CHAPTER 2

BORON DEFICIENCY IN MAIZE

2.1 Introduction

Maize (*Zea mays*, L.) is spread to the rest of the world after European contact with the Americas in the late 15th century and early 16th century. It is widely cultivated throughout the world, and one of the most productive food plant in which being the leading cereal in term of world production. Total world maize production was over 600 million tons. It is multi-used use plant that can be as food at various stage of the development of plant from young to maturity (Chapter 1). World population in present is approximately 6000 millions and is expected to reach 8000 millions in 2030. About 2000 millions are increasing in developing countries in which tropical maize will continue to play a major role to feed as the increasing population and the demand for maize will continue to increase in the future (FAO, 2000). Maize is an important cereal for human consumption in particular in Africa and Latin America. In many Asian countries, the demand for maize as animal feed will continue grow very fast rate. The demand for feed will reach approximately 400 million tons in 2030 compared with 165 million tons in the present levels. This a big challenge to produce enough of maize for human consumption and animal feed. It is very difficult to increase the area in some countries under cultivation and maize production from increased productivity and intensification of cropping system. Most of maize-growing areas are located under unsuitable condition i.e. infertile soil, produced low yield. Most grain yield of maize in Africa is lower 2 tons ha^{-1} compared with 3.41 tons ha^{-1} of the word average maize yield (FAO, 2005). Maize yield is low when plants grown in infertile soils with low boron (B) in soil or B potential to be limiting yield (Sherrocks, 1997). Early work by Vaughan (1977) in Africa demonstrated that poorly-grain set came from B deficiency in silk associated with non- receptive of these silks did not fertilized of pollen from high-B plant and suggested that the concentration of B in all plant parts was about 3 ppm necessary for growth and normal function of silk. The application of B in some soil can increased maize yield about 9% (Chapter 1). By contrast, B application on a low B soil resulting increased grain yield about two times in wheat and barley (Rerkasem and Jamjod, 1989). B concentration below 4 mg B kg^{-1} in barley was associated with grain reduction in barley (Jamjod and Rerkasem, 1999). Boron requirement for reproductive development in wheat had critical concentration of B in anthers was 10 mg B kg^{-1} and in carpel was 8 mg B kg^{-1}. Moreover, grain set was closely correlated with the concentration of B in both (Rekasem *et al.*, 1997). With knowledge of B response of maize is little. Therefore, the present experiment was set out to evaluate the level of B application and to explore whether B concentrations in some plant parts can predict grain yield. B uptake and distribution in maize grown in sand culture without and with added B were determined.

2.2 Materials and methods

These experiments were conducted from November 2001 to March 2002 at Multiple Cropping Center, Chiang Mai University, Thailand. This chapter is made up of two experiments. First experiment was conducted in field and another was in sand culture.

2.2.1 Experiment 1 : Response of maize to soil B application

Maize (*Zea mays* L.) cv. NS72 were grown on a sandy loam of the San Sai series of the Typic Torpaqualf. The design was based on a RCB model with four replicate plots. Each replicate were set in 3 m wide and 9 m long plot. Treatments consisted of applying lime at 1000 kg ha^{-1} (BL) and borax ($Na_2B_4O_7.10H_20$) at rates of 0, 5, 10, 20 and 30 kg ha^{-1} (designated as B0, B1, B2, B3 and B4), respectively. Maize (*Zea mays* L.) cv. NS72 were sown at 0.4 x 0.4 m spacing. Basal fertilizers were banded at the rate of 50 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1} as 16-20-0 and 50 kg K ha^{-1} K as muriate of potash. Urea at the rate of 100 kg ha^{-1} were re-applied after the first harvesting. Plants were harvested at three development stages: during vegetative development at the 5-leaf stage, anthesis, and at maturity. Plants were separated into the YEB (youngest emerged leaf blade),YEB+1 (next leaf older than the YEB), YEB-1 (next leaf younger than the YEB), rest of tops and ear from each plant, dried at 75 °C to constant weight then B were analysed by dry ashing followed by the azomethine–H method (Lohse, 1982). At maturity, grain and straw dry weight were determined, yield components were obtained (e.g. the number of grain per ear and grain weight) and shoot and leaf B concentrations will be measured.

