Chapter 5

DISCUSSION

5.1 Relationship between B supply and grain set

Different responses to low B supply among wheat genotypes were observed in both field and pot studies. The results from field study have clearly shown that SW 41 and BL 1022 responded to B deficiency. In both genotypes, the grain yield (Table 11) was depressed by low soil B through poor grain set, *e.g.* basal floret fertility(r=0.7154)(Figure 5), percentage of fertile florets (r=0.8281) (Table 7). Response of BL 1022 to B supply was less marked than that in SW 41. In genotypes that are tolerant to B deficiency, *e.g.* Sonora 64, KUHR 12, CMU 26 and NL 460, the yield and grain set were not influenced by low soil B supply. These genotypes (responsive and nonresponsive B) were consistent with previous reports (Rerkasem, 1989). However, the response of CMU 285, previously defined as sensitive, was not apparent. Rerkasem (1992) has also demonstrated that the response of CMU 285 to B was sometimes slight.

Genotypes that are moderately sensitive to B deficiency, SW 23, Sonalika and Kanchan, were somewhat variable. The response of Kanchan was slight. Yield and grains per ear, in Kanchan, varied little among various soil B supplies although basal floret fertility was depressed by low soil B. The responses of SW 23 and Sonalika were the same as that of tolerant genotypes. In genotypes of moderately sensitive to B deficiency, however, seed and yield were reduced only with an application of lime to accentuate B deficiency (Rerkasem, *et al.*, 1989). The previous ranking of genotypes of their responses to B was derived from several observations, so this is now under further investigated.

The pot results of grain set (Figure 17, Figure 18 and Figure 19) for SW 41 and Sonora 64 were in line with the field study.

The relation between vegetative development and grain set was obvious in pot study. The plants with failure to set grain continued to grow vegetatively (late ears) (Figure 21). Similar effects have been reported in China (Li, *et al.*, 1978) and in Thailand (Rerkasem, 1989).

The plants produced higher number of late ears at sowing date 1 than that at sowing date 2. The patterns of mature ears (Figure 21) as well as 1000 grain weight (Figure 20) were also higher at sowing date 1 than that at sowing date 2. Schlehuber and Tucker (1967) have suggested that the minimum growth temperature for wheat is 3 to 4°C, the optimum growth temperature is about 25°C and the maximum about 32 °C. He also indicates that temperatures materially above the optimum do not produce normal, vigorous plants and root systems. As shown in Table 16, monthly maximum temperature drastically increased from 28.7 to 38.3 °C during Jan.-April, 1992, *e.g.* the period of plant growth at sowing date 2. The results of pot study, in terms of mature ears, late ears and 1000 grain weight, could be accounted for by the growth depression at the high maximum temperature.

The relative effects between grain filling and flowering was observed. Superfluous spikelets produced in plants with failure to set grain (low B supply plants) (Table 25), and, as a result, they also became sterile. Therefore, grains per ear, in SW 41, were still significantly lower in B0 plants than that in high B supply plants (Figure 19) although the highest spikelets per ear was recorded.

Additionally, the production of superfluous spikelets also caused low percentage of fertile florets (Figure 18).

Meyer, *et al.*, (1960) has reported that if the blossoms of the sweet pea (*Lathyrus odoratus)* are allowed to develop, for example; flowering soon ceases. However, if they are picked from time to time, flower primordia and blossoms develop continually throughout the growing season.

In lower B supply plants, the superfluous spikelets evidently produced evidently more at sowing date 1 (pot study) than that at sowing date 2 (Table 25) and also in field study (Table 8) for SW 41 and Sonora 64. At sowing date 2, the superfluous spikelet growth might be affected by environmental conditions, which might be similar to the effects on late ear growth. For field study, the superfluous spikelet growth might limit either environmental condition, or soil fertility or water supply.

