CHAPTER 6

Enhanced boron transport into the ear of wheat as a mechanism for boron efficiency

6.1 Introduction

There are numerous reports of genetic variation in B efficiency among wheat genotypes (Li *et al.*, 1978; Rerkasem and Jamjod, 1997; Rerkasem *et al.*, 1993; Subedi *et al.*, 1993; Tandon and Naqvi, 1992) and other cereals such as barley (Rerkasem and Jamjod, 1989). Differences in B efficiency in wheat are generally associated with differences in the degree of pollen sterility or the ability to set grain in low B soil (Rerkasem and Jamjod, 1997; Rerkasem and Loneragan, 1994; Subedi *et al.*, 1993). Pollen development in B-inefficient cultivars is impaired by B limitation resulting in pollen grains that are small and misshapen and do not accumulate starch (Rerkasem *et al.*, 1989; Anantawiroon *et al.*, 1997; Subedi *et al.*, 1997). It has been suggested (Chapter 5) that the critical phase of anther development is a period of a few days surrounding pollen meiosis (Rawson., 1996), especially the period from premeiotic interphase through meiosis to late tetrad development (Dell and Huang., 2002; Huang *et al.*, 2000). However, the mechanisms underlying cultivar differences

in B efficiency in wheat remain unknown, but clearly they are related to B supply to the ear during critical stages of microsporogenesis.

In broccoli (*Brassica oleracea* var. *italica* Plenck), greater remobilization of B into the inflorescence under conditions of low B supply was responsible for the avoidance of anatomical disorders in the inflorescence in some cultivars (Shelp *et al.*, 1992). Similarly, Stangoulis (2001) recently reported that greater B efficiency in oilseed rape (*Brassica napus* L. cv. Huashuang) was associated with retranslocation of ^{10}B from old leaves whereas B-inefficient cultivars lacked this capacity. However, both of these studies were carried out with *Brassica* species which exhibit a degree of phloem mobility of B in contrast to many other species which do not (Brown and Shelp, 1997). Huang *et al.* (2001) showed that little ^{10}B, sequestered in vegetative plant parts following absorption from the external solution, was later partitioned to the ear of wheat following transfer from adequate to low external B supply. Thus there was no evidence for significant retranslocation of ^{10}B from leaves in the phloem. Since a B-inefficient cultivar, Wilgoyne (Rerkasem unpublished), was used by Huang *et al.* (2001), the possibility remains that the capacity to partition or retranslocate B into the ear may differ across cultivars, and that this difference may be a mechanism for B efficiency in wheat. We hypothesise that avoidance of male sterility in B-efficient wheat cultivars involves the ability to supply B adequately into the anthers of non-transpiring ears. To test this hypothesis, we examined B uptake, distribution and

redistribution of an efficient and an inefficient wheat genotype during microsporogenesis, the period that is most sensitive to B deficiency.

6.2 Materials and Methods

6.2.1 Plant material and culture

Two spring wheat cultivars were selected from the efficient (cv. Fang 60) and inefficient (cv. SW 41) classes of B efficiency determined by Rerkasem and Jamjod, (1997). Seeds were imbibed in aerated 2 *m*M $CaSO_4$ solution for 24 hours and germinated on paper towels moistened with 2 *m*M $CaSO_4$ for 48 hours in the dark at 25 °C. Seeds of SW41 were germinated 1 day before seeds of Fang 60 in order to synchronize the critical stage of ear development at microsporogenesis. Seedlings were transferred into trays containing 8 L of 1/3 strength nutrient solution (as described in Chapter 3 solution culture experiment) with a concentration of 0.1 μM ^{11}B and 5 *m*M 2-[N-Morpholino]ethanesulfonic acid. The pH was adjusted daily to 6.0 ± 0.2 with 1 M KOH or 10 % H_2SO_4. Four days after germination, the plants were transferred to a 5 L full-strength basal nutrient solution that contained 10 μM ^{11}B and aliquots of all nutrients were added to each pot during the experiment by programmed nutrient addition (Asher and Blamey, 1987) as described in Huang *et al.* (1996). The amount of nutrients added to each treatment and genotype was varied according to their growth rate. Tillers were restricted to a maximum of 4 tillers plant^{-1}

[1], by removing the 5 th and subsequent tillers as they emerged. The stage of ear development was determined by dissection of spare plants at the 6, 7 and 8 leaf stages and the stage of pollen development was determined on extra B-adequate plants using DAPI (4'-6-Diamidino-2-phenylindole 2HCl, Sigma Lot 104F-0542) fluorescence (Vergne et al., 1987). Ten μM ^{11}B was supplied continuously up to 38 days after germination when premeiotic interphase (white anther stage) in the main stem had occurred (Bennett et al., 1973). The plants were then treated with either 0.1 or 10 μM of 99.43 % ^{10}B-enriched boric acid continuously up to the late tetrad / young microspore stage (early green anther stage) at day 42, after which the plants were returned to solutions with 10 μM ^{11}B up to anthesis. ^{10}B was used as a tracer for B distribution to the ear during critical stage of microsporogenesis, while ^{11}B was used as a tracer for B remobilization to the ear.