2.2.2 Experiment 2 : Response of maize to two B levels in sand culture

The experiment was based on a randomized complete block (RCB) model with two treatments (2 B levels), three replicates and three harvests. Seeds of maize (*Zea mays* L.) cv. NS72 were sterilized with Na-hypochlorite (10% of the commercial product) for 15 min washed several times with tap water, then soaked in water at 50 °C for 30 min and placed on a moistened paper aluminum tray until germination (approximately two days). Germinants were transplanted to pots (0.30 m diameter and 0.30 m deep) filled with washed river quartz sand at 4 plants per pot. Pots were supplied twice daily with complete nutrient solution, adapted from Broughton and Dilworth (1971) and Mozafar (1989), with two levels of B (0 and 20 μM B: B0 and B20). The complete nutrient solution were consisted of KNO_3 15000 μM; $CaCl_2$ 1000 μM; $Mg(SO_4)_2.7H_2O$ 2000 μM; KH_2PO_4 1000 μM; Fe-EDTA 100 μM; K_2SO_4 250 μM; $MnSO_4.H_2O$ 9 μM; $ZnSO_4.7H_2O$ 0.76 μM; $CuSO_4.5H_2O$ 0.31 μM; $CoSO_4$ 0.1 μM and $Na_2MoO_4.2H_2O$ 0.1 μM respectively. All pots were flushed with water every week to avoid accumulation of salts in the sand. Plants were harvested during vegetative development at the 5-leaf stage and during reproductive growth at anthesis. They were separated into root, YEB, YEB+1, YEB-1, rest of tops and ear from each pot, dried at 75 °C to constant weight then B were analysed by dry ashing followed by azomethine–H method determination (Lohse, 1982). At maturity, grain and straw dry weight were determined, yield and yield components were recorded and analysed for B.

2.3 Results

3.3.1 *Experiment 1: Response of maize to soil B application*

The consideration of plant growth in term of dry weight accumulation, it was found that total shoot plant dry weight was not affected by B treatment at vegetative growth or 5-leaf stage (Figure 2.2, a), anthesis stage (Figure 2.6) and at maturity

(Table 2.3). However, examination of the dry weight in differ plant part revealed that of the younger leaf (YEB-1) had a tendency to increase in the dry weight when application of B at rate of 10 (B2) and 20 (B3) kg borax ha^{-1} (Figure 2.1).

The concentration of B in plant parts such as YEB-1, YEB and YEB+1 were increased significantly at the highest of borax application (30 kg ha^{-1}:B4) approximately 9.2-11.5 mg B kg^{-1} or in the average of shoot was 10.4 mg B kg^{-1} (Figure 2.3). There were no differed the concentration of B in shoot and these leaves at lower borax application except the YEB in which its B concentration increased with applied borax at rate of 20 (B3) and 30 kg ha^{-1} (B4) resulting increased from 5.2 mg B kg^{-1} to 6.8-9.2 mg B kg^{-1}. However, the B content in differ part or shoot was not affected by B treatment. For example, shoot plants contained B about 42.6-54.5 μg $plant^{-1}$ (Figure 2.4).

At anthesis stage, soil application of boron was only increased the dry weight of tassel. Maximum tassel DW was 11.49 g $plant^{-1}$ at the highest of B application (B4) whereas without B application was about 8.5-9.0 g $plant^{-1}$ (Figure 2.5). Boron treatments were effect on B concentration in the reproductive plant parts is showed in Figure 2.6. Plant tissues B increased with soil B application, the highest tissue B tissue was found at the highest of application of B. For example, in silks, flag leaf and tassel had 11.4, 9.8 and 9.0 mg B kg^{-1} compared with no supplied B plant had about 4.3-5.4, 6 and 4.9-5.0 mg B kg^{-1} respectively. There was highly a significant between B treatment in their effect on the content of B in some reproductive parts such as silks and tassel but not in flag leaf (Figure 2.8). At the highest of B supplied (B4), tassel B had approximately twice the boron content that of the lower than 20 kg borax ha^{-1} application.

