CHAPTER 3

MORPHOLOGICAL AND PHYSIOLOGICAL RESPONSES OF MAIZE TO LOW BORON

3.1 Introduction

Along with most cereals, maize has generally been considered to have a relatively low boron (B) requirement compared with other crops (Marten and Westermann, 1991). Boron deficiency in field grown maize was first observed in 1960s in the United States associated with increasing grain yield by B application (Shorrocks and Blaza, 1975). In the previous chapter I found that dry weight of young leaf (i.e. YEB-1) increased with the addition of B at rate of 20 kg borax ha^{-1} and declined at higher soil B application, but this effect was not found in sand culture (at 0 and 20 μM B levels). Furthermore, B deficiency was found to have no effect on total shoot dry weight in maize grown in both field and sand culture. By contrast, reproductive development of maize plants grown in sand culture was adversely affected when B was omitted from the nutrient solution. Symptoms of B deficiency observed included abnormal anthers (thin and dead anthers) and ears. Boron deficiency also markedly depressed grain yield. The symptom of B deficiency has been described as male sterility in wheat, resulting in grain set failure (Rerkasem *et al*., 1993). Moreover, grain set of wheat has been correlated with B concentration in the ear and flag leaf (Rerkasem and Lordkaew, 1992). I therefore postulate that B deficiency may depress development of the anthers and pollen in maize. B deficiency has also been reported to depress grain set through impaired development and function

of the stigma or silk (Agarwala *et al*., 1981; Vaughan 1977). Thus, the objectives of this set of experiments were to examine the response to B of maize in term of vegetative and reproductive development which may provide a basis for an understanding of the role of B in yield formation. Four experiments were conducted to evaluate the following. The first experiment examined vegetative and reproductive responses of maize to low B in sand and solution culture. The second experiment examined the effect of low B on viability of the pollen and function of the silk by manual pollination between B deficient plants (B0) and B sufficient plants (B20). The third experiment studied effects of B deficiency on micro-structure of silks and anthers and pollen. The fourth experiment examined the effect of B on *in vitro* pollen germination.

3.2 Materials and methods

3.2.1 Experiment 1: Responses of maize to low boron

This experiment consisted of two sub-experiments, including sand culture and nutrient solution culture.

3.2.1.1 Experiment 1.1: Responses of maize to low boron in sand culture

Maize genotype cv. NS72 was grown at two levels of B (0 and 20 μMB) in sand culture as in Chapter 2. This experiment was conducted at Chiang Mai University during September-December 2002. There were three replicate pots per treatment for each of the 3 harvests. Two day-old seedlings were transplanted to freely drained (0.30 m diameter and 0.30 m deep) pots filled with washed river quartz

sand at 2 plants per pot. Pots were supplied twice daily with complete nutrient solution with two levels of B (0 and 20 μM B) as experiment 2 of Chapter 2. Boron deficiency symptoms were observed as they developed throughout the experiment. Plants were harvested during vegetative and reproductive growth. At silking stage, florets were collected from the central of the main tassel axis and the central of the branches tassel. Then, 100 florets were separated into anthers and chaff (remaining lemma and palea after anthers were removed) and measured for dry weight and B concentration.

Plant samples were dried at 75 °C to constant weight, then B were analysed by dry ashing followed by azomethine–H method determination as in Chapter 2. At maturity, grain and straw dry weight were determined; yield and yield components were recorded.

The objective of the present study was to examine the effect of B deficiency on reproductive and vegetative development and also to define B-deficiency symptoms.

3.2.1.2 Experiment 1.2: Responses of maize to low boron in nutrient solution during early vegetative growth.

This experiment was conducted to examine the effect of B deficiency on maize and any possible difference between genotypes in early vegetative response to B deficiency. This experiment was conducted from August to September 2006 at Multiple Cropping Center, Chiang Mai University, Thailand. Seeds of two maize genotypes, NS72 and SC, were germinated for two days and transplanted to 5 L-plastic pots containing continuously aerated triple deionized water (TDI) for 2 day. Four seedlings were transferred to each of the hydroponic plastic pots containing full-strength basal nutrient solution that were continuously aerated with an air pump. The

nutrient solution was based on Huang *et al*. (2000) and a range of B (0, 0.01, 0.03, 0.10 and 1 μM B) treatments were applied. The experiment design was a factorial in RCB combination of treatment involving two maize genotypes and five B levels. The experiment had three replicates pots. Solution pH was adjusted to 6.0 $\pm$0.2 every day with 4% H_2SO_4 or 2% NaOH. Analytical grade chemicals were used to make up the nutrient solutions. Triple deionized water and nutrient solutions were purified to remove B by passing drop wise (Dura, silicone tube with size 8x12 mm, 80 cm in length) through a B-specific resin (IRA-743, Sigma chemical Co.) column three times with new resin. Plants were harvested at two stages about 10 and 20 days after transplanting. Plants were separated into leaf, rest of top and root components. Plant tops were rinses with TDI water and roots were washed with running tap water and rinsed with TDI water. Parts were dried at 75 °C to constant weight for dry weight and B determination.

3.2.2 Experiment 2: Manual pollination between B0 and B20

This experiment was conducted from November 2001 to March 2002 in sand culture at Multiple Cropping Center, Chiang Mai University, Thailand. Maize (cv. NS72) was grown with two B level (0 and 20 μM B: DB for B deficient and SB for B deficient) as experiment 1.1. This experiment was conducted to determine the response of the male and the female flower to low B. Cross-pollination was done by applying pollen from BD and BS plants to silk of plants in BD and BS, in complete set of crosses of silk x pollen, i.e. DBxSB, DBxDB, SBxDB and SBxSB. Silks were bagged to prevent cross fertilization as soon as they appeared. At anthesis, freshly shedding pollen grains were collected on white paper (A4 size) during 9.00-11.30 am and immediately applied to the silks. Each manual pollination was done three times to

ensure sufficient supply of pollen. Separate samples of silks and pollen grains were also collected during this time for B determination. At maturity, grain weight, grain number and straw were determined and analyzed of B concentration.

3.2.3 Experiment 3: Effect of B deficiency on anatomy and morphology of the silk and pollen

The objective of this experiment was to determine which male or female development is sensitive to B supply. Plant samples were prepared at Chiang Mai University in northern Thailand from November 2002 to March 2003. Then, samples were evaluated at School of Biological Sciences and Biotechnology, Division of Science and Engineering, Murdoch University, Australia, during November-December 2003.

Seeds of maize (*Zea mays* L., cv. NS72) and plants were grown in sand culture and supplied with nutrient solution at the same as experiment 2 Chapter 2. Plants were harvested during vegetative development at the 5-leaf stage, during reproductive growth at anthesis and separated into root, leaves (ear leaf, flag) rest of tops and ear from each pot, dried at 75 °C, dry weight and B were determined.

Samples from anthesis were prepared for two purposes as follows:

1. Light microscopy

Tassels and ears of maize (cv. NS72) were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer, pH 7, at 4 ^{o}C overnight. The samples will be rinsed in 0.025 M phosphate buffer, 3 times each for 30 min, and dehydrated in an alcohol series (100% methoxyethanol, 100% ethanol, 100% 1-n-propanol, 100% 1-n-butanol; 2 changes each; 5 hr each) at room temperature. Anthers were infiltrated with glycol methacrylate [syn. 2-hydroxyethyl methacrylate, GMA (Pro Sci Tech)] for 2 weeks at

room temperature with 2 changes of resin before flat embedding in fresh purified GMA. The resin was polymerised in an oxygen-free oven at 60 ^{o}C overnight. Sections (2.5 μm thick) were cut (3 anthers for each treatment) using glass knives (25 x 6.4 mm) on a Sorvall-microtome and stained (see below). Images of representative areas were captured with a digital camera attached to a compound microscope.

The following staining procedures were carried out:

(1.1). Toluidine blue O (TBO: C.I. 52040): 0.025% w/v in benzoate buffer, pH 4.4. Sections were stained at room temperature for 5 min, rinsed in distilled water and, when dry, mounted in DPX (Depex; mounting medium 'Gurr').

(1.2). Periodic Acid-Schiff's (PAS) Reaction. The slides were placed in a solution of the blocking agent-a fresh solution of DNPH (2, 4-dinitrophenyl-hydrazine) in 15% acetic acid in water for 30 min. After rinsing in running water for 3 min the slides were placed in 1% periodic acid solution for 10 min, followed by running water for 5 min. The slides were stained in Schiff's reagent for 30 min, rinsed in running water for 3 min and, when slides dry at room temperature, mounted in DPX.

(1.3) Pollens were examined for starch accumulation with KI/I_2 staining solution, then observed under the microscope at 10-40X magnification and was recorded as stained or not stained with iodine.

2. Scanning electron microscope (SEM)

Silks were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer, pH 7.0, at 4 ^{o}C overnight, and then washed several times with 0.025 M phosphate buffer. The specimens were dehydrated in an acetone series (30, 50, 70 and 90%) with two

changes of each solution for 15 min., and then replaced with 100% acetone. The acetone were substituted with Spurrs (Spurr, 1969) resin (5, 10, 20, 40, 60, 80, 90% resin in acetone; 5 hr each). The specimens were transferred into 100% resin for 2 days, before embedding with 100% resin and polymerising at 60 ^{o}C overnight. Ultra-thin sections (90-150 nm; gold interference colour) were cut using an ultramicrotome with a diamond knife (Diatome 3 mm width). The sections were transferred to 100 mesh, formvar-coated copper grids and stained with 0.1-0.4% lead citrate and saturated uranium acetate. The specimens were examined in a SEM (Philips XL20; Bio-transmission electron Microscope) at 80 kv.

3.2.4 Experiment 4: pollen viability

This experiment was conducted from November 2005 to February 2006 in sand culture at Multiple Cropping Center, Chiang Mai University, Thailand. Seeds of maize (*Zea mays* L.) cv. NS72 were germinated for two days, then were transplanted to pots (0.30 m diameter and 0.30 m deep) filled with washed quartz river sand at 2 plants per pot. Pots were supplied twice daily with a complete nutrient solution with two levels of B (0 and 20 μM B) as in experiment 2 Chapter 2. There were ten pots per treatment and two plants per pot. They were grown until harvested. This experiment was made with 2 sets.

First set, tassels were collected during pollen shedding between 09.00 and 11.30 am from the main axis. Fresh pollen, anther just emerged from the upper floret at each main tassel were sprinkled onto a germination medium contained 15% sucrose, 0.6% bacto-agar, 0.03% calcium nitrate and with or without added B (0 or 0.01% boric acid, respectively) (Pfahler, 1967). After a 2-h germination period, pollen grains were classified under a microscope as germinated if they had a pollen

tube which was at least as long as the diameter of the pollen grain. In calculation of percentage germination, the germinated grain was taken as the percentage of total. Pollen grains were scored in each of five sectors on each petri dish.

The second set, the whole tassel were collected for measuring the number of floret, anther and including pollen grain per ear. The starch accumulation was also determined by iodine solution (KI/I_2). Normal anthers were only chosen from main axis to stain with iodine solution. The pollen was put on 0.5 ml iodine solution. Then a drop of pollen suspension is place on slide about 5 min. The pollen was observed under microscope at 35x magnification and count in ten drops. Pollen was dark with iodine solution was classified as stained or a starch deposit in which remained transparent was designated as dead pollen. Total number of pollen grains in a 0.5 ml-suspension was calculated to the number pollen per anther. In tassel, the number of floret, anther and the number of branch were determined.

The objective of the present study was to examine the fertility of pollen grains (in the media) by germination on agar, specifically to test the hypothesis that B deficiency does not affect viability of pollen.

Statistic analysis

The results were analysed statistically by analysis of variance (ANOVA). Significantly different means were separated at the 0.05 probability level by LSD. Data of statistical analyses were done by using commercial software (Statistix V. 8, Analytical Software, Inc.).

3.3 Results

3.3.1 Responses of maize to low boron

3.3.1.1 Experiment 1: Responses of maize to low boron in sand culture

At vegetative development (Harvest 1), B did not effect plants growth when measured in term of total dry weight or in each plant parts (Figure 3.1). There was a highly significant effect of B on the concentration of B in shoot and in various plant parts (YEB-1, YEB, YEB+1 and root) at vegetative stage (Figure 3.2). When plants grown in B0, the concentration of B in shoot was only 4.5 mg B kg^{-1} compared with 16.8 mg B kg^{-1} in B20, and in the roots they were 6.1 mg B kg^{-1} compared with 10.0 mg B kg^{-1} in B20. The B application also increased B contents in all plant parts (Table 3.1). The B content ratio of shoot per root was also increased from 3.0 in B0 to 5.5 with B20.