The pH was adjusted to 6.0 ± 0.2 with 1 M KOH or 10 % H_2SO_4. The pots were randomly distributed in temperature-controlled water baths (18-22 °C) and repositioned daily within the baths and shifted between baths every 3 days. The growth conditions in the glasshouse were: mean air temperature 27.5 °C (range: 20-35); mean photosynthetic active radiation 1165 μmol m^{-2} s^{-1} (range: 960-1900). Nutrient solutions were aerated with filtered air and the dry weight increments of extra plants were used to calculate the amount of nutrients required to maintain nutrient supply by programmed nutrient addition (Asher and Blamey, 1987). Analytical grade chemicals were used to make up the nutrient solutions and water,

and purified by passing through a column packed with B-specific resin (IRA-743, Sigma Chemical Co.). Between the treatments, the roots were washed with DI water, then rinsed three times in 5 *m*M $CaSO_4$ solution, in order to remove unbound B from root free space.

6.2.2 Sampling and plant analysis

The first harvest was taken before the ^{10}B treatment at the beginning of the critical stage of pollen development in the main stem (Huang *et al.*, 2000). The second and third harvests were taken 1 and 5 days (late tetrad stage) after the ^{10}B treatment began. At each harvest, three pots (replicates) of plants (two plants each) were taken for each cultivar. The plants were subdivided into flag leaf, ear, penultimate leaf, and stem between the flag and penultimate leaves of the main stem. All samples were analysed for ^{11}B and ^{10}B content. The fourth harvest was taken at anthesis. From three replicate pots (two plants per pots) of each cultivar, pollen was taken from the central 4 spikelets of the main shoot ear. Pollen at anthesis was tested for viability with the fluorochromatic (FCR) method (Heslop-Harrison and Shivanna, 1984). The plant samples were oven-dried (70 °C). Dry samples were ground in a stainless steel mill and dry-ashed in 1 % nitric acid as described in Huang *et al.* (2001) and B concentration was determined using an inductively coupled plasma atomic emission spectrometer (ICP-AES) (Zarcinas *et al.*, 1987) and B isotopes by

inductively coupled plasma mass spectrometer (ICP-MS, Perkin Elmer, Elan 6000, USA).

6.2.3 Statistical analysis

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

6.3 Results and discussion

6.3.1 Pollen viability

Pollen viability in B-inefficient SW 41 was nearly halved when the external B supply was interrupted during the premeiotic interphase to the late tetrad stage of pollen development (Figure 6.1). The same treatment had no effect on pollen viability of Fang 60. Huang *et al.* (2000) suggested that the period of pollen development from the premeiotic interphase to the late tetrad was the main phase of reproductive development in wheat that was sensitive to B deficiency. They also postulated that B deficiency during meiosis might impair the formation of pollen cell walls and cell expansion, leading to a reduction in pollen viability in wheat. The differential B response in pollen viability of Fang 60 and SW 41 is consistent with known and repeatable differences in the sensitivity to B deficiency of the genotypes as represented by their male fertility and grain set in a range of low B conditions in both sand culture and the field (Anantawiroon *et al.*, 1997; Subedi *et al.*, 1999).