Despite, B treatment was affects on the dry weight and tissue B of some plant parts, there were not affected on yield, plant height and yield components of maize at maturity (Figure 2.9). For example, the number of grain per ear of no-supplied B treatment (B0 and BL) was 501-507 in which similar to the highest of B application was 515. The grain size or 100 grain weight was about 24.1-27.6 g and grain yield was approximately 100-116g plant $^{-1}$ (6141-7241 kg ha^{-1}) at all treatment.

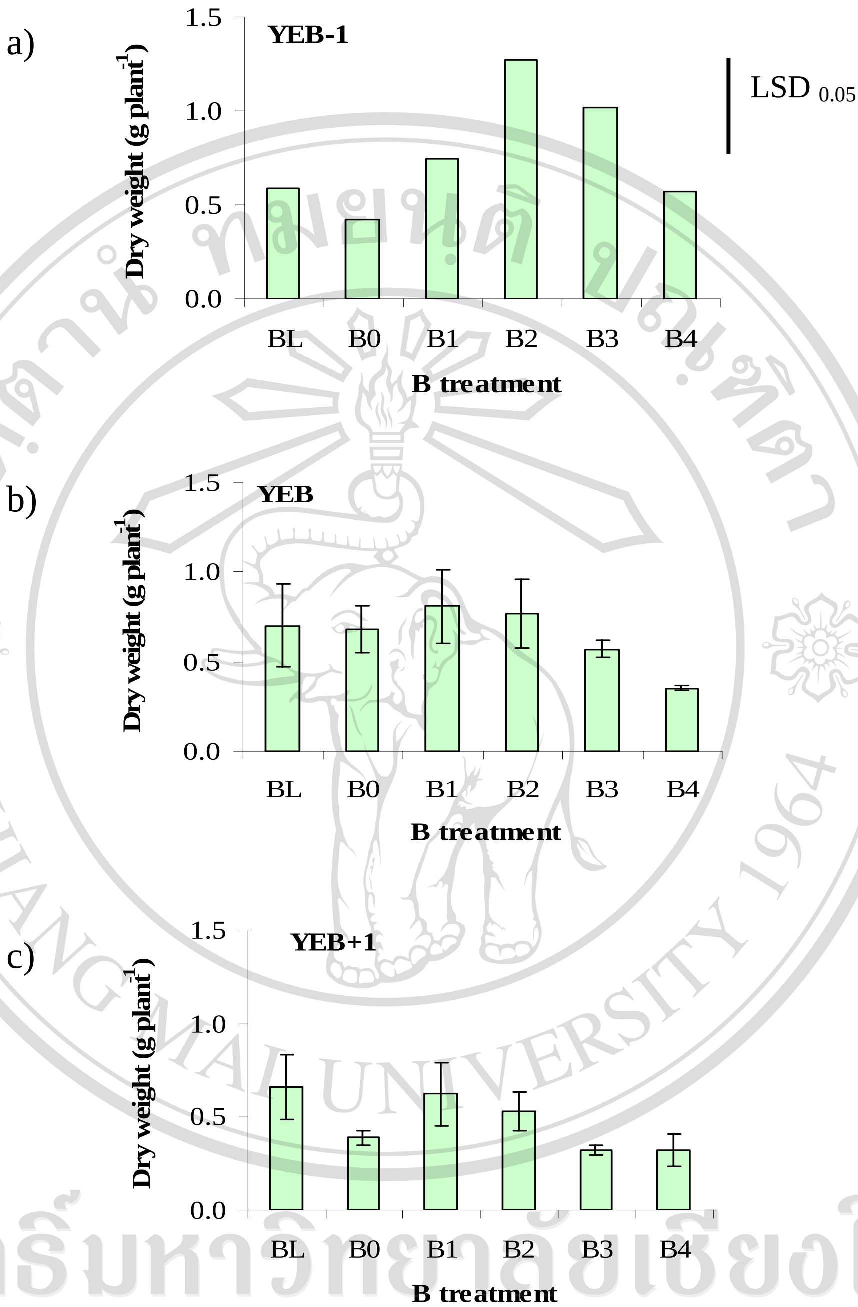