At day 40, dry weight in various plant parts, root and total shoot dry weight were significantly higher in B20 than in B0 (Figure 3.2). B0 maize plant had total shoot dry weight about 16.3 g $plant^{-1}$ and slightly increased to significantly higher at 21.1 g $plant^{-1}$ in B20. Boron did not affect in the shoot: root dry weight ratio at this harvest. The effect of B on B concentration in different plant parts was similar to those in the first harvest, in that the values in B0 were much lower than in B20. However, the difference in B concentration between B0 and B20 was varied considerably between the various plant parts. The whole shoot had only approximately 4 mg B kg^{-1} DW which was half that in B20 (8.2 mg B kg^{-1} DW). In case of leaves, higher B concentration were found in the older leaf in B20 the B concentration in YEB+1, YEB and YEB-1 were 8.3, 7.8 and 6.9 mg B kg^{-1} DW, respectively, but they were all about the same in B0 at 4 mg B kg^{-1} DW. Similarly, the B content in each plant parts increased with supply B. B0-plant contained about half of the B in B20-plant. For example, the B content in YEB of B0 was 4.9 μg

compared with 10.9 μg in B20 (Figure 3.5). Without added B in the nutrient solution, the B content ratio of shoot to root was increased from 5.7 to 9.1.

At early tassel emergence (visible about 50% of tassel just emerged, 63 DAS), dry weight in each part was not affected by B except tassel dry weight. In B0, dry weight of tassel was 3.3 g plant^{-1} compared with decreased from 5.1 g plant^{-1} in B20 (Figure 3.5). B concentration increased significantly in all parts of maize in the addition of B treatment (Figure 3.7). Individual parts of plant in B0 had B concentration below 4 mg B kg^{-1} DW and the lowest B concentration was found in tassel only about 2.5 mg B kg^{-1} DW compared with 6.2 mg B kg^{-1} DW in B20-tassel. Among plant parts in B20, leaves (YEB-1, YEB, YEB+1) had higher B concentration approximately 13-16 mg B kg^{-1} DW whereas root had only 5 mg B kg^{-1} DW. B0-tassel contained B about 7 μg (Figure 3.7, a) in which less than the B20-tassel contained about 4 times (29 μg B). Similarly, the content of B in leaves were about 4-6 μg in B0-plants compared with 20-25 μg at the B20 plant (Figure 3.7). At anthesis (75 DAS) dry weight of anthers and chaff per 100 florets were not significantly different between plants in B0 and B20 for those florets from those from the main axis of the tassel (Table 3.7). However, for those florets from the tassel branches, dry weight of anthers and chaff per 100 florets in B0 were only about 60% of those in B20. Highly significant the effects of B level on the concentration of B and B content in anther and chaff in both located in the main and branch of tassel. Anther B and chaff B in B0 were generally only half those in B20.

Plant growth and visible B-deficiency symptom

While no symptom was ever observed in B20, plants in B0 developed B deficiency symptoms in young leaves after 22 days (Harvest 1). These leaves first showed white spots (Figure 3.9, a) which later joined to become short white stripes. At the 40 day, these symptoms developed to few transparent streaks on the lamina (Figure 3.9, b). Subsequently, these leaves development of the symptom were severely transparent streaks at surrounding tassel emergence (Figure 2.2, Chapter 2). Severe B deficiency symptom remained expressed during pollen shedding time resulted in more transparent streaks on the upper leaf (Figure 2.2, c) and small tassel (Figure 2.3, c; Chapter 2). In B0 tassel showed shrivelled anthers and thin anthers (Figure 2.4 a and b; Chapter 2) whereas B20-anthers were healthy (Figure 2.4, c; Chapter 2). Moreover, some B0 plants produced multiple ears with short silks or no silk (Figure 2.3, a), with some of these plant showing abnormal looking ears when the husk was removed (Figure 3.9, c).

Table 3.1 Boron concentration (mg B kg^{-1} DW) and boron content (µg $plant^{-1}$) in various plant part of maize (cv. NS72) at vegetative growth (5-leaf stage) without B (B0) and with added B (B20).

Added B (µB)	YEB-1	YEB	YEB+1	Shoot	Root	Shoot: root
			B concentration			
B0	3.8	4.7	5.1	4.5	6.1	
B20	16.6	18.1	17.9	16.8	10.0	
F-test	***	**	***	***	**	
$LSD_{0.05}$	1.8	5.0	1.8	2.0	1.8	
			B content			
B0	1.7	1.3	1.0	12.5	6.2	3.0
B20	8.9	7.2	4.5	62.2	12.9	5.5
F-test	**	**	**	**	**	**
$LSD_{0.05}$	2.8	3.0	1.4	22.2	4.1	1.3

** and *** significant at $P< 0.01$ and 0.001, respectively.

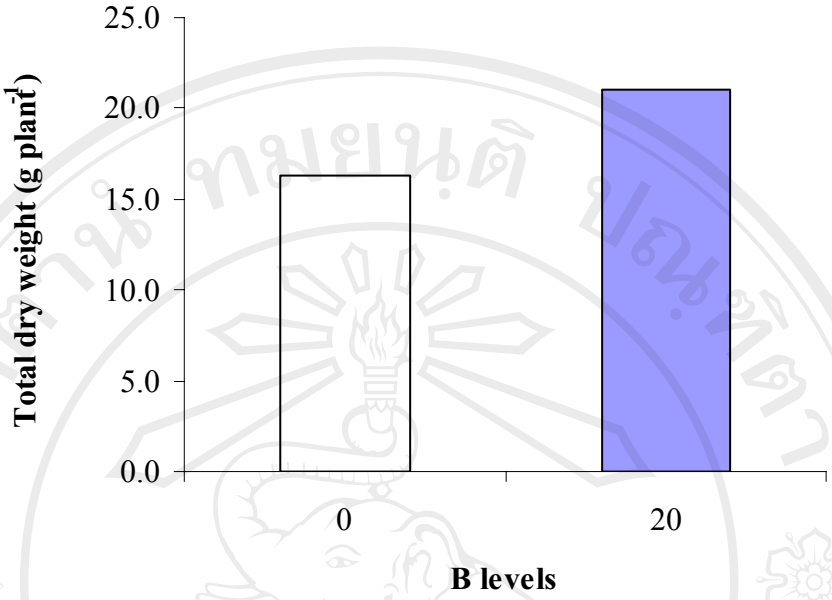


Figure 3.1 Total dry weight of maize at 5-leaf stage (27DAS) grown in sand culture without B (B0) and with added B (B20). Bar represent mean of three replicates (±SE).

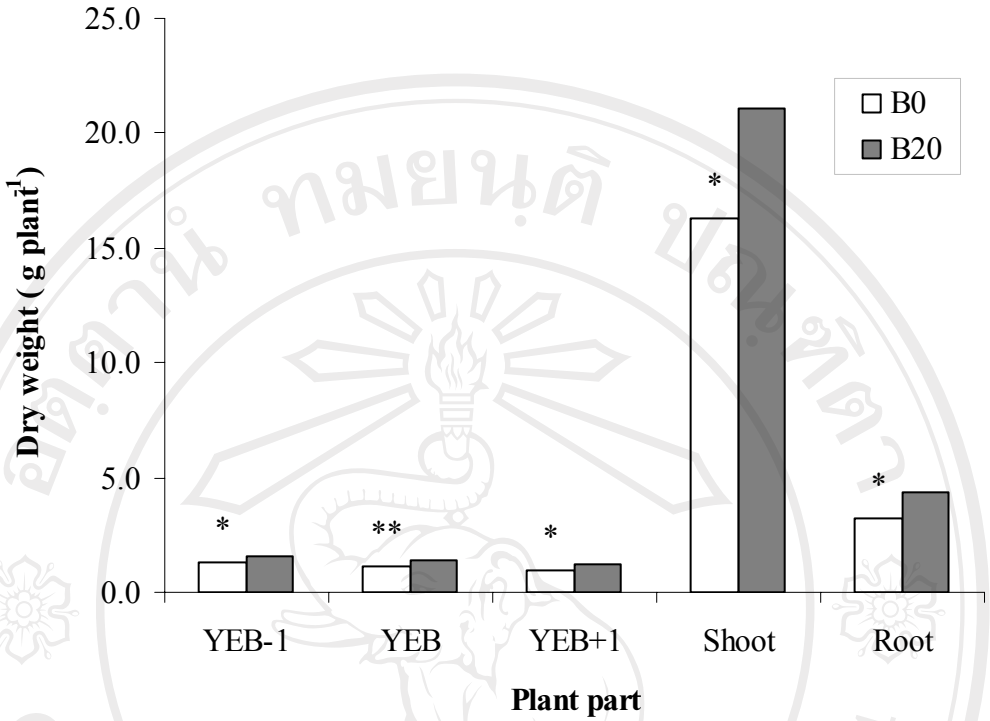


Figure 3.2 Dry weight (g plant^{-1}) of plant parts at vegetative growth (40 DAS, before tassel emergence) grown in sand culture without B (B0) and with added B (B20).

* and ** significant at $P< 0.05$ and 0.01, respectively.

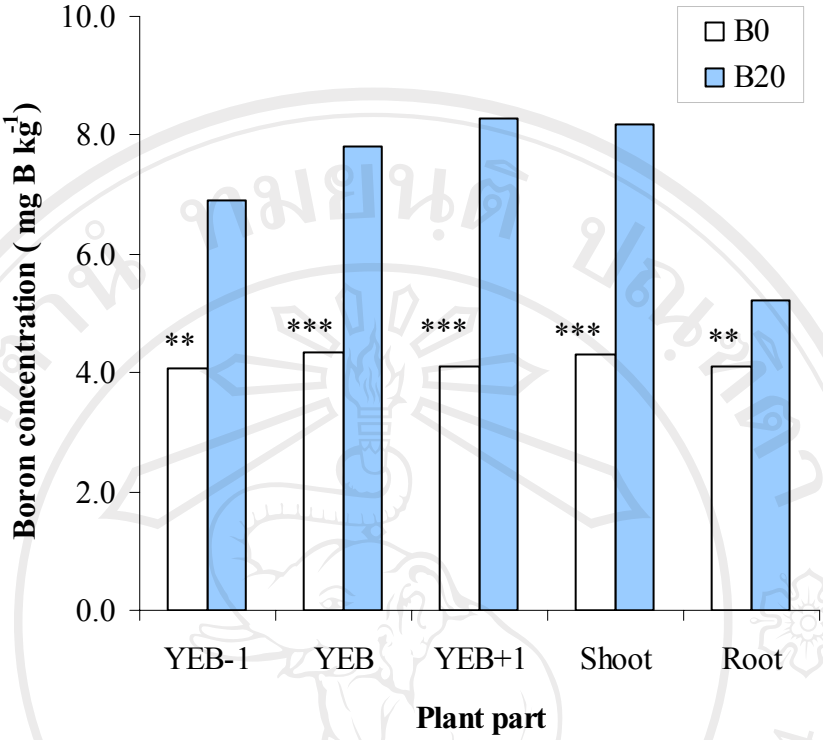


Figure 3.3 B concentration (mg B kg^{-1} DW) in plant parts at vegetative growth (40 DAS, before tassel emergence) grown in sand culture without B (B0) and with added B (B20). * and *** significant at $P< 0.01$ and 0.001, respectively.

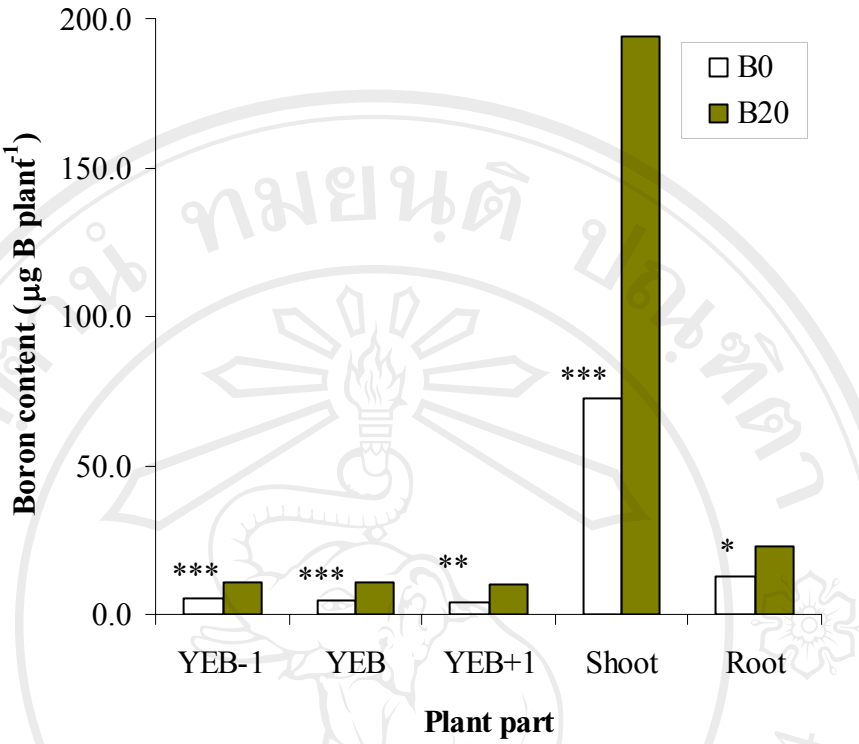


Figure 3.4 B content (µg B plant^{-1}) in plant parts at vegetative growth (40 DAS, before tassel emergence) grown in sand culture without B (B0) and with added B (B20). *, ** and *** significant at $P< 0.05$, 0.01 and 0.001, respectively.

