CHAPTER 5

GENERAL DISCUSSION

5.1 Response of maize to boron application

At vegetative growth (30DAS), boron (B) treatments had no effect on maize plant (cv. NS72) growth in term of total shoot dry weight, but addition of B at rate of 30 kg borax ha^{-1} resulted in increasing of B concentration in leaf and shoot of maize and produced the maximum dry weight of tassel at anthesis. However B treatments were not effect on grain yield. At the same location (field experiment) has ever been reported that some crops grown without added B depressed grain yield by 60% in soybean (cv. NW1), 45% in peanut (cv. Tainan 9) and 93% in black gram (cv. Regur). Especially, B deficiency in peanut and soybean expressed hollow heart symptom (Rerkasem *et al*., 1993). In case of wheat (cv. SW41), soil B application increased grain yield from about 680 to 1380 kg ha^{-1} (Rerkasem *et al*, 1997). For maize, the application of boron in certain soils can increased the grain yield of maize by 70% in the United States (Berger *et al*., 1957), by 26% in India (Shorrocks and Blaza, 1973), by more than 10% in Switzerland (cited by Mozafar,1987) and by 9% in China (Li and Liang, 1997). For this study, B treatment was not effect on grain yield. The evidences above indicated that there were the diversity of effect of B-deficiency on reproductive among species between sites and season. By contrast, the growth of plant in sand culture was affected with B treatments. Without added B (B0), concentration of B in tassel and silks about 4-5 mg B kg^{-1}DW was associated with significant depressed in the dry weight of both that there were produced less silks or not emerged out from the husk. Moreover, the symptom of B deficiency showed white strips and transparent streaks on the leaf and abnormal anthers, including thin and dead anthers. B-deficient plants produced multiple ears with short silks at pollen shedding time. These evidences pointed out that B deficient plant may not produced enough pollen to fertilization and set grain during fertilization time. In B-deficient wheat and barley grown in low available B, the concentration of B in the flag leaf was below 4 mg B kg^{-1} DW associated reduction of grain yield (Jamjod and Rerkasem, 1999). Rerkasem *et al*. (1997) reported that grain set of wheat was closely correlated with B concentration in anther (r=0.77) that of the concentration of B was about 7 mg B kg^{-1} DW.

It is worth nothing that the evaluation of boron (B) in the field do not always signify that the application of B can increase yield because this results showed that the concentration of B in the ear leaf ranging from about 4 to 7 mg B kg^{-1} DW at anthesis was adequate for plant growth that agreement with an adequate range of 4 to 26 mg B kg^{-1} DW that was reported by Touchton and Boswell (1975). Moreover, tassels of no-B applied looked so healthy and dry weight quite high about 9 g $plant^{-1}$ with contained B ranging from 5-9 mg B kg^{-1} DW compared with about only ≤ 4 g $plant^{-1}$ when grown in sand culture without added B (B0) and tassel B concentration ≤ 4 mg B kg^{-1} DW at the same physiological stage. Despite, the concentration of B in silk of both experiments was similar about 4 mg B kg^{-1} DW. The concentration of B in some plant parts in the field and in sand culture suggested that substantial differences can occur such as the concentration of B in flag leaf were lower in the sand culture than in the field is probably because some plant roots in the lower B treatments had the opportunity of grew continuously supply of B. Response of maize to B varied to sites, season and environmental control (Dell and Huang, 1997).

5.2. Morphological and physiological response of maize to low boron

Without added B (B0) in sand culture, growth of maize (cv. NS72) was normally without any the symptom of B deficiency from day 1 (transplanted to pot) to harvest 1 (5-leaf stage). The first symptom of B deficiency occurred at 27 days after transplant including white spot and partially became short white strips. Subsequently, these leaves were severely deficiency symptom, white strip became transparent streaks at tassel emergence (63DAS). The leaf symptoms of B deficiency was white spots that merge into transparent short longitudinal streaks are unlike symptoms of other nutritional disorder. These could therefore be used to diagnose for B deficiency in the field. Boron deficiency, however, adversely affects vegetative growth of maize only when deficiency is severe. Such extreme B deficiency has been created in this study with a solution culture which has had B carefully removed with B specific resin. In such situation, shoot growth responded more strongly to increasing B than root growth, with the result of the dry weight shoot: root ratio increasing with increasing B. The result of this solution culture experiment indicated that only about 0.1 μM B in the external solution is sufficient for vegetative growth of maize which nearly the requirement for wheat (Asad *et al*., 2001).