In SW 41, grain set in pot study was lower at sowing date 1 than that at sowing date 2 (Figure 17, Figure 18 and Figure 19). The absorption of B by roots is thought to be predominantly a passive process with a small metabolic component and greatly influenced by transpiration rates (Raven, 1980). The distribution of B is related to the loss of water from the shoot organs (through transpiration) (Michael, *et al.*, 1969). In present study, it might be affected either by uptake B or by distribution of B in plant due to high temperature (Table 16) to induce high transpiration rates at sowing date 2 during wheat plant growth.

5.2 Development of anther and pollen

The results of field study showed that wheat grain set was related to starch deposit in pollen (r=0.8525)(Table 6) and anther length (Table 5) in SW 41 and BL 1022. However, from pot experiment results of SW 41, grain set (Figure 17, Figure 18 and Figure 19) was strongly influenced by plant B treatments although effects of plant B treatments on anther length (Table 17) or starch deposit in pollen (Figure 8) was not evident. Li, *et al.*, (1978) has also observed that some of florets had healthy looking anthers and pollens in wheat ear that were late only partially filled. The development degree of anther and pollen might associate with either the time when B deficiency occurred or the extent of B deficiency. Li, *et al.*, (1978) has reported that the positive reaction to iodine of wheat pollen with KI/I_2 solution is nil or weak as pollen development is affected by stress as early as the pollen mother cell stage (PMC) through to the two-celled stage; the ripe pollen of wheat contains three functional cells (three-celled stage). At the three-celled stage, pollen has strongly positive reaction to iodine. Perhaps, the development of pollen in pot experiment might reach three-celled stage.

A problem with starch stain technique is that many immature or aborted pollen grains contain levels of constitutive chemicals sufficient to yield positive results in stain tests (Stanley and Linskens, 1974). Kihara (1959) has observed that non-viable pollen grains of hybrid wheat stain just as readily as normal mature grains. Jovancevic (1962) has reported that oak pollen may stain poorly for starch and yet be highly viable.

Furthermore, under some conditions, the environment can induce greater changes in pollen starch level than genetic factors. Mature pollen of *Corylus avellana, Cornus mas* and *Poa annua* on flowers developed in high humidity contained less starch, or even no starch, compared to pollen on flowers reaching anthesis in low relative humidity (Kaufman, 1920). *Pelargonium* pollen, matured at 25°C, contained less starch than that maturing at 15°C (Sears and Metcalf, 1926).

The present study agrees that starch stain in KI/I_2 solution is not sufficiently accurate when compared to germination tests since it give only crude estimates of pollen viability (Vazhnitskaya, 1960; Nagorajan, *et al.*, 1965).

The results of pot experiment showed that the development of anther (Table 17) and pollen (Figure 8) in the middle part of ear were much better than that in top and bottom parts of ear. The same results have been reported in China (Li, *et al.*, 1978). This may have been related to the rule for nutritional distribution in the ear, which may correspond with the order of spikelet initiation and development. Therefore, the rule of nutritional distribution causes that result in B deficiency in top and bottom parts of ear may be more severe than that in middle part of ear.

The effects of plant B treatments on the number of pollen was not found at both sowing dates (Table 18). The relationship of B and pollen production was not clear. Experimental evidence to support this study is lacking.

Stanley and Linskens (1974) have suggested that differences in the amount produced on the same plant in successive years can be quite

great. In the top and bottom ear part for Sonora 64, differences in the number of pollen were found between two sowing dates. Thus, quantities measured in one growing season or one year may not be always a good index of producing capacity of wheat plants in successive seasons or years.

The capacity to produce pollen, however, is primarily under genetic and physiological control. From this study, the number of pollen per anther ranged from 947 to 2072 at both sowing dates for SW 41 and Sonora 64. The results fell within the ranges of pollen grains per anther which varied from 581 to 2153 in comparing 22 varieties of wheat (Beri and Anand, 1971).

