longest stigma. Extents of stigma exertion were between 49-100%, with RD10, RD7 and Sakonakorn 1 (SKN1) showed 100% stigma exertion. NP1 and PTT1 exhibited the longest stigma among all varieties but turn out to possessed the lowest rate of stigma exertion. Among the modern varieties, RD7 and SKN1 had higher potential of outcrossing than the others. For traits require for male parents, R258 and PTT1 exhibited the largest anthers. Number of pollen grains per anther was highest in PTT1. Fertilization was depended on number of pollen deposited on stigma. Most of varieties deposited more than 40 pollen grains per stigma and ensured fertilization. The highest pollen grain per stigma (79) was found in SKN1. For flowering behavior, opening of the spikelet or blooming duration, the period from opening to closing of floret, was varied with genotypes and within the range of 35-50 min. For the long duration genotypes, RD10, R258 and PTT1 lasted for 50 min. Pollen viability test by agar method was developed and can be used to screen for pollen viability in breeding program or other physiological and biological studies in rice.

2. **To identify genotypic variation in B response rice**

Three rice genotypes, KDML 105, Suphanburi 1 (SPR1) and Chianat 1 (CNT1), were evaluated for response to B in sand culture for two seasons. Genotypic variation in response to B was found. B deficiency depressed pollen viability and grain set in rice but no effect on any vegetative part was observed. Concentrations of B in anther and flag leaf can be used to indicate B status that affected grain set in rice. Between the three genotypes, SPR1 was the most efficient and CNT1 was the most inefficient and KDML 105 was intermediate between the former two genotypes. For response of hybrid rice to B levels, four varieties were tested at two B treatments (with and without B) in sand culture. Genotypic variation in hybrid rice was found in B requirement for pollen germination in

agar media. Low germination of pollen in media without added B indicated external B was required for pollen germination. B deficiency reduced average grain set at about 12%.

3. To identify genotypic variation in grain set under high temperature

Variation in grain set was evaluated in 12 rice varieties at three planting dates in dry season (mid-January, late January and mid- February 2009) at two locations, Chiang Mai and Suphanburi. There were genotypic differences in response to high temperature. Differential genotype response to high temperature was attributed to grain set failure and unfilled grain. At mid-February planting date, grain set of the sensitive genotypes, SPT1, RD4, RD10 and RD21 were reduced to 75-78% at The smallest decrease in grain set with planting dates in both locations were found in RD29, SPR1 and PTT1 suggested that they were tolerant to high temperature in this study.

4. To evaluate and select for traits promote outcrossing

Evaluation and selection for traits promote outcrossing, by the method developed from objective 1, were conducted in two sets of crosses: (1) F_4 generation from common wild rice x SPR1 (2) Backcross populations derived from common wild rice (*Oryza rufipogon* Griff.) x local rice and then backcrossed to Suphanburi 1 (*O. sativa* L., cv SPR 1). For the first set, selection and evaluation were carried on until F_6, selected lines showed desirable recombination of many traits, e.g. photoperiod insensitivity, semi-dwarf plant type, large panicle, high number of spikelets/panicle and high seed fertility. However, seed shattering were found in all lines when compared with SPR1 parent. For the second set, backcrossing, evaluation and selection were practiced until BC_2F_4. Sixteen lines with non-seed shattering type, large anther/stigma and desirable agronomic traits were selected. These lines can be used as parents in breeding for male or female parents in hybrid seed production.

Implication of main research findings

1. From variation in floral characteristics in rice the project has contribute to the extent of variation found in rice varieties commonly grown in Thailand. This will help to set the goal of improvement, source of variation and correlation of traits, to be used in breeding program.
2. Selection and screening method for pollen viability has been developed.
3. B deficiency affected rice yield via pollen germination and grain set. Pollen germination of sensitive genotypes were affected by both internal B in pollen and external B in stigma. Applying B in soil has a potential to improve rice yield in areas with low B soil. The results have led to the foliar B application study in hybrid rice evaluation at Bayer research station.
4. Critical value for B established for reproductive development and can be used to diagnose for B status and possibility of B deficiency in rice.
5. Grain set, number of filled grain can be used as screening tools for heat tolerance during reproductive phase. Results on genotypic variation in response to high temperature have led to further research on response to high temperature in rice. This was part of the project funded by TRF and a PhD scholarship funded by The Office of the Higher Education Commission.
6. Advanced lines with large anther and/or large pistil, transferred from common wild rice to cultivated, high yielding rice variety, were developed. They can be used as parents in breeding for large anther or large stigma in hybrid seed production.

การเพิ่มประสิทธิภาพการผลิตเมล็ดพันธุ์ข้าวลูกผสมด้วยการสร้างความเข้าใจในความหลากหลายของกระบวนการสืบพันธุ์ด้วยเมล็ดและการใช้ธาตุอาหารโบรอน

Executive summary

การใช้เมล็ดพันธุ์ลูกผสม (F_1 hybrids) เป็นเทคโนโลยีเพิ่มผลผลิตข้าว ที่ได้ผลมาแล้วในหลายประเทศ อาทิ จีน อินเดีย และเวียดนาม การได้ผลผลิตสูงของเมล็ดพันธุ์ในชั่วแรก เป็นเป้าหมายสำคัญของการผลิตเมล็ดพันธุ์ลูกผสม เพราะจะส่งผลประโยชน์ให้แก่เกษตรกรผู้ปลูกไม่ต้องซื้อเมล็ดพันธุ์ในราคาแพง แต่ข้อจำกัดของการผลิตเมล็ดพันธุ์ลูกผสมในพืชที่ตามธรรมชาติเป็นชนิดผสมตัวเอง จึงทำให้มีอัตราการผสมข้ามต่ำและขึ้นกับปัจจัยภายนอกด้วย ข้าวปลูกปกติเป็นพืชผสมตัวเอง แต่บรรพบุรุษของมันคือข้าวป่านั้นเป็นพืชชนิดผสมข้าม ดังนั้น ลักษณะการผสมเกสรที่เอื้อต่อการผสมข้ามที่นำไปใช้ประโยชน์ได้สามารถคัดเลือกจากลูกผสมข้าวปลูก x ข้าวป่า ส่วนปัจจัยภายนอกที่เป็นตัวจำกัดการผสมเกสรและการติดเมล็ดในธัญพืชที่แพร่หลายที่สุดปัจจัยหนึ่งคือการขาดธาตุอาหารโบรอน เพราะโบรอนมีบทบาทสำคัญต่อพัฒนาการของละอองเรณูและการผสมเกสร งานวิจัยนี้ได้การสร้างความเข้าใจในความหลากหลายของกระบวนการสืบพันธุ์ด้วยเมล็ดและการใช้ธาตุอาหารโบรอน และนำไปใช้ประโยชน์ในการเพิ่มอัตราการผสมข้ามในการสร้างลูกผสมโดยแบ่งตามวัตถุประสงค์ย่อยแต่ละข้อดังนี้

1. บ่งชี้ความแตกต่างทางพันธุกรรมของลักษณะที่เอื้อต่อการผสมข้ามในข้าวปลูก

ข้าวปลูกเป็นพืชผสมตัวเอง แต่มีรายงานว่าในบางเงื่อนไขหรือบางพันธุ์พบว่าข้าวปลูกมีอัตราการผสมข้ามสูงได้ถึง 7% งานทดลองนี้จึงได้ศึกษาพบความแตกต่างทางพันธุกรรมของลักษณะดอกที่เกี่ยวกับโอกาสการผสมข้ามในข้าวปลูกพันธุ์ไทยที่เป็นพันธุ์สมัยใหม่ ไม่ไวต่อช่วงแสง ในลักษณะเกสรตัวเมียพบว่าข้าวพันธุ์ปทุมธานี 1 และเหนียวแพร่ 1 มีขนาดยอดเกสรตัวเมียใหญ่ที่สุด บางพันธุ์ขณะผสมเกสรมียอดเกสรตัวเมียโผล่ออกมานอกกลีบดอกโดยพันธุ์ กข 10 กข 7 และสกลนคร 1 มียอดเกสรตัวเมียโผล่ออกมาทั้งหมด 100% ส่วนลักษณะเกสรตัวผู้นั้นพบว่าข้าวพันธุ์ R258 และปทุมธานี 1 มีอับละอองเรณู (anther) ใหญ่ที่สุด พันธุ์ปทุมธานี 1 มีจำนวนละอองเรณูต่อ anther มากที่สุด ทุกพันธุ์ที่ติดเมล็ดเป็นปกติพบว่ามีการโปรยละอองเรณูบนยอดเกสรตัวเมียไม่น้อยกว่า 40 ละอองเรณูขึ้นไป พันธุ์สกลนคร 1 รับละอองเรณูได้สูงสุดถึง 79 ละอองต่อยอดเกสรตัวเมีย สำหรับพฤติกรรมการบานดอกพบว่ากลีบดอกจะบานแยกออกจากกันประมาณ 35-50 นาทีโดย กข 10 R258 และปทุมธานี 1 บานดอกนานที่สุด ได้พัฒนาวิธีการตรวจสอบความมีชีวิตของละอองเรณูโดยการเพาะในวุ้นและเป็นประโยชน์ในการศึกษาทางด้านสรีรวิทยาและปรับปรุงพันธุ์

2. บ่งชี้ความแตกต่างทางพันธุกรรมของการตอบสนองต่อธาตุอาหารโบรอนในข้าว

ศึกษาในข้าวปลูก 3 พันธุ์คือขาวดอกมะลิ 105 สุพรรณบุรี 1 และชัยนาท 1 ทำซ้ำ 2 ฤดู ปลูกปลูกในกระถางบรรจุทรายรดด้วยสารละลายธาตุอาหารครบแต่ผสมโบรอนให้เพียงพอ (B10) และไม่ให้ (B0) ในสารละลายพบว่าการขาดโบรอนมีผลทำให้ลดความมีชีวิตของละอองเรณูส่งผลให้ลดการติดเมล็ดในข้าวแต่ไม่พบผลกระทบใดๆ ต่อการเจริญเติบโตทางลำต้นและใบ การวัดความเข้มข้นของโบรอนในอับละอองเรณูและในใบธงสามารถใช้บ่งชี้สถานะของโบรอนที่มีผลต่อการติดเมล็ดได้ พบความแตกต่างระหว่างพันธุ์ในการตอบสนองต่อโบรอนในการติดเมล็ดของพันธุ์ที่ศึกษา โดยพันธุ์สุพรรณบุรี 1 มีสมรรถนะการใช้โบรอนได้สูงสุด รองลงมาได้แก่ขาวดอกมะลิ 105 และต่ำสุดได้แก่ชัยนาท 1 นอกจากนั้นยังได้ศึกษาในข้าวพันธุ์ลูกผสม 4 พันธุ์พบว่าการขาดโบรอนในสารละลายที่ให้ทางรากทำให้อัตราการติดเมล็ดลดลงประมาณ 12% มีความแตกต่างระหว่างพันธุ์ในความต้องการโบรอนในการงอกของละอองเรณูเมื่อเพาะในวุ้น แสดงว่าการงอกของละอองเรณูนั้นนอกจากต้องการโบรอนภายในตัวละอองเรณูเองแล้ว ยังต้องการโบรอนภายนอกในยอดเกสรตัวเมียให้เพียงพออีกด้วย

3. บ่งชี้ความแตกต่างทางพันธุกรรมของการติดเมล็ดในสภาพอุณหภูมิสูง

ประเมินการติดเมล็ดของข้าวปลูก 12 พันธุ์โดยจัดวันปลูก 3 ช่วงในฤดูนาปรัง เพื่อให้ระยะเวลาออกดอกกระทบอุณหภูมิสูง ศึกษาในแปลงทดลองที่มหาวิทยาลัยเชียงใหม่และแปลงทดลองของภาคเอกชนที่สุพรรณบุรี จากผลการทดลองทั้งสองแห่งพบความแตกต่างระหว่างพันธุ์ในการตอบสนองต่ออุณหภูมิโดยเมื่อปลูกกลางเดือนกุมภาพันธ์พบว่าพันธุ์ที่ไม่ทนร้อน ได้แก่สันป่าตอง 1 กข 4 กข 10 และกข 21 ติดเมล็ดเพียง 75-78% ผลกระทบต่อการติดเมล็ดในข้าวหรือการเกิดเมล็ดลีบเป็นผลมาจากการไม่ผสมเกสรในระยะออกดอกหรือถึงแม้ผสมเกสรได้แต่เกิดเมล็ดลีบไม่เต็มเมล็ดเป็นผลต่อการสะสมแป้งในเมล็ด พันธุ์กข 29 สุพรรณบุรี 1 และปทุมธานี 1 ถูกจัดเป็นพันธุ์ทนร้อนและติดเมล็ดเป็นปกติ

4. ประเมินและคัดเลือกลักษณะที่เอื้อต่อการผสมข้าม

ดำเนินงานจากคู่ผสม 2 ชุด คือ (1) ลูกผสมชั่วที่ 4 ระหว่างข้าวป่าสามัญกับข้าวปลูก (2) ลูกผสมกลับจากคู่ผสมระหว่างข้าวป่าสามัญ x สุพรรณบุรี 1 แล้วผสมกลับไปยังสุพรรณบุรี 1 ได้ใช้วิธีการที่พัฒนามาจากวัตถุประสงค์ที่ 1 ในการคัดเลือก ในชุดแรกได้ประเมินและคัดเลือกได้ลูกที่มีลักษณะใหม่ๆ จนถึงชั่วที่ 6 สายพันธุ์ที่คัดเลือกมีขนาดเกสรใหญ่และมีลักษณะอื่นๆ ดีตามต้องการ แต่ยังพบว่ามีอัตราการร่วงของเมล็ดสูงอยู่ ในชุดที่ 2 ได้คัดเลือก ประเมิน ผสมกลับจนถึงผสมกลับครั้งที่ 2 ชั่วที่ 4 ได้คัดเลือกสายพันธุ์ที่มีขนาดเกสรใหญ่และมีลักษณะอื่นๆ ดีตามต้องการและเมล็ดไม่ร่วงได้ 16 สายพันธุ์ ซึ่งสามารถนำไปใช้เป็นพ่อแม่พันธุ์ในการปรับปรุงพันธุ์เพื่อคัดเลือกลักษณะที่เอื้อต่อการผสมข้ามได้ต่อไป

การใช้ประโยชน์จากผลงานวิจัย

1. จากงานศึกษาความแตกต่างระหว่างพันธุ์ข้าวปลูกในลักษณะที่เกี่ยวข้องกับการผสมเกสรทำให้ทราบถึงขอบเขตความสามารถของพันธุ์ต่างๆ สามารถกำหนดเป้าหมายของงานปรับปรุงพันธุ์ ลักษณะที่ต้องการปรับปรุง ความสัมพันธ์ของลักษณะต่างๆ จะช่วยให้ทราบถึงทิศทางของการตอบสนองต่อการคัดเลือกเพื่อปรับปรุงลักษณะดอกต่างๆ ให้ไปในทางที่ต้องการ

2. ได้พัฒนาวิธีการตรวจสอบความมีชีวิตของละอองเรณูอย่างง่าย รวดเร็ว แม่นยำ สามารถใช้ในงานศึกษาผลกระทบจากสภาพแวดล้อมต่างๆ ได้อย่างกว้างขวางรวมทั้งช่วยในการคัดเลือกพันธุ์

3. งานศึกษาการตอบสนองต่อธาตุอาหารโบรอนนำไปสู่ความเข้าใจในผลกระทบของการขาดโบรอนต่อผลผลิตข้าวผ่านทางการลดการงอกของละอองเรณูส่งผลให้การผสมเกสรล้มเหลว และลดการติดเมล็ดในที่สุด ในพื้นที่ที่มีโบรอนในดินต่ำการใส่โบรอนในดินมีโอกาสช่วยลดผลกระทบต่อผลผลิตข้าว ในพันธุ์ที่มีสมรรถนะการใช้โบรอนต่ำยังพบว่าการงอกของละอองเรณูต้องการโบรอนภายในตัวมันเองและโบรอนภายนอกในบริเวณเกสรตัวเมียด้วย ผลการทดลองนำไปสู่งานวิจัยเรื่องการจัดการธาตุโบรอนในการผลิตเมล็ดพันธุ์ข้าวลูกผสมในภาคเอกชน

4. นอกจากนั้นงานศึกษาด้านโบรอนยังได้ทราบและกำหนดค่าวิกฤตของความเข้มข้นโบรอนที่มีผลต่อพัฒนาการด้านสืบพันธุ์และสามารถใช้ระบุสถานะของธาตุอาหารโบรอนและการขาดโบรอนในข้าว

5. งานศึกษาการตอบสนองต่ออุณหภูมิสูงในข้าวทำให้เข้าใจและสามารถใช้ลักษณะการติดเมล็ด จำนวนเมล็ดเต็มในการคัดเลือกพันธุ์เพื่อปลูกในสภาพอากาศร้อน ผลจากการศึกษาความแตกต่างระหว่างพันธุ์ได้พัฒนาต่อไปเป็นส่วนหนึ่งของงานวิจัยข้าวไทยเพื่อโลกร้อน (ทุนสนับสนุนจากสกว.) และโครงการวิจัยเรื่องการถ่ายทอดทางพันธุกรรมของการตอบสนองต่ออุณหภูมิสูงในข้าว (ทุนวิจัยระดับปริญญาเอกจากสกอ.)

6. ใช้ความเข้าใจและวิธีการตรวจสอบที่พัฒนาในการคัดเลือกและผสมพันธุ์ข้าว ได้ลูกผสมสายพันธุ์ก้าวหน้าที่มีลักษณะเอื้อต่อการผสมข้ามที่พัฒนามาจากข้าวป่าสามัญและข้าวปลูก สายพันธุ์เหล่านี้สามารถนำไปใช้ในโครงการปรับปรุงพันธุ์เพื่อสร้างพ่อแม่ในการเพิ่มผลผลิตเมล็ดลูกผสมได้

Improving Hybrid Rice Seed Production by Better Understanding of Diversity in the Breeding System and Boron Nutrition

การเพิ่มประสิทธิภาพการผลิตเมล็ดพันธุ์ข้าวลูกผสมด้วยการสร้างความเข้าใจในความหลากหลายของกระบวนการสืบพันธุ์ด้วยเมล็ดและการใช้ธาตุอาหารโบรอน

Hybrid seed technology has been successfully deployed to increase efficiency in maize production in Thailand and Southeast Asia. The private sector in this country is now embarking in research in hybrid rice, which promises to raise farmer's as well as potential yield of irrigated rice by 30-50%.

Once the basic requirements for superior performance in production (wide adaptation, high yield, resistance to major diseases etc) are met, the primary target in the development of hybrid seed technology is high yield of the F_1 hybrid seed. The higher yield will translate into lower cost per kg of the seed, which will in turn help to lower farmers' input cost. However, the key process in hybrid seed production is the pollination and fertilization. This project has contributed to knowledge in genotypic variation in breeding system and boron response to improve outcrossing in hybrid seed production, in four sets of objectives as follows.

I. TECHNICAL REPORT

1. To identify genotypic variation in traits promote outcrossing in cultivated rice

This part was conducted in two main experiments, namely, variation in floral organs and flowering behavior and pollen viability study.

Variation in floral organs and flowering behavior

Rice is a self-pollinated crop. Up to 7% of outcrossing has been reported in specific varieties or conditions. Genotypic variation for floral morphology relation to outcrossing was studied in 12 modern, photoperiod insensitive, Thai rice varieties during dry season 2008/2009. The experiment was carried out in pots, 10 plants per pot for each variety with three replicates. At flowering, floral traits those reported to influence outcrossing were measured as (1) Stamen traits: anther length and anther width, number of pollen per anther, pollen viability, (2) Pistil traits: degree of exerted stigma, number of exerted stigma per floret, stigma length, style length and number of germinated pollen on a stigma, (3) flowering behavior: time at blooming and blooming duration. Stamen and pistil traits were collected from 27 florets per genotype. Anther and pistils were collected and fixed in acetic-alcohol then the length and width were measured under stereomicroscope. For pollen number, nine florets from each variety were collected, stained with 1% potassium iodine (KI/I) and counted for number of pollen grains per anther. Late in the afternoon when the florets have complete fertilization, three florets on the top, middle and bottom of three panicles were collected and determined for number of pollen on the surface of stigma. The florets were excised on slide and stained with cotton blue. Pollen number and viability were counted under stereomicroscope.