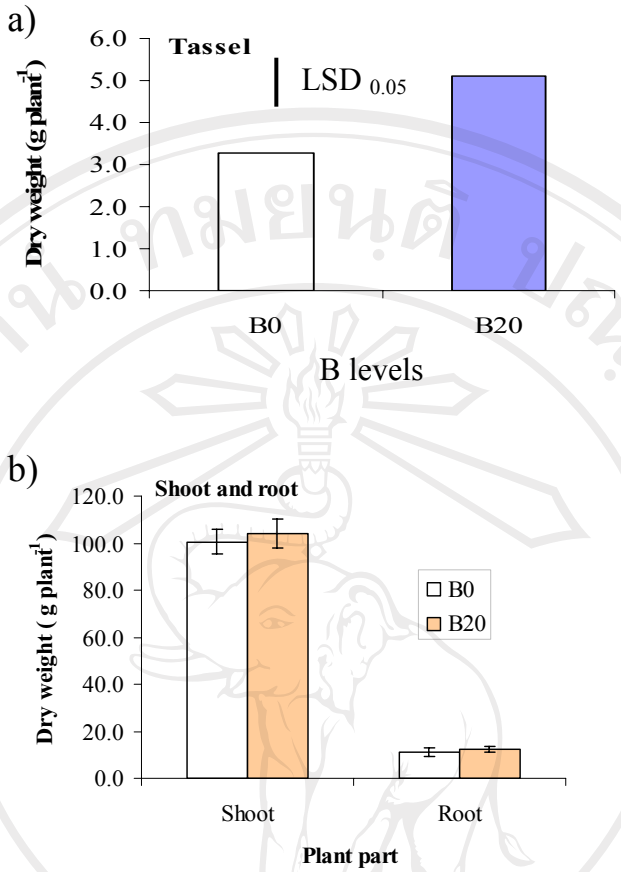


Figure 3.5 Dry weight (g plant^{-1}) in plant parts of maize at early tassel emergence (63 DAS) grown in sand culture without B (B0) and with added B (B20). Bars represent means of three replicates (±SE). The vertical bars represent the LSD (P<0.05).

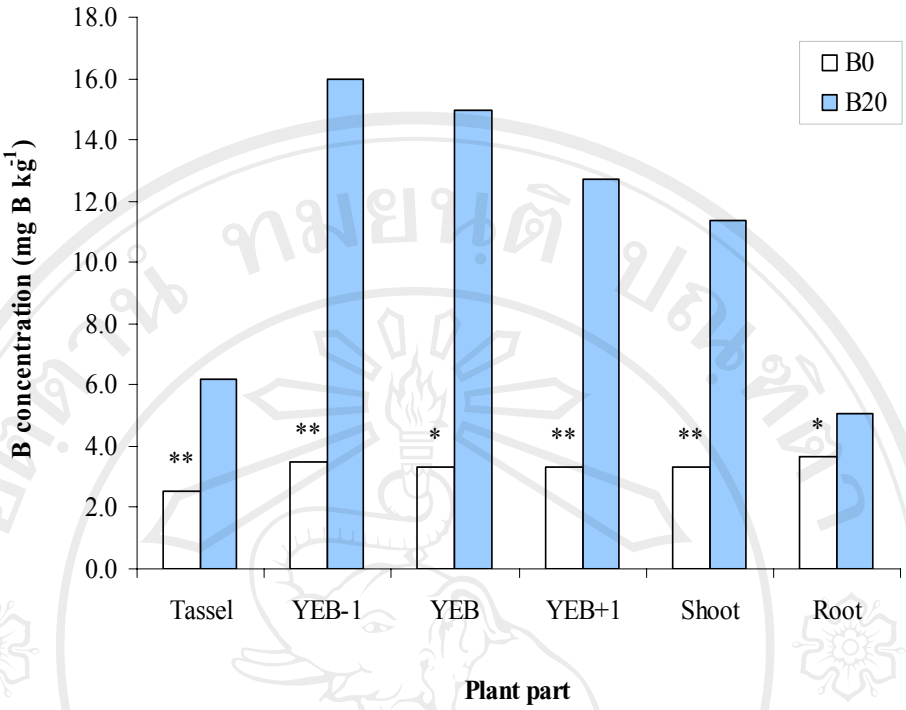


Figure 3.6 B concentration (mg B kg^{-1} DW) in plant parts of maize at early tassel emergence (63 DAS) grown in sand culture without B (B0) and with added B (B20). * and ** significant at $P< 0.05$ and 0.01, respectively.

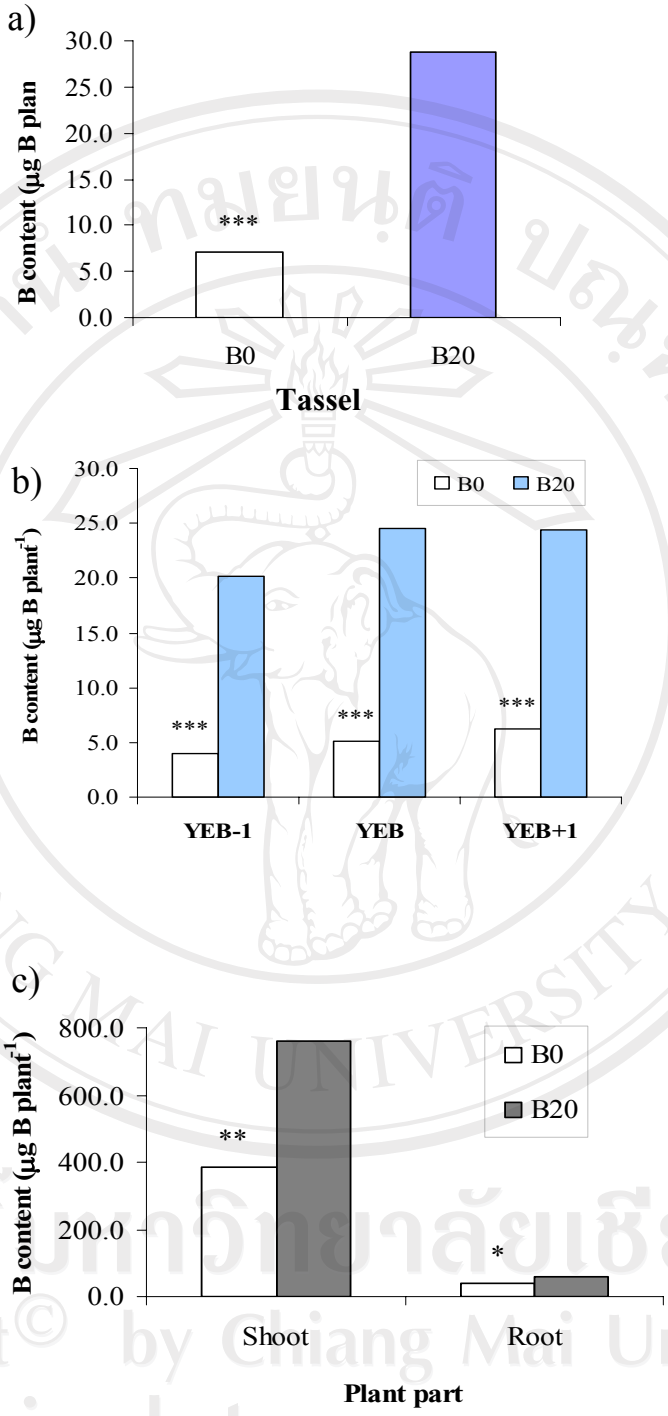


Figure 3.7 B content (µg B plant^{-1}) in parts of maize at early tassel emergence (63 DAS) grown in sand culture without B (B0) and with added B (B20). *, ** and *** significant at $P< 0.05$, 0.01 and 0.001, respectively.

Table 3.2 Dry weight (g) and B concentration (mg B kg^{-1} DW) in anther and chaff of maize from 100 florets at reproductive development (silk emergence) grown in sand culture with and without added B.

Added	Dry weight				B concentration			
B (μM)	anther		chaff		anther		chaff	
	main	branch	main	branch	main	branch	main	branch
B0	0.24	0.10	0.12	0.08	2.9	3.5	3.4	3.6
B20	0.28	0.18	0.14	0.14	6.4	7.4	5.2	5.2
F-test	NS	*	NS	*	**	**	***	**
$LSD_{0.05}$		0.06		0.04	1.4	2.3	0.6	0.6

NS = not significant *, ** and *** significant at $P<0.05$, 0.01 and 0.001 respectively.

Figure 3.8 Boron deficiency symptom : white spot (a) at 27 DAS and white strips (b) at 40DAS.

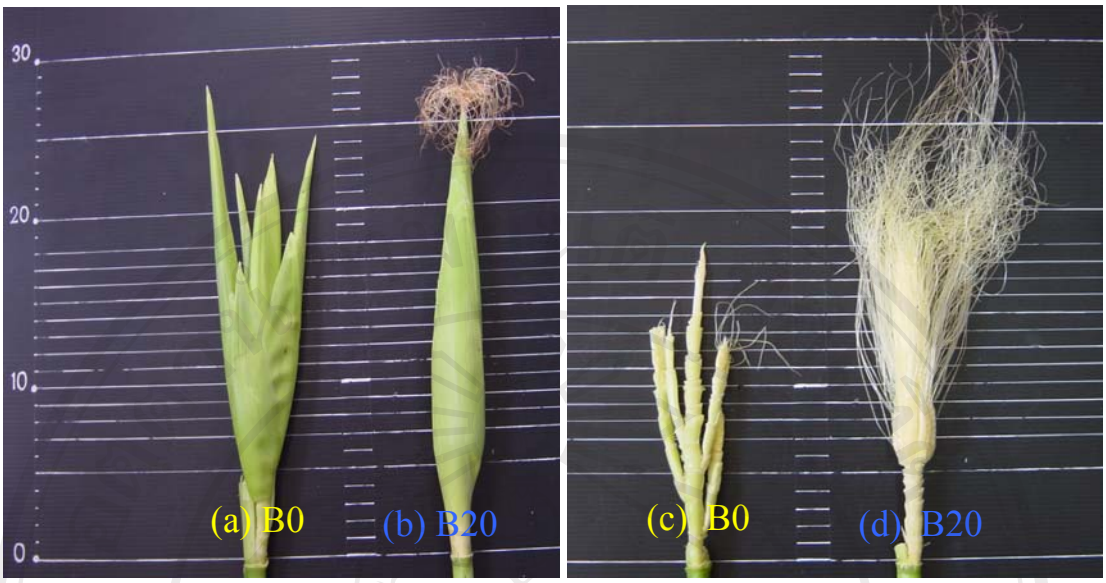


Figure 3.9 Multiple ears and short silks in B0 maize (a and c: removed husk) and normal ear in B20 (b and d: ear after removed husk) at anthesis.

In B20 the maize plants set grain normally, but in B0 there was almost no grain (Table 3.3). In B20 each plant produced an average of 410 grains, with grain yield of 72.3 g plant^{-1} but in B0 it was only 0.5 g or 0.4 grain plant^{-1}. This depression in grain yield in B0 was associated with arrested development of the tassel and silk (Table 3.4). Dry weight of tassel and silk in B0 were much lower than those in B20. Plants in B0 also produced shorter silks, 5.3 cm compared with 13.2 cm in B20, that were fewer in number with 121 ear^{-1}, compared with 417 silks ear^{-1} in B20. The B concentration of tassels, silks and pollen in B0 plants was only 3.9, 4.4 and 4.4 mg B kg^{-1}DW, compared to 8.1, 11.3 and 9.0 mg B kg^{-1} DW in B20 plants (Table 3.4).