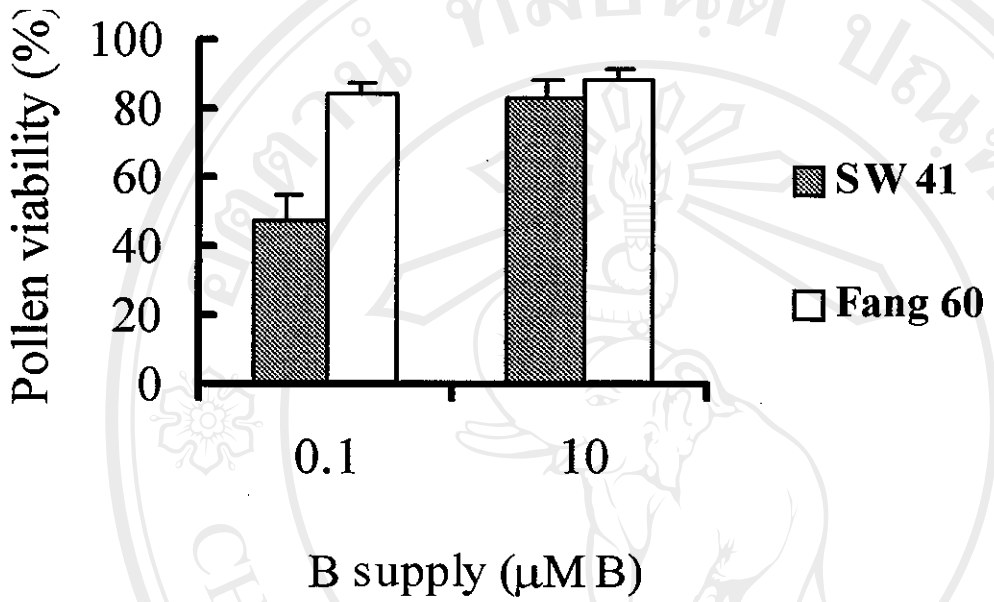

Figure 6.1 Effect of short term (5 days) B deficiency during microsporogenesis on pollen viability in two wheat cultivars at anthesis by fluorochromatic (FCR) test. Bars are means of three replicates ± se.

6.3.2 Ear boron concentration

The differential response to short-term B deficiency in SW 41 and Fang 60 was associated with significantly lower concentration of B in the ear of SW 41 (Table 6.1). Ear-dry weight in the two genotypes increased by about the same amount over the 5 days of treatment (Table 6.2). Therefore, the lower ear B concentration in SW 41 was not a dilution effect. Thus, the B-efficient and inefficient genotypes in this study were distinguishable by the B concentration in their ears. This difference was not detected by others who have reported genotypic variation in responses to low B for male fertility and grain set (Rerkasem and Lordkaew, 1996), including a comparison between SW 41 and Fang 60 (Subedi *et al.*, 1999). In these previous studies, B in the ear was not measured until booting. By this stage of ontogeny, the B-deficient wheat ear may have continued to accumulate B in the palea, lemma and other nonsexual parts of the ear even though damage to the pollen had already occurred. The present results suggest that it may be feasible to distinguish between the B-efficient and inefficient genotypes by early B analysis of the ear. However, timing of the analysis will be critical. Sampling for the ear has to be done immediately upon the completion of the critical meiotic stage of pollen development.

6.3.4 Leaf boron concentration

Boron concentrations in upper canopy leaves of Fang 60 were greater than those in SW 41 after 5 days of low B supply (Table 6.1). The greater ability of Fang

60 to distribute B into the developing ear may contribute to its tolerance to low external B. The reproductive parts of wheat, the anthers and carpels, were found to require B at greater concentration for their normal development than leaves (Rerkasem *et al.*, 1997; Huang *et al.*, 2000). The greater amount of B distributed into the apical regions of the plant may increase the opportunity for reproductive success.

6.3.5 Shoot B and pattern of B distribution

Total shoot B under low B supply did not differ between Fang 60 and SW 41 (Table 6.1). Others who have compared these two genotypes also found no difference in their B uptake ability (Subedi *et al*, 1999). The contribution of flag leaf and stem between flag leaf and penultimate leaf to the whole shoot plant B of Fang 60 were higher than that in SW 41 at both adequate and deficient B treatments (Table 6.1). By contrast, the percentage of B in penultimate leaf did not differ between the two cultivar in both B treatments. At adequate B supply, ear of both cultivars contributed the same to the total plant B, but under low B supply, ear of Fang 60 contributed more (1.9 %) to the total shoot B, compared to (1.1 %) the ear of SW 41. The results demonstrate that low B supply changed the pattern of B distribution in the ear between Fang 60 and SW 41, resulting in greater proportion of B partitioning into the ear of Fang 60 than SW 41. This suggests that cultivar difference in sensitivity to B deficiency may be associated with the pattern of B distribution within the plant when B was limited.

Table 6.1. Total B concentration in plant parts (mg kg^{-1} dry weight) and relative B distribution (% total shoot B) after 5 days of varied B supply.[1]

Plant part	B treatment (μM ^{10}B)	Total B concentration (mg kg^{-1})		Relative B distribution (% of total shoot B)[2]	
		Fang 60	SW 41	Fang 60	SW 41
Ear	0.1	6.8 ± 0.7	3.8 ± 0.3	1.9 ± 0.2	1.1 ± 0.1
	10	12 ± 1.4	7.8 ± 0.5	1.6 ± 0.4	1.5 ± 0.2
Flag leaf	0.1	13 ± 2.3	9.3 ± 0.6	15 ± 1.8	9.2 ± 1.4
	10	21 ± 1.4	20 ± 0.5	14 ± 1.3	11 ± 0.5
Penultimate leaf	0.1	17 ± 0.4	13 ± 0.7	12 ± 0.4	12 ± 1.4
	10	19 ± 0.5	20 ± 0.8	12 ± 1.6	11 ± 0.6
SSFP[3]	0.1	4.0 ± 0.4	4.7 ± 0.3	0.8 ± 0.1	0.6 ± 0.1
	10	6.2 ± 0.9	5.9 ± 0.6	0.7 ± 0.01	0.6 ± 0.05
[2]Total shoot B content (μg $plant^{-1}$)	0.1			15 ± 0.4	16 ± 0.8
	10			22 ± 0.8	24 ± 0.9

[1]Values are means of three replicates ± SE.