For stigma exertion, data were recorded from 45 florets/genotype. Exertion types were rated as no exertion, single stigma exertion and dual stigma exertion. For flowering behavior, time when the glumes started opening until closing for each floret were recorded from 15 florets/genotype.

Outcrossing in rice was influenced by floral traits and flowering behavior. Expected traits influenced outcrossing for genotypes used as female parents are stigma size and exertion, style length and duration of spikelet blooming. On the other hand, traits promoted outcrossing for the pollen, or male, parent are anther size, number of pollen grains per anther, pollen viability and duration of spikelet blooming . Range of variation of these traits among modern, high yielding cultivated Thai rice varieties found in this study is summarized in Table 1.1 and genotypic variation for each traits in Tables 1.2-1.3 and Figure 1.1.

Table 1.1 Summary of flower characteristics of 12 rice varieties, grown at Chiang Mai, Thailand.

Characters	Mean ± SE	Min	Max
Female flower			
Pistil length (mm)	1.9 ± 0.05	1.7	2.4
Stigma length (mm)	0.9 ± 0.03	0.7	1.1
Style length (mm)	1.0 ± 0.05	0.8	1.3
Total stigma exertion (%)	79.1 ± 4.9	48.9	100
Single stigma exertion (%)	52.2 ± 3.4	28.9	71.1
Dual stigma exertion (%)	26.9 ± 5.8	4.4	71.1
Male flower			
Anther length (mm)	2.5 ± 0.03	2.3	2.7
Anther width (mm)	0.8 ± 0.01	0.8	0.9
Pollen/anther	1509 ± 17	1220	1960
Pollen/stigma	58.7 ± 1.1	34.7	81.0
Pollen viability (%)	94.7 ± 1.1	87.7	97.1
Flower behavior			
Time at blooming (a.m.)	10:18	9:30	11:20
Blooming duration (min)	37 ± 1.3	28	50

For traits require for the female parents, the range of variation for stigma and style lengths were between 1.7-2.4 mm and 0.7-1.1 mm, respectively. Pathumtani 1 (PTT1) and Neaw Phrae 1 (NP1) were the varieties with the longest stigma while, Chainat 1 (CNT1) had the longest style (Tables 1.1 and 1.2). Extents of stigma exertion were between 49-100%, with RD10, RD7 and Sakonakorn 1 (SKN1) showed 100% stigma exertion. As each floret of rice has two stigma (Picture 1.1), we found variation in rice varieties of exertion for single and dual stigma per floret (Picture 1.2). The highest dual stigma exertion was found in RD10 and highest single stigma exertion in SKN1 (Figure 1.1). NP1 and PTT1 exhibited the longest stigma among all varieties but turn out to be the lowest rate of stigma exertion. Among the modern varieties, RD7 and SKN1 had higher potential of outcrossing than the others.

For male flower of rice, anther length and width were between 2.3-2.7 mm and 0.8-0.9 mm, respectively. R258 and PTT1 exhibited the largest anthers. Number of pollen grains per anther were between 1,220 in RD4 to 1,960 in PTT1 (Table 1.3). Fertilization was depended on number of pollen deposited on stigma. Most of varieties deposited more than 40 pollen grains per stigma and ensured fertilization. The highest pollen grain per stigma (79) was found in SKN1 (Table 1.3). For flower behavior, opening of the spikelet or blooming at Chiang Mai University, Chiang Mai started at 9.30 to 10.20 am. Blooming duration, the period from opening to closing of floret, was varied with genotypes and within the range of 35-50 min. For the long duration genotypes, RD10, R258 and PTT1 lasted for 50 min (Figure 1.2).

Variations for some flower traits of the 13 genotypes were correlated. Variation in pistil length was more influenced by style length than stigma length (Table 1.4). Positive correlation between anther length was found with style length and anther width. Improvement of floral traits involving size of male or female organ can be done together. However, pistil length tended to show negative correlation with blooming duration. Therefore, improvement of floral traits and duration of blooming should be done separately.

Table 1.2 Variation in female reproductive traits of 12 rice varieties.

Genotypes	Stigma length (mm)	Style length (mm)	Pistil length (mm)	Stigma exsertion (%)
NP1	1.01 ab	1.00 cde	2.02 ab	51 e
R258	0.74 cd	1.02 cde	1.76 abc	67 de
SPT1	0.83 cd	0.98 def	1.81 ab	73 cd
RD4	0.76 cd	1.27 ab	2.03 a	76 bcd
RD7	0.86 bcd	1.17 bc	2.03 a	100 a
RD10	0.73 cd	1.10 bcd	1.83 ab	100 a
RD21	0.81 cd	1.17 bc	1.98 ab	67 de
RD29	0.91 abc	1.03 cde	1.94 ab	78 bcd
CNT1	0.71 d	1.35 a	2.06 a	91 ab
SPR1	0.88 bcd	0.81 fg	1.69 bc	91 ab
SKN1	0.81 cd	0.64 g	1.45 c	100 a
PTT1	1.07 a	0.81 fg	1.89ab	49 e
F-test	**	****	*	***

*, ** and *** significant at p<0.05, 0.01 and 0.001, respectively.

Means followed by the same letters within column do not differed significantly by LSD at p=0.05

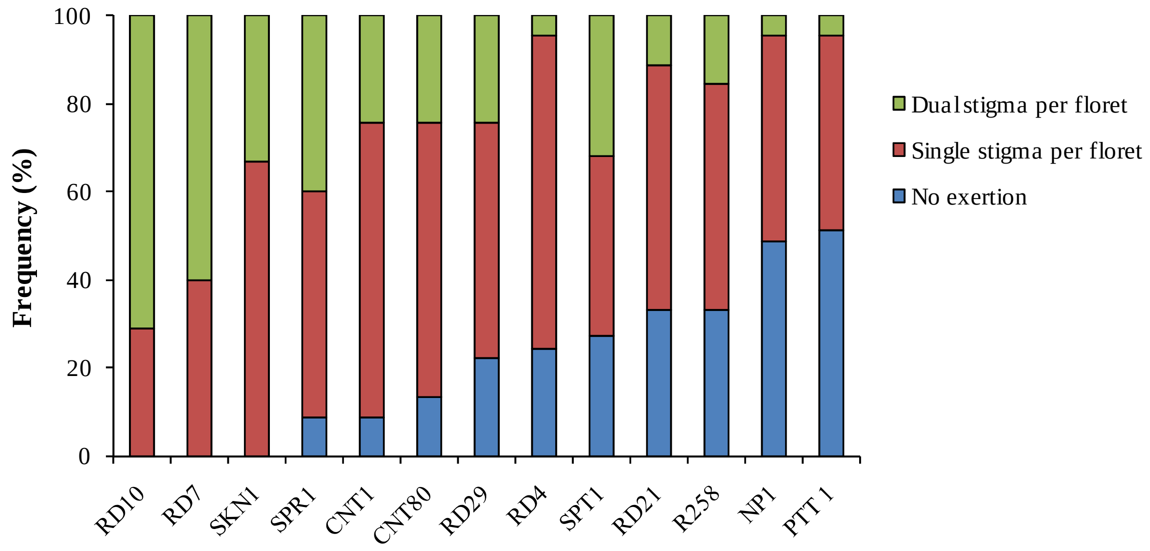


Figure 1.1 Frequency (%) of spikelets with no, single and dual stigma exertion per floret of 12 rice varieties. Data were average from 27 florets per variety.

Table 1.3 Variation in male reproductive traits of 13 rice varieties.

Genotypes	Anther length (mm)	Anther Width (mm)	Number of pollen per anther		Number of pollen deposited per stigma	
			Total	KI/I[a] stained (%)	Total	Viability[b] (%)
NP1	2.40 c	0.86 a	1610 bc	88 f	50	54 c
R258	2.74 a	0.82 a	1490 cd	91 e	81	66 b
SPT1	2.44 bc	0.86 a	1470 cd	97 a	64	78 a
RD4	2.57 abc	0.82 a	1220 e	95 c	63	78 a
RD7	2.43 bc	0.79 a	1620 bc	96 ab	61	76 ab
RD10	2.54 abc	0.81 a	1320 de	95 c	55	75 ab
RD21	2.55 abc	0.78 a	1330 de	96 ab	67	80 a
RD29	2.56 abc	0.77 a	1470 cd	96 ab	44	79 a
CNT1	2.44 bc	0.82 a	1460 cd	96 ab	53	74 ab
SPR1	2.33 c	0.80 a	1367 de	96 ab	35	74 ab
SKN1	2.01 d	0.64 b	1795 ab	95 cd	79	78 a
PTT1	2.69 ab	0.80	1960 a	94 d	56	73 ab
F-test	**	*	***	**	ns	***

[a] Pollen viability test by iodine (KI/I) staining.
[b] Pollen viability test by cotton blue staining.
*, ** and *** significant at p<0.05, 0.01 and 0.001, respectively.
Means within a column followed by the same letters do not differed significantly by LSD at p=0.05

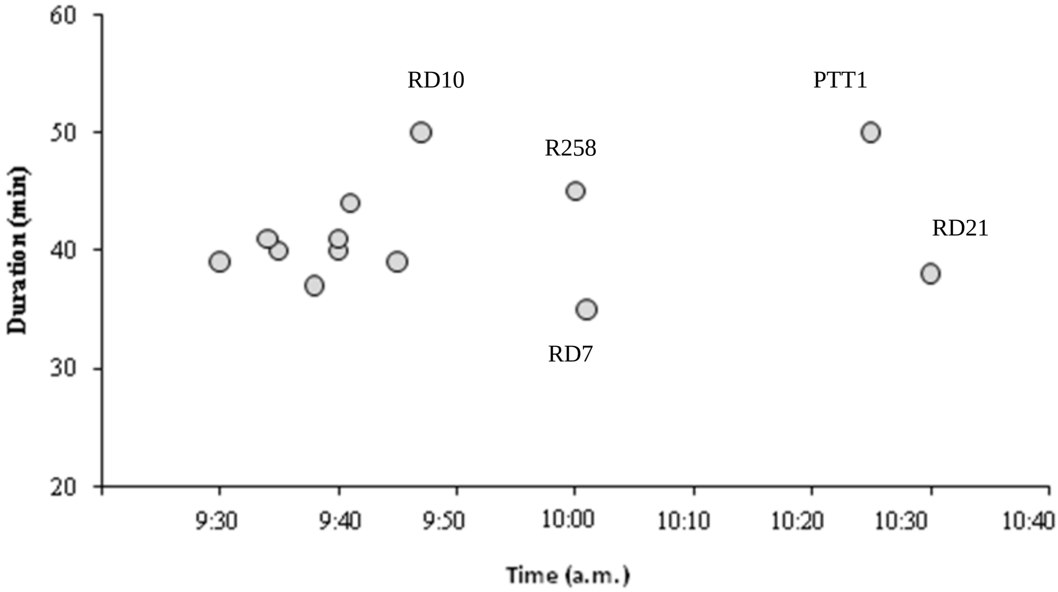
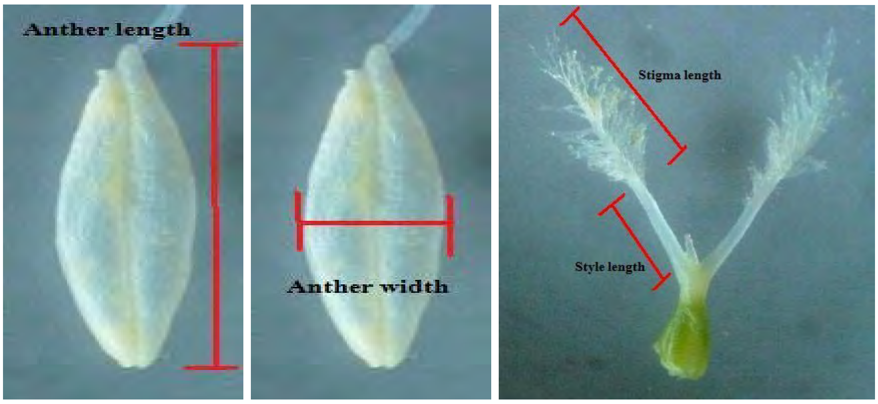


Figure 1.2 Time (a.m.) at first floret started blooming and blooming duration (min) of 12 rice varieties.

Table 1.4 Correlations between floral characteristics among 12 rice varieties.

Traits	Stigma length	Style length	Pistil length	Anther length	Anther width
Style length	-0.156				
Pistil length	0.446**	0.815***			
Anther length	0.283	0.527***	0.643***		
Anther width	0.367*	0.555***	0.718***	0.705***	
Blooming duration	0.062	-0.399*	-0.325*	-0.101	-0.148

*, ** and *** significant at $p<0.05$, 0.01 and 0.001, respectively.



Picture 1.1 Anther and pistil measurements in this study.

(a) (b) (c)

Picture 1.2 Types of stigma exertion in rice a) no exertion b) single stigma exertion and c) dual stigma exertion.

Pollen viability

Pollen viability was studied in agar media in petri-dishes. Fresh pollen was shaken directly to media after the anther just emerged from the glumes. This was done during 10.30-11.45 a.m. The media contained 0.70% agar, 0.50 M sucrose, 0.03 % $CaCl_2.2H_2O$ and 0.01 % boric acid gave the highest pollen germination when pollens were collected immediately after anther dehiscence. Pollen germination was scored microscopically after 4h. Pollen was recorded as germinated if the pollen tube was longer than half of the diameter of the pollen grain. Germination of rice pollen was depended on time of collection, genotypes and other environmental factors. Rice pollen grains after shedding were short life. For example, germination of CNT1 after anther dehiscence was lost about 70% of pollen viability at 3 min and nearly 100% about 9 min (Figure 1.3).

Pollen viability test by agar developed in this study was validated further in three rice varieties (CNT1, SPR1 and SPT1). Sowing dates were set to cover from November to January 2008 to cover a range of seed set. Plants were flower during February to April 2009. Maximum and minimum temperatures during this period were between 30-40 ºC and 20-25 ºC, respectively. At flowering, anthers were collected and germination tested in agar. In addition, stigmata were also collected from six florets, stained with cotton blue. Number of pollen grains deposited on each stigma were counted. Those stained with cotton blue were rated as viable and those with pollen tube were rated as germinated. At maturity, plant were harvested and determined for seed set. Significant correlations between seed set at maturity and number of pollen germinated on stigma (r = 0.583**) and between seed set and pollen germination on agar media (r = 0.841***, Figure). Therefore, pollen germination tested was reliable, and can be used to screen for pollen viability in breeding program or other physiological and biological studies in rice.

Implications of main research findings

- From variation in floral characteristics in rice the project has contribute to the extent of variation found I rice varieties commonly grown in Thailand. This will help to set the goal of improvement, source of variation and correlation of traits, to be used in breeding programe.
- Selection and screening method for pollen viability has been developed. Training for students and researchers at CMUPNlab was done in 2008. Training for staffs in private partner is scheduled in July 2010.

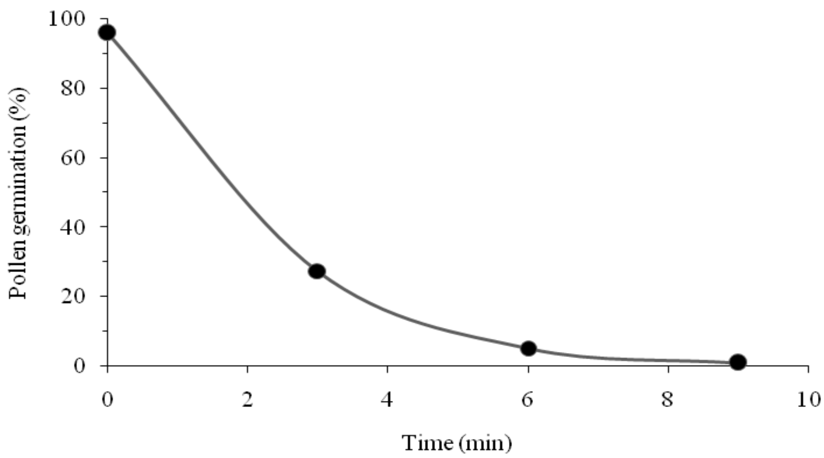
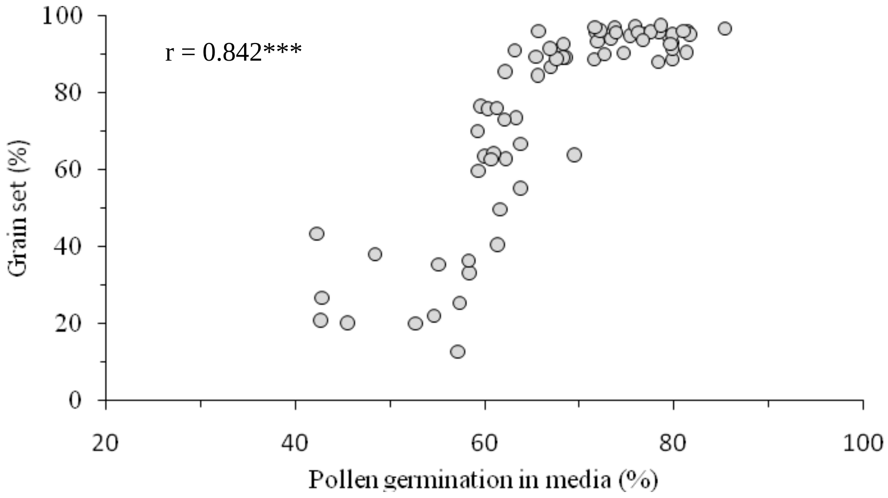


Figure 1.3 Pollen germination (%) of CNT1 rice, on agar media at different times (min) after anther dehiscence.

a)



b)

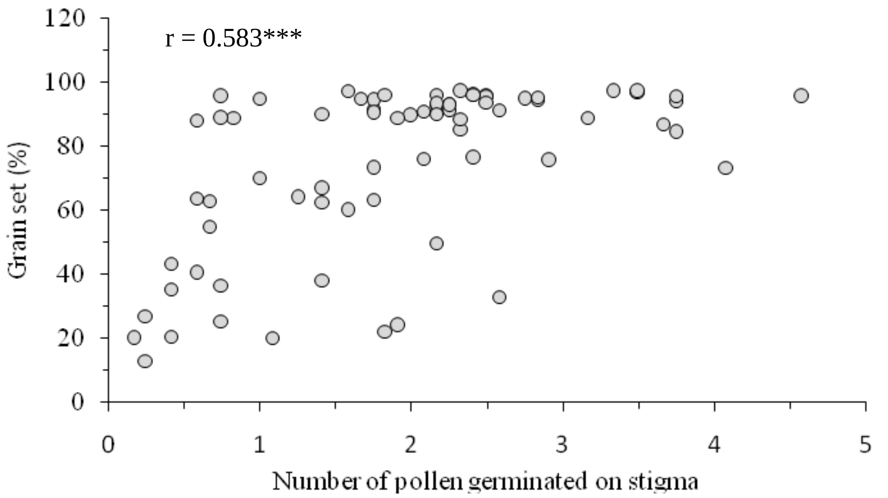


Figure 1.5 Relationship between grain set (%) at maturity and a) pollen germination (%) on agar media b) number of pollen germinated on stigma.

To identify genotypic variation in B response rice

A major constraint to successful pollination and seed set in cereals is boron deficiency. Cereals, along with other grasses, require very little boron for their vegetative growth. It is, however, well established that successful seed set requires many times more boron per unit dry weight in specific tissues such as pollen grain (ten times or more) and pistil or silk in maize (3-4 times) than in somatic tissues like root, leaf and stem.