Table 3.3 Effects of B on grain yield and the number per ear of maize (cv. NS 72) grown in sand culture without B (B0) and with added B (B20).

B level (μM)	Grain DW (g plant^{-1})	Grain no. plant^{-1}
B0	0.5	0.4
B20	72.3	410.0
F-test	***	***
LSD $_{0.05}$	11.7	72

*, *** significant at $P < 0.05$ and 0.001, respectively.

Table 3.4 Dry weight (g plant^{-1}) of tassel, silk and the number of silk, silk length (cm) and B concentration (mg B kg^{-1} DW) in tassel, silk and pollen of maize (cv. NS72) at anthesis (75DAS).

Added B (μM)	Dry weight		Silk no ear^{-1}	Silk length (cm)	B concentration		
	Tassel	Silk			tassel	silk	pollen
0	3.7	0.52	121	5.3	3.9	4.4	4.4
20	8.8	0.93	417	13.2	8.1	11.3	9.0
F-test	*	***	*	**	**	***	**
LSD 0.05	1.6	0.10	234	2.7	2	1.8	0.8

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

3.3.1.2 Experiment 2: Responses of maize to low boron grown in nutrient solution

At seedling stage, the two maize genotypes grew differently in nutrient solution from which B has been removed. Shoot DW were not different, but SC produced only about 1/3 of the root DW of NS72 (Table 3.5). This shoot: root ratio for DW of SC was double that in NS72. By contrast, the concentration of B in the root and shoot of SC was higher than NS72 about 2-3 folds. The concentration of B in shoot and root of NS72 was similar (3.1 and 3.8 mg B kg^{-1} DW), but in SC the B concentration in shoot had about half of root. Although, the ratio of shoot: root dry weight of SC (3.4) was more than NS72 (1.7), but the content of B in shoot or root of both genotypes was similar. However, the ratio of B contents in shoot: root of SC (1.9) higher than NS72 (1.2). Some plants in both genotypes showed the symptom of B-deficiency i.e. yellow green between the vein, interveinal chlorosis and marginal strips on the leaves (Figure 3.10).

At 10 days after transplanting (DAT, H1), dry weight of shoot in both of NS72 and SC was increased significantly when increasing B in solution at 1.0 μM B (Table 3.6). During 10-d growth period, increasing B increased dry weight of roots in NS72 but not in SC. The dry weight of NS72 root (0.08-0.13 g $plant^{-1}$) was higher than SC root in which the dry weight of SC root was 0.05 g $plant^{-1}$. At H2 (18 DAT), the dry weight of root in SC was increased with adding B in nutrient solution at ≥ 0.03 μM B whereas the dry weight of NS72 root increased with increasing B concentration in solution to 0.01 μM B (Table 3.6). The maximum dry weight of shoot in NS72 was found when adding B at 1.0 μM B about 3.1 g $plant^{-1}$ whereas SC the maximum dry weight was found at ≥ 0.10 μM B (1.67 g $plant^{-1}$).

The ratio of shoot: root dry weight at H1 of NS72 plant was not increased with increasing B in nutrient solution and remained the same value at lower than 1 μM B whereas slightly increased in SC plant (Table 3.6). However, the ratio was markedly increased at 1 μM B of SC about 2-4 folds as compared with the lowest solution B that of the shoot: root dry weight ratio in NS72 slight increased from about 4-5 to 7. At day 18 (H2), the ratio of shoot: root dry weight in both of NS72 and SC plants remained the same when compared to H1, excepted this ratio of SC plant at 1 μM B decreased from 12.6 at H1 to 5 at H2.

B concentration in shoot and YEB of NS72 were slightly increased with increasing B in nutrient solution and the maximum concentration of B in YEB of NS72 and SC. was 5.8 mg B kg^{-1} at 1 μM B on day 10 (Table 3.7). At H2, the concentration of B in YEB of both genotypes was similarly increased with increasing B in nutrient solution like as H1 and also sharp increased at 1 μM B. The concentration of B in YEB was 3.4 mg B kg^{-1} that associated with maximum shoot growth of SC plant (1.6 g $plant^{-1}$) grown at ≥ 0.10 μM B compared with the maximum shoot dry weight of NS72 was 3.2 g $plant^{-1}$ with the concentration 5 mg B kg^{-1} in YEB (Figure 3.11).

There was effected of B on plant height, the plant height in NS72 and SC increased with increasing solution B at H1 and H2. On day 18 (H2), NS72 plant had highest about 63 cm when grown in nutrient solution at ≥ 0.10 μM B whereas SC plant had 57.9 cm at 1 μM B (Table 3.8). Similarly, root length in both of NS72 and SC increased with increasing the B concentration in solution at ≤ 0.10 μM B grown for 10 and 18 days (Figure 3.12). Root length of NS72 was longer than SC in each level of B in nutrient solution, however at ≥ 0.10 μM B in both of maize genotypes

root length was not increased significantly. For example, root length at H2 of NS72 was about 56 cm compared with 40 cm of SC plant. The growths of plants in both genotypes were similar that the root length was correlated with the dry weight of whole plant (Shoot + root) dry weight in H1 and H2 (Figure 3.13).

Uptake of B in shoot (defined as B efficiency: shoot B per g root dry weight) by two maize genotypes increased with increasing the concentration of B in nutrient solution in which B uptake of SC plant was greater than NS72 plant especially at ≥ 0.1 μM B on day 10 (H1) (Figure 3.14). For example, the uptake rate of B in shoot about 65 μM B g^{-1} root DW of SC plant compared with 30 μM B g^{-1}root DW in NS72 plant. At H2, there was found that uptake rate of B in SC plant at ≤ 0.10 μM B (excepted at 0 and 1 μM B) was greater than SC plant (Figure 3.15). B was affected on relative growth rate that of it increased with increasing B in solution. Although, relative growth rate of NS72 and SC plant at H1 was very similar to that in H2 (Figure 3.16), but considering in relative shoot dry weight (defined as shoot dry weight in each B level divided by that of B1) was found that relative shoot dry weight at H2 of SC plant had higher than NS72 plant at 0.10 μM B (Figure 3.17).

Table 3.5 Dry weight (mg plant^{-1}), B concentration (mg B kg^{-1} DW),B content (µg plant^{-1}) and ratio of shoot and root in two maize seedlings (7-day old) grown in nutrient solution without B.

Genotypes	Dry weight			B concentration		B content		
	Shoot	Root	Shoot: root	Shoot	Root	Shoot	Root	Shoot: root
NS72	34.7	20.9A	1.7B	3.1B	3.8B	0.1	0.1	1.2B
SC	21.8	6.4B	3.4A	6.5A	11.6A	0.1	0.1	1.9A
F-test	NS	*	**	*	***	NS	NS	*
LSD $_{0.05}$		10.5	0.7	2.8	0.5			

NS = not significant. *, ** and *** significant at $P< 0.05$, 0.01 and 0.001, respectively.

The difference between genotypes in the same column is indicated by upper case letters.

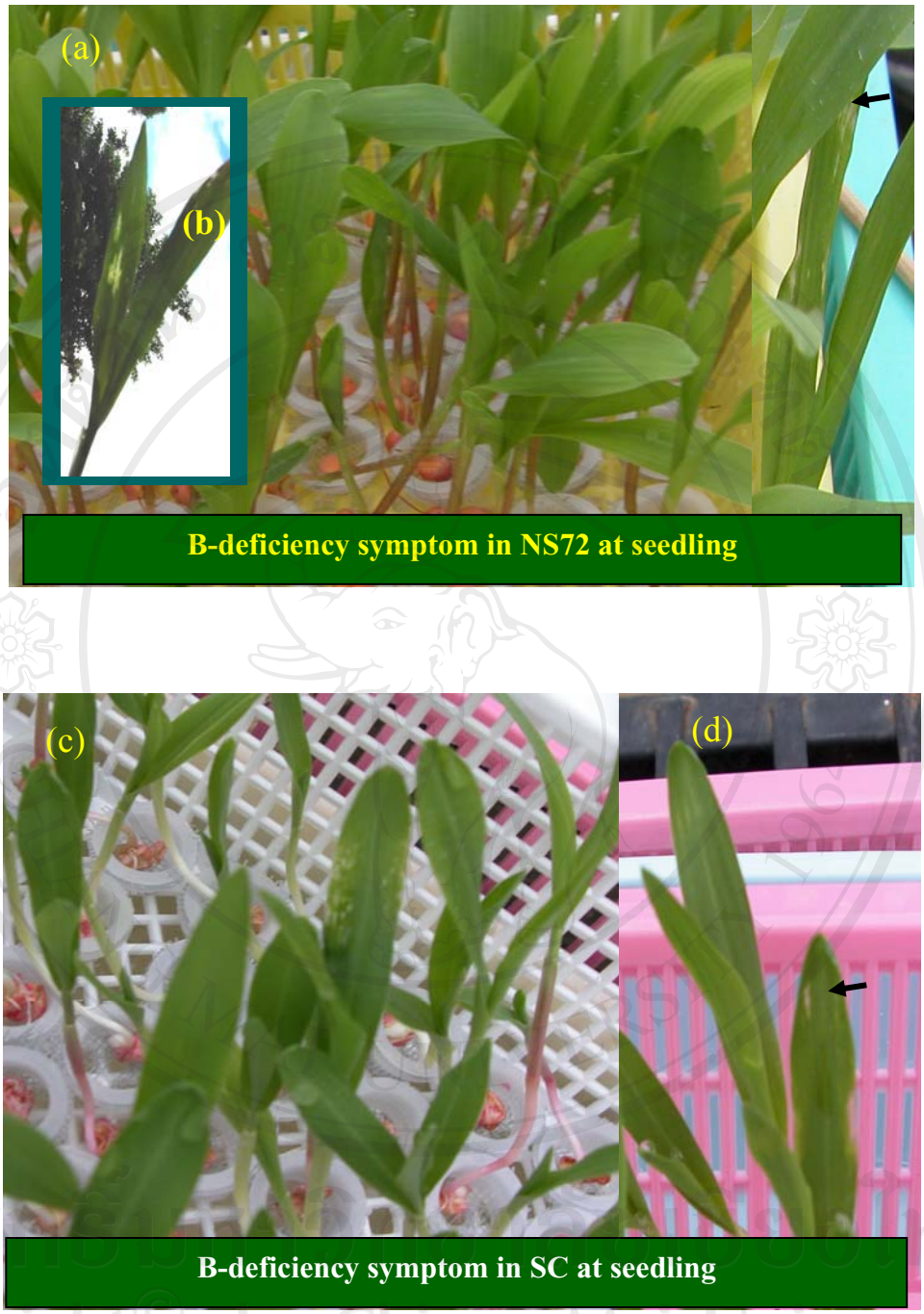


Figure 3.10 Boron deficiency symptom in leaves of two genotypes of maize (NS 72: a and b; SC: c and d): transparent streak at 7-day old seedlings grown in the free B nutrient solution.

Table 3.6 Dry weight (g plant^{-1}) in shoot and root of maize grown in nutrient solution for10 days (H1) and 18 days (H2).

G	B levels	Shoot		Root		Shoot : Root	
	(μM)	H1	H2	H1	H2	H1	H2
NS72	0	0.31ef	0.51f	0.08c	0.16ef	4.1cd	3.23def
	0.01	0.37d	0.90e	0.10b	0.28cd	3.9cd	3.19ef
	0.03	0.44c	1.46d	0.10b	0.36b	4.7cd	4.05d
	0.10	0.57b	2.67b	0.13a	0.67a	4.5cd	4.02de
	1.00	0.65a	3.16a	0.10b	0.63a	6.9b	5.08bc
SC	0	0.16g	0.26g	0.04d	0.10f	3.8d	2.64f
	0.01	0.20g	0.50f	0.05d	0.14f	4.0cd	3.72de
	0.03	0.26f	0.84e	0.05d	0.14f	5.1c	5.90b
	0.10	0.32e	1.67c	0.05d	0.24de	6.4b	7.08a
	1.00	0.52b	1.61cd	0.04d	0.32bc	12.6a	5.00c
$LSD_{0.05}$							
B levels(B)		0.04***	0.13***	0.01***	0.05***	0.87***	0.59***
Genotypes(G)		0.02***	0.08***	0.01***	0.03***	0.55***	0.37***
BxG		0.05***	0.19***	0.01***	0.08***	1.22***	0.83***

*** significant at $P< 0.001$.

Differences (by LSD $P < 0.05$) in the same column are indicated by different letters.