[3]Stem segment between flag leaf and penultimate leaf node

Table 6.2 Dry weight of ear on the main stem (g plant^{-1}) at day 0 (D0), day 1 (D1) and day 5 (D5) under different boron supply.

Boron treatment	Fang 60			SW 41		
(μM ^{10}B)	D0	D1	D5	D0	D1	D5
0.1	0.0097	0.029	0.085	0.019	0.035	0.095
10	0.0097	0.027	0.055	0.019	0.043	0.091

$LSD_{(0.05)} = 0.014$

6.3.6 Long distance transport of boron

It has been suggested that B accumulated during vegetative growth might be redirected to the ear of B-efficient genotypes, such as Fang 60, when external supply becomes limited during reproductive growth (Rawson, 1996; Subedi *et al.*, 1999). We found this to be unsupportable by two sets of results, which instead suggest that the primary mechanism for B efficiency in Fang 60 was associated with its long distance transport directly from the root. Firstly, accumulation of ^{11}B in the ear of both SW 41 and Fang 60 ceased after ^{11}B in the nutrient solution was replaced by ^{10}B. The content of ^{11}B in the ear of either SW 41 or Fang 60 did not increase after the ^{10}B treatments were imposed (Table 6.3), indicating an absence of any ^{11}B retranslocation from other plant parts after cessation of external ^{11}B supply. Secondly, in the low B treatment, the ^{10}B content in the ear of B-efficient Fang 60 at day 5 was three times

that of the B-inefficient SW 41 (Table 6.4). The greater amount of ^{10}B in the ear of Fang 60 reflected more ^{10}B transported into the ear, since there was no difference in ear biomass increment between Fang 60 and SW 41 over the 5 days of B interruption (Table 6.2). The difference between both cultivars became clearer when ^{10}B and ^{11}B in the ear were considered relative to one another (Table 6.5). The ratio of ^{10}B:^{11}B increased significantly in the ear with time during the 5 days that the B^{11} supply was replaced by ^{10}B but at different rates in the two genotypes. The increase in the ratio of ^{10}B:^{11}B with time after the transfer from ^{11}B to ^{10}B was much stronger in Fang 60 than SW 41, especially with lower external B. During the 5 days in which external B was lowered to 0.1 μM, the ^{10}B:^{11}B ratio in Fang 60 increased from 0.17 to 1.15, but from 0.11 to 0.38 in SW 41.

Table 6. 3 The content of ^{11}B in the ear on the main stem (μg ear^{-1}) at day 0 (D0), day 1 (D1) and day 5 (D5) under different boron supply.

Boron Treatment	Fang 60			SW 41		
(μM ^{10}B)	D0	D1	D5	D0	D1	D5
0.1	0.117	0.098	0.108	0.130	0.100	0.104
10	0.117	0.079	0.070	0.130	0.086	0.140

$LSD_{(0.05)} = 0.038$

Table 6.4. The content of ^{10}B in the ear on the main stem ($\mu g\ ear^{-1}$) at day 1 (D1) and day 5 (D5) under different boron supply.

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

$LSD_{(0.05)} = 0.028$

Table 6.5 The ratio of ^{10}B : ^{11}B in the ear on the main stem at day 1 (D1) and day 5 (D5) under different boron supply.

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

$LSD_{(0.05)} = 0.021$

6.3.7 Conclusions

Pollen viability of the B-efficient Fang 60 was not affected by withholding B during the critical stage of microsporogenesis while pollen viability in inefficient SW 41 was nearly halved. The genotypic difference in B efficiency is related to the greater ability of Fang 60 to accumulate and distribute B into the developing ear than SW41. A greater precision in diagnosis for B deficiency that can distinguish between B efficient and inefficient genotypes may be possible by ear B analysis if sampling is done immediately upon the completion of the critical meiotic stage of pollen development. By using ^{11}B and ^{10}B, we were able to demonstrate that net B movement into the ear, when external supply was restricted, did not come from the ^{11}B previously taken up by the plant. The greater amount of ^{10}B accumulated by Fang 60 in low B further confirmed that a primary mechanism for B efficiency in Fang 60 is its greater capacity to supply adequate B to the ear concurrently from B uptake by roots, enabling Fang 60 to avoid pollen sterility. Boron efficiency appears to be related to meet B demand in the ear directly from the root when external supply is low. The potential now exists for breeding for cultivars in which this trait is expressed.