Soils low in boron are widespread in Thailand, so the risk of boron deficiency depressing the yield of hybrid seed is real, especially on highly leached upland soils of the north and northeast. Better understanding of how boron nutrition affects male fertility/sterility in rice may also offer another option for controlling male sterility in the process of hybrid seed production. In this part, there are two sets of studies for a) response to B in three commercial, popular Thai rice varieties and b) response to B in hybrid rice varieties

Response to B in Thai rice varieties

Boron deficiency adversely affects grain yield of cereals including wheat, barley triticale and maize. It has been reported that B deficiency caused male sterility and reduced grain set via pollen sterility. In rice, B deficiency was confounded with seasonal effect. Preliminary study found that grain set in wet season was not affected and no genotypic variation was detected. Therefore, the experiments were conducted in dry season in two years. Three rice varieties commonly grown in Thailand were grown in sand culture, pot experiments. B treatments were set as with (10 M kg B, designated as B10) or without added B (B0) to the nutrient solution. As found in other cereals, vegetative growth and number of spikelets per panicle of all rice varieties were not affected by low B supply (Tables 2.1 and 2.2). Significant interaction BxG effect was found in pollen viability, grain set and B concentrations in flag leaf, YEB+1 and anthers (Tables 2.1 and 2.3).

Table 2.1 Analysis of variance results for effects of boron (B), genotype (G) and boron x genotype interaction (BxG) on vegetative response, reproductive response and B concentrations of rice grown in two seasons (2007/08 and 2008/09).

Characters	2007/08			2008/09		
	B	G	B x G	B	G	B x G
Vegetative response						
Tillers plant^{-1}	ns	**	ns	ns	**	ns
Panicles plant^{-1}	ns	**	ns	ns	**	ns
Culm length (cm)	ns	ns	ns	ns	ns	ns
Straw dry weight (g)	ns	**	ns	ns	**	ns
Root dry weight (g)	ns	**	ns	ns	**	ns
Reproductive response						
Spikelets panicle^{-1}	ns	**	ns	ns	**	ns
Pollen viability (%)	**	*	*	**	**	**
Grain set (%)	**	**	*	**	**	**
B concentrations (mg B kg DW^{-1})						
Flag leaf [B]	**	**	**	**	*	*
YEB+1 [B]	*	ns	**	**	*	**
Anthers [B]	**	**	**	**	*	**

* and ** significant at p<0.05 and 0.01, respectively; ns not significant.

In these experiments, B deficiency can be seen in B concentrations in anther or flag leaf. A reduction in grain set, compared with B10, was involving B concentrations in anther and flag leaf less than 25 and 8 mg kg B^{-1}, respectively (Figure 2.1). B in flag leaf can be used as a general indicator for overall genotype for B status at early stage of plant growth. Between the three genotypes, SPR1 was the most efficient and CNT1 was the most inefficient and KDML 105 was intermediate.

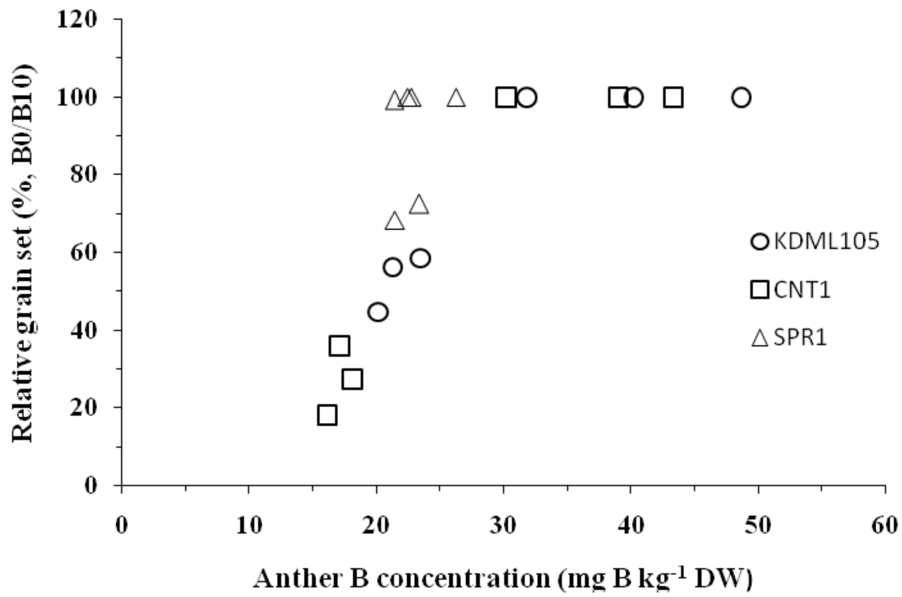
Table 2.2 Number of panicles $plant^{-1}$ and spikelets $panicle^{-1}$ of three rice genotypes at two levels of B supply.

B level (µM)	Genotype	2007/08		2008/09	
		Panicles $plant^{-1}$	Spikelets $panicle^{-1}$	Panicles $plant^{-1}$	Spikelets $panicle^{-1}$
0	KDML105	7	129	11	107
	CNT1	8	113	13	85
	SPR1	8	161	14	118
10	KDML105	7	125	11	125
	CNT1	9	103	16	85
	SPR1	10	146	13	134
F-test					
Genotype (G)		**	**	*	*
B (B)		ns	ns	ns	ns
BxG		ns	ns	ns	ns

Table 2.3 Pollen viability and grain set (%) of three rice genotypes at two levels of B supply.

B level (µM)	Genotype	2007/08		2008/09	
		Pollen viability (%)	Grain set (%)	Pollen viability (%)	Grain set (%)
0	KDML105	28.6 a	10.9	25.2 a	11.5 a
	CNT1	33.2 a	6.7	55.1 bc	14.6 bc
	SPR1	27.4 a	27.9	54.0 b	47.2 c
10	KDML105	36.9 a	18.7	65.1 c	21.7 b
	CNT1	59.9 b	12.9	59.6 bc	53.9 cd
	SPR1	70.3 b	38.4	80.9 d	59.7 d
F-test					
Genotype (G)		*		**	**
B (B)		**		**	**
BxG		*		**	**
$LSD_{0.05}$		18.8		10.0	9.2

a)



b)

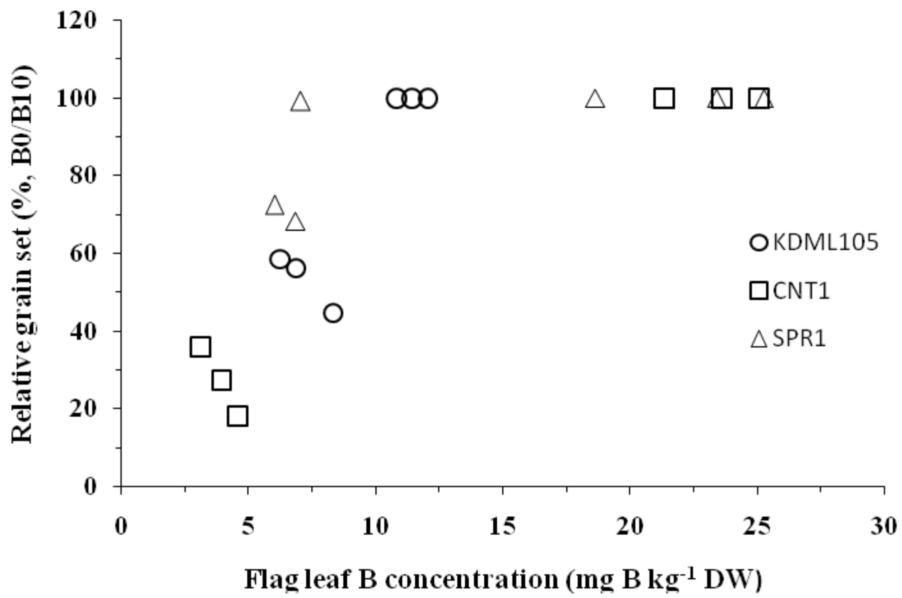


Figure 2.1 Relationship between grain set (%) and B concentrations in a) anther and b) flag leaf of three rice genotypes, Pot Experiment 2008/09.

Response to B in hybrid rice varieties

For hybrid rice, four varieties were tested at the same two B treatments in sand culture (B0 and B10) in 2008/2009. Genotypic variation in hybrid rice was found for B requirement for pollen germination in agar media (Table 2.4). Low germination of pollen in media without added B indicated external B was required for pollen germination. At maturity, no BxG interaction was significant for all characters. All hybrids responded to B similarly. B deficiency reduced average grain set at about 12% (Table 2.5).

Table 2.4 Genotypic variation of pollen germination (%) in media with and without added B of three hybrid rice and CNT varieties at two B supply (0 and 10 uM B; designated as B0 and B10).

Plant	Media	HR 107-3-3	HR 107-3-5	HR 107-4-5	HR 107-4-5		
B0	Without added B	8.4	0.6	3.5	33.7		
	Added B	46.1	41.5	20.1	56.5		
B10	Without added B	6.3	18.3	9.3	31.0		
	Added B	38.4	73.5	61.9	49.7		
Mean		24.8	33.5	23.7	42.7		
	B	G	Media	B x G	B x media	G x media	B x G x media
F-test	***	***	***	***	NS	***	***
LSD $_{0.05}$	4.0	5.7	4.0	8.0		8.0	11.3

Table 2.5 Average culm length (cm) and number of tillers, panicles and spikelets and grain set of four hybrid rice genotypes at two B supply (0 and 10 uM B; designated as B0 and B10).

B treatment	Culm length (cm)	Tillers plant^{-1}	Panicles plant^{-1}	Spikelets panicle^{-1}	Grain set (%)
B0	52	28	15	151	43
B10	53	25	19	157	54
F-test	ns	ns	ns	ns	**
LSD0.05					7

Implications of the main research findings

- B deficiency affected rice yield via pollen germination and grain set. Pollen germination of sensitive genotypes were affected by both internal B in pollen external B in stigma. Applying B in soil has a potential to improve rice yield in areas with low B soil. The results have led to the foliar B application study in hybrid rice evaluation at Bayer research station.
- Critical value for B established for reproductive development and can be used to diagnose for B status and possibility of B deficiency in rice.

3. To identify genotypic variation in grain set under high temperature

Variation in grain set was evaluated in 12 rice varieties at three planting dates in dry season (PD1: mid-January, PD2: late January and PD3: mid- February 2009) at two locations, Chiang Mai and Suphanburi. The planting dates were set to allow genotypes to flower during high temperature in summer. At Chiang Mai, genotypes were sown in pots on Jan 17 2009 (PD1), Jan 31 2009 (PD2) and Feb 14 2009 (PD3). At Bayer Research Station in Suphanburi, genotypes were sown in field experiments on Jan 13 2009 (PD1), Jan 20 2009 (PD2) and Feb 10 2009 (PD3). Plants were flowering during mid-April to late May 2009 which maximum temperatures were above 35°C and minimum temperatures were above 20°C (Figure 3.1).

Numbers of spikelets, fertilized, filled and empty grains were recorded from both locations. There were genotypic differences in response to high temperature.

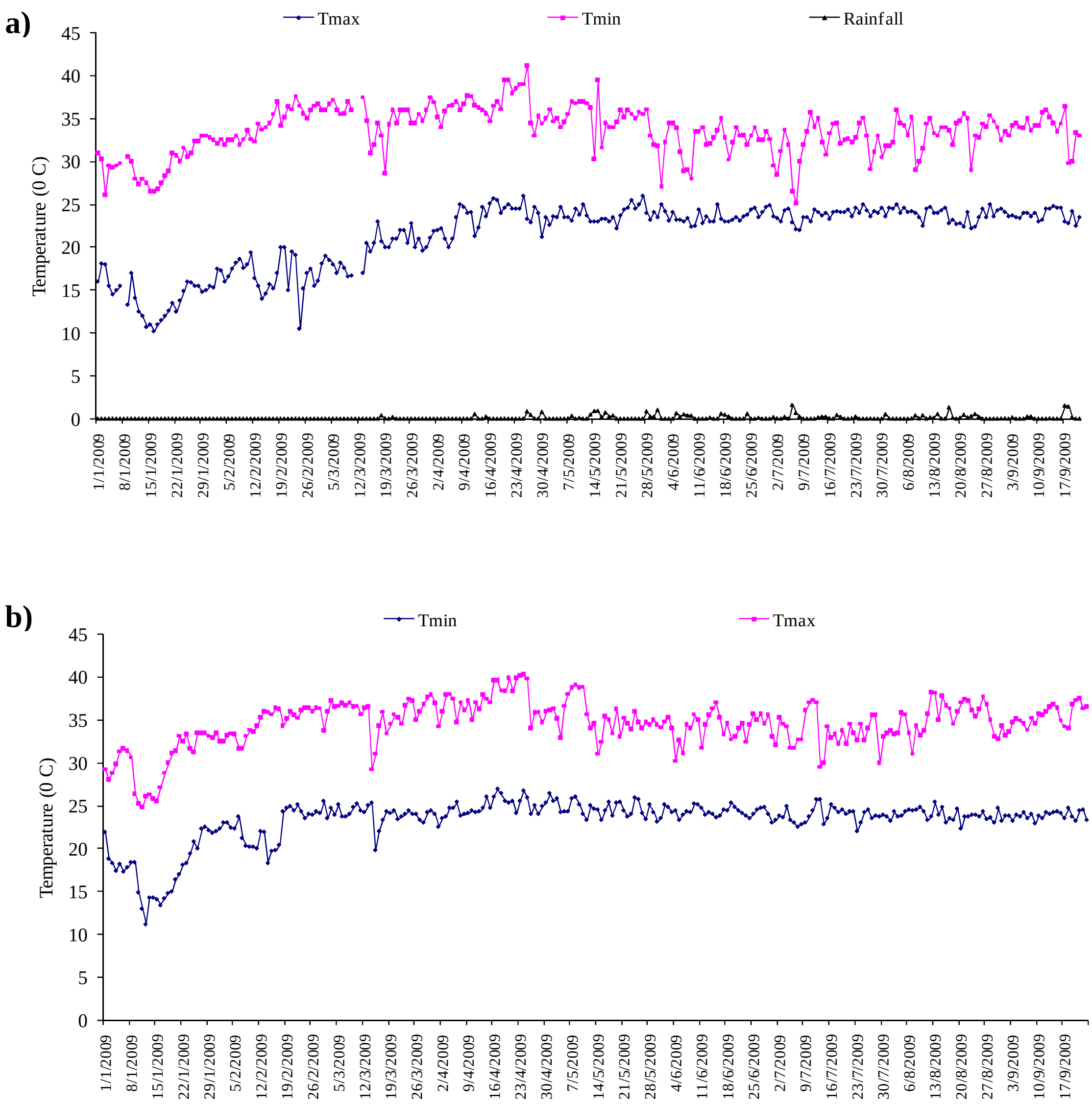


Figure 3.1 Daily minimum and maximum temperatures at Chiang Mai (a) and maximum temperature at Suphanburi (b) during January 2009 to September 2009. Flowering times of rice varieties of the three planting dates were between early April and late May 2009.

Differential genotype response to high temperature was attributed to grain set failure and unfilled grain. Significant effects of genotypes and planting date x genotype interaction ($p<0.001$) were found for all variables (Table 3.1). For number of spikelets per panicle RD21 displayed the highest spikelet number which were increased with planting dates. There were different types of response of genotypes to planting date (Table 3.2).

Table 3.1 Analysis of variance results for effects of planting date (PD), genotype (G) and planting date x genotype interaction (PDxG) on six traits of 12 rice varieties grown at two locations, Chiang Mai and Suphanburi.

Characters	Chiang Mai			Suphanburi		
	PD	G	PDxG	PD	G	PDxG
Spikelets panicle^{-1}	***	***	***	ns	***	***
Grains panicle^{-1}	***	***	***	***	***	***
Grain set (%)	***	***	***	***	***	***
Unfertilized grain (%)	***	***	***	***	***	***
Unfilled grain (%)	***	***	***	***	***	***

*** significant at $p<0.001$; ns not significant.

Small but significant effect of planting date x genotype on grain set was observed. Delaying sowing dates to the end of January or mid February decreased grain set on average at 3-4%. All genotypes, except SPT1, RD4, RD10 and RD21, set grain between 80-96% at all planting dates. Grain set of the sensitive genotypes, SPT1, RD4, RD10 and RD21, were between 85-94% at PD1 and PD2 and those of SPT1 and RD4 were reduced to 76% at PD3 in Chiang Mai and of SPT1, RD4, RD10 and RD21 reduced to 75-78% at PD3 grown in Suphanburi (Table 3.3). They were classified as sensitive to high temperature. The smallest decrease in grain set with planting dates in both locations were found in RD29, SPR1 and PTT1 suggested that they were tolerant to high temperature in this study.

The negative effect of high temperature on rice yield not only came from grain set failure alone but also included the effect on grain filling. These can be seen by number of empty grains which resulting from both unfertilized and fertilized but unfilled grains. All genotypes except RD29 and CNT1 had >30% empty grains when grown at Chiang Mai or Suphanburi. For the effect on unfilled grain, about 20% unfilled grain were found in NP1 and RD21 at PD1 and SPR1 and PTT1 at PD3 grown in Chiang Mai (Figure 3.2). In Suphanburi, unfilled grain of RD21 jumped to as high as 35% at PD3 (Figure 3.3). RD29 had the lowest unfilled grain in all planting dates and locations. Among genotypes those bred and adapted to high temperature in the Central Plain, RD29 and CNT1 were the most tolerant when flowering in summer of both locations. Further studies on effect of high temperature on pollen production, fertilization and grain filling processes will provide more knowledge on mechanism of heat tolerance in these germplasms.

Implications of the main research findings

- Grain set, number of filled grain can be used as screening tools for heat tolerance during reproductive phase.
- Results on genotypic variation in response to high temperature have led to further research on response to high temperature in rice. This was part of the project funded by TRF (Prof. Dr. Benjavan Rerkasem at CMU) and a PhD scholarship funded by , The Office of the Higher Education Commission Thailand (Ms Suphansa Sukkaew) started from 2009, titled “Genetic control of response to high temperature in rice”.

Table 3.2 Number of spikelets per panicle of 12 rice genotypes grown in dry season 2009 at three planting dates and two locations, Chiang Mai and Suphanburi.

Genotype	Chiang Mai			Suphanburi		
	PD1[#]	PD2	PD3	PD1	PD2	PD3
NP1	91 ab	97 a	84 b	120 c	140 b	160 a
R258	74 a	82 a	81 a	95 a	109 a	102 a
SPT	93 a	99 a	95 a	139 b	108 c	159 a
RD4	93 a	98 a	97 a	111 a	107 a	116 a
RD7	85 ab	88 a	77 b	103 a	94 a	96 a
RD10	82 c	110 b	119 a	110 b	105 b	135 a
RD21	98 b	106 b	134 a	139 b	172 a	181 a
RD29	90 a	96 a	92 a	121 b	134 a	103 c
CNT1	84 b	103 a	98 a	121 a	101 b	89 c
SPR1	79 b	110 a	104 a	121 a	107 ab	96 b
SKN1	95 b	111 a	77 c	110 a	96 a	93 a
PTT1	94 b	100 ab	113 a	89 a	99 a	77 a
Mean	88 B	100 A	98 A	115	114	117
	PD	G	PDxG	PD	G	PDxG
LSD(0.05)	2	4	8	ns	10	17

[#] Sowing dates at Chiang Mai were Jan 17, Jan 31 and Feb 14 2009; at Suphanburi were Jan 13, Jan 27 and Feb 10 2009 and designated as PD1, PD2 and PD3, respectively. Means within each row follows by the same letter are not differed signicantly at p =0.05.

Table 3.3 Grain set (%) of 12 rice genotypes grown in dry season 2009 at three planting dates and two locations, Chiang Mai and Suphanburi.

Genotype	Chiang Mai			Suphanburi		
	PD1[#]	PD2	PD3	PD1	PD2	PD3
NP1	91 a	80 b	85 b	88 b	93 a	80 c
R258	92 a	86 b	89 ab	86 a	83 b	85 ab
SPT	96 a	85 b	76 c	87 b	94 a	76 c
RD4	89 a	83 b	76 c	90 a	82 b	86 a
RD7	94 a	84 b	91 a	88 a	85 b	88 ab
RD10	87 b	86 a	89 a	86 a	94 a	78 c
RD21	81 a	86 a	87 a	93 a	90 a	75 b
RD29	88 a	87 ab	84 b	89 a	85 b	87 ab
CNT1	94 a	91 ab	88 b	89 b	96 a	95 a
SPR1	86 b	90 ab	93 a	93 a	91 ab	90 b
SKN1	89 ab	92 a	87 b	95 a	86 c	90 b
PTT1	91 a	91 a	93 a	94 a	91 a	93 a
Mean	90 A	87 B	87 B	90 A	89 A	86 B
	PD	G	PDxG	PD	G	PDxG
LSD(0.05)	1	2.1	3.6	1	1.5	2.7

[#] Sowing dates at Chiang Mai were Jan 17, Jan 31 and Feb 14 2009; at Suphanburi were Jan 13, Jan 27 and Feb 10 2009 and designated as PD1, PD2 and PD3, respectively. Means within each row follows by the same letter are not differed signicantly at p =0.05.

