CHAPTER 5

The effect of B on pollen development in B-efficient (cv. Fang 60) and B-inefficient (cv. SW 41) wheat (*Triticum aestivum* L.) genotypes.

5.1 Introduction

Boron deficiency causes male sterility in wheat but sensitivity differs among cultivars. In previous studies, a B- inefficient cultivar (cv. SW41) growing in sand culture at low B supply produced pollen that appeared normal at vacuolated young microspore stage, but by anthesis, had become deformed and empty containing no starch (Rerkasem *et al.*, 1997). It has been suggested that the adverse effect of B deficiency may be related to the B requirement during the critical phase of anther development surrounding pollen meiosis: the period from premeiotic interphase through meiosis to late tetrad (Rawson, 1996; Huang *et al.*, 2000). This study was to test the effect of short term B deficiency on pollen quality of B efficient and inefficient wheat cultivars to determine whether different sensitivities to B deficiency during critical stages of pollen microsporogenesis might explain the known cultivar differences in B efficiency.

5.2 Materials and Methods

Seed of wheat (Fang 60-B efficient and SW 41-inefficient: see Rerkasem et al. 1997) were imbibed in aerated 2 mM $CaSO_4$ solution for 24 hours in the dark at 25 °C. Seedlings were then transferred into trays containing 8 L 1/3 strength nutrient solution with 10 μM H_3BO_3 and MES (2-[N-Morpholino]ethanesulfonic acid) solution, and pH was adjusted to 6.0 ± 0.2 everyday with 1 M KOH or 10 % H_2SO_4. Four days after germination, uniform seedlings were transferred to pots containing 5 L of complete nutrient solution with adequate B (10 μM). Nutrient solution was continuously aerated with filtered air and the dry weight increment of extra plants was used to calculate the amount of nutrients for maintaining nutrient supply with programmed nutrient addition (Asher and Blamey, 1987). Seedling roots were rinsed in three changes of 5 mM $CaSO_4$ solution in order to remove B adsorbed on the root surface before transplanting. Two uniform plants per pot were transferred into the B treatments: either low B (0.1 μM B, referred to as -B) or adequate B (10 μM B, referred to as +B) during the critical stage of pollen development (premeiotic to late tetrad). Pollen developmental stages were identified by dissecting extra plants and staining the microspores with DAPI (4'-6-Diamidino-2-phenylindole·2HCl, Sigma Lot 104F-0542) and examining them under a UV-fluorescence microscope (Vergne *et al.*, 1987). After 5 days of treatment, plants were transferred back to adequate solution B supply (10 μM) and harvested at anthesis. Anthers were collected and fresh pollen examined for viability by the fluorochromatic (FCR) test (Heslop-

Harrison et al, 1984) and absence or presence of nuclei by DAPI. Starch accumulation in pollen was assessed by the iodine (KI/I_2) test.

5.3 Results and discussion

Withholding B during the period from premeiotic interphase through meiosis to late tetrad depressed pollen viability at anthesis in the B-inefficient wheat cultivar (cv. SW 41) by 40-70 % (Figures 5.1 and 5.2). In contrast to previous reports, starch accumulation in both cultivars was not affected by the temporary B deficiency (Table 5.1). Furthermore, the pollen of SW41 in -B also appeared to differ from SW41 in +B and Fang 60 in -B and +B in two other respects. Many of the pollen of SW41 in -B remained attached in pairs and their mitotic nuclei were fewer (Figure 5.3).

The cultivar SW41 was more sensitive to B deficiency during the critical stage of microsporogenesis than Fang 60. B deficiency during meiosis has been previously shown to inhibit anther elongation and severely depressed pollen viability (*Huang et al.*, 2000). In SW 41, B deficiency decreased B content in anthers (Rerkasem *et al.*, 1997). It is possible that the adverse effect of pollen development is caused by inadequate supply of B to the ear and anthers. Rerkasem and Loneragan (1994) and Rerkasem *et al.* (1997) could not detect any difference in flag leaf and whole spike B concentrations between tolerant and susceptible cultivars and Subedi *et al.* (1999) even found that a tolerant cultivar had lower B in the flag leaf. Therefore, it is unclear whether cultivars differ in B demand or ability to deliver B into the ear. However, B

deficient Fang 60 and SW 41 did not differ in their pattern of B partitioning after flag leaf emergence (Subedi *et al.*, 1999). This contradicts a conclusion drawn by Rawson (1996) that the tolerant genotypes can utilise previously stored B when uptake is limited during the critical reproductive stage. Therefore, the mechanism for B efficiency is still unclear.

Unlike in previous reports (e.g. Li *et al.*, 1978; Da Silva and da Andrade, 1980), this study found the effect of low B on pollen viability without any effect on starch accumulation. Starch accumulation was not sensitive to B withdrawal in the 5 days during premeiotic to late tetrad. In wheat, starch is normally visible about 12 to 24 h after pollen grain mitosis I and the microspore was packed with numerous starch at mitosis II (Bennett *et al.*, 1973). In this study, the B supply would have been restored during starch accumulation. It is unclear what role B plays in starch accumulation, if any but clearly withdrawal of B before the starch accumulation phase was not harmful to starch accumulation. Possibly more significant is the finding that the presence of starch is not an unambiguous indicator of viable pollen. On the other hand, it is interesting that inviable pollen can continue to accumulate starch. Huang *et al.* (2002) found that withdrawal B during mitosis I impairs pollen viability, possibly by interfering with starch accumulation.