Table 3.7 B concentration (mg B kg^{-1} DW) in various plant parts and the ratio of B content in shoot and root of two maize genotype grown in nutrient solution for 10 (H1) and 18 days (H2).

G	B levels	YEB		Shoot		Root		Shoot: root	
	(μM B)	H1	H2	H1	H2	H1	H2	H1	H2
NS72	0	1.6e	1.1g	2.1	1.7d	4.8	6.9b	1.8de	0.8
	0.01	2.2d	2.4ef	2.4	1.7d	8.6	5.7cd	1.1e	1.0
	0.03	1.9de	3.2cd	2.5	2.9bc	8.5	4.9de	1.3de	2.5
	0.10	2.2d	3.4c	2.7	3.4b	5.8	4.0e	2.1cd	3.5
	1.00	5.8a	5.0b	4.8	5.7a	5.0	4.8de	6.8b	6.0
SC	0	1.9de	1.8f	3.0	2.5c	7.7	5.9bc	1.5de	1.1
	0.01	2.0de	2.5e	3.1	2.7c	10.6	6.9b	1.2de	1.5
	0.03	2.2d	2.7de	3.1	2.9bc	10.6	8.3a	1.5de	2.0
	0.10	3.0c	3.4c	3.2	2.8bc	7.1	6.8b	2.9c	3.0
	1.00	5.8a	6.1a	5.7	6.2a	7.3	6.0bc	9.5a	5.2
$LSD_{0.05}$									
B levels(B)		0.30***	0.42***	0.33***	0.40***	1.0***	0.72**	0.64***	0.63***
Genotypes(G)		0.19**	0.27*	0.21***	0.27*	0.6***	0.46***	0.41**	NS
BxG		0.42***	0.60**	NS	0.6**	NS	1.02***	0.91***	NS

NS = not significant *, ** and *** significant at $P < 0.05$, 0.01 and 0.001 respectively. Differences (by LSD $P < 0.05$) in the same column are indicated by different letters.

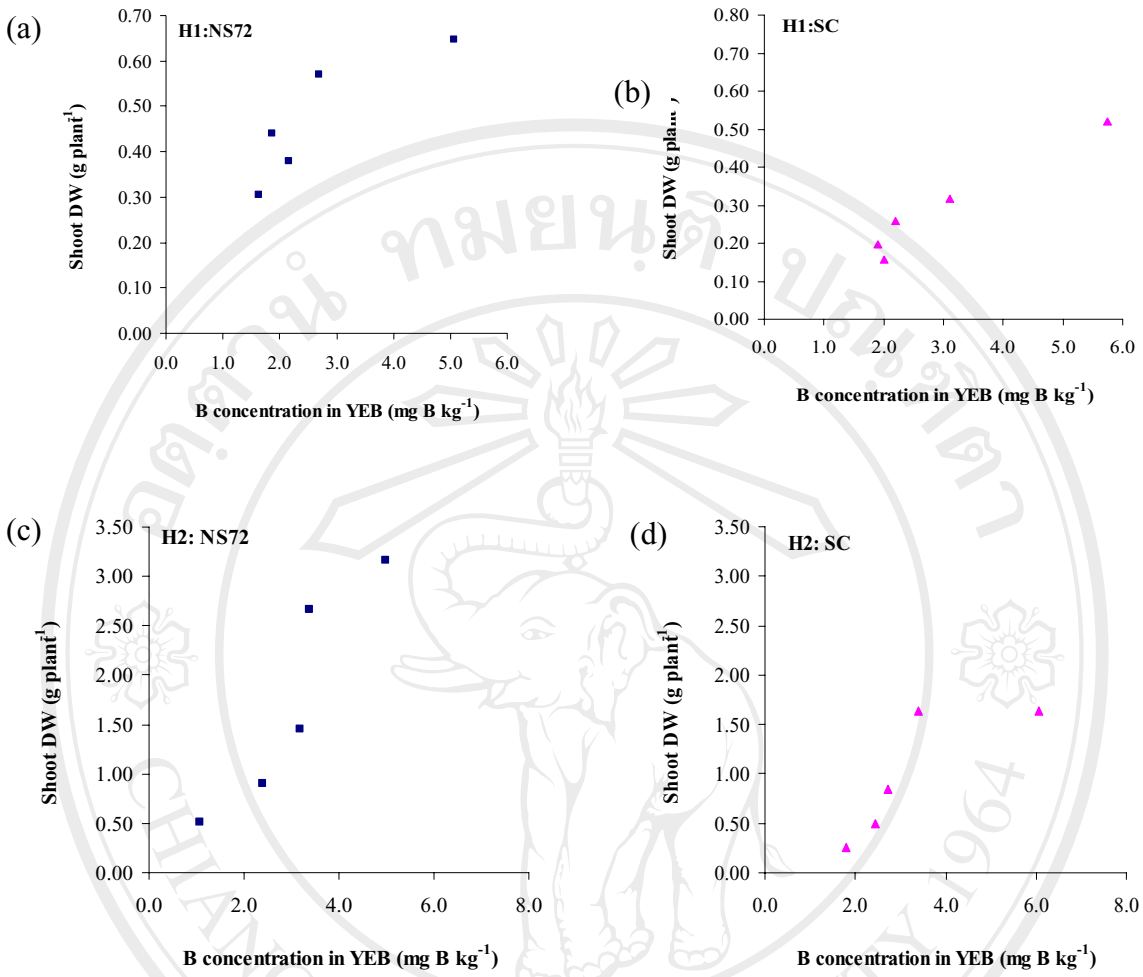


Figure 3.11 The relationship between B concentration in YEB (mg B kg^{-1}) and shoot dry weight (g $plant^{-1}$) of two maize genotypes (cv. NS72 and SC) grown in nutrient solution for 10 (H1) and 18 days (H2).

Table 3.8 Effects of B on plant height (cm), number of leaf per plant of two maize genotypes grown in nutrient solution for 10 (H1) and 18 days (H2).

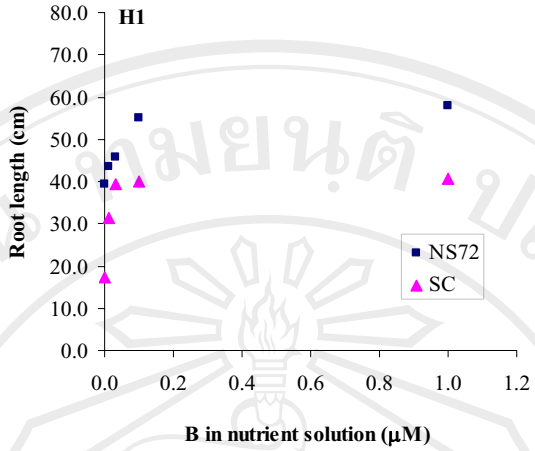
Genotypes	B levels (µM)	The number of leaf per plant [a]		Plant height (cm)	
		H1	H2	H1	H2
NS72	0.00	3	4	26.8d	22.3ef
	0.01	4	5	22.8e	24.8e
	0.03	4	6	28.3d	37.1d
	0.10	4	6	48.7a	62.6a
	1.00	4	6	46.0b	63.2a
SC	0.00	4	4	12.7h	16.2g
	0.01	5	4	14.1gh	19.7fg
	0.03	4	6	18.2f	23.3ef
	0.10	4	6	16.3fg	51.5c
	1.00	4	5	38.3c	57.9b
$LSD_{0.05}$					
B levels(B)		0.5**	0.5**	1.8***	3.1***
Genotypes(G)		NS	0.3***	1.1***	1.9***
BxG		NS	NS	2.6***	4.4*

NS = not significant. *, ** and *** significant at $P < 0.05$, 0.01 and 0.001, respectively.

Differences (by LSD $P < 0.05$) in the same column are indicated by different letters.

[a] leaf number was counted that of the leaf collar was appeared clearly (blade and leaf sheath).

(a)



(b)

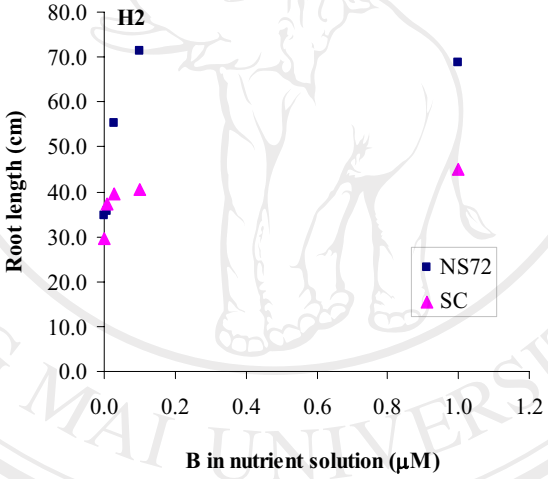


Figure 3.12 The relationship between root length (cm) and B in nutrient solution (0, 0.01, 0.03, 0.10 and 1.00 µM B) of two maize genotypes (cv. NS72 and SC) grown in nutrient solution for 10 days (H1) and 18 days (H2).

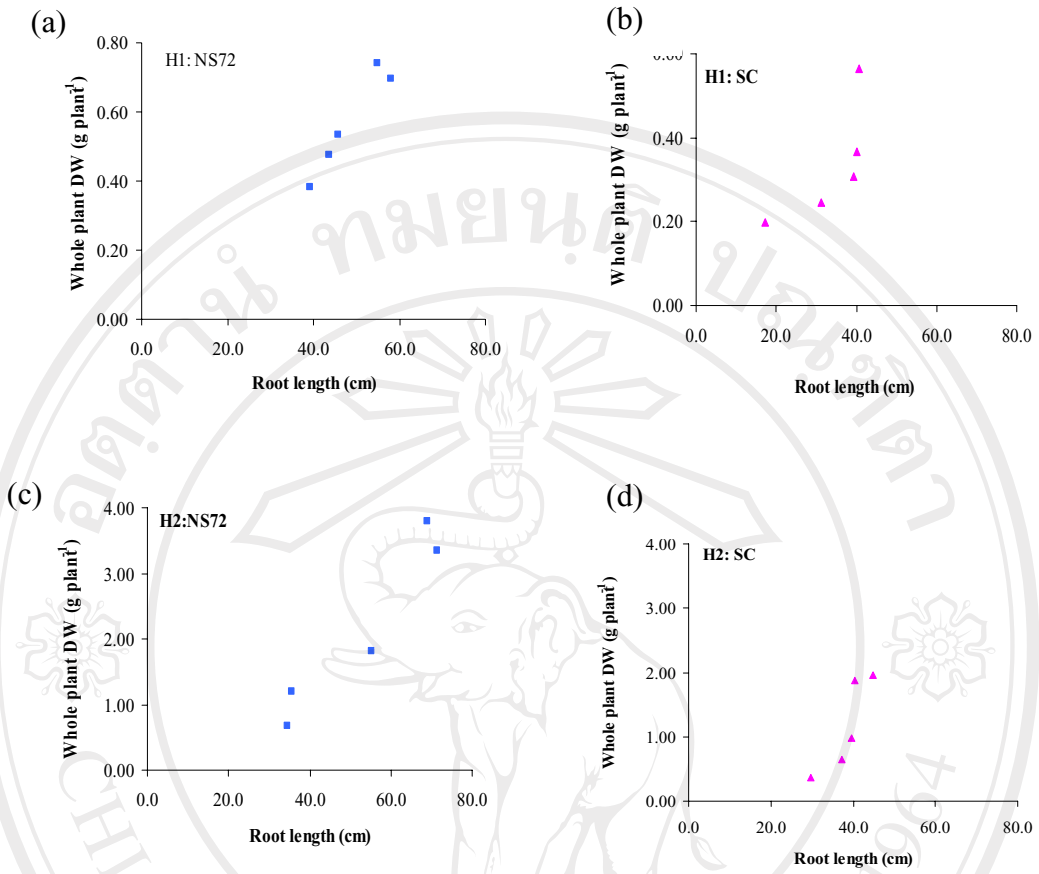


Figure 3.13 The relationship between root length (cm) and whole plant (total+ root) dry weight (g plant^{-1}) of two maize genotypes (cv. NS72 and SC) grown in nutrient solution for 10 (H1) and 18 days (H2).