Figure 2.1 Dry weight (g plant^{-1}) in various plant parts of maize at 5-leaf stage grown in the field with limed soil (BL : 1000 kg ha^{-1}) and boron treated at 0, 5, 10, 20 and 30 kg borax ha^{-1} (designated as B0, B1, B2, B3 and B4), respectively. Bars are mean of four replicates ± SE.

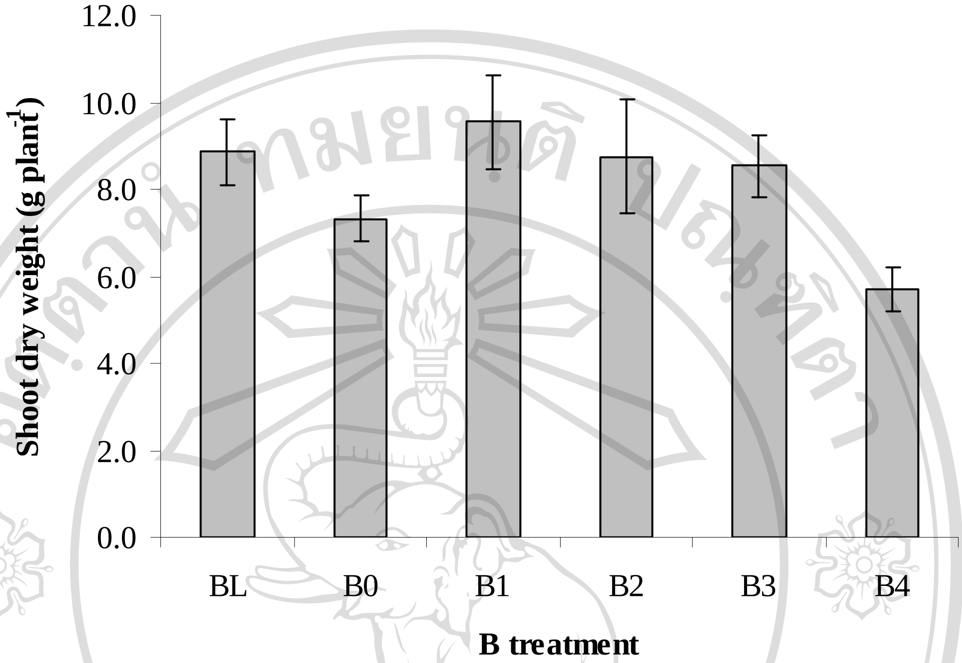

Figure 2.2 Total dry weight (g plant^{-1}) of maize at 5-leaf stage grown in the field with limed soil (BL : 1000 kg ha^{-1}) and boron treated at 0, 5, 10, 20 and 30 kg borax ha^{-1} (designated as B0, B1, B2, B3 and B4), respectively. Bars are mean of four replicates ± SE.