Most reports of B deficiency in maize described effects on reproduction. For example, Agarwala *et al.* (1981) showed that under low B condition the emergence of tassel and silk was suppressed and delayed. The most common B deficiency symptom is small, misshapen cobs with missing kernels, resulting in significantly decreased yields. The results of this set of experiments have also shown the adverse effect of B deficiency and symptoms on reproductive development in maize even when no effect on vegetative growth was observed. Most significantly, this study has shown the comparative effects of B deficiency on different growth processes. Reproductive growth has been shown to be more sensitive to B deficiency than vegetative growth, in the same way that has been reported for wheat (Rerkasem and Loneragan, 1994). However, unlike wheat which exhibit the symptom of B deficiency primarily as male sterility, the symptoms of B deficiency in maize found in this study were on both the male and female flowers, which are summarized as follows.

Boron deficiency symptoms of the male flower:

The male flower or tassel may be depressed in dry weight, with some branches degenerated into white, papery dead tissues; the tassel is more severely affected in its branches than the main axis, with low B depressing dry weight of the whole floret as well as the dry weight of anthers in each floret more in those florets on the branches and much less in those florets in the main axis; the pollen grains are fewer per anther in B deficient plants, with some to most of the pollen showing sign of sterility by the absence of starch deposit and germination failure; of the two staminate florets on each spikelet of the tassel, the lower floret is more prone B deficiency that causes pollen sterility than the upper floret; boron deficiency symptoms were associated with tassels that contained 4-5 mg B kg^{-1} DW and pollen that contained 3 mg B kg^{-1} DW, whereas normally function tassels contained 8-18 mg B kg^{-1} DW and normally function pollen contained 7 mg B kg^{-1} DW.

Boron deficiency symptoms of the female flower: boron deficient maize may develop multiple ears at the same node; these B deficient ears may no longer have the typical morphology of the maize ear, but develop into branches that look more like tassels; those B deficient ears that keep normal morphology of the maize ear may not show any adverse effect of B deficiency on the ear dry weight, but may produce silk threads that are much shorter, much fewer in numbers, and appear to be thinner with collapsed internally under the microscope, boron deficient silk may not function properly- grain set may still fail when healthy pollen is applied to these, as healthy pollen requires sufficient external B supply to germinate fully; boron deficiency symptoms were associated with silk that contained 4 mg B kg^{-1} DW, whereas normally function 15-18 mg B kg^{-1} DW.

B-deficient plant, decreased in tassel size (dry weight) and showed abnormal anthers (thinned and dead anthers) including shrivelled pollen grains. The B0-anthers was not accumulated starch in pollen grains, anther wall and connective tissues and also contained less vascular tissues compared with B20 plant. Moreover, the shape and orientation of B0-pollen grains was irregular. In germination of pollen grains in media of the B0 plant was very small about 6% compared with 19% of the B20 plant similar to the iodine staining. Under B deficiency, plants also showed markedly decreased the number of pollen grain per anther. In female flower development, (about 2 cm silk in long), although less clear effect of B deficiency on development that of the B0-silk at the upper part of young ear was thin shape compared with thick silk of B20 plant. Silk tip from top of young ear was collapsed (from cross section). Subsequently, B-deficient plants produced more ears with short silks or not emerged from the husk. Moreover, this ear became to the small ears with short silks like branches of tassel.

In contrast to the report on B deficiency causing yield loss in wheat primarily through male sterility (Rerkasem *et al*, 1989; Rerkasem and Loneragan, 1994), this study has found that in maize B deficiency first depresses development of the female flower, specifically through function of the silk. The conclusive explanation for complete or partial failure of grain set in low B was provided by cross-pollination experiments (Chapter 3). These evidences included anthers from B-deficient plants were small, thinned, dead, small amount of starch deposited and produced far fewer pollen grains, particularly in the pollen grain from the B-deficient plants were demonstrated to be non-viable by iodine staining (100%) and germinating in media (> 95%). This is supported by the cross pollination experiment, in which application of B sufficient pollen on B deficient silk had no effect on improving grain set, whereas application of pollen from the same B deficient plants on B sufficient silk succeeded in significantly increasing grain set. As result of *in vitro* pollen germination has been shown to require external supply of B (media act as style or silk), it is most likely that pollen germination is inhibited on B deficient silk. These are in agreement with earlier reports of B deficiency limiting silk function (Agarwala *et al.*,1981; Vaughan, 1979). The current study has definitively established that B deficiency in maize is different from that in wheat in three respects. Firstly, the effect of B deficiency is visible in the female flower as well as on the male flower. Secondly, on the same B deficient plant, the female flower may be adversely affected more severely than the male flower. The ear may develop abnormally, with multiple ears on the same node with appearance of tassel-like branches, instead of the typical maize ear. The silk threads may be fewer and much shorter and fail to function completely even with healthy pollen. On the same plants, there may still be a few pollen grains that are viable, especially those in the upper florets of the staminate spikelets. Thirdly, B supply for pollen germination in the silk appears to be the limiting step for B deficiency in maize, as shown in the cross pollination and *in vitro* pollen germination experiment.