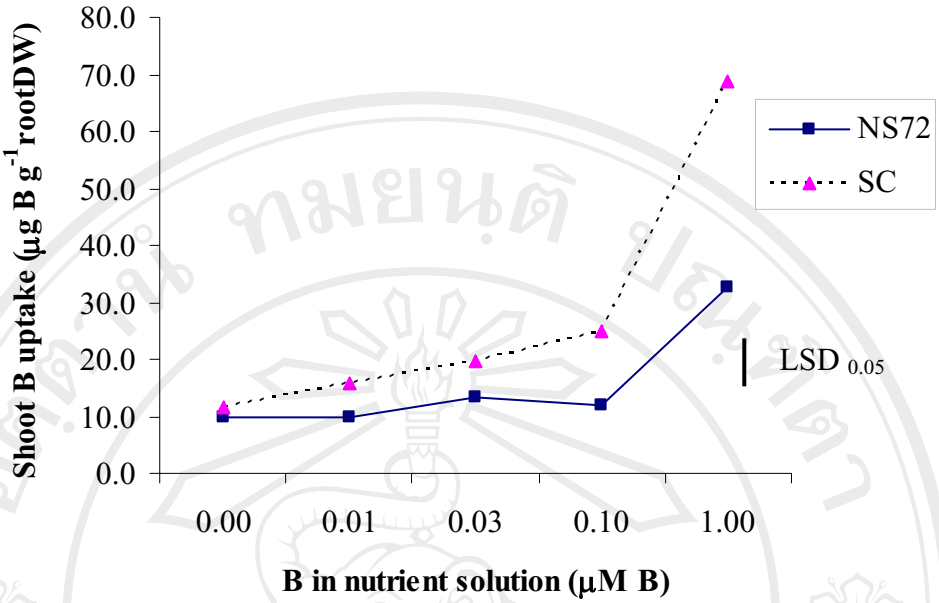


Figure 3.14 The relationship between B uptake in shoot (µg B g^{-1}root DW) and B concentration in nutrient solution (0, 0.01, 0.03, 0.10 and 1.00 µM B) of two maize genotypes (cv.NS72 and SC) at day 10 (H1).

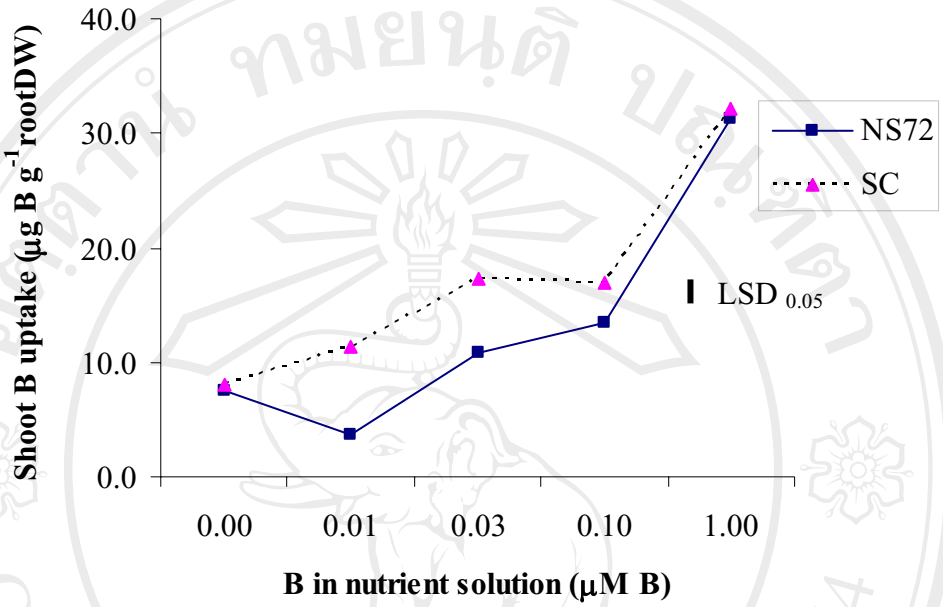


Figure 3.15 The relationship between relative shoot dry weight (relative shoot dry weight: shoot DW in each B levels/shoot DW of B1) and B concentration in nutrient solution (0, 0.01, 0.03, 0.10 and 1.00 µM B) of two maize genotypes (cv.NS72 and SC) grown for 10-18 days (H2).

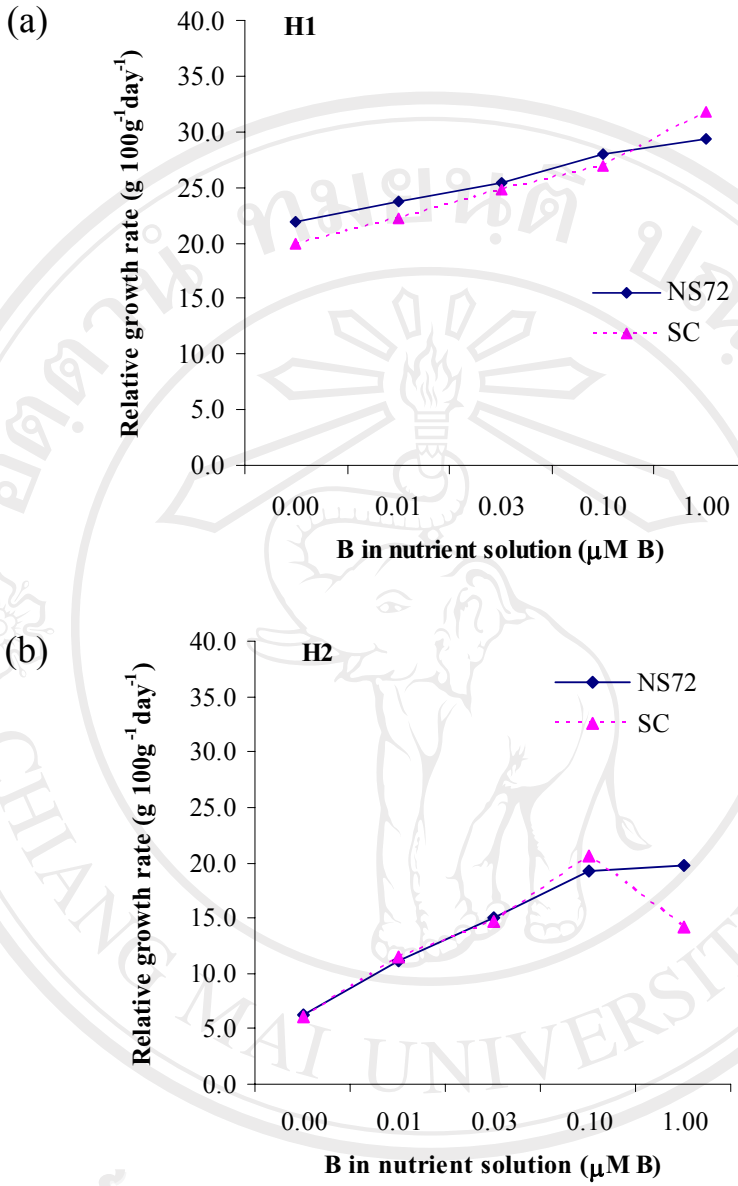


Figure 3.16 Relative growth rate (g $100g^{-1}day^{-1}$) of two maize genotypes (cv. NS72 and SC) grown in nutrient solution (0, 0.01, 0.03, 0.10 and 1.00 µM B) for 10 (H1) and 18 days (H2).

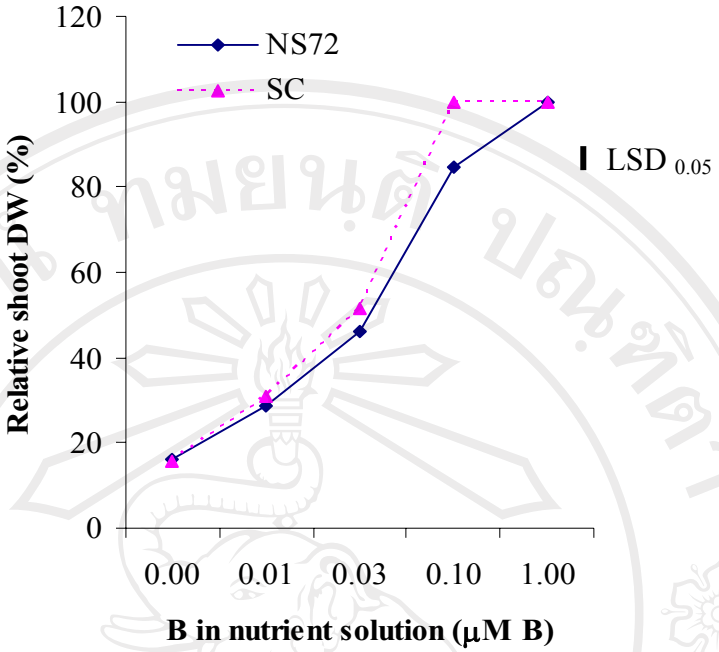


Figure 3.17 The relationship between relative shoot dry weight (dry weight of each B level divided at B1) and B concentration in nutrient solution (0, 0.01, 0.03, 0.10 and 1.00 µM B) of two maize genotypes (NS72 and SC) grown for 18 days (H2).

3.3.2 Experiment 2: Manual pollination between B0 xB20 maize plant

The adverse effect of B deficiency on grain set and grain yield of maize has been shown by cross-fertilization to be primarily through the silk (Figure 3.18). Boron sufficient pollen applied to B sufficient silk (SB x SB: designates silk x pollen) produced complete grain set with 452 grains plant^{-1} (Table 3.9). When B sufficient silk was pollinated with pollen from B deficient plants (BS x BD) 169 grains plant^{-1} were produced; but when B sufficient pollen was applied to B deficient silk (BD x BS) only 2 grains plant^{-1} (0.4%), which was almost the same as when B deficient pollen was applied to B deficient silk (BD x BD). This demonstrates that silk B at 4 mg B kg^{-1} is clearly limiting the fertilization process even with pollen from healthy plants.

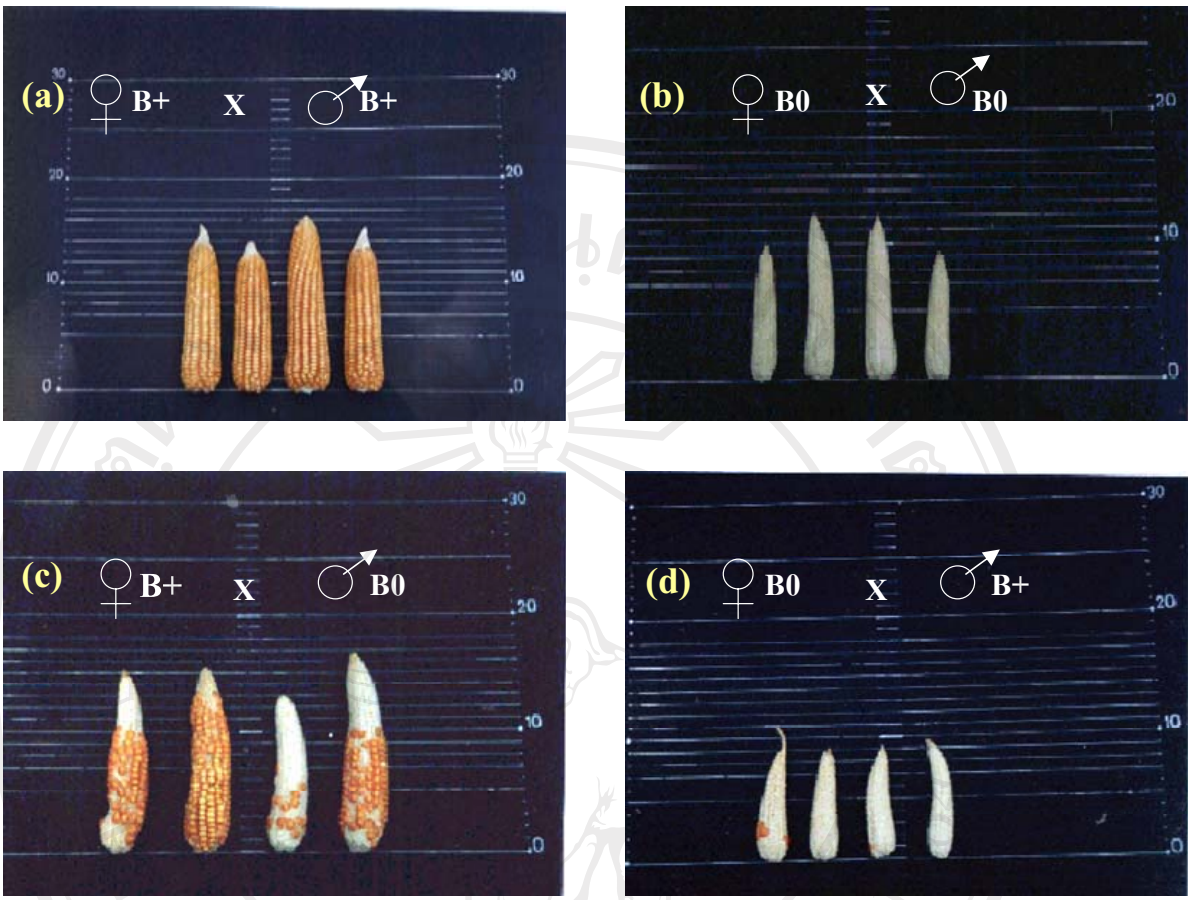


Figure 3.18 Effects of B on grain set in maize (cv. NS72): a) B20xB20; b) B0xB0; c) B20xB0 and d) B0xB20. Female (♀) and male (♂) is silk and pollen respectively, B+ is represented B-sufficient plant (B20) and B0 is B-deficient plant.