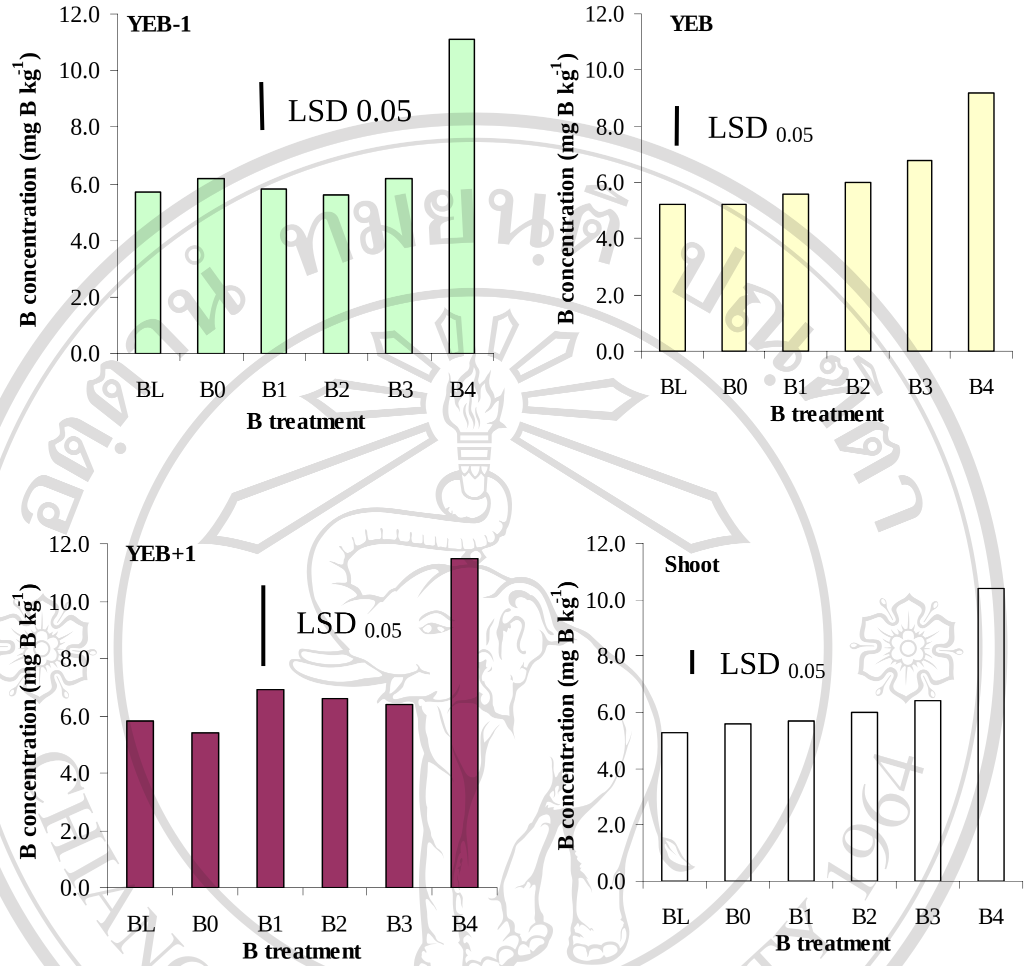

Figure 2.3 Boron concentration (mg B kg^{-1}) in various plant parts of maize at 5-leaf stage grown in the field with limed soil (BL: 1000 kg ha^{-1}) and boron treated at 0, 5, 10, 20 and 30 kg borax ha^{-1} (designated as B0, B1, B2, B3 and B4), respectively. Bars are mean of four replicates $\pm$ SE.