5.3 Pollen germination and fertilization

The pot results of both sowing dates clearly showed that the percentage of germinated pollen increased as medium B supply increased (Figure 9 and Figure 10). These results showed that B is an essential ingredient on raffinose medium for pollen germination of wheat (Cheng and McComb, 1992). The length of pollen tube was longer at M100 than that at M20 (Figure 14). Vasil (1964) and Dickinson (1978) proved that the addition of boric acid to the basic sugar medium not only improves the percentage of pollen germination but also has a marked effect on the growth of the pollen tubes. Experiments on petunia hybrida suggested a role for B in the control of protein secretory activity in pollen tubes (Johnson, 1989).

The percentage of germinated pollen was low at M0 and M10, they ranged from 2.2-15.5%, regardless of B supply to the plants (Figure 9

and Table 19). Pollen grains of many plants are deficient in B (O'Kelley, 1957). However, this deficiency is often met by the high levels of B in the stigmatic and styler tissues in nature (Vasil, 1963). Schumucker (1935) found that the stigmatic extract of *Nymphaea* has an appreciable quantity of boric acid. Vasil (1963) has also demonstrated that plants which are deficient in B do not show good germination of pollen but show considerably improved germination after B has been supplied either through the roots or through cut ends of branches.

At higher media B treatments (M15, M20 and M100)(Figure 9 and Table 19), the percentage of germinated pollen was markedly lower in B0 plants than that in higher B supply plants. The results might imply that pollen viability in B0 plant might be lower than that in higher B supply plants. Abortion of pollen was caused by B deficiency in wheat (Li, *et al.*, 1978; Silva and Andrade, 1983; and Rerkasem, 1989). Garg (1979) has demonstrated that germination capability was considerably improved as a result of B application in rice.

The physiological mechanisms associated with B are not completely understood. The stimulative effect of B on pollen germination and tube growth may be due to: 1) an increased absorption, translocation and metabolism of sugars as due to formation of sugar borate complexes (Gauch and Duggar, 1953; Linskens, 1955; O'Kelley, 1957; Pfahler, 1966); 2) increasing oxygen uptake (O'Kelley, 1957); 3) the formation of pectic materials for the wall of rapidly growing pollen tubes (Vasil, 1964; Augsten and Eichhorn, 1976); 4) proteins inserted into the membrane and/or the tube wall built up as pollen tube elongation proceeds as due to borate control of protein secretion (Jackson, 1989); and 5)

maintaining at a low level of phenols in order to present the formation of damaging concentration of oxygen free radicals during pollen tube growth and also maintaining an acid pH (Amberger, *et al.*, 1990).

In Table 20, the percentage of burst pollen was the highest in B0 plants at M0. The excessive bursting of pollen grains in the absence of B may be due to a close negative correlation between tissue hydration and the supply of B (Schmucker, 1935; and Vasil, 1964). Leakage of sugars from the pollen decreases with increasing external B concentration (Dickinson, 1978). In *Pyrus communis* pollen, more B was found in wall fraction (Dugger, 1983). The rapid cell enlargement might either be due to the excessive water imbibition or to the mechanical weakness of the cell wall (Vasil, 1964). The status of the burst pollen is unclear; whether it would be viable pollen in an *in vivo* situation remains unknown (Herrero and Johnson, 1980).

Herrero and Johnson (1980) have suggested that the ungerminated pollen was considered nonviable and incapable of fertilization due to the lack of visible activity of this pollen. From this study, however, the relationship between the percentage of ungerminated pollen, non-viable pollen, and plant B treatments or medium B treatments was not evident (Table 21).

The tube length was increased as plant B supply was increased. The longest one was record in B3 plants at M100 (Figure 14). The length of the pollen tube is directly related to the concentration of boric acid while the percentage of bursting is inversely related to it (Visser, 1955). The pollen tube formed in many species is a massive structure related to the reserved materials in the pollen grain, and the reserves

often are quickly consumed (Vasil, 1960). For this study, [B] of Pollen in B3 plants might be higher than that in other lower B supply plants, which might be more viable compared with pollen in lower B supply plants. Agarwala, *et al.*, (1981) has reported that B concentration of pollen was higher in freshly shed pollen grains from normal plants than that pollen from B deficient plants of maize.