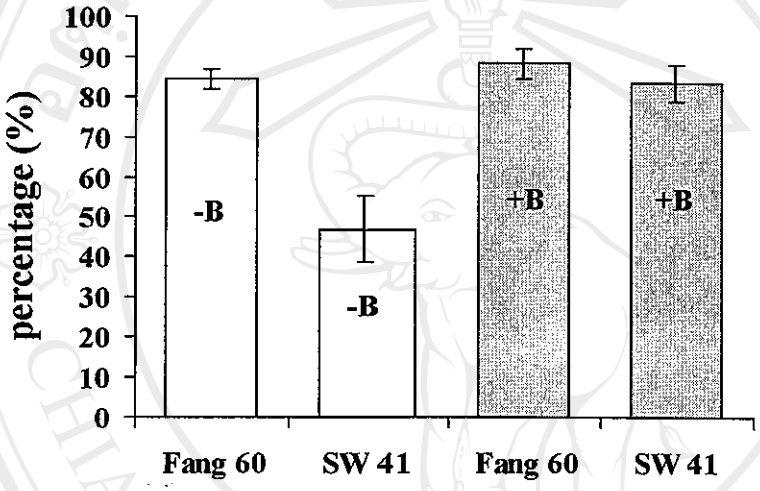

Figure 5.1 The effect of short term B deficiency (5 days corresponding to the tetrad – young microspore) on pollen viability (%) in two wheat cultivar by fluorochromatic (FCR) test.

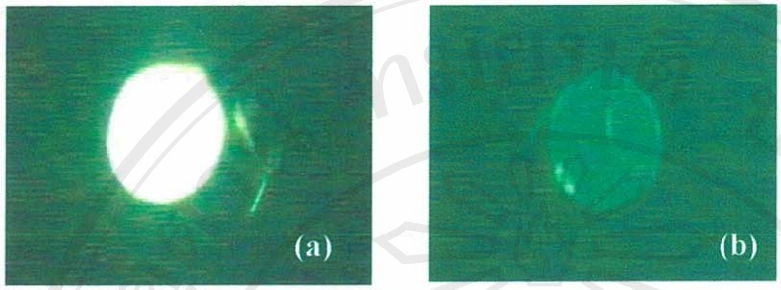

Figure 5.2 The pollen at anthesis by FCR test. (a) viable pollen (b) nonviable pollen showing lack of fluoresence in the non-viable pollen.

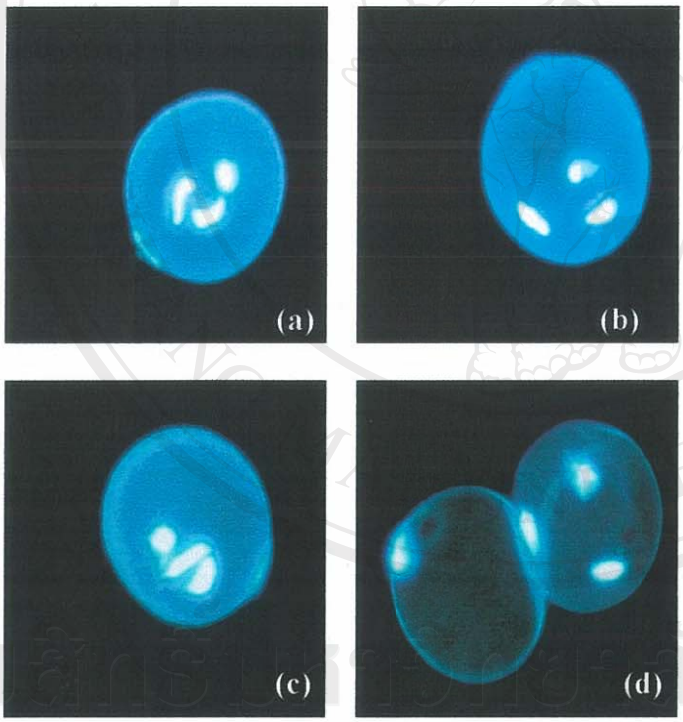

Figure 5.3 Pollen at anthesis showing the nuclei by DAPI.
(a) +B, Fang 60. (b)+B, SW 41. (c) –B, Fang 60.
(d) –B SW 41 (showing failure of cell walls to separate normally, and fewer nuclei in –B SW 41, but normal pollen in other treatments)

Table 5.1 Reaction to KI/I_2 staining for starch in the pollen of two wheat cultivars at anthesis after 5 days of B treatment earlier at tetrad-young microspore stages..

Gentoype	Boron treatment	
	-B	+ B
Fang 60	+++	+++
SW 41	+++	+++

+++ = most pollen were stained black.