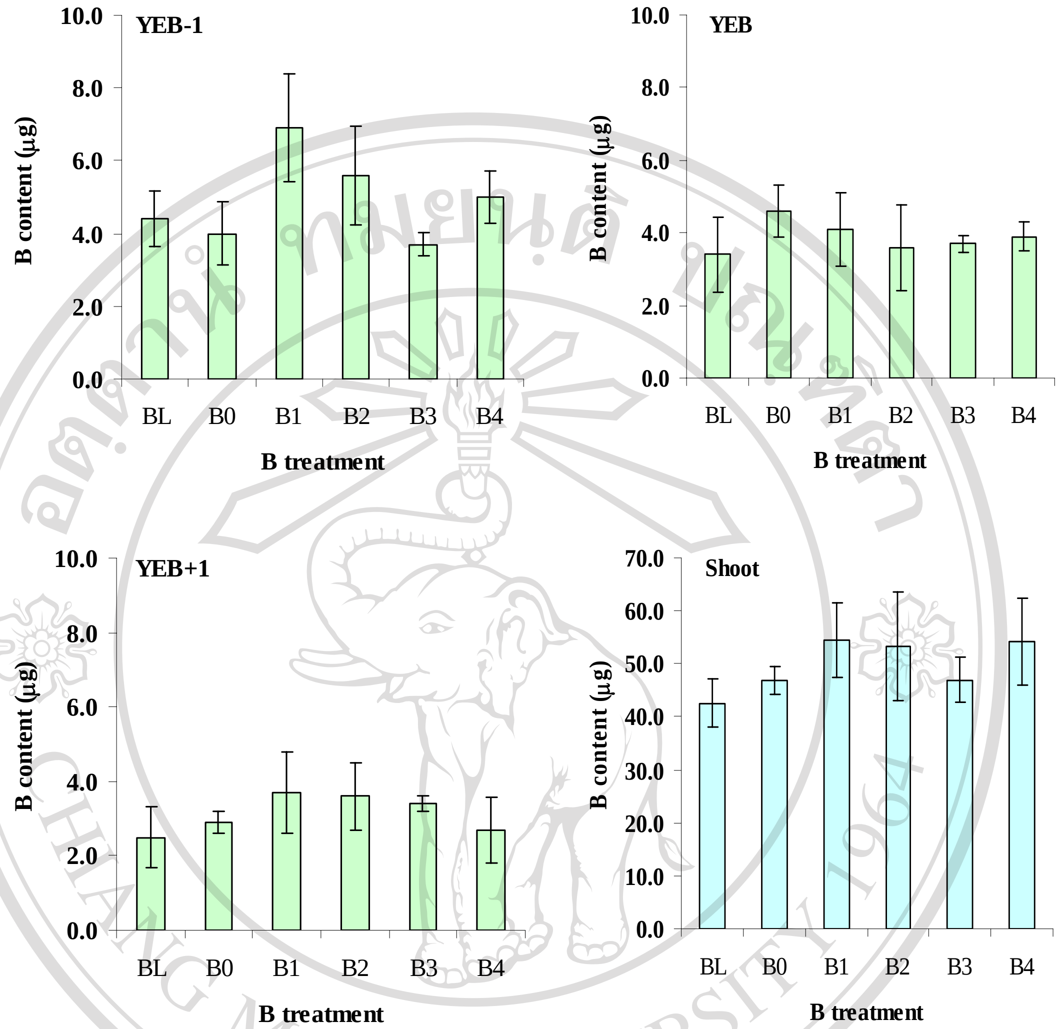

Figure 2.4 Boron content (µg plant^{-1}) in various plant parts of maize at 5-leaf stage grown in the field with limed soil (BL: 1000 kg ha^{-1}) and boron treated at 0, 5, 10, 20 and 30 kg borax ha^{-1} (designated as B0, B1, B2, B3 and B4) respectively. Bars are mean of four replicates ± SE.

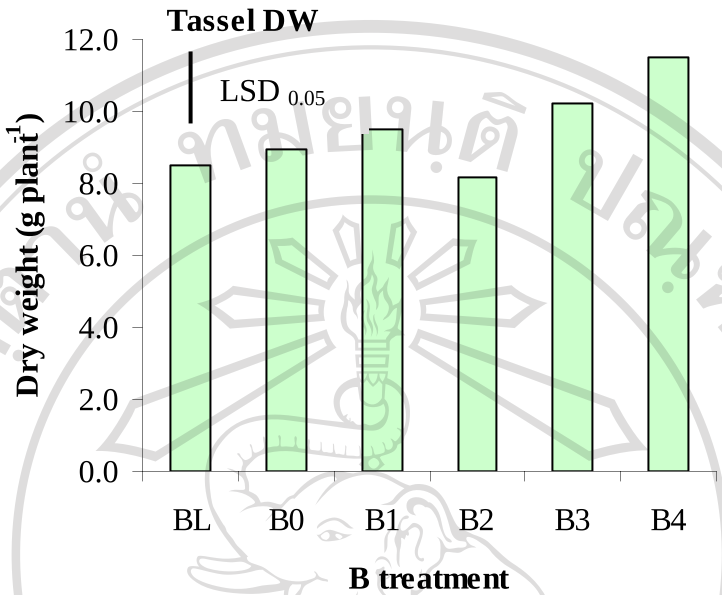

Figure 2.5 Tassel dry weight (g plant^{-1}) of maize at anthesis grown in the field with limed soil (BL: 1000 kg ha^{-1}) and boron treated at 0, 5, 10, 20 and 30 kg borax ha^{-1} (designated as B0, B1, B2, B3 and B4), respectively.