Table 3.9 Grain set by crossing pollination of pollen and silk from B deficient plants (DB) and sufficient plants (SB) of NS72.

Cross	**Grain no.** ($plant^{-1}$)	**Grain set** (%)
Female x Male [a/]		
SB x SB	452.0	100.0
SB x DB	169.0	37.4
DB x SB	2.0	0.4
DB x DB	0.0	0.0

[a/] Female and male is silk and pollen respectively ; SB is represented B- sufficient plant (B20) and DB is B-deficient plant (B0).

3.3.3 Experiment 3: Effect of B deficiency on anatomy and morphology of the silk and pollen

In previous experiment, B-deficient maize was associated with malformation of some reproductive parts, such as showing 3-5 small abnormal ears, with short silks, at the first ear node (Figure 3.9). This experiment further examines effects of B deficiency on anatomy and morphology of the silk and pollen.

The SEM images showed different effect of B deficiency on the silk located at different position on the pre-anthesis female flower of maize, or young ear. The silks located at the tip of young ear were thinner in B0 (Figure 3.19, a) than in B20 (Figure 3.19, b). However, the silk located at the middle and basal parts of the young ear did not appear to differ between B0 and B20, both of which quite hairy (Figure 3.19, c and d). Moreover, silk tip from the top of young ear (same located at figure 3.19, a and b) in which the starch accumulation of B0-silk (Figure 3.20, a) was similar at B20-silk (Figure 3.20, b), however some cells was collapsed (a) compared with the B20-silk (b).

In male flower development with anther or pollen grains, cross section of anther showed no starch was observed in pollen grains and anther wall in B0-plants (Figure 3.21, a) where as starch deposited (PAS staining reagent) in pollen grains and anther wall (endothecium) of B20 plant (Figure 3.21, b). Starch accumulation (PAS and TBO staining) was also observed in the connective tissue, starch deposited (starch granule) was showed in the B20 anther (Figure 3.22, b) but not in B0-anther (Figure 3.22, a). Moreover, development or amount of vascular tissue of the B0-anther was less than B20-anther (Figure 3.22, b). In case of pollen grain, floret site was also tested pollen fertility by iodine staining. Because of iodine staining (dark: starch

deposit) from B20 (Figure 3.21, a) and B0-maize plants could be stained with iodine and some pollens remained transparent are classified dead or aborted. Apart from B20 and B-deficient plant (B0) had a highly significant stained-pollen (Table 3.10), the position of floret differed in starch deposited (Table 3.10) in which the pollens at the lower floret (F2) were totally absent (100% sterility, lacked starch deposit) (Figure 3.23, c) whereas in the upper position were 44.7% sterile. However, pollen of the B20-maize plant was mainly fertile (Table 3.10).

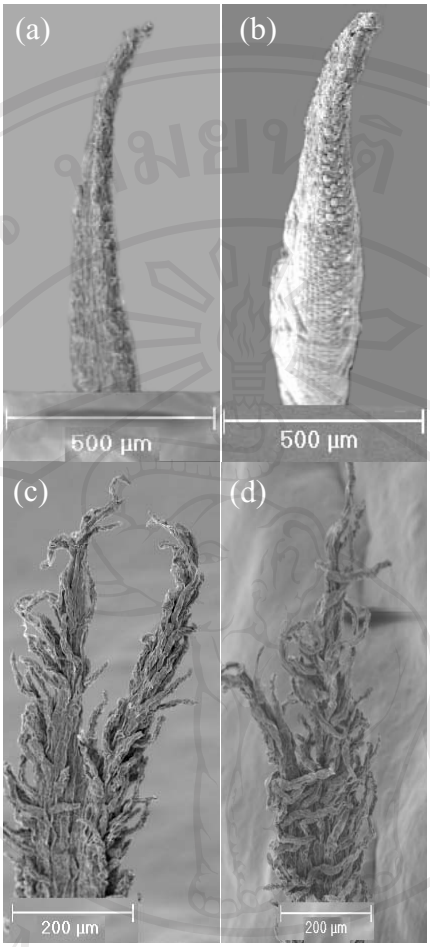


Figure 3.19 SEM showed a silk from the upper (a=B0 and b=B20) and lower part (c=B0 and d=B20) of young ear at anthesis stage. Clear effect of B on the silk, a thin silk in B0 (a) and a thick silk (b). No effect of B on the lower silk (c and d).

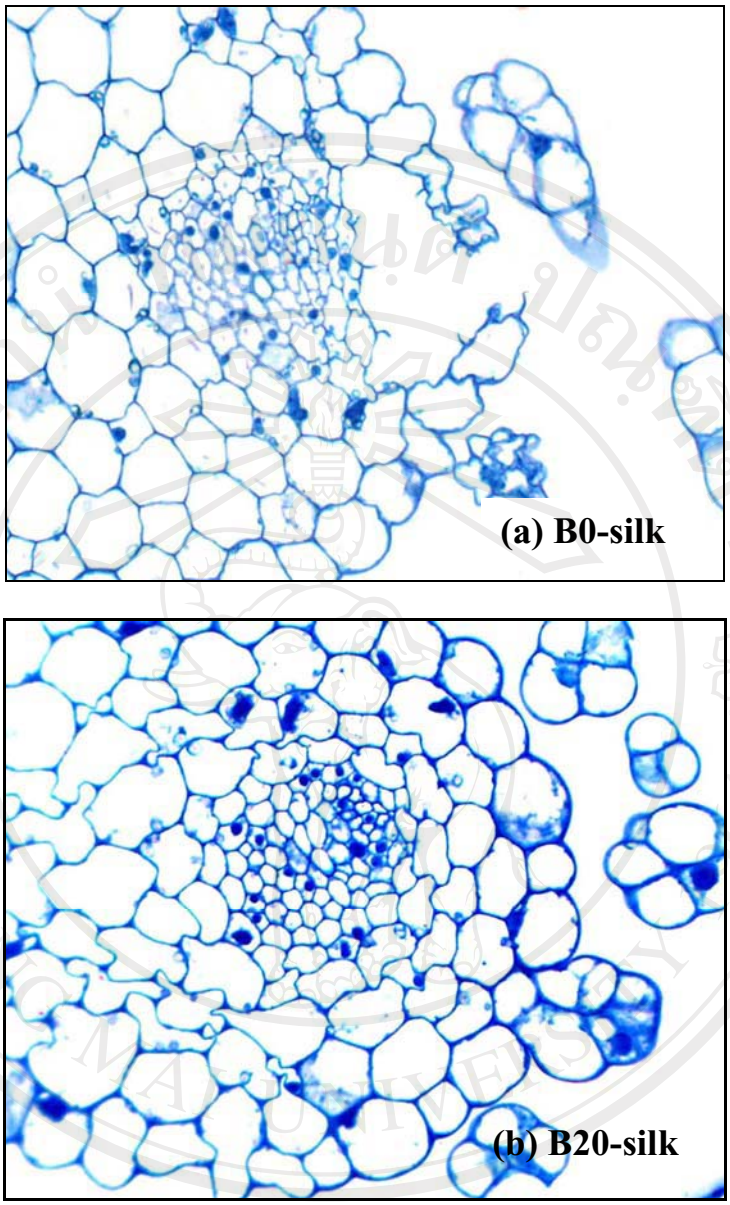


Figure 3.20 Cross section of silk tip from top of young ear stained with PAS- TBO to indicate the accumulation of starch of B20-maize plant (b) and the B-deficient maize plant (a). Mag. 40 x 1.25.

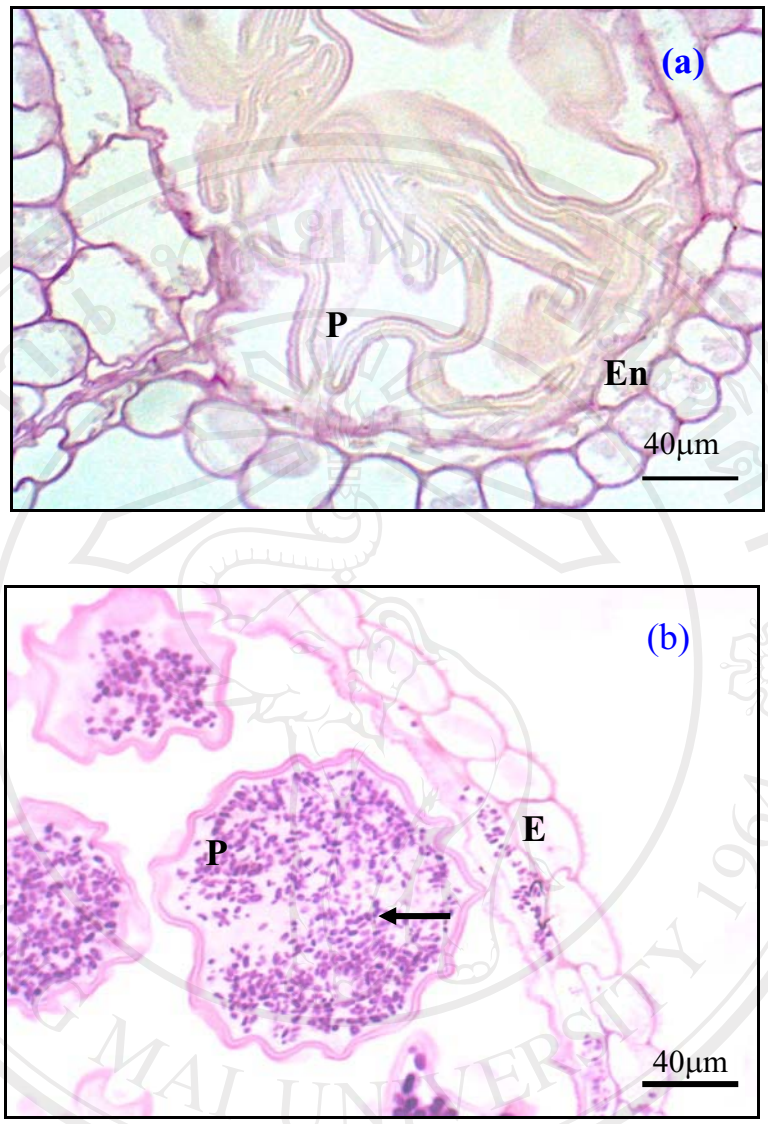


Figure 3.21 Cross section of anthers stained with PAS to indicate the accumulation of starch (arrow) in pollen grains and anther wall of B20-maize plant (b) and the absence of starch in B-deficient maize plant (a). Abbreviations, E: epidermis En: endothecium and P: pollen grain. Arrow indicated starch deposition.