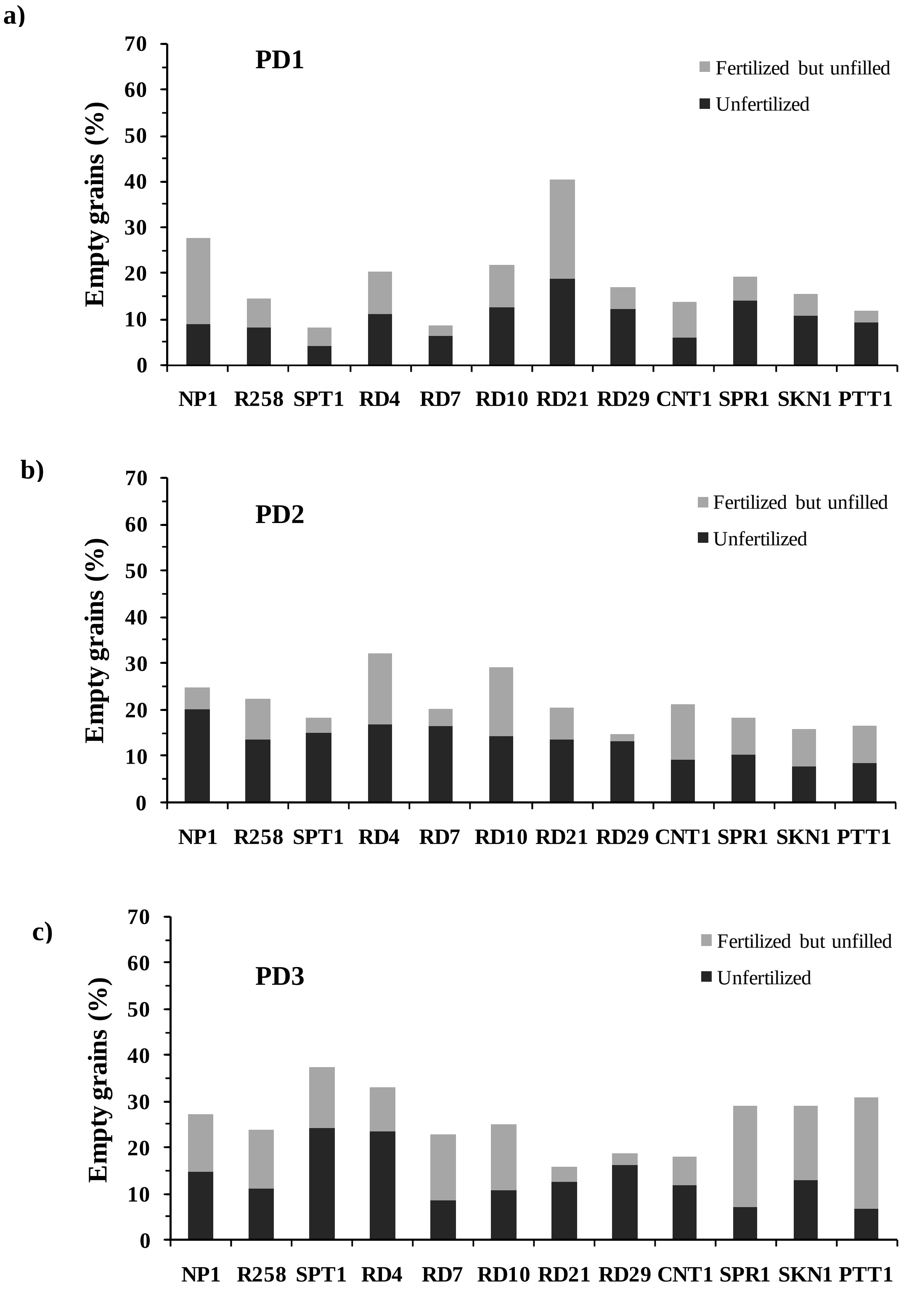


Figure 3.2 Empty grains (%) of 12 rice genotypes at three planting dates: a) Jan 17 2009 (PD1) b) Jan 31 2009 (PD2) and c) Feb 14 2009 (PD3) at Chiang Mai. $LSD_{0.05}$(PDxG) for unfertilized and unfilled grains were 3.6 and 3.0% , respectively.

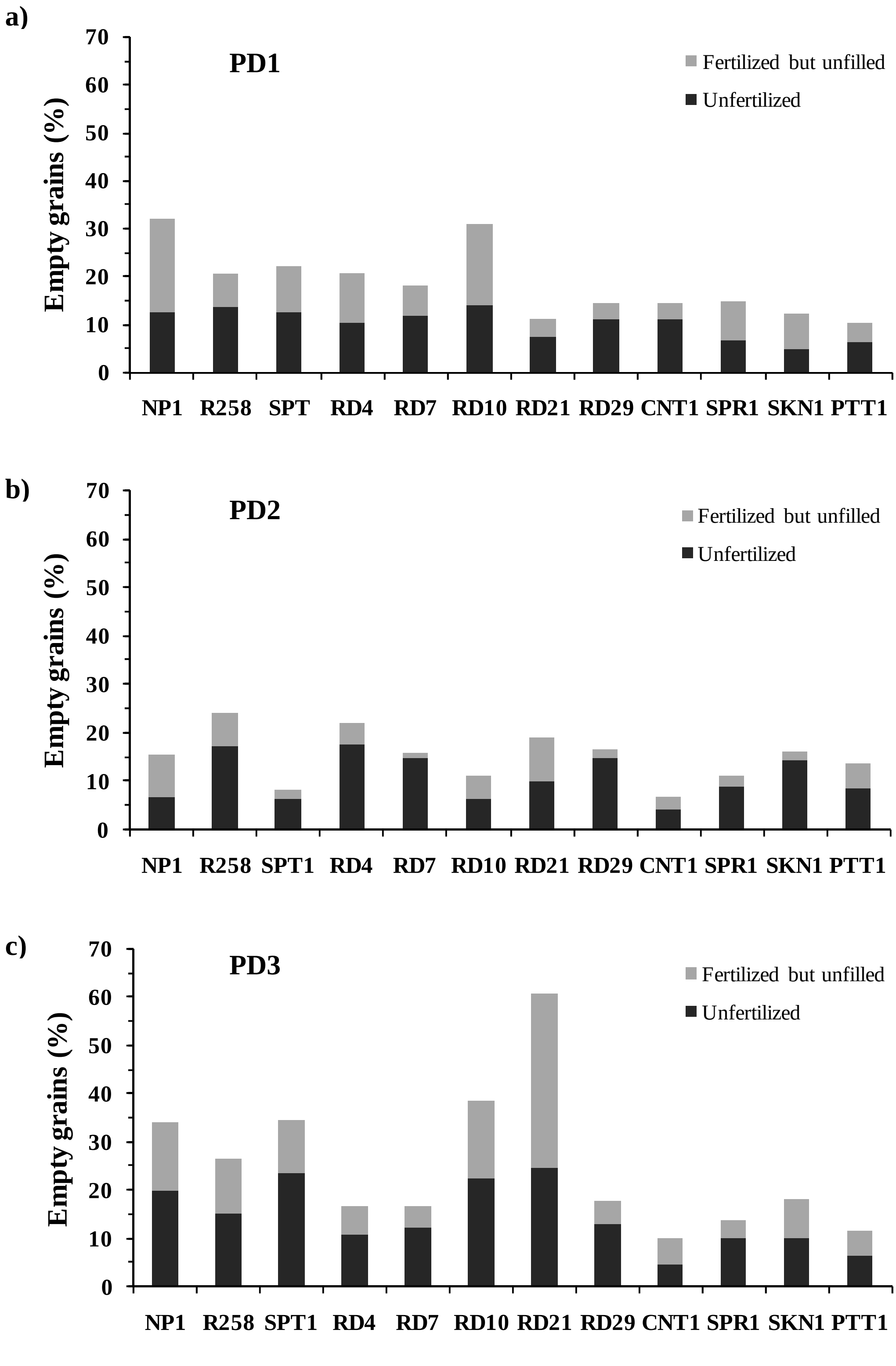


Figure 3.3 Empty grains (%) of 12 rice genotypes at three planting dates: a) Jan 13 2009 (PD1) b) Jan 20 2009 (PD2) and c) Feb 10 2009 (PD3) at Suphanburi. $LSD_{0.05}$(PDxG) for unfertilized and unfilled grains were 2.7and 3.1% , respectively.

4. To evaluate and select for traits promote outcrossing

Although rice is self-pollinated crop. High rate of cross pollination was found in its progenitor, common wild rice (*Oryza rufipogon* Griff.). Research results from Thai Rice Germplasm Project at CMU showed that cultivated rice had lower stigma exertion, shorter stigma and anthers than common wild rice. Therefore, an attempt was made to transfer long stigma and anther traits from common wild rice collected in Thailand to cultivated rice. Populations derived from crosses between crop rice and common wild rice were kindly provided by the Thai Rice Germplasm Unit of Plant Nutrition and Genetic Resource Laboratory at Chiang Mai University (CMUPN*lab*) directed by Professor Benjavan Rerkasem. Evaluation and selection were conducted in two sets of crosses: (1) F_4 generation from common wild rice x SPR1 (2) Backcross populations derived from common wild rice (*Oryza rufipogon* Griff.) x local rice and then backcrossed to Suphanburi 1 (*O. sativa* L., cv SPR 1). The objectives of this part was to develop lines possess floral traits from common wild rice those increase outcrossing and can be transferred to parental lines in hybrid seed production. Therefore, selection was aimed for two sets of floral traits, large and exerted anthers for pollen parents; and large and exerted stigma for female parents.

Populations were evaluated in the fields. Evaluation, selection and backcrossing were done in four consecutive seasons. For each generation, about 600-800 plants were sown for F_3-F_5 populations, and 100 plants for BCF_1, 200-300 plants for BCF_2 and >1500 plants for BCF_3 populations. Before anthesis, anther and stigma size and exertion were rated and selected visually. Then five florets from each selected plant were collected and kept in eppendorf tube and fixed with acetic:alcohol. Anther, stigma and style lengths were measured microscorpically, two anthers and stigma per floret. As wild rice possess some undesirable linkage between long stigma or long anther with low pollen viability and seed shattering. At anthesis, pollen grains from selected plants were collected and viability tested by iodine and agar methods. After anthesis, two panicles of each selected plants were bagged to recover the shattered seeds. The same procedure was carried out in all generation.

Evaluation of F_4-F_6 derived from common wild rice x SPR1

Seven hundred F_4 plants of cross between common wild rice and SPR1 were grown in the experimental field in wet season 2007. Single plant selection was made based on reproductive traits including anther size, stigma size, stigma exertion, pollen shedding and also some other agronomic traits that involved yield components. F_5 lines were grown in pot experiment in dry season 2007/08. Lines were evaluated for the reproductive traits, plant type, days to flowering and plant height. All selected F_5 lines exhibited large anthers and well exerted, large stigma (Figure 4.1). F_5 lines with photoperiod insensitivity (evaluated from pot experiment) were sown in experimental field, with more plants per lines (60) in wet season 2008. All selected F_5 lines exhibited large anthers and well exerted, large stigma (Figure 4.2). Selected lines showed other desirable traits, e.g. photoperiod insensitivity, semi-dwarf plant type, large panicle, high number of spikelets/panicle and high to intermediate seed fertility when tested in the following season (Tables 4.1 and 4.2). However, seed shattering were found in all lines when compared with SPR1 parent. To recover non-seed shattering, the progenies between common wild rice and SPR1 were backcrossed to SPR1 and described in the next section.

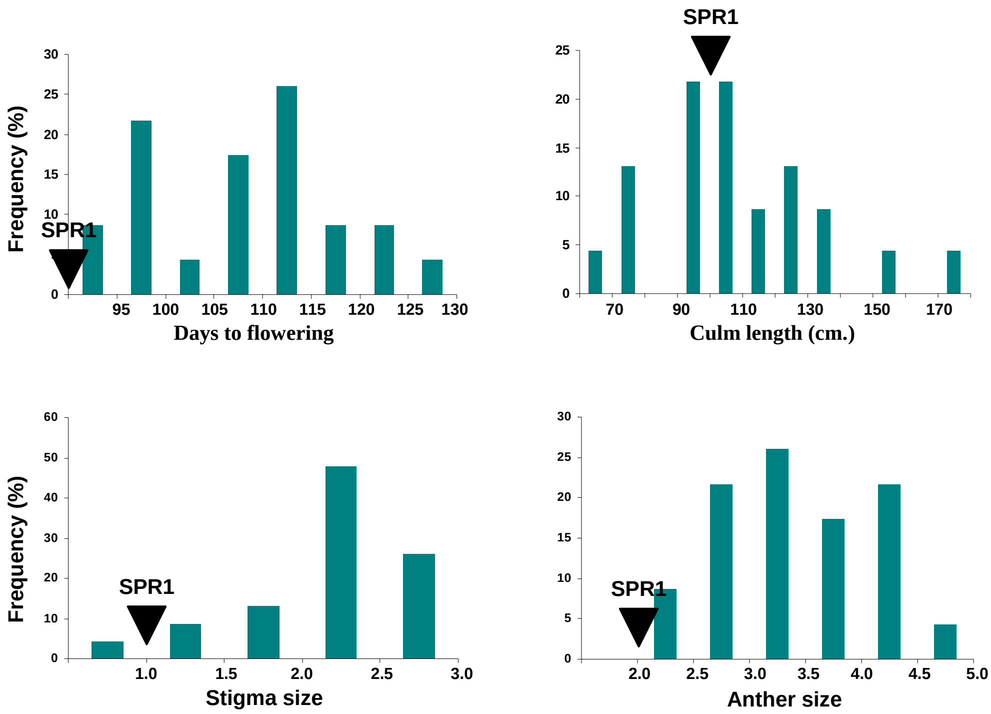
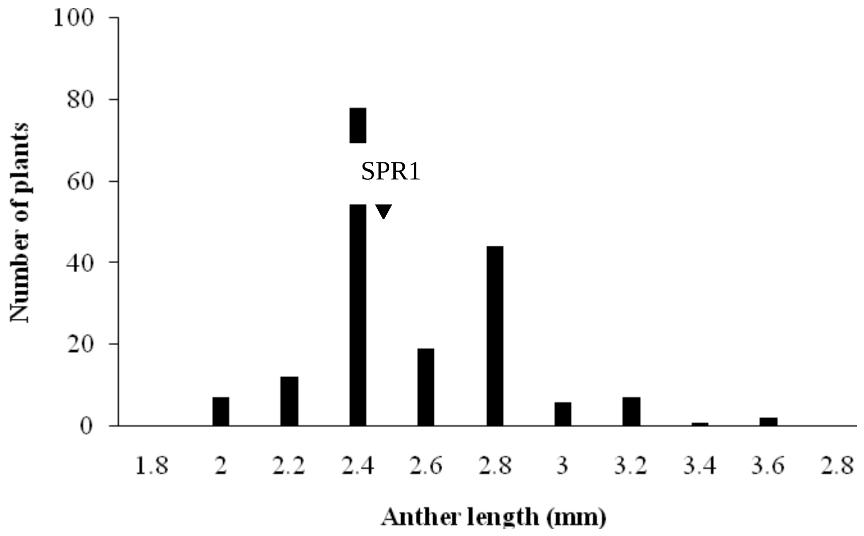


Figure 4.1 Frequency distribution of days to flowering , culm length, stigma size and anther size of F_5 lines from cross between *O. rufipogon* and SPR1, Dry

a)



b)

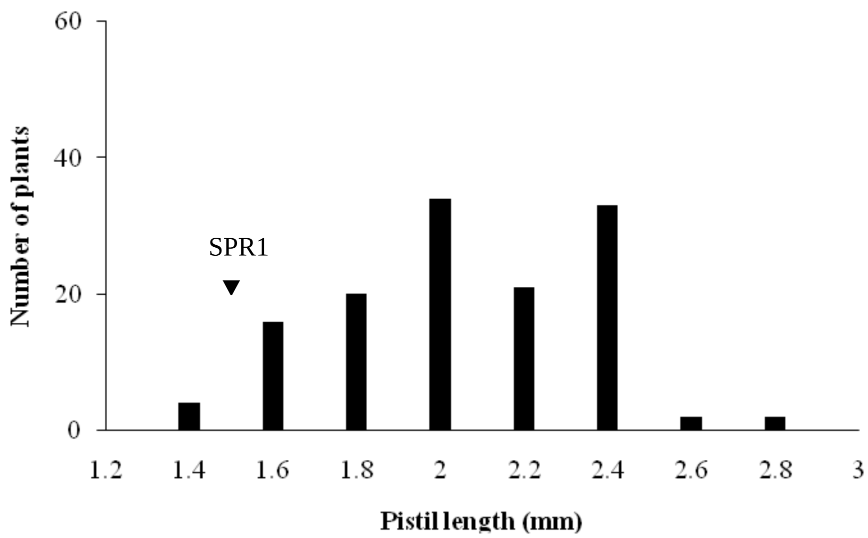


Figure 4.2 Distibution of a) anther length (mm) and b) pistil length (mm) of selected F5 plants from crosses derived from common wild rice and cultivated rice, Wet season 2008.

Table 4.1 Characterisitics of five lines selected for large pistil, Dry season 2008/09.

Code	1-1	1-2	1-3	1-4	1-5	SPR1
Stigma length (mm)	2.00	1.65	1.62	1.54	1.5	0.9
Style length (mm)	0.80	0.85	0.78	0.84	0.85	0.8
Pistil length (mm)	2.80	2.50	2.40	2.38	2.35	1.7
Anther length (mm)	2.75	2.57	2.59	2.52	2.66	2.30
Anther width (mm)	0.75	0.78	0.79	0.78	0.79	0.80
Plant height (cm)	85	110	85	88	73	87
Panicles/plant	11	16	12	16	17	13
Panicle length (cm)	29	33	26	32	26	26
Branches/panicle	14	15	13	12	12	12
Spikelets/panicle	171	203	149	160	121	134
Grains/panicle	126	154	108	109	105	125
Grain set (%)	74	76	72	68	87	92
Grain weight (g/plant)	28	50	28	35	35	32

Table 4.2 Characterisitics of four lines selected for large anther, Dry season 2008/09.

Code	2-1	2-2	2-3	2-4	SPR1
Anther length (mm)	3.17	2.9	2.9	2.75	2.30
Anther width (mm)	0.81	0.85	0.8	0.75	0.80
Stigma length (mm)	1.4	1.15	1.1	2	0.9
Style length (mm)	0.78	0.63	1.05	0.8	0.8
Pistil length (mm)	2.18	1.78	2.15	2.8	1.7
Plant height (cm)	83	108	93	85	87
Panicles/plant	16	15	25	11	13
Panicle length (cm)	28	30	25	29	26
Branches/panicle	13	13	9	14	12
Spikelets/panicle	160	143	115	171	134
Grains/panicle	78	117	58	126	125
Grain set (%)	49	82	51	74	92
Grain weight (g/plant)	22	32	38	28	32

Selection and evaluation of backcross populations

BC_1F_1s were sown in 2007/08. Plants with anther or pistil longer than SPR1 were selected and backcrossed to SPR1. BC_2F_1s were sown in 2008 to produce F_2 seeds. Two BC_2F_2 populations (70034 and 70036) were evaluated in the field in 2008/09. Anther and pistil length of SPR1 crop rice were 2.2 and 1.7 mm, respectively. BC_2F_2 populations of both crosses were segregating in a large range of anther and stigma sizes. All plants had longer anther and stigma than SPR1 (Figure 4.3). For both crosses, significant positive correlations between anther length and anther width (r = 0.51**and 0.76** for 70034 and 70036, respectively), and between pistil length and stigma length (r = 0.89** and 0.88***) were found (Table 4.3). This indicated that selection for anther length and width can be done together and selection for long pistil resulted from long stigma. As the crosses were derived from common wild rice, many undesirable traits were transferred together with floral characteristics. These characters included poor pollen viability and seed shattering. BC_2F_2 plants also segregating for pollen viability. Significant relationship between anther length and pollen viability was found between crosses. For 70034, no correlation for the two traits was found and pollen viability were between 40-100% with three plants had pollen viability less than 40% (Figure 4.4a). In contrast, positive correlation (r=0.55**) between anther length and pollen viability was shown for 70036 (Figure 4.4b). Therefore, there was a higher possibility to select plants with large anther with viable pollen grains.