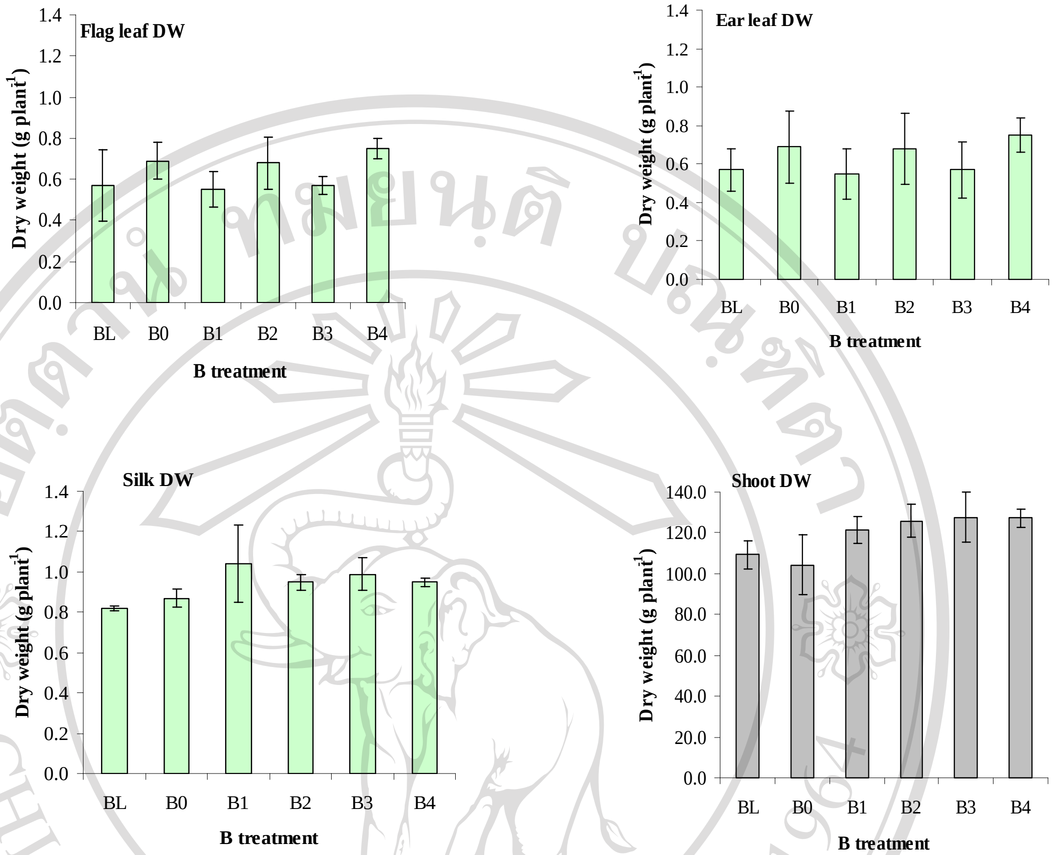

Figure 2.6 Dry weight (g plant^{-1}) in various plant parts of maize at anthesis grown in the field with limed soil (BL: 1000 kg ha^{-1}) and boron treated at 0, 5, 10, 20 and 30 kg borax ha^{-1} (designated as B0, B1, B2, B3 and B4), respectively. Bars are mean of four replicates ± SE.