No differences in pollen viability examined *in vitro* were found between Sonora 64 and SW 41. Fertilization *in vivo*, however, was quite different between both genotypes. Grain set (Figure 17 and Figure 18) in Sonora 64, *e.g.* basal floret fertility, floret fertility, was not influenced by lower plant B treatments although pollen viability was low. It may be because numerous pollen grains may alight on or be deposited on a single stigma. Under the conditions of abundant pollination, from 600 to 900 pollen grains may be present (Meyer, *et al.*, 1969). However, grain set in SW 41 was evidently depressed in B0 plants. The silks of B deficient plants of maize were non-receptive and fertilization did not occur even with pollen from high-B plants (Vaughan, 1977). According to Lewis (1980) high B levels in the stigma and style are required for physiological inactivation of callose from the pollen tube walls by the formation of borate-callose complexes. In SW 41, the failure of fertilization might be either because B concentration in stigma and style was low or because sugars as nutrients during pollen tube growth was insufficient. Sugar insufficiency in B deficient plants may be caused by either low absorption, translocation and metabolism of sugar (Gauch and Duggar, 1953; Linskens, 1955; O'Kelley,1966) or low invertase activity in pollen which may contribute

to loss of viability (Agarwala, *et al.*, 1981). Thus, pollen germination and pollen tube growth might fail in *vivo*.

Results from the pot study showed that pollen viability *in vitro* was higher at sowing date 1 than that at sowing date 2, namely, the percentage of germinated pollen (Figure 11) was higher, whereas the percentage of ungerminated pollen (Table 21) was lower at sowing date 1. Rerkasem (1989) demonstrated that B deficiency was more severe in the November sowing than that in the October sowing in Thailand. Environmental effects on pollen germination, for this study, may be related to the weather pattern of the cool season (Nov.-Dec., sowing date 1) (Figure 6a) and the hot season (Feb.-March, sowing date 2) (Figure 7a) from booting to flowering. It may be possible that the plants adapted to grow during the cool season, thus improved their germination. In respect of the percentage of burst pollen (Table 20), humidity might be one of main effects. Vasil (1959) reported that germination is the high relative humidity which is responsible for widespread bursting of pollen grains in situ. At sowing date 1, humidity was higher than that at sowing date 2 (Figure 6 and Figure 7), which might cause low fertilization. Generally, pollen tube length, which was longer at sowing date 1 than that at sowing date 2, may also be related to the weather pattern (Figure 15).

The results of temperature treatments showed that, at 30°C, the percentage of germinated pollen (Figure 12) was suppressed and the percentage of burst pollen (Figure 13) was increased; the pollen tube was considerably short (Figure 16). With Petunia pollen germination, Jackson (1989) exhibited peak pollen tube length at 25°C. He has also

reported that protein loss to the medium rises at high temperature, and the rate of pollen tube elongation falls. Herrero and Johnson (1980) have reported that pollen viability does not appear as temperature is up to 30°C in maize.

At 30°C, the percentage of germination in Sonora 64 was higher in B3 plant than that in B0 plants at M100 (Figure 12), in responses to the percentage of burst pollen going in opposite direction (Figure 13). In SW 41, pollen tube length, at 30°C, was markedly longer in B3 plants than that in B0 plants (Figure 16). Temperature limitations of pollen germination are alleviated by B, especially in the higher temperature ranges (around 30°C) in Clapps Favorite pear (Visser, 1955). These results might also imply that pollen in B3 plants might have relatively high B concentration.