Thirty-one BC_2F_2 families were selected (Table 4.4). The F3 families were evaluated in the field experiment in Wet season 2009. To select for lines with large anther or pistil with desirable agronomy traits and non-seed shattering types, larger number of plants were screened than that used in the former section. Families were grown in rows, 50-60 plants per family, total 1680 plants. About 124 plants (7.4%) from 12 families were selected visually. Then the anthers and pistils from the selected plants were collected for size and the panicles were bagged. At maturity, plants with large anther or pistil with good agronomic characters were selected. Sixteen plants with non-seed shattering type and desirable flowering and agronomic traits were recovered (Tables 4.5-4.6). These plants will be multiplied and the progeny lines can be used as parents in breeding for large anther or large stigma in hybrid seed production.

Implications of the main research findings

- Advanced lines with large anther and large pistil, transferred from common wild rice, were developed. They can be used as parents in breeding for large anther or large stigma in hybrid seed production.
- New project is being developed with Bayer in which the screening and selection method will be applied to outcrossing rate study and development of parents.

a)

b)

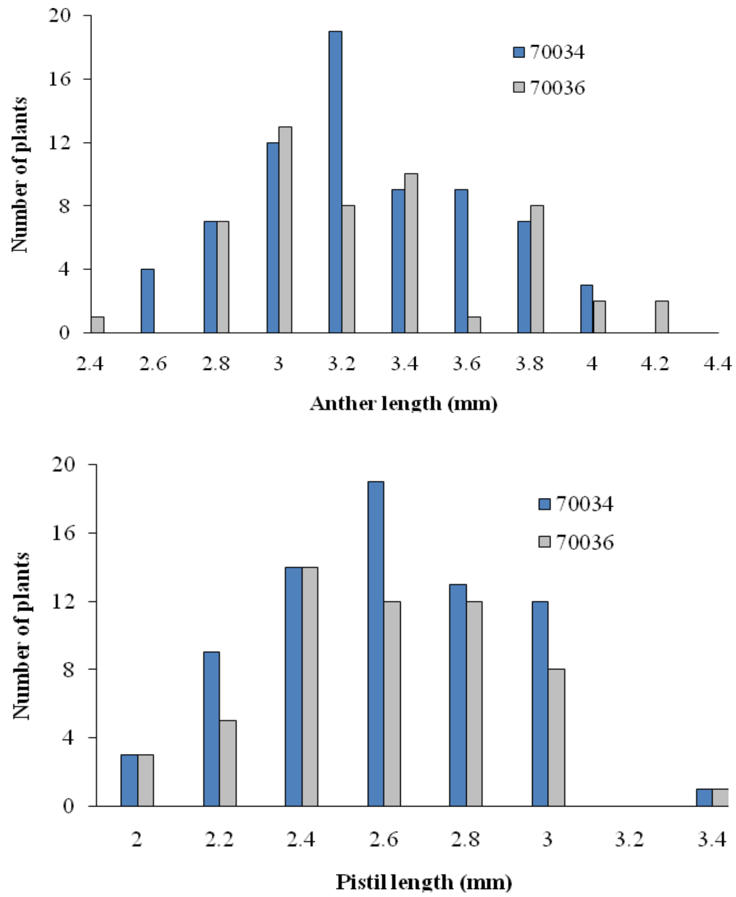
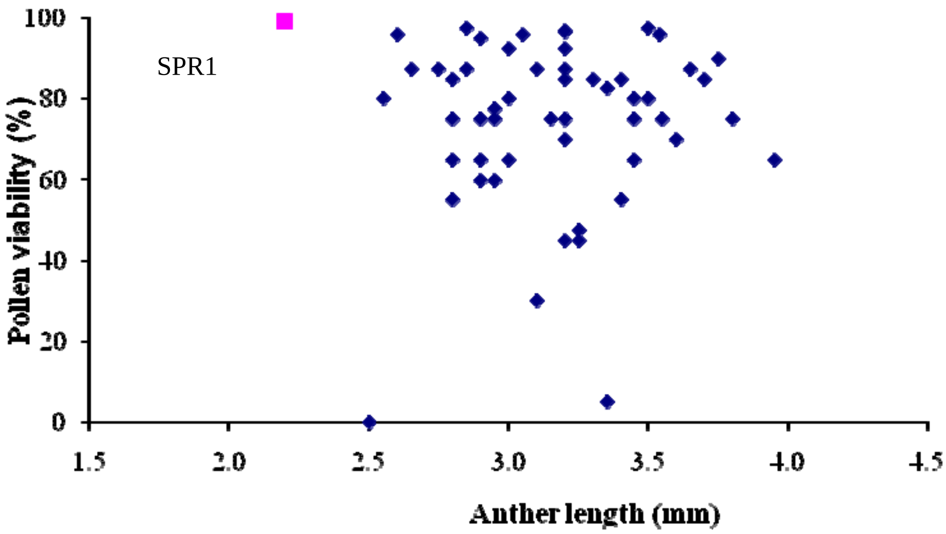


Figure 4.3 Distibution of a) anther length (mm) and b) pistil length (mm) of BC_2F_2 populations from crosses derived from common wild rice and cultivated rice, 70034 and 70036. Dry season 2008/09. Note: anther and pistil length of SPR1 parent were 2.2 and 1.8 mm, respectively.

Table 4.3 Correlation coefficients (r) between floral traits of BC_2F_2 populations from crosses derived from common wild rice and cultivated rice;70034 and 70036, Dry season 2008/09.

	Anther length	Anther width	Stigma length	Style length	Pollen viability
70034					
Anther width	0.51**				
Stigma length	0.34	0.12			
Style length	-0.34	-0.43*	-0.40*		
Pollen viability	-0.22	-0.16	-0.20	0.10	
Pistil length	0.23	-0.08	0.89***	0.05	-0.19
70036					
Anther width	0.76**				
Stigma length	-0.18	-0.47*			
Style length	-0.31	-0.21	-0.04		
Pollen viability	0.55**	0.50*	-0.09	-0.48*	
Pistil length	-0.32	-0.56**	0.88***	0.41	-0.34

*, ** and *** significant at p<0.05, 0.01 and 0.001, respectively.



b)

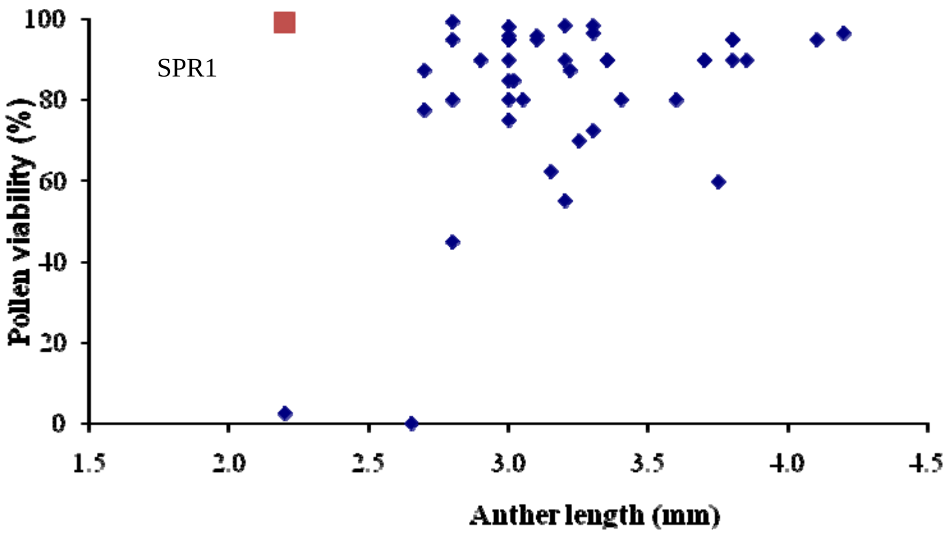


Figure 4.4 Distribution of anther length (mm) and pollen viability of F_2 plants from crosses derived from common wild rice and cultivated rice; a) 70034 and b) 70036, Dry season 2008/09.

Table 4.4 Anther length, width and pistil length (mm) and pollen viability (%) of selected BC_2F_2 plants and characteristics of BC_2F_3 seeds from Cross 70034 and 70036. Dry season 2008/09.

Selected No.	Anther		Pistil length			Pollen viability (%)	Seed shattering	Pericarp color
	Length (mm)	Width (mm)	Stigma (mm)	Style (mm)	Total (mm)			
92	3.4	0.8	1.7	0.8	2.5	-	no	white
65	3.3	0.8	1.3	0.8	2.1	45	no	white
94	3.2	0.6	1.7	0.7	2.4	62	no	red
101	3.2	0.9	2.1	0.8	2.9	-	no	white
119	3.0	0.5	1.8	0.7	2.5	-	no	white
58	2.9	0.8	1.4	1.0	2.4	87	no	white
121	2.7	0.5	1.6	0.6	2.2	-	no	white
79	3.7	0.8	2.0	0.7	2.7	85	yes	white
102	3.6	0.8	1.9	0.7	2.6	-	yes	white
48	3.5	-	1.4	1.1	2.5	96	yes	white
63	3.5	0.8	1.4	0.9	2.3	65	yes	white
76	3.5	0.8	2.0	0.8	2.8	75	yes	white
85	3.4	0.8	1.7	0.7	2.4	90	yes	white
89	3.4	0.8	1.5	0.9	2.4	90	yes	-
73	3.3	0.6	2.1	1.0	3.1	70	yes	white
54	3.2	0.5	1.6	1.2	2.8	75	yes	white
59	3.2	0.8	1.3	1.0	2.3	87	yes	white
104	3.2	0.8	1.5	1.0	2.5	-	yes	white
122	3.2	0.8	1.5	1.2	2.7	-	yes	-
62	3.2	0.8	1.5	1.2	2.7	96	yes	-
56	3.0	0.9	1.4	1.0	2.4	90	yes	white
64	3.0	0.8	1.3	0.9	2.2	60	yes	white
83	3.0	0.8	1.5	0.8	2.3	80	yes	white
117	2.9	0.4	2.2	0.7	2.9	-	yes	red
57	2.8	0.7	1.2	0.8	2.0	82	yes	white
17	2.8	-	1.6	1.1	2.7	80	yes	white
99	2.8	0.4	1.9	1.0	2.9	45	yes	white
14	2.6	-	2.0	0.7	2.7	96	yes	white
SPR1	2.3	0.8	0.9	0.8	1.7	98	no	white

Table 4.5 Floral size (mm), plant hight, number of panicles and seed weight (g) per plant of 16 selected F_3 families, Wet season 2009.

Selection no.	Anther size (mm)		Pistil size (mm)			Height (cm)	Panicles/ plant	Seed wt/plant (g)
	Length	Width	Stigma	Style	Pistill			
5	4.0	0.9	1.8	0.8	2.6	147	4	9.5
4	4.0	0.8	1.8	0.9	2.7	77	6	7.3
1	3.9	0.9	2.2	1.0	3.2	138	3	4.7
7	3.8	0.8	1.6	0.7	2.3	60	9	9.4
6	3.8	0.8	1.8	0.7	2.5	117	8	16.0
13	3.4	0.8	1.7	1.0	2.7	87	5	13.8
10	3.3	0.8	1.8	1.0	2.8	132	4	4.8
8	3.2	0.7	1.8	1.0	2.8	91	4	4.9
11	3.2	0.8	2.1	0.8	2.9	118	9	12.6
12	3.1	0.8	1.8	1.0	2.8	80	3	5.6
18	3.0	0.8	2.1	0.8	2.9	69	5	8.5
16	2.9	0.8	2.0	1.0	3.0	120	5	5.5
19	2.9	0.8	2.0	0.7	2.7	94	4	6.8
15	2.8	0.7	2.3	1.0	3.3	112	7	9.8
17	2.8	0.7	2.0	1.0	3.0	131	4	4.1
20	2.4	0.7	2.1	0.8	2.9	139	5	6.6
SPR1	2.3	0.8	0.9	0.8	1.7	-	-	-

Table 4.6 Seed characteristics of 16 selected F_4 seeds, Wet season 2009.

Line no.	Shattering	Hull color	Pericarb color	Awning	Seed size (mm)		
					Length	Width	Thickness
5	no	straw	white	awnless	10.61	2.49	1.82
4	no	straw	white	awnless	10.63	2.36	1.86
1	no	straw	white	awnless	10.7	2.44	1.73
7	no	straw	white	awnless	10.77	2.4	1.79
6	no	straw	white	awnless	11.04	2.29	1.82
13	no	straw	white	awnless	11.20	2.6	1.85
10	no	straw	white	awnless	10.59	2.33	1.76
8	no	straw	white	awnless	10.91	2.37	1.75
11	no	straw	white	tip awned	10.77	2.48	1.92
12	no	straw	white	awnless	10.88	2.35	1.84
18	no	straw	white	awnless	10.62	2.42	1.82
16	no	straw	white	tip awned	10.97	2.48	1.81
19	no	straw	white	tip awned	10.74	2.43	1.74
15	no	straw	white	awnless	10.38	2.43	1.83
17	no	straw	white	awnless	10.75	2.34	1.81
20	no	straw	white	awnless	10.77	2.22	1.81
SPR1	no	straw	white	awnless	9.33	2.56	1.99

Appendix

ภาคผนวก

1. บทความสำหรับการเผยแพร่

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To be submitted to: Plant and Soil

Genotypic variation of boron deficiency in rice

Sawika Konsaeng, Sittichai Lordkaew, Benjavan Rerkasem and Sansanee Jamjod

Abstract

Boron (B) deficiency adversely affects growth and development of various crop species. The variation among plant genotypes of response to B deficiency has been reported, but little is known about B deficiency in rice. Thus, the objective of this experiment was to determine how B deficiency affects rice and to examine genotypic variation in the response to B deficiency. Two repeated experiments were conducted in sand culture during October to February in 2007/08 and in 2008/09. Pots were applied with nutrient solution with B added to 10 µM (sufficient, B10) or without boron (deficient, B0). Three rice varieties, KDML105, CNT1 and SPR1, were sown in freely drained pot containing washed river sand, five plants per pots with 3 replications. At anthesis, anthers from each treatment were collected for determining pollen viability by iodine solution (KI/I_2). Growth responses (number of tillers, plant height, root length, shoot dry weight and root dry weight) were collected at 2 harvests (anthesis and grain maturity). Yield and yield components were also recorded at maturity. Flag leaf, the first leaf below flag leaf (FL+1) and anther were collected at anthesis for B analysis. The parallel results were found in two experiments of both years. It was shown that vegetative growth of rice was not affected by low B supply at both harvests. The difference in growth was due only to the variation of rice varieties. For reproductive responses, it was found that B had no effect on number of spikelets/panicle while significant difference in response to B on grain set was shown by the significant B x G interaction of % grain set. The percentage of grain set in 2007/08 and 2008/09 of three rice varieties in sufficient B was between 13-39% and 22-60%, respectively . In B0, % grain set was decreased to 7-28% in 2007/08 and 12-47% in 2008/09 with the highest in SPR1. Additionally, significant B X G interactions were found for B concentrations in flag leaf, FL+1 and anther at anthesis. The results also showed genotypic variation of B deficiency on pollen viability by using KI/I_2 staining. In both B treatments, pollen viability of all three varieties which was indicated by %KI/I_2 staining, had significant correlation with anther B concentration in 2007/08 (r = 0.414*) but not in 2008/09 ($r = 0.287^{ns}$). When considered the correlation between %KI/I_2 staining with % grain set from all three varieties of both B treatments, positive correlation (r = 0.412* for 2007/08 and r = 0.653** for 2008/09) was shown in this experiment. In conclusion, the responses of B deficiency varied among rice genotypes. The response to B increase from B0 to B10 and the low pollen fertility and grain set in B10 suggest that the B supply in B10 may still be insufficient for maximum reproductive development in rice under the condition of this study. The most tolerant to B deficiency was SPR1 while the most sensitive to B deficiency was KDML105. The method used in this experiment could be used for determining the responses to B deficiency in other rice varieties of interests. The different response to B deficiency, especially in pollen viability and % grain set, of rice genotypes will be useful for controlling male sterility in hybrid rice production.

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Abstract

Boron (B) deficiency adversely affects growth and development of various crop species. The variation among plant genotypes of response to B deficiency has been reported, but little is known about B deficiency in rice. Thus, the objective of this experiment was to determine how B deficiency affects rice and to examine genotypic variation in the response to B deficiency. Two repeated experiments were conducted in sand culture during October to February in 2007/08 and in 2008/09. Pots were applied with nutrient solution with B added to 10 µM (sufficient, B10) or without boron (deficient, B0). Three rice varieties, KDML105, CNT1 and SPR1, were sown in freely drained pot containing washed river sand, five plants per pots with 3 replications. At anthesis, anthers from each treatment were collected for determining pollen viability by iodine solution (KI/I_2). Growth responses (number of tillers, plant height, root length, shoot dry weight and root dry weight) were collected at 2 harvests (anthesis and grain maturity). Yield and yield components were also recorded at maturity. Flag leaf, the first leaf below flag leaf (FL+1) and anther were collected at anthesis for B analysis. The parallel results were found in two experiments of both years. It was shown that vegetative growth of rice was not affected by

low B supply at both harvests. The difference in growth was due only to the variation of rice varieties. For reproductive responses, it was found that B had no effect on number of spikelets/panicle while significant difference in response to B on grain set was shown by the significant B x G interaction of % grain set. The percentage of grain set in 2007/08 and 2008/09 of three rice varieties in sufficient B was between 13-39% and 22-60%, respectively . In B0, % grain set was decreased to 7-28% in 2007/08 and 12-47% in 2008/09 with the highest in SPR1. Additionally, significant B X G interactions were found for B concentrations in flag leaf, FL+1 and anther at anthesis. The results also showed genotypic variation of B deficiency on pollen viability by using KI/I_2 staining. In both B treatments, pollen viability of all three varieties which was indicated by $\%KI/I_2$ staining, had significant correlation with anther B concentration in 2007/08 (r = 0.414*) but not in 2008/09 ($r = 0.287^{ns}$). When considered the correlation between $\%KI/I_2$ staining with % grain set from all three varieties of both B treatments, positive correlation (r = 0.412* for 2007/08 and r = 0.653** for 2008/09) was shown in this experiment. In conclusion, the responses of B deficiency varied among rice genotypes. The response to B increase from B0 to B10 and the low pollen fertility and grain set in B10 suggest that the B supply in B10 may still be insufficient for maximum reproductive development in rice under the condition of this study. The most tolerant to B deficiency was SPR1 while the most sensitive to B deficiency was KDML105. The method used in this experiment could be used for determining the responses to B deficiency in other rice varieties of interests. The different response to B deficiency, especially in pollen viability and % grain set, of rice genotypes will be useful for controlling male sterility in hybrid rice production.

Introduction

Boron (B) deficiency adversely affects growth and development of various crop species (Shorrocks, 1997). The higher impact of B deficiency was found during reproductive development than in vegetative growth. It has been reported that B deficiency caused male sterility and reduced grain set in wheat (Rerkasem and Jamjod, 1997) and barley (Jamjod and Rerkasem, 1999). In rice, Garg et al. (1979) suggested that pollen fertility was decreased. Rice yield could be increased 10-46% with B application (Rashid et al., 2000). The susceptible to B deficiency was found to be varied among plant genotypes, e.g. in wheat (Rerkasem and Jamjod, 1997), barley (Jamjod and Rerkasem, 1999) and rice (Rashid et al., 2000). Therefore, this study aimed to determine responses of B deficiency in rice and indicate its genotypic variation. The different response to B deficiency, especially in pollen viability and seed sterility of rice genotypes will be useful for controlling male sterility or enhancing seed set in hybrid rice or cultivated rice production.