In conclusion, B deficiency markedly depressed reproductive development in maize more than vegetative growth. Furthermore, B deficiency depresses grain set by its adverse effect on pollen viability and development and function of the ear female flower. On the same B deficient plant, the effect on the female flower is more severe than on the male flower. The ear may develop abnormally into tassel-like branches. Normal looking ears may have poorly developed silk that fail to function even with healthy pollen. Some evidence of differential response to B was found between the two genotypes in the early vegetative growth study. Since genotypic variation in response to low B has been reported in many crop species (Rerkasem and Jamjod, 1997).

5.3 Genotypic variation in vegetative and reproductive responses to boron in maize

The interaction between B and genotypes was not affected on vegetative growth (dry weight of shoot), but varied between genotypes. SC had higher proportion shoot and root in dry weight and the content of B than other genotypes

(NS1, Pioneer, CM, GC, NS72 and WSC). B treatment was affected on the concentration of B in various plant parts such as YEB in NS1, GC, CM, SC and WSC but not in Pioneer and NS72 (Table 4.2). However, the addition of B (B20) was significantly increased B concentration in shoot of all genotypes, but were no differed at B0.

Highly significant genotype x boron interaction were observed for grain yield. Considerable genotypic variation in B efficiency was base on relative grain yield (B0/B20). This results indicated that most efficiency genotypes were SC and WSC exceeded 70% relative grain dry weight. Moderate efficiency was GC had 46% whereas inefficiency genotypes were NS1 and NS72. These results can be ranked from SC and WSC>GC>pioneer, NS1 and NS72. The high potential grain yield SC genotype (B0/B20, 84%) was associated with higher the concentration of B in silk (7 mg B kg^{-1}) and pollen (5 mg B kg^{-1}) and also higher the number of grain per ear. For WSC was similar reacted of SC, but the concentration of B in pollen and relative grain yield was lower by 2 mg kg^{-1} and 74%, respectively. The others three of the most susceptible to low boron were NS1, GC and NS72 associated with low B concentration in both the silk was about 3 mg B kg^{-1} and the pollen was 2 mg B kg^{-1}, particularly in B-deficient NS72 was also markedly reduced the number of silk per ear. This concentration of B was approximately 3 mg B kg^{-1} of plant tissues that agreement with results reported by Vaughan (1977) that of this minimum concentration necessary for growth and normal function in all parts. Under B deficiency, the four of all maize genotype produced more ears at the first node in NS72, Pioneer, GC and N1.

Differences plant genotypes in grain yield production allocated to the male (pollen) and the female (silk) development. Grain yield was closely correlation (r= 0.81**) between grain number and silk B. Moreover, these evidences showed that SC and WSC were able to produced grain yield at low boron in which to lower requirement at the functional sites. Two sweet corn genotypes (SC and WSC) may be related the possibility that B movement into the sink. In broccoli flowers acquire some of their B from phloem transport (Shelp *et al*., 1995) in which B can move in the phloem with carbohydrate in those species containing with sugar alcohol (particularly sorbitol and manitol) (Brown and Hu, 1996) that majority role of B in transportation and redistribution in some higher plant (Raven, 1980; Brown and Hu, 1996; Hu *et al*, 1997). Generally, the results indicated that differences among genotypes in during reproductive development, including days to tassel and silk emergence, number of branches-tassel $plant^{-1}$ and silk number. Only two genotypes of all were not affected by B treatment included CM and SC whereas WSC was affected by B in all of observed parameters.

5.4 General conclusion

In this thesis I have delineated how B deficiency can affect maize yield through impairment of the reproductive process and that silk function is probably the most sensitive to B deficiency among all the different physiological processes. A very large genotypic variation in response to low B has also been demonstrated. Sensitivity to B deficiency in maize genotypes was closely correlated to B concentration, especially in reproductive organs such as silk, anthers and pollen. It is therefore postulated that genotypic variation in B efficiency is maize is associated with the ability to supply B to these organs.

5.5 Further research

Results from this thesis indicated that there are genotypic variation in response to boron deficiency in maize, and that tolerant genotypes may be better at providing sufficient B supply for reproductive development. Further work could attempt to find the genetic control of B efficiency so that the relevant genes may be deployed. Another direction of future research could be to better define how B efficient maize genotypes are able to supply sufficient B to the reproductive tissues while supply to B to the root is limited. Candidate mechanisms are (1) phloem B mobility, possibly mediated by some sugar or sugar alcohol, or (2) better ability to deliver B into developing ears, silk and tassel.