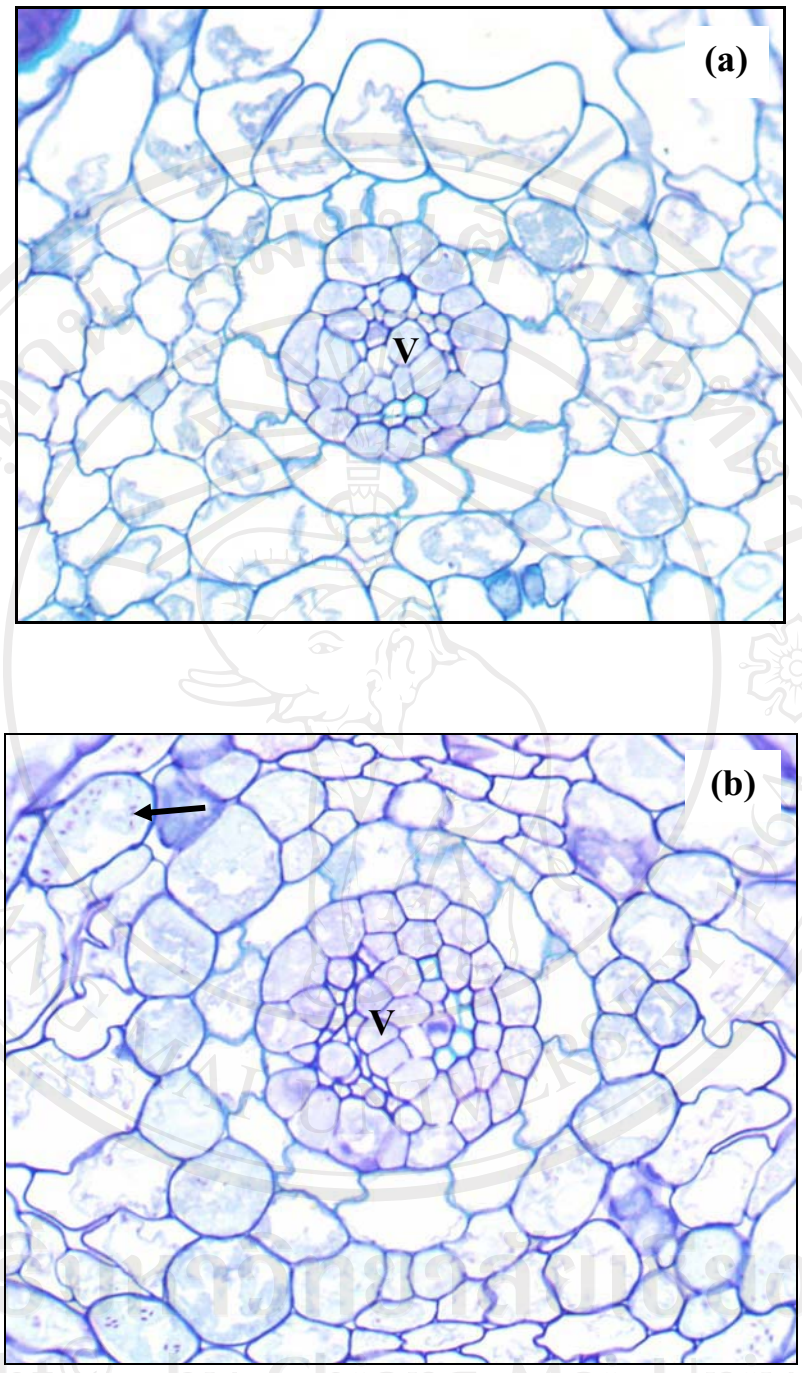


Figure 3.22 Cross section of connective tissue of anther stained with PAS and TBO to indicate the accumulation of starch in B20-maize plant (b: B20) and B-deficient maize plant (b: B0). Abbreviations, V: vascular cell. Arrow indicated starch deposition. Mag. 40 x 1.25.

Table 3.10 Pollen sterility (%) of maize (cv.NS72) determined by iodine staining.

Boron level (μM)	Floret [1/]	
	F1	F2
0	44.7b	100.0a
20	1.0c	2.9c
F-test	B*** F***	BxF ***
$LSD_{0.05}$	4.8 2.7	3.9

Numbers followed by the same letter do not significantly differ at 5%.

[1/] These staminate flowers were taken from central part of main axis of tassel at pollen shedding. Each of tassel contains numerous spikelets, each of which have two staminate flowers, including F1 is the upper flower and F2 is the lower flower.

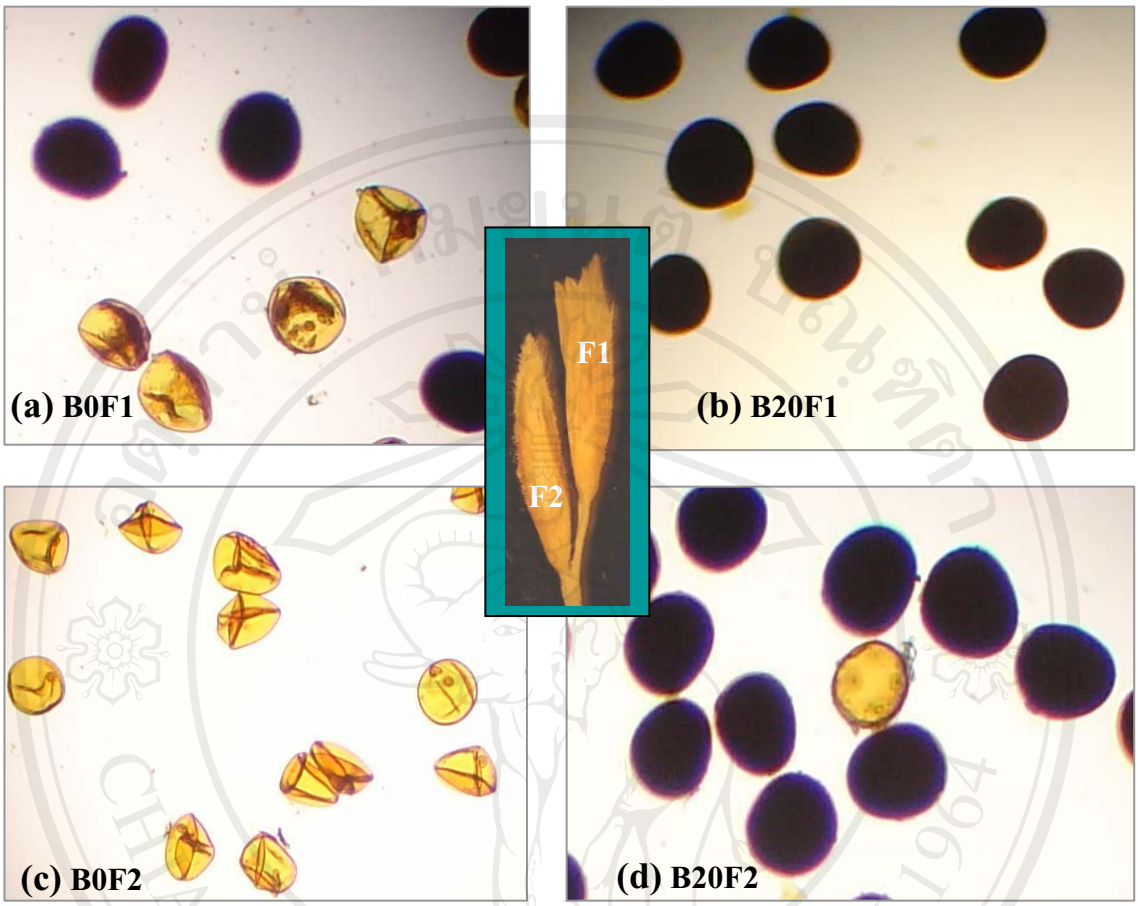


Figure 3.23 Pollen stained with iodine staining solution. Stained pollen grains were black (starch deposit) and not stained with iodine solution were yellow (starch absence), F1: the upper floret and F2: the lower floret of each spikelet from main axis of tassel.

3.3.4 Experiment 4: pollen viability/germination in media

This experiment showed that external supply of B is essential for maize pollen germination (Table 3.11). Pollen grains from B0 plants germinated only 5.5 % in germinating media without added B, and adding B to the germinating media increased germination to 25.5%. Normal pollen grain from B20 plants (see above section) germinated only 19% without B added to the germinating media, only when B was added to the germinating media was germination increased to 97.5%. The B0 plants that produced these B deficient silk and pollen with impaired functions produced silk and tassel that were only a fraction of their dry weight in B20, 16% for silk and 61% for tassel. There was no dry weight difference between B0 and B20 plants in shoot, root, and all other plant parts (Table 3.12).

The lower dry weight of tassel in B0 was associated with fewer pollen per anther (Table 3.13). There were 1386 pollen grains per anther in B0, compared with 3000 in B20. Three quarters of the B0-pollen showed absence of starch deposit by not staining with iodine solution, while the starch deposit was present in 81% of the pollen from B20 (Table 3.13). However, there was no significant difference in the number of floret per plant, tassel length, the number of branches of tassel and days to tasseling between B0 and B20. The much lower silk dry weight in B0 was also associated with much fewer silk threads per ear, only 30 in B0 and 206 in B20. The dry weight of young ear and husk, however, were not significantly different between B0 and B20.

The concentration of B in B0-pollen grains was 2.8 mg B kg^{-1} and in the B0 silk was 4.3 mg B kg^{-1}, which were only about one third of the B concentration in these tissues in B20 (Table 3.14). The same or even greater order of magnitudes of

difference in B concentration between B0 and B20 was also found in the tassel, ear leaf, flag leaf and whole shoot. The B concentration in young ear, husk and shank were also significantly lower in B0 than B20, though much less pronounced. The root B concentration did not differ significantly. The content of B in all parts was also in B0 than in B20, although the difference was not significant in young ear, shank and (Table 3.15). The ratio of B content in shoot: root was 17 in B20, and only one quarter of that in B0.

Table 3.11 Pollen germination (%) in media with and without added B.

Added B	Pollen germination in media (%)	
(μM B)	Without added B	Added B
B0	5.5c	25.5b
B20	19.3b	97.5a
F-test	***	
LSD $_{0.05}$	6.3	

*** significant at $P < 0.001$.

Number followed with the different letters indicated significantly different by LSD ($P < 0.05$)

Table 3.12 Effects of B deficiency on dry weight (g plant^{-1}) in various plant parts of maize and shoot: root dry weight at maturity.

Plant part	Added B (μM)		LSD $_{0.05}$
	0	20	
Tassel	2.78	4.56	1.0**
Flag leaf	0.39	0.45	NS
Ear leaf	3.54	3.60	NS
Silk	0.14	0.87	0.2***
Young ear	2.86	2.67	NS
Husk	14.35	20.85	NS
Shank	3.03	3.87	NS
Shoot	167.48	183.78	NS
Root	24.72	30.22	NS
Straw: root ratio DW	6.7	6.3	NS

NS = not significant ** and *** significant at $P < 0.01$ and 0.001, respectively.

Table 3.13 Effects of B deficiency on B concentration (mg B kg^{-1} DW) in various plant parts of maize (cv. NS72) at pollen shedding.

Plant part	Added B (μM)		
	0	20	$LSD_{0.05}$
Pollen	2.8	7.3	1.2***
Tassel	4.7	18.0	3.0***
Flag leaf	5.1	35.8	6.3***
Ear leaf	5.3	28.5	5.7***
Silk	4.3	17.7	3.0***
Young ear	3.8	6.7	1.2***
Husk	3.1	5.1	1.1**
Shank	4.1	5.7	0.8**
Shoot	4.2	16.9	0.8***
Root	4.5	5.6	NS

NS = not significant ** and *** significant at $P < 0.01$ and 0.001, respectively.

Table 3.14 Effects of B deficiency on B content (μg B plant^{-1}) in various plant parts of maize (cv. NS72).

Plant part	Added B (μM)		LSD $_{0.05}$
	0	20	
Tassel	13.2	83.5	27.2***
Flag leaf	2.1	15.5	5.1***
Ear leaf	20.1	101.9	239.0***
Silk	0.6	14.2	3.4***
Young ear	10.9	17.0	NS
Husk	47.9	100.9	43.3*
Shank	14.6	21.5	NS
Straw	443.1	2861.7	789.0***
Root	117.1	171.7	NS
B content of whole: root ratio	3.8	17.0	2.7**

NS = not significant * and *** significant at $P< 0.05$ and 0.001 respectively.

Table 3.15 Effects of B deficiency on reproductive development of maize (cv. NS72).

Plant part	Added B (μM)		
	0	20	$LSD_{0.05}$
Tassel length (cm)	39	44	NS
The number of floret $plant^{-1}$	1109	1208	NS
The number of branch of tassel $plant^{-1}$	18	18	NS
The number of pollen $anther^{-1}$	1386	2999	1268*
Pollen KI/I_2 positive (%)	24.1	81	27**
Plant height (cm)	142	163	NS
Silk number ear^{-1}	30	206	125*

NS = not significant * and ** significant at $P< 0.05$ and 0.01 respectively

3.5 Discussion

3.5.1 Plant growth and boron deficiency symptom

Description of B deficiency symptoms on maize leaves presented in this thesis is the most comprehensive among the very rare report of B deficiency on vegetative growth of maize that is available in the literature. The leaf symptoms described, 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 agrees with the requirement for (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 Borax, 2007). 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 exhibits 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. It has been previously reported that in maize the upper floret tend to develop more full than the lower floret (Martin et al., 1976).
- Boron deficiency symptoms were associated with tassels that contained 5 mg B kg^{-1} DW and pollen that contained 3 mg B kg^{-1} DW, whereas normally function tassels contained 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 18 mg B kg^{-1} DW.

3.5.2 Comparing effects of B deficiency on the male and female flower

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. 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, 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), the next set of experiments will evaluate genotypic variation response to low B

in maize order to get some information in which may be useful in maize breeding program on low B soils.