Materials and Methods

Genotypes

Three Thai rice varieties, KDML105, Chainat1 (CNT1) and Suphanburi1 (SPR1) were used. KDML105 is the jasmine rice improved by pure line selection from local jasmine rice. CNT1 and SPR1 are semi-dwarf, photoperiod insensitive high yield varieties bred and released from Department of Rice, Thailand. Seeds were pre-geminated in petri dish for 48h before sowing to ensure uniform germination.

Experimental procedure

Two experiments were carried out in dry season, between October to February, for two years, 2007/08 and 2008/09. Sand culture was used. Rice seeds were sown in freely drained earthenware pots (30 cm diameter, 30 cm deep) containing washed river sand, five plants per pot. Pots were applied with basal nutrient solution [containing (ppm): NH_4NO_3, 40; $NaH_2PO_4.2H_2O$, 10; K_2SO_4, 40; $CaCl_2.2H_2O$,40; $MgSO_4.7H_2O$, 40; Fe-EDTA, 7; $MnCl_2.4H_2O$, 0.5; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.05; ZnSO4.$7H_2O$, 0.01; $CuSO_4.5H_2O$, 0.01; $CuSO_4.5H_2O$, 0.01, modified from Yoshida (1976)] with added 10 µM B (sufficient B, B10) or without added boron (deficient B, B0). Boron treatments and genotypes were arranged factorially in a completely randomized design with three replications. At anthesis, anthers were collected for B concentration analysis and pollen viability determination. For B analysis, anthers were collected from 10 panicles from 5 plants. Boron concentration was determined by dry ashing and azomethine-H method (Lohse, 1982). For pollen viability test, anthers from top, mid and bottom of five panicles from each treatment were randomly collected. Each anther was placed into an eppendorf tube containing 500 µL of KI/I. The tube was vortexed and pollen were counted from five replicates of 10 µL. Pollen viability was estimated as the percentage of stained pollen to total number of pollen grains counted. At maturity, flag leaf and the first leaf below flag leaf (YEB+1) of all plants were collected for B analysis. The rest of each pot were measured for culm length, number of tillers plant^{-1}, number of panicles plant^{-1}. Then each plant was harvested and separated into panicles, straw and root. Straw and root were oven dried and dry weight determined. Panicles of each plant were counted for number of spikelets panicle^{-1}, filled grains panicle^{-1}. Grain set was calculated from percentage of filled grains per panicle.

Data analysis

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

Results and discussion

Vegetative responses to boron deficiency

In both years, vegetative growth was not affected by low B supply at all harvests. The difference in growth was only due to the variation of rice varieties (data shown only the second harvest at grain maturity, Table 1 and Table 2). Remarkably, B-deficient plants (B0) of year 2 (2008/2009) produced more tillers per plant than B-sufficient plants (B10) (Table 2).

Table 3 showed panicles per plant and tillers bearing panicles of rice varieties grown in both years. In year 1 (2007/2008), the difference in panicles per plants was either from rice varieties or B levels. When considered tillers bearing panicles (%), it was found to that B deficiency did not affect tillers bearing panicles. KDML105 had 38.3 % of tillers bearing panicles which was higher than the other two varieties. In year 2 (2008/2009), panicles per plant ranged 11 -16 panicles which was due to only from the variation of rice varieties (Table 3). However, significant B x G interaction was shown for the percentage of tillers bearing panicles. In B-deficient plants (B0), the percentage of tillers bearing panicle was between 35-71% with the lowest in RD29. The supply of B added to nutrient solution increased the percentage of panicles bearing tillers of all rice varieties except KDML105.

Reproductive responses to boron deficiency

In 2007/2008, B had no effect on number of spikelets/panicle (Table 4). However, significant difference in response to B on grain set was shown by the significant B x G interaction of % sterility. Sterility percentage of three varieties in sufficient B was between 61-87%. In B0, sterility was increased to 72-93% with the highest in CNT1 and KDML105. Differently, number of spikelets/ panicle of 2008/2009 was affected by either rice varieties or B levels (Table 5). However, the significant B x G interaction was found in the percentage of seed sterility (Table 5). In B-sufficient rice plants, % sterility was between 40-79%. It was found that B deficiency increased the percentage of seed sterility of all four varieties to 53-97% with the highest in RD29.

Boron concentration in plant parts

Significant B x G interactions were found for B concentrations in flag leaf, YEB+1 and anther at anthesis in both years (Table 6). In Year 1 (2007/2008), B concentration of flag leaf and YEB+1 in B10 of CNT1 and SPR1 were 21.8-27.6 and 11.2-13.3 mg B/kg DW, respectively, while those of KDML105 were 8.5 and 8.4 mg B/kg DW, respectively. Anther B concentrations in B10 of CNT1 and SPR1 were 18.3 and 28.2 mg B kg/DW, respectively whereas those of B0 were 13.2 – 13.9 mg B kg/DW. It was found that anther B concentration of KDML105 in B10 and B0 were not significantly different. In Year 2 (2008/2009), B concentration of flag leaf YEB+1 of B10 plants were 11.4 – 23.3 mg B/kg DW and 8.0 – 9.3 mg B/kg DW, respectively. Boron concentrations in B0 plant of all four varieties in both years were reduced to about 28 – 80%, compared with B10 (Table 6).

Pollen viability and the seed sterility

There was genotypic variation of B deficiency on pollen viability by using KI/I_2 staining in both years (Table 4 and Table 5). At sufficient B in 2007/2008, about 59-70% of pollen from SPR1 and CNT1 and 36% of KDML105 were stained with KI/I_2. At B0, those of both CNT1 and SPR1 were reduced significantly, compared with B10 (Table 4). In 2008/2009, the staining with KI/I_2 of B-sufficient pollen was 15%, 60%, 65% and 81% in RD29, CNT1, KDML105 and SPR1, respectively. Boron deficient condition decreased %KI/I_2 staining to 1%, 25% and 54% in RD29, KDML105 and SPR1 but did not affect CNT1 (Table 5).

These responses were supported by the reduction of B concentration at anthesis in flag leaf, YEB+1 and anther of CNT1 and SPR1 in B0 compared with B10 in 2007/2008, while that of KDML105 was still the same (Table 5). In both B treatments, pollen viability of all three varieties, which was indicated by %KI/I_2 staining, had significantly correlation with anther B concentration (R = 0.414*) (Figure 1a). Unlikely, no correlation between anther B concentration and %KI/I_2 staining was found in 2008/2009 (Figure 1b). Although seed sterility in B10 of all four varieties was reduced compared with B0, it was found that anther B concentration of SPR1 and RD29 had no difference (Table 6). When considered the correlation between %KI/I_2 staining with % seed sterility from all three varieties of both B treatments in Year 1 (2007/2008), negative correlation (R= - 0.412*) was shown in this experiment (Figure 2a). In the same way, it was also found negative correlation (R= -0.734**) between %KI/I_2 staining with % seed sterility in 2008/2009 (Figure 2b).

The present study suggested that B deficiency affected on reproductive development in rice without any responses during vegetative growth. This was as previously reported in wheat (Rerkasem and Jamjod, 1997; Huang et al., 2000) and barley (Jamjod and Rerkasem, 1999). Reproductive stage has been found to be more sensitive to low B supply than vegetative growth (Dell and Huang, 1997), especially to male sterility which cause grain set failure (Rerkasem and Jamjod, 1997). Similarly, the results gained from this study showed that seed set of all rice varieties in both two years was depressed in B0 treatment (Table 4 and Table 5). The main cause of this phenomenon was related to pollen viability which was shown by the significant correlation between %KI/I_2 (Figure 2a).

The response to B increase from B0 to B10 and the low pollen fertility and grain set in B10 suggest that the B supply in B10 may still be insufficient for maximum reproductive development in rice under the condition of this study. The most tolerant to B deficiency was SPR1 while the most sensitive to B deficiency was KDML105. The method used in this experiment could be used for determining the responses to B deficiency in other rice varieties of interests. The different response to B deficiency, especially in pollen viability and % grain set, of rice genotypes will be useful for controlling male sterility in hybrid rice production.

Acknowledgement

This work was supported by a grant from the Thailand Research Fund (TRF).

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Table 1. Analysis of variance results for effects of boron (B), genotype (G) and boron x genotype interaction (BxG) on vegetative response, reproductive response and B concentrations of rice grown in two seasons (2007/08 and 2008/09).

Characters	2007/08			2008/09		
	B	G	B x G	B	G	B x G
Vegetative response						
Tillers plant^{-1}	ns	**	ns	ns	**	ns
Panicles plant^{-1}	ns	**	ns	ns	**	ns
Culm length (cm)	ns	ns	ns	ns	ns	ns
Straw dry weight (g)	ns	**	ns	ns	**	ns
Root dry weight (g)	ns	**	ns	ns	**	ns
Reproductive response						
Spikelets panicle^{-1}	ns	**	ns	ns	**	ns
Pollen viability (%)	**	*	*	**	**	**
Grain set (%)	**	**	*	**	**	**
B concentrations (mg B kg DW^{-1})						
Flag leaf [B]	**	**	**	**	*	*
YEB+1 [B]	*	ns	**	**	*	**
Anthers [B]	**	**	**	**	*	**

* and ** significant at $p<0.05$ and 0.01, respectively; ns not significant.

Table 2 Numbers of tillers plant^{-1}, panicles plant^{-1} and spikelets panicle^{-1} of three rice genotypes at two levels of B supply in two seasons 2007/08 and 2008/09.

B level (µM)	Genotype	2007/08				2008/09		
		Tillers plant^{-1}	Panicles plant^{-1}	Spikelets panicle $^{-1}$		Tillers plant^{-1}	Panicles plant^{-1}	Spikelets panicle $^{-1}$
0	KDML105		7	129			11	107
	CNT1		8	113			13	85
	SPR1		8	161			14	118
10	KDML105		7	125			11	125
	CNT1		9	103			16	85
	SPR1		10	146			13	134
F-test								
Genotype (G)				**	**		*	*
B (B)				ns	ns		ns	ns
BxG				ns	ns		ns	ns

* and ** significant at p<0.05 and 0.01, respectively; ns not significant.

Table 3 Pollen viability and grain set (%) of three rice genotypes at two levels of B supply.

B level (µM)	Genotype	2007/08		2008/09	
		Pollen viability (%)	[1]Grain set (%)	Pollen viability (%)	[1]Grain set (%)
0	KDML105	28.6 a	10.9 d	25.2 a	11.5 a
	CNT1	33.2 a	6.7 d	55.1 bc	14.6 bc
	SPR1	27.4 a	27.9 b	54.0 b	47.2 c
10	KDML105	36.9 a	18.7 c	65.1 c	21.7 b
	CNT1	59.9 b	12.9 cd	59.6 bc	53.9 d
	SPR1	70.3 b	38.4 a	80.9 d	59.7 d
F-test					
Genotype (G)		*	**	**	**
B (B)		**	**	**	**
BxG		*	**	**	**
$LSD_{BxG(0.05)}$		18.8	8.1	10.0	9.2

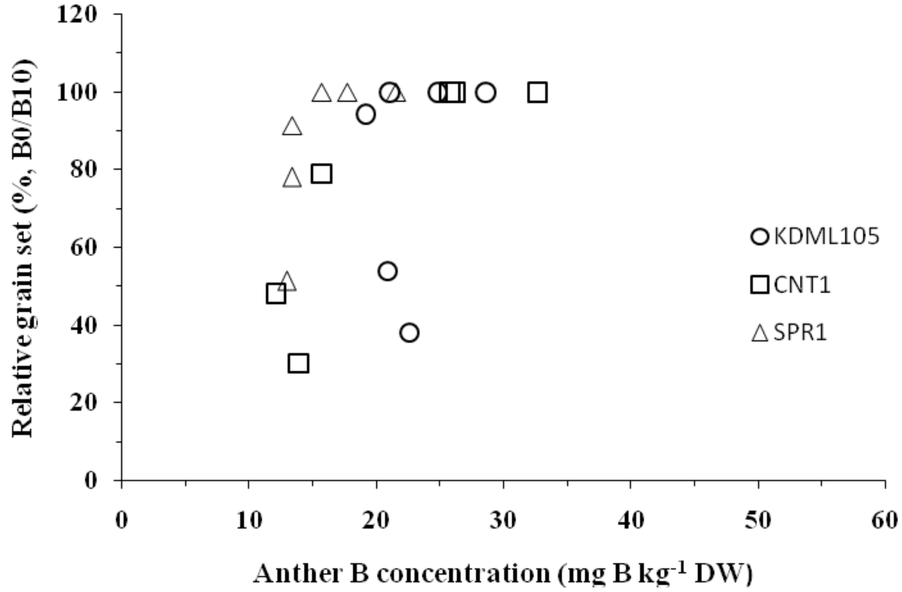
Table 4 Boron concentrations (mgB kg^{-1}) in flag leaf (FL), first leaf below flag leaf (YEB+1) at maturity and in anther at anthesis of three rice genotypes at two levels of B supply in two seasons 2007/08 and 2008/09.

B level (μM)	Genotype	2007/08 FL	2007/08 YEB+1	2007/08 Anther	2008/09 FL	2008/09 YEB+1	2008/09 Anther
0	KDML105	4.64 a	6.19 bc	20.85 bc	7.12 b	7.25 b	21.57 a
	CNT1	6.40 a	5.49 bc	13.88 a	3.86 a	3.72 a	17.09 a
	SPR1	5.49 a	5.06 a	13.21 a	6.64 ab	4.14 a	22.06 a
10	KDML105	8.53 a	8.40 cd	24.76 cd	11.42 c	8.10 bc	40.19 b
	CNT1	21.82 b	13.32 d	28.18 d	23.32 d	9.26 c	37.49 b
	SPR1	27.56 b	11.24 de	18.33 b	22.42 d	8.01 bc	20.47 a
F-test							
Genotype (G)		**	ns	**	**	*	*
B (B)		**	**	**	**	**	**
BxG		**	**	**	**	**	**
$LSD_{BxG(0.05)}$		7.35	2.98	4.10	2.99	1.55	9.02

* and ** significant at $p<0.05$ and 0.01, respectively; ns not significant.

Mean within a column with the same letter do not differ significantly at 5% level with LSD.

a) 2007/08



b) 2008/09

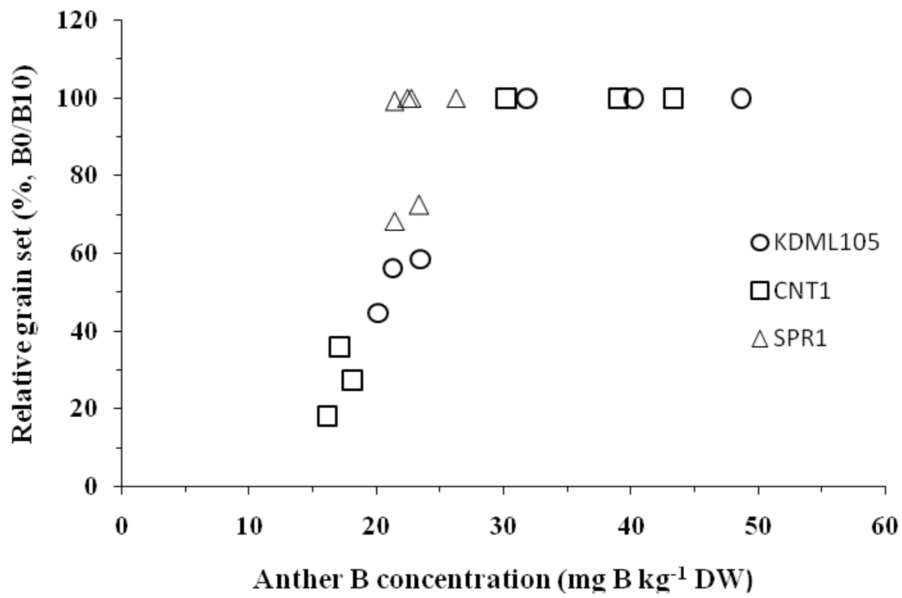
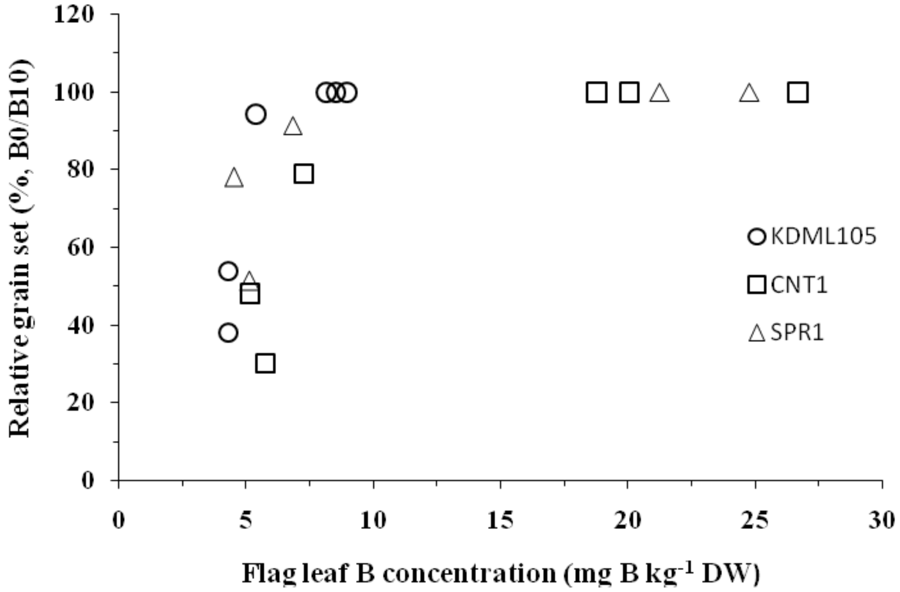


Figure 1 Anthers

a) 2007/08



b) 2008/09

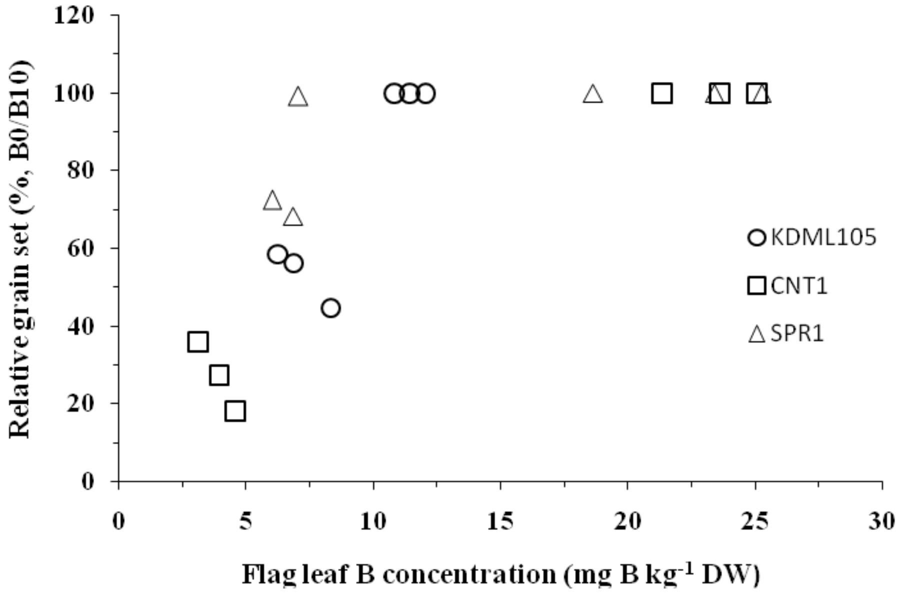
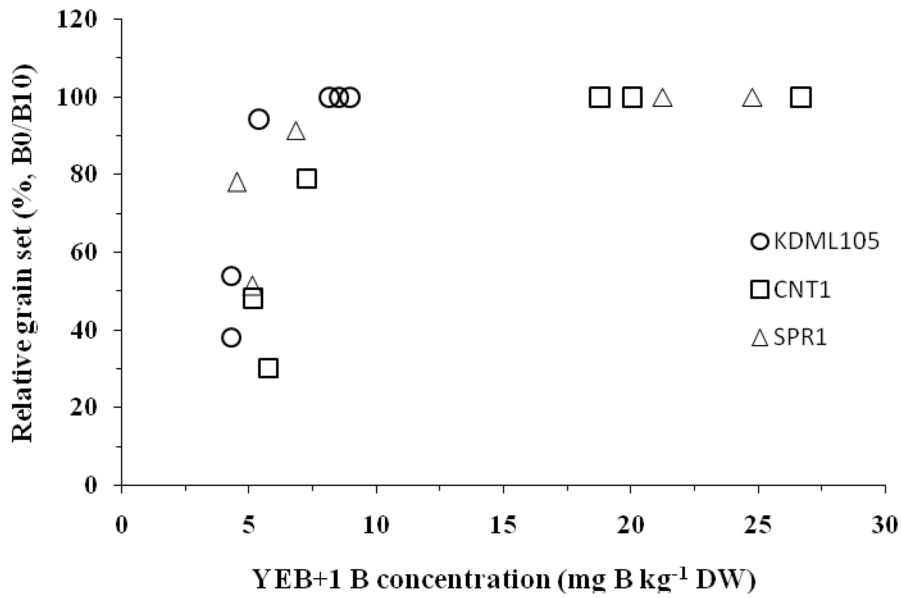