5.4 Relationship between tissue [B] and grain set

Bergmann (1983) suggested that [B] in the range of 5-10 mg Bkg^{-1} dry wt are adequate for growth of wheat plants. The results from field study showed that, in B0 plants, [B] in whole tops during vegetative stage was adequate for growth of wheat plants; the amounts of [B] ranged 7.4-12.1 mg Bkg^{-1} dry wt for 9 genotypes (excluding NL 460) at double ridge stage (Table 12). In pot study, the results were similar to that in field study; [B] of SW 41 and Sonora 64, at both sowing dates, ranged from 5.8 to 7.8 mg BKg^{-1} dry wt at tillering stage (Figure 22) and at double ridge stage (Figure 23). It was also found in China (Li, *et al.*, 1978) and in Indian (Ganguly, 1979) that plant with failure to set grain had a normal vegetative growth. Marschner (1986) has suggested that B

requirement for seed production is usually higher than that needed for vegetative growth.

In general, the results from field and pot studies showed that [B] in tissue (whole tops at tillering and at double ridge, flag leaf and developing ear at booting) increased as B supply increased. Relationships between [B] of tissue and its response to B in terms of grain set, however, were unclear. In B0 plants, tissue [B] did not differ significantly between responsive and nonresponsive genotypes, with a strong genotype effects.

From field and pot studies, no relationships between grain set and [B] in whole tops at tillering and at double ridge stage or [B] in flag leaf at booting stage were found. It might be because B is immobile, thus, tending to be poorly redistributed from one tissue or organ to a young one (Salisbury, *et al.*, 1969). The limiting factor in the long-distance transport of B is most likely the high permeability of the sieve tube plasma membrane to B and a correspondingly high leakage of B out of sieve tubes (Oertli and Richardson, 1970; Raven, 1980).

[B] in developing ear (Table 14), from field study, was closely related to grain set in SW 41 and BL 1022 and Kanchan. The relationships were also investigated in pot study (Figure 25) for SW 41 at sowing date 2. [B] in developing ear was less than or equal to 4.5 mg Bkg^{-1} dry wt, causing poor grain set. Similar result was reported early by Rerkasem, *et al.*, (1990). Pollen development is associated with booting stage. [B] in developing ear at booting may directly affect grain set. Hence, it may predict effects of B deficiency on grain set and grain yield.

In Sonora 64, KUHR 12, CMU 26 and CMU 285, grain set was not affected by low [B] in developing ear (3.8-4.2 mg Bkg^{-1} dry wt). Therefore, SW 41, BL 1022 and Kanchan might have a high internal B requirement, which might result largely from either a poorer distribution (SW 41 and Kanchan although high [B] was detected at vegetative growth) or a poorer ability of uptake B (BL 1022, [B] in tissue was relatively low). In other words, Sonora 64, KUHR 12, CMU 26 and CMU 285 may be high B efficient genotypes with either low internal B requirement or the better ability to get B into the developing spike during booting.

In B0 plants of SW 23, Sonalika and NL 460, [B] in developing ear was relatively higher than that in others, which was 5.0 mg Bkg^{-1} dry wt for SW 23 and Sonalika, and 5.9 mg Bkg^{-1} dry wt for NL 460. Hence, no effects of B on grain set were found among these genotypes. It might either be better at getting B into the developing spike during booting, such as SW 23, although [B] of whole tops at double ridge was relatively low; or reflect the capacity for B uptake, such as Sonalika, [B] of tissue (whole tops at double ridge and flag leaf at booting) was relatively higher in B0 plants compared with BL 1022, SW 23 and Sonora 64. However, no information is available to support the present study on how these characteristics (internal B requirement, the capacity for B uptake and getting B into the developing spike during booting) may vary among genotypes.

5.5 Weakness and further studies

Since root system was not concentrated on in present study, the type of mechanism on tolerant to B deficiency could not be distinguished well. Secondly, germination pollen was not confirmed in *vivo*.

Further studies will be necessary to determine mechanisms on tolerant to B deficiency in wheat genotypes and to understand B physiological mechanisms on pollen viability, germination as well as fertilization. The future studies will identify and manipulate wheat genotypes of tolerant to B deficiency.

Moreover, time-of-application B study will also be important to manage wheat production in low B soils. Hence, potential contribution towards sustainability of cropping systems can be defined.