Figure 2 Flag leaf

a)



b)

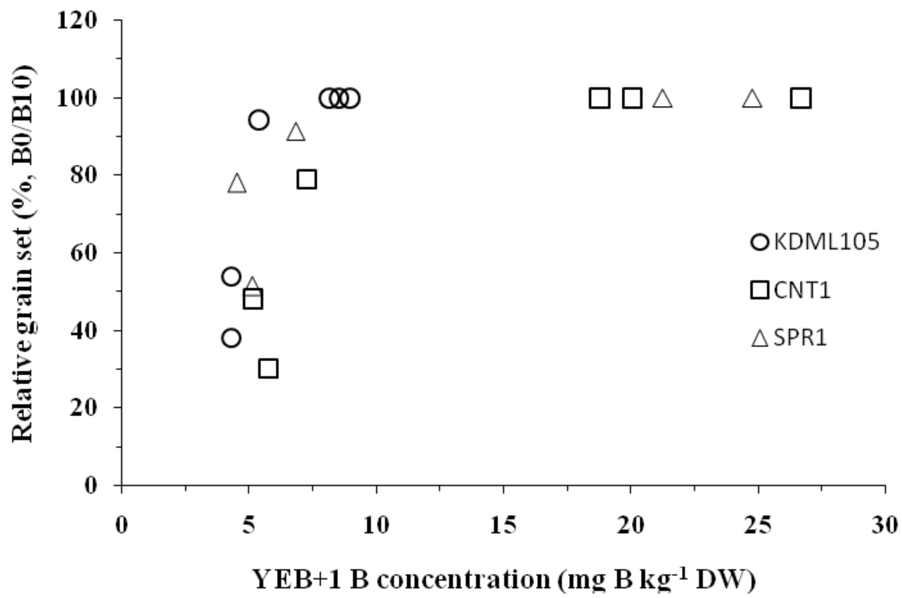


Figure 3 YEB

Figure 2.1 Relationship between relative grain set (% B0/B10) and B concentrations in a) anther and b) flag leaf of three rice genotypes, Pot Experiment 2008/09.

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Genotypic variation in floral morphology traits of Thai rice (*Oryza sativa* L.) varieties

Sansanee Jamjod, Nipim Paramee, Thitinan Sreethong and Sunisa Nirantrayakul.

Abstract

Variation in natural outcrossing in rice (*Oryza sativa* L.) can be attributed to variation in flowering behavior, floral traits, pollen parents and variation in environmental factors. These reproductive traits play an important role to seed production in hybrid rice varieties. This experiment aimed to evaluate reproductive traits contributing cross pollination in 12 cultivated rice varieties bred and released in Thailand. Plants of each variety were grown in pots, 10 plants per pot. There were three replicates. At flowering, data for flowering behavior (time at blooming and duration of blooming), pistil traits (degree of stigma exertion, stigma and style length), stamen traits (anther length and width) and pollen germination were collected. Results from the experiment indicated that differences in blooming duration and flowering behavior were found between varieties. The blooming for most varieties occurred between 9:30 A.M. to 11:00 A.M. and the peak of were at 10:00 A.M. The average blooming duration ranged from 33–43 minutes. Varieties with the longest blooming duration were RD10, PTT1 and SKN1. For stigma exsertion, the highest stigma exsertion rate was found in RD7, RD10, SKN1 and the lowest in PTT1, NP1 and R258.

For floral traits, genotypic variation in pistil and stamen lengths were found. The longest anther were observed in R258 and PTT1 and the longest stigma and longest style lengths found in PTT1 and CNT1. For pollen study, number of pollen grains per anther of all varieties were between 1,220-1,960. At anthesis, about 35-81 of pollen grains per stigma were found with about 73-80% viability. The variety with the highest percent pollen viability on stigma was RD21 and the lowest was NP1. Information on variation of floral traits of the local varieties will assist in targeting of characteristics to be improved and parental selection in hybrid seed production for Thailand and Southeast Asia.

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For floral traits, genotypic variation in pistil and stamen lengths were found. The longest anther were observed in R258 and PTT1 and the longest stigma and longest style lengths found in PTT1 and CNT1. For pollen study, number of pollen grains per anther of all varieties were between 1,220-1,960. At anthesis, about 35-81 of pollen grains per stigma were found with about 73-80% viability. The variety with the highest percent pollen viability on stigma was RD21 and the lowest was NP1. Information on variation of floral traits of the local varieties will assist in targeting of characteristics to be improved and parental selection in hybrid seed production for Thailand and Southeast Asia.

Introduction

In recent years, private sectors have invested in hybrid rice research in Thailand. Development for commercial production is now underway by breeding to improve adaptation to local area such as cooking quality, pest and disease resistance. Major limitation of hybrid seed production is low outcrossing rate in rice because it is predominately self-pollinated crop. However, outcrossing were observed under specific forms and environmental conditions (Virmani, 1994). Variation in natural outcrossing in rice can be attributed to variation in flowering behavior, floral traits, pollen parents and variation in environmental factors. These reproductive traits play an important role to seed production in hybrid rice varieties. Genotypic variation for traits contributing outcrossing in rice have been studied inter- and intra-specifically (Kato and Namai, 1987; Uga et al., 2003). However, no information of flowering traits involving outcrossing in Thai rice genetic background is available. Therefore, the purpose of this study was to evaluate genotypic variation in floral morphology traits of Thai rice varieties focus on those contributing to outcrossing.

Materials and Methods

Genotypes and experimental procedure

Twelve rice varieties (Table 1) were used, including Neaw Phrae 1 (NP1), R258, Sun Pa Thong 1 (SPT1), RD4, RD7, RD10, RD21, RD29, Chainat 1 (CNT1), Chainat 80 (CN80), Suphanburi 1 (SPR1), Sakonnakorn 1 (SKN1) and Pathumthani 1 (PTT1). Seeds of each variety were pre-germinated in petri dishes. After 5 days germination in the petri dishes, 10 seedlings of each variety were transplanted in an un-drained, plastic pot (30 cm diameter, 30 cm deep), containing soil of the San Sai series. When the rice seedlings were about 10 cm tall, the pots were kept flooded with about 5 cm of water above the soil surface until maturity. Fertilizers and pesticides were applied uniformly to avoid nutrient deficiency and insect infestation. Genotypes were arranged in a Completely Randomized Design with three replications.

Flowering duration and stigma exertion

At flowering, three panicle from each pot was randomly selected. Five florets per panicle, 45 florets per variety, were marked and recorded for time that lemma and palea began to open and duration of blooming. In the afternoon, stigma exertion of the marked florets were determined as single, dual or no stigma exertion and then converted to rate of stigma exertion (%) as described by Yan et al. (2009).

Anther and pistil sizes

Three panicles per replication were randomly selected. Three florets, at the top, middle and bottom of each panicle were collected and kept in a tube containing 95% alcohol. For each floret, stigma length, style length, anther length and anther width were measured microscopically.

Pollen number and pollen viability

Five florets from five panicles from each variety were randomly collected. Number of pollen grains per anther and number of pollen grains on a stigma were counted. For pollen grains per anther, anthers were collected before anthesis. For each panicle, anthers from the top, middle and bottom were collected. Each anther was placed into an eppendorf tube containing 500 uL of KI/I. The tube was vortexed and pollen were counted from five replicates of 10 uL. For pollen grains on stigma, the florets were collected about 3 hours after anthesis and fixed in 90% ethanol. Stigma from these florets were excised on a glass slide and stained with cotton blue. Pollen viability was estimated as the percentage of stained pollen to total number of pollen grains counted.

Grain set

At maturity, two panicles from each plant were harvested. Data on spikelets panicle^{-1}, grains panicle^{-1}, empty grains panicle^{-1}. For empty grains, each spikelet was opened by foceps and determined as unfertilized or fertilized but unfilled grains. Grains set was determined as the percentage of number of fertilized grains to total number of spikelets. Therefore, grain set included both completely filled and partially filled grains.

Data analysis

Data of all traits were analysed as CRD design with three replications. Relationship between characters was determined by correlation coefficient (r).

Results

Flowering duration and stigma exertion

Opening of the spikelet or blooming at Chiang Mai University, Chiang Mai started at 9.30 to 10.20 am (Table 1). Most genotypes started opening at close to 10 am except SPT1, RD7, RD10, RD21 and PTT1 which were half an hour later. Blooming duration, the period from opening to closing of floret, was varied with genotypes and within the range of 35-50 min (Table 2). RD10, R258 and PTT1 were among the group of long duration genotypes, lasted for 50 min (Figure 1). Extents of stigma exertion were between 49-100%, with RD10, RD7 and SKN1 showed 100% stigma exertion. As each floret of rice has two stigma, we found variation in rice varieties of exertion for single and dual stigma per floret (Table 3 and Figure 2). The highest dual stigma exertion was found in RD10 and highest single stigma exertion in SKN1 (Figure 1.1). NP1 and PTT1 exhibited the longest stigma among all varieties but turn out to be the lowest rate of stigma exertion.

Anther and pistil sizes

For male flower of rice, anther length and width were between 2.3-2.7 mm and 0.8-0.9 mm, respectively. R258 and PTT1 exhibited the largest anthers. Number of pollen grains per anther were between 1,220 in RD4 to 1,960 in PTT1 (Table 4). Fertilization was depended on number of pollen deposited on stigma. Most of varieties deposited more than 40 pollen grains per stigma. The highest pollen grain per stigma (79) was found in SKN1. The range of variation for stigma and style lengths were between 1.7-2.4 mm and 0.7-1.1 mm, respectively. Those two made up for the length of pistils between 1.45-2.06 mm (Table 5). PTT1 and NP1 were the varieties with the longest stigma while, CNT1 had the longest style. When sum up the length of stigma and style, CNT1, RD4 and RD7 had the longest pistils.

Pollen number and pollen viability on stigma

Fertilization was depended on number of pollen deposited on stigma. All variety deposited more than 40 pollen grains per stigma and no significant different between genotypes was found. On the stigma, NP1 and R258 expressed the lowest pollen germination rate, 54% and 66%, respectively. While those of the other genotypes were at 74-80% (Table 7).

References

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Table 1 Distribution of time of blooming for 12 rice varieties.

Genotypes	Time (AM)								
	9:00-9:30	>9:30-10:00	>10:00-10:30	>10:30-11:00	>11:00-11:30	>11:30	Mean	Min	Max
NP1		6	4	5			10:06	9:35	10:38
R258			15				10:02	10:00	10:05
SPT1		3		12			10:28	9:40	10:43
RD4		9	5	1			10:01	9:38	10:37
RD7			4	8	3		10:46	10:10	11:15
RD10		2	2		11		10:51	9:47	11:20
RD21				15			10:38	10:30	10:49
RD29		6	6	3			10:12	9:40	10:55
CNT1		6	4	5			10:03	9:30	10:35
SPR1		3	12				10:00	9:34	10:10
SKN1		10	5				9:53	9:41	10:15
PTT1			2	7	6		10:51	10:25	11:15

Table 2 Distribution of blooming duration for 12 rice varieties.

Genotypes	Duration (min) 20 - 29	30 - 39	40 - 49	50 - 59	Mean[#]	Min	Max
Neaw Phrae		12	3		36 cde	33	40
R 258		14	1		35 de	31	45
SPT 1		9	6		36 cde	30	40
RD 4		15			33 e	30	37
RD 7		15			33 e	32	35
RD 10			12	3	46 a	43	50
RD 21		15			34 de	30	38
RD 29	1	12	2		34 de	28	41
CNT 1		15			33 e	30	39
CNT 80		15			37 cd	35	39
SPR 1		13	2		37 cd	35	41
SKN 1		7	8		40 bc	36	44
PTT 1		4	8	3	43 ab	35	50

[#] Means followed by the same letters within column are not differed significantly by LSD at p=0.05.

Table 3 Distribution of stigma exertion from florets of 12 rice varieties.

Genotype	Frequency of florets (%)			
	No exertion	With exertion		
		Single	Dual	Total
NP1	49 ab	49 bcd	4 e	51 e
R 258	33 bc	51 abcd	16 de	67 de
SPT 1	27 cd	41 cd	32 cd	73 cd
RD 4	24 cde	71 a	4 e	76 bcd
RD 7	0 f	40 cd	60 ab	100 a
RD 10	0 f	29 d	71 a	100 a
RD 21	33 bc	56 abc	11 de	67 de
RD 29	22 cde	53 abc	24 cde	78 bcd
CNT 1	9 ef	67 ab	24 cde	91 ab
CNT 80	13 def	62 abc	24 cde	87 abc
SPR 1	9 ef	51 abcd	40 bc	91 ab
SKN 1	0 f	67 ab	33 cd	100 a
PTT 1	51 a	44 bcd	4 e	49 e

Means followed by the same letters within column are not differed significantly by LSD at p=0.05.

Table 4 Variation in female reproductive traits of 12 rice varieties.

Genotypes	Stigma length (mm)	Style length (mm)	Pistil length (mm)
NP1	1.01 ab	1.00 cde	2.02 ab
R258	0.74 cd	1.02 cde	1.76 abc
SPT1	0.83 cd	0.98 def	1.81 ab
RD4	0.76 cd	1.27 ab	2.03 a
RD7	0.86 bcd	1.17 bc	2.03 a
RD10	0.73 cd	1.10 bcd	1.83 ab
RD21	0.81 cd	1.17 bc	1.98 ab
RD29	0.91 abc	1.03 cde	1.94 ab
CNT1	0.71 d	1.35 a	2.06 a
SPR1	0.88 bcd	0.81 fg	1.69 bc
SKN1	0.81 cd	0.64 g	1.45 c
PTT1	1.07 a	0.81 fg	1.89ab
F-test	**	****	*

*, ** and *** significant at p<0.05, 0.01 and 0.001, respectively.

Means followed by the same letters do not differed significantly by LSD at p=0.05

Table 5 Variation in male reproductive traits of 12 rice varieties.

Genotypes	Anther length (mm)	Anther Width (mm)	Number of pollen per anther		Number of pollen deposited per stigma	
			Total	KI/I_2 [a] stained (%)	Total	Viability [b] (%)
NP1	2.40 c	0.86 a	1610 bc	88 f	50	54 c
R258	2.74 a	0.82 a	1490 cd	91 e	81	66 b
SPT1	2.44 bc	0.86 a	1470 cd	97 a	64	78 a
RD4	2.57 abc	0.82 a	1220 e	95 c	63	78 a
RD7	2.43 bc	0.79 a	1620 bc	96 ab	61	76 ab
RD10	2.54 abc	0.81 a	1320 de	95 c	55	75 ab
RD21	2.55 abc	0.78 a	1330 de	96 ab	67	80 a
RD29	2.56 abc	0.77 a	1470 cd	96 ab	44	79 a
CNT1	2.44 bc	0.82 a	1460 cd	96 ab	53	74 ab
SPR1	2.33 c	0.80 a	1367 de	96 ab	35	74 ab
SKN1	2.01 d	0.64 b	1795 ab	95 cd	79	78 a
PTT1	2.69 ab	0.80	1960 a	94 d	56	73 ab
F-test	**	*	***	**	ns	***

[a] Pollen viability test by iodine (KI/I_2) staining.

[b] Pollen viability test by cotton blue staining.

*, ** and *** significant at $p<0.05$, 0.01 and 0.001, respectively.

Means within a column followed by the same letters do not differed significantly by LSD at p=0.05

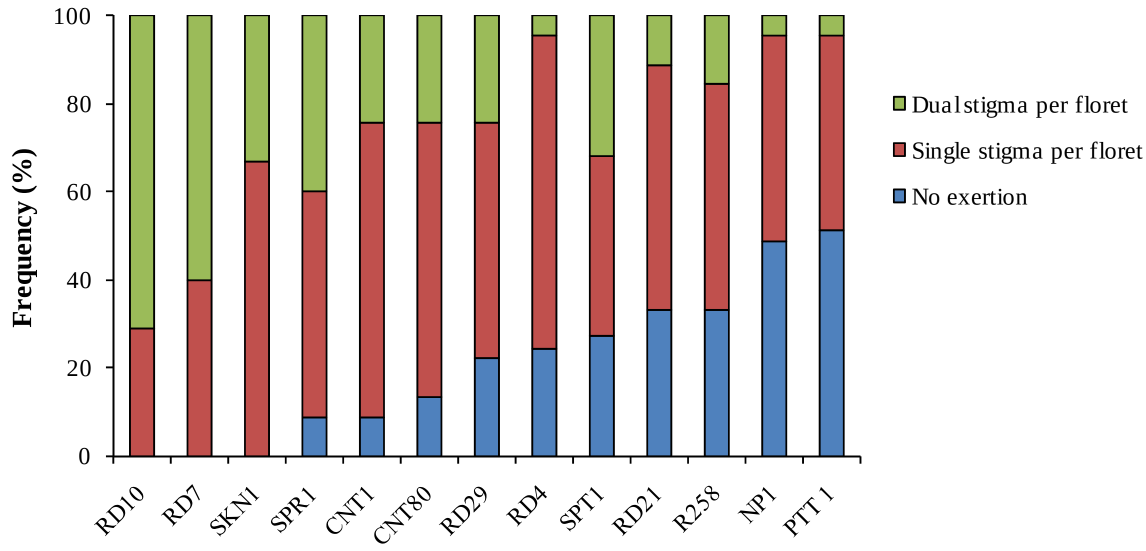

Figure 1 Frequency (%) of spikelets with no, single and dual stigma exertion per floret of 12 rice varieties. Data were average from 27 florets per variety.

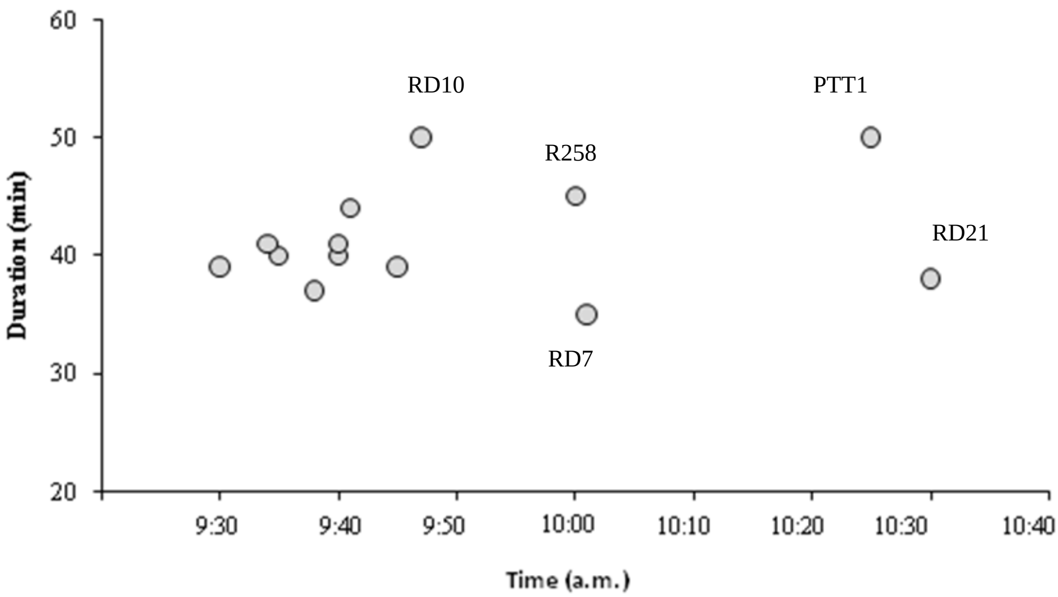

Figure 2 Time (a.m.) at first floret started blooming and blooming duration (min) of 12 rice varieties.

ความแปรปรวนทางพันธุกรรมของการติดเมล็ดในข้าว

Genotypic Variation of Grain Set in Rice

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Abstract: Effect of increasing temperature as a consequence of global warming on grain set reduction in rice is a serious problem on rice productivity. Therefore, the objective of this study was to evaluate the variation of grain set among rice varieties in summer season. Eleven photoperiod insensitive rice varieties were grown on 9 January 2009 in plastic pots, 10 plants per pot in 3 replications. At maturity, two panicles from each plant were randomly selected. Number of spikelets/panicle, number of filled grains/panicle, number of unfertile grains/panicle and number of unfilled grains/panicle were recorded. The results illustrated that there was genotypic variation in grain set among rice varieties. RD21 and NP1 had the lowest percentage of filled grain (59.5 and 72.5%, respectively). More than 20% of empty grains were found in NP1, RD4, RD10 and RD21 which were between 20-40%. Variation of grain set among 11 rice varieties under high temperature resulted from both unfertilized and unfilled grains.

Keywords: Improved rice variety, grain set, high temperature

บทคัดย่อ: การเพิ่มขึ้นของอุณหภูมิในปัจจุบันอันเนื่องมาจากสภาวะโลกร้อนกำลังเป็นปัญหาสำคัญที่ทำให้การติดเมล็ดของข้าวลดลง ซึ่งมีผลกระทบทำให้ผลผลิตข้าวลดลง ดังนั้นการศึกษาครั้งนี้มีวัตถุประสงค์เพื่อประเมินความสามารถในการติดเมล็ดของข้าว ศึกษาในข้าวพันธุ์ไม่ไวต่อช่วงแสงทั้งหมด 11 พันธุ์ โดยปลูกวันที่ 9 มกราคม 2552 ในกระถางพลาสติกบรรจุดินขนาดเส้นผ่าศูนย์กลาง 30 เซนติเมตร จำนวน 10 ต้นต่อกระถาง พันธุ์ละ 3 ซ้ำ เมื่อถึงระยะเก็บเกี่ยว สุ่มเก็บตัวอย่าง 2 รวงต่อต้น นำแต่ละรวงมานับจำนวนดอกต่อรวง จำนวนเมล็ดดีต่อรวง จำนวนเมล็ดลีบแบบผสมไม่ติด และเมล็ดลีบแบบผสมติดแต่ไม่เต็มเมล็ด พบว่าข้าวแต่ละพันธุ์มีความสามารถในการติดเมล็ดแตกต่างกันเมื่อปลูกในสภาพอุณหภูมิสูง ซึ่งอุณหภูมิสูงสุดและต่ำสุดของวันในช่วงออกดอกคือ 40 และ 22 องศาเซลเซียส ตามลำดับ พบว่าพันธุ์เหนียวแพร่ 1 และ กข21 มีอัตราการติดเมล็ดต่ำที่สุด และพันธุ์ที่มีเปอร์เซ็นต์เมล็ดลีบรวมมากกว่า 20% ขึ้นไปได้แก่พันธุ์เหนียวแพร่ 1, กข4, กข10 และ กข21 มีค่าอยู่ระหว่าง 20-40% การเกิดเมล็ดลีบในพันธุ์ข้าวที่ศึกษาเกิดจากทั้งการผสมเกสรไม่ติดและผสมเกสรติดแต่ไม่เต็มเมล็ด

คำสำคัญ: ข้าวพันธุ์ปรับปรุง การติดเมล็ด อุณหภูมิสูง

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คำนำ

ข้าวเป็นธัญพืชที่ปลูกกันมากในประเทศแถบเอเชีย เนื่องจากนิยมบริโภคเป็นอาหารหลักมาตั้งแต่อดีตจนถึงปัจจุบัน ผลผลิตของข้าวนั้นขึ้นอยู่กับปัจจัยหลายอย่างทั้งที่จำเป็นต่อการเจริญเติบโตและการให้ผลผลิต เช่นความสูงของพื้นที่ ลักษณะทางกายภาพของดิน แสง ความชื้นสัมพัทธ์ ความเร็วลม และสภาพแวดล้อมอื่น ๆ อีกมากมาย อุณหภูมิก็เป็นปัจจัยหนึ่งที่สำคัญในการให้ผลผลิตของข้าว โดยพบว่าทั้งการปลูกข้าวในที่ที่มีอุณหภูมิต่ำและสูงเกินไปมีผลทำให้ผลผลิตของข้าวลดลง สำหรับผลของอุณหภูมิต่อการลดลงของผลผลิตข้าวพบจากรายงานการวิเคราะห์สถานการณ์การเพิ่มขึ้นของอุณหภูมิและผลผลิตจากอดีตถึงปัจจุบันทำให้ทำนายผลผลิตของข้าวเมื่ออุณหภูมิเพิ่มขึ้นได้ โดยพบว่าผลผลิตข้าวจะลดลง 10 เปอร์เซ็นต์เมื่ออุณหภูมิเพิ่มขึ้น 1 องศาเซลเซียส ซึ่งมีสาเหตุหลักมาจากการผสมเกสรและการเติมเต็มเมล็ด (Peng *et al.*, 2004) นอกจากนี้ยังพบว่าการที่ข้าวได้รับอุณหภูมิสูงในช่วงดอกบาน ส่งผลให้เปอร์เซ็นต์การติดเมล็ดลดลงด้วย (Suzuki *et al.*, 1981, 1982; Tanno *et al.*, 1999) การทดลองนี้เป็นส่วนหนึ่งของการประเมินลักษณะการติดเมล็ดของข้าว เมื่อปลูกในช่วงเวลาต่าง ๆ เพื่อนำไปศึกษาถึงพันธุกรรมที่ควบคุมลักษณะการตอบสนองต่ออุณหภูมิที่มีการเปลี่ยนแปลงอยู่ตลอดเวลา งานทดลองนี้มีวัตถุประสงค์เพื่อประเมินความแตกต่างทางพันธุกรรมของความสามารถในการติดเมล็ดของพันธุ์ข้าวชนิดไม่ไวต่อช่วงแสง โดยจัดการทดลองให้วันออกดอกตรงกับช่วงที่คาดว่าอุณหภูมิจะสูงกว่าอุณหภูมิปกติในช่วงระยะออกดอก

อุปกรณ์และวิธีการ

ทดลองที่ภาควิชาพืชศาสตร์และทรัพยากรธรรมชาติ คณะเกษตรศาสตร์ มหาวิทยาลัยเชียงใหม่ ใช้พันธุ์ข้าวชนิดไม่ไวต่อช่วงแสงทั้งหมด 11 พันธุ์ คือ เหนียวแพร่ 1 (NP1), อาร์ 258 (R 258), สันป่าตอง 1 (SPT1), กข4 (RD4), กข7 (RD7), กข10 (RD10), กข21 (RD21), กข29 (RD29), ชัยนาท 1 (CNT1), สุพรรณบุรี 1 (SPR1) และสกลนคร 1 (SKN1) ปลูกเมื่อวันที่ 9 มกราคม 2552 เพื่อให้พบอุณหภูมิสูงในช่วงออกดอก โดยอุณหภูมิสูงสุดและต่ำสุดของวันในช่วงดังกล่าวคือ 40 และ 22 องศาเซลเซียส ตามลำดับ วางแผนการทดลองแบบสุ่มสมบูรณ์ (Completely Randomized Design) จำนวน 3 ซ้ำ ปลูกในกระถางพลาสติกบรรจุดินขนาดเส้นผ่าศูนย์กลาง 30 เซนติเมตร จำนวน 10 ต้นต่อกระถาง ใส่ปุ๋ยสูตร 16-20-0 อัตรา 25 กก. /ไร่ หลังย้ายปลูกในกระถาง 20 และ 60 วัน และ 46-0-0 อัตรา 5 กก./ไร่ ในระยะกำเนิดช่อดอก เมื่อถึงระยะเก็บเกี่ยว สุ่มเก็บตัวอย่าง 2 รวงต่อต้น นำแต่ละรวงมานับแยกเมล็ดดี (filled grain) เมล็ดลีบแบบผสมไม่ติด (unfertilized grain) เมล็ดลีบแบบผสมติด (unfilled grain) วิเคราะห์ผลการทดลองโดยวิธีการวิเคราะห์ความแปรปรวน (Analysis of Variance) เปรียบเทียบความแตกต่างโดยใช้ค่า Least Significant Difference (LSD) ที่ P=0.05

ผลการทดลอง

พบความแตกต่างระหว่างพันธุ์ในลักษณะจำนวนดอกต่อรวงและอัตราการติดเมล็ด (ตารางที่ 1) โดยพันธุ์ที่มีจำนวนดอกต่อรวงสูงที่สุดคือ กข21 และสกลนคร 1 มีค่าอยู่ระหว่าง 95-98 ดอกต่อรวง ส่วนพันธุ์ที่มีจำนวนดอกต่อรวงน้อยที่สุดได้แก่ พันธุ์อาร์ 258, กข10, ชัยนาท 1 และสุพรรณบุรี 1 มีค่าอยู่ระหว่าง 73-84 ดอกต่อรวง สำหรับอัตราการติดเมล็ด พันธุ์ที่มีอัตราติดเมล็ดน้อยที่สุดคือ เหนียวแพร่ 1 และ กข21 มีค่าอยู่ที่ 72.5 และ 59.5% ตามลำดับ ส่วนพันธุ์ที่เหลือมีอัตราการติดเมล็ดอยู่ที่ 79-91% ส่วนเปอร์เซ็นต์เมล็ดลีบรวมนั้น พบว่า พันธุ์ที่มีเมล็ดลีบรวมมากกว่า 20% ขึ้นไปได้แก่พันธุ์ เหนียวแพร่ 1, กข4, กข10 และ กข21 มีค่าอยู่ระหว่าง 20-40% เมื่อจำแนกเมล็ดลีบออกเป็นสองส่วนคือ ส่วนที่เกิดจากการผสมไม่ติด (unfertilized grain) และส่วนที่เกิดจากการผสมติดแต่ไม่เต็มเมล็ด (unfilled grain) พบว่าในพันธุ์ที่ติดเมล็ดน้อยที่สุด คือ เหนียวแพร่1 ส่วนใหญ่เกิดจากการผสมติดแต่ไม่เต็มเมล็ด เท่ากับ 18.7% เมื่อเทียบกับผสมไม่ติดเพียง 8.8% ส่วนอัตราเมล็ดลีบในพันธุ์กข21 นั้นเกิดจากทั้ง 2 แบบในสัดส่วนใกล้เคียงกัน ในพันธุ์ที่เหลือส่วน

ใหญ่เมล็ดลีบจะเป็นผลมาจากการผสมไม่ติดมากกว่าผสมติดแต่ไม่เต็มเมล็ด เมื่อวัดความสัมพันธ์ระหว่างลักษณะที่ศึกษาพบว่าลักษณะจำนวนดอกต่อรวงไม่มีผลต่ออัตราการติดเมล็ดของข้าว พบความสัมพันธ์ในทางบวก (r=0.46*) ระหว่างเมล็ดลีบชนิดไม่ผสมและเมล็ดลีบชนิดผสมแต่ไม่เต็มเมล็ด การเกิดเมล็ดลีบในพันธุ์ข้าวที่ศึกษานี้เกิดจากทั้งการไม่ผสมเกสรและผสมเกสรแต่ไม่เต็มเมล็ด โดยมีค่าความสัมพันธ์กับการเกิดเมล็ดลีบเท่ากับ 0.81** และ0.89** ตามลำดับ (ตารางที่ 2)

Table 1 Number of spikelets per panicle filled grain (%) unfertilized grain (%) and unfilled grain (%) of eleven varieties.

Variety	Spikelet/panicle	Filled grain (%)	Empty grain (%)		
			Unfertilized grain	Unfilled grain	Total33333
Neaw Phrae 1 (NP1)	91.1 ab[1/]	72.5 c	8.8 bc	18.7 a	27.5 b
R-258	73.5 b	85.8 ab	8.2 bc	6.0 bc	14.2 cd
Sanphatong 1 (SPT1)	92.8 ab	91.2 a	4.2 c	3.9 c	8.1 e
RD4	93.3 ab	79.7 b	11.0 bc	9.3 b	20.3 bc
RD7	84.9 ab	91.3 a	6.3 c	2.4 c	8.7 d
RD10	82.2 b	78.4 bc	12.6 b	9.0 b	21.6 bc
RD21	98.0 a	59.5 d	18.8 a	21.8 a	40.5 a
RD29	90.0 ab	82.9 b	12.0 bc	5.1 bc	17.1 c
Chainat 1 (CNT1)	84.4 b	86.2 ab	6.0 c	7.8 bc	13.8 cd
Suphanburi 1 (SPR1)	78.7 b	80.8 b	13.9 ab	5.3 bc	19.2 c
Sakonnakhon 1 (SKN1)	95.4 a	84.4 ab	10.6 bc	5.0 bc	15.7 cd
F-test	**	***	**	***	***
LSD (0.05)	12.2	7.8	5.8	4.5	7.9
CV (%)	8.2	5.7	33.4	31	24.7

and * Significant at P < 0.01 and P < 0.001, respectively.

[1/]Means within a column with the same letter do not difference significantly at 5% level with LSD.

Table 2 Correlation coefficients (r) between spikelets per panicle, filled grain, unfilled grain, unfertilized grain and empty grain.

	Spikelet/panicle	Filled grain (%)	Unfilled grain (%)	Unfertilized grain (%)
Filled grain (%)	-0.26			
Unfilled grain (%)	0.31	-0.89**		
Unfertilized grain (%)	0.13	-0.81**	0.46*	
Empty grain (%)	0.27	-1.00	0.89**	0.81**

*and** Significant at P < 0.05 and P < 0.01, respectively.

วิจารณ์

จากการศึกษาพบความแตกต่างทางพันธุกรรมในการติดเมล็ดของพันธุ์ข้าวที่ศึกษา พันธุ์ที่ติดเมล็ดสูงสุดในช่วงดอกบานซึ่งตรงกับเดือนเมษายน เป็นช่วงที่มีอุณหภูมิสูงสุดของกลางวันสูงถึง 40 องศาเซลเซียส และกลางคืน 20 องศาเซลเซียส ได้แก่พันธุ์สันป่าตอง 1 และ กข7 เป็นพันธุ์ที่คิดว่าน่าจะทนต่อสภาพอากาศร้อนได้ดี แต่ควรจะมีการทดสอบยืนยันในพื้นที่อื่น ๆ ถึงความสามารถในการติดเมล็ด ส่วนพันธุ์ที่ไม่แนะนำให้ปลูกในสภาพอากาศร้อนคือพันธุ์ กข21 และเหนียวแพร่ 1 เพราะมีอัตราการติดเมล็ดที่ต่ำ สำหรับพันธุ์ กข4, กข10, ชัยนาท 1, สุพรรณบุรี 1 และ สกลนคร 1 มีอัตราการติดเมล็ดอยู่ที่ 78-86 เมื่อเทียบกับพันธุ์สันป่าตอง 1 และ กข7 ที่มีอัตราการติดเมล็ดสูงสุดแล้ว พันธุ์เหล่านี้ถือได้ว่ามีอัตราการติดเมล็ดอยู่ในระดับปานกลาง Prasad *et al.* (2005) พบว่าอุณหภูมิสูงทำให้อัตราการติดเมล็ดในข้าวลดลง ซึ่งจะแตกต่างกันออกไปในแต่ละพันธุ์ โดยแบ่งออกได้เป็น 3 กลุ่มคือ พันธุ์ที่ทนต่อสภาพอุณหภูมิสูงได้ดีที่สุด พันธุ์ที่ทนได้ปานกลาง และพันธุ์ที่อ่อนแอที่สุด สำหรับเมล็ดลีบรวมที่พบจากพันธุ์ที่ศึกษาครั้งนี้มาจาก 2 ส่วนคือ ส่วนที่ผสมไม่ติด และส่วนที่ผสมติดแต่ไม่เต็มเมล็ด จากงานทดลองของ Peng *et al.* (2004) พบว่าอุณหภูมิที่สูงในตอนกลางวันมีผลต่อการผสมเกสรของข้าว และจากงานของ Jagadish *et al.* (2007) ที่สรุปว่าอุณหภูมิกลางคืนมีผลต่อการเติมเต็มเมล็ด อย่างไรก็ตามการทดลองนี้เป็นการทดลองเริ่มต้นที่ศึกษาเพียงวันปลูกเดียว การเพิ่มวันปลูกในการทดลองต่อไปจะสามารถยืนยันสาเหตุความแปรปรวนและความสามารถในการติดเมล็ดของข้าวพันธุ์ต่าง ๆ ได้มากขึ้น

สรุป

จากการประเมินความแตกต่างในการติดเมล็ดของข้าวทั้งหมด 11 พันธุ์ พบว่าข้าวแต่พันธุ์มีอัตราการติดเมล็ดภายใต้สภาพที่มีอุณหภูมิสูงต่างกัน และพบว่าผลของการผสมเกสร และความสามารถในการเติมเต็มเมล็ดมีผลต่อความแตกต่างในการติดเมล็ดในสภาพที่มีอุณหภูมิสูงของข้าว

กิตติกรรมประกาศ

งานวิจัยนี้ได้รับทุนสนับสนุนจากสำนักงานกองทุนสนับสนุนงานวิจัย (สกว.) และมูลนิธิ McKnignt ผู้วิจัยแรกได้รับทุนสนับสนุนจากโครงการเครือข่ายเชิงกลยุทธ์เพื่อการผลิตและพัฒนาอาจารย์ในสถาบันอุดมศึกษา

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2. กิจกรรมที่เกี่ยวข้องกับการนำผลงานไปใช้ประโยชน์

2009	เข้าร่วมประชุมเสนอผลงานในการประชุมร่วมระหว่างกลุ่มเมธีวิจัยอาวุโส สาขาพืชไร่ โดยมีนักวิจัยในกลุ่มและนักศึกษาบัณฑิตศึกษาร่วมเสนอผลงานจำนวน 4 เรื่อง โดยเสนอแบบปากเปล่า 2 เรื่องและโปสเตอร์ 2 เรื่อง
2009	นักวิจัย (รศ.ดร.ศันสนีย์ จำจด) ได้รับเชิญให้เข้าร่วมประชุมเสนอบทความเรื่อง Genetic diversity and gene flow of common wild rice in Thailand, organized by Collaborative Investigation to Research Wild Rice Committee ระหว่างวันที่ 22-24 พฤศจิกายน 2552
2008	นักวิจัย (ดร. สาวิกา กอนแสง) เดินทางไปปฏิบัติการวิจัย (วิเคราะห์โบรอนในละอองเรณูข้าว) ณ Murdoch University, Western Australia (Prof. Bernie Dell) April-May 2008
2007	นักวิจัย (ดร. สิทธิชัย ลอดแก้ว) จัดอบรมวิธีการทดสอบความมีชีวิตของละอองเรณูข้าวในวุ้นให้แก่นักวิจัยและนักศึกษาบัณฑิตศึกษา CMUPN*lab* ภาควิชาพืชศาสตร์และทรัพยากรธรรมชาติ มหาวิทยาลัยเชียงใหม่ ประมาณ 25 คน

3. Expected and realized project output

Project outputs	Proposed	Achieved
1. Publications	4	
Manuscripts under preparation		2
Paper in Thai		1
2. New varieties	2	
Advanced lines		16
3. Protocol		
Method to evaluation pollen germination in agar	1	1
4. Researchers and postgraduate students gain experience in R&D with industrial section	20	20