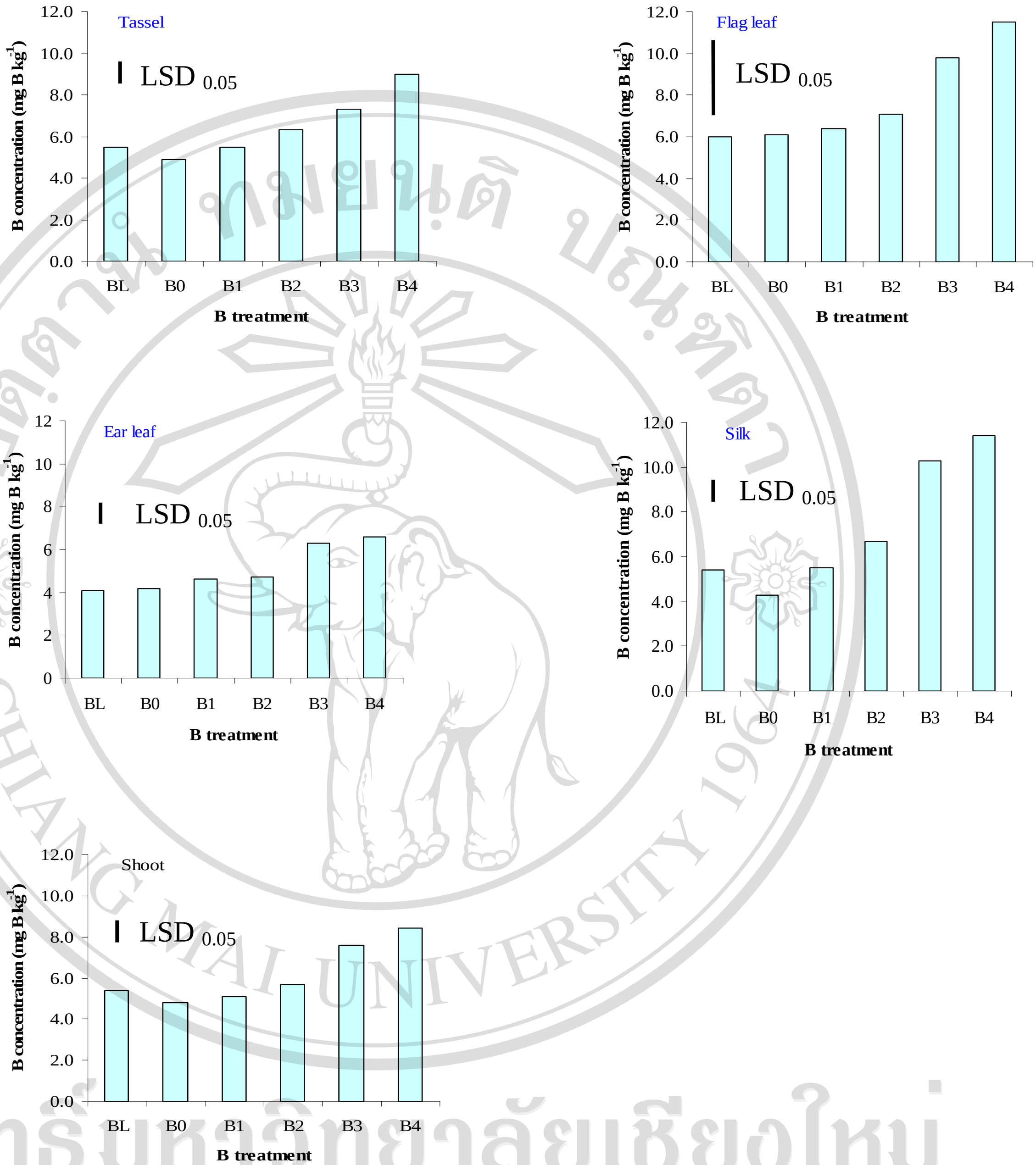

Table 2.7 Boron concentration (mg B kg^{-1}) in various plant parts of maize at anthesis grown in the field with limed soil (BL: 1000 kg ha^{-1}) and boron treated at 0, 5, 10, 20 and 30 kg borax ha^{-1} (designated as B0, B1, B2, B3 and B4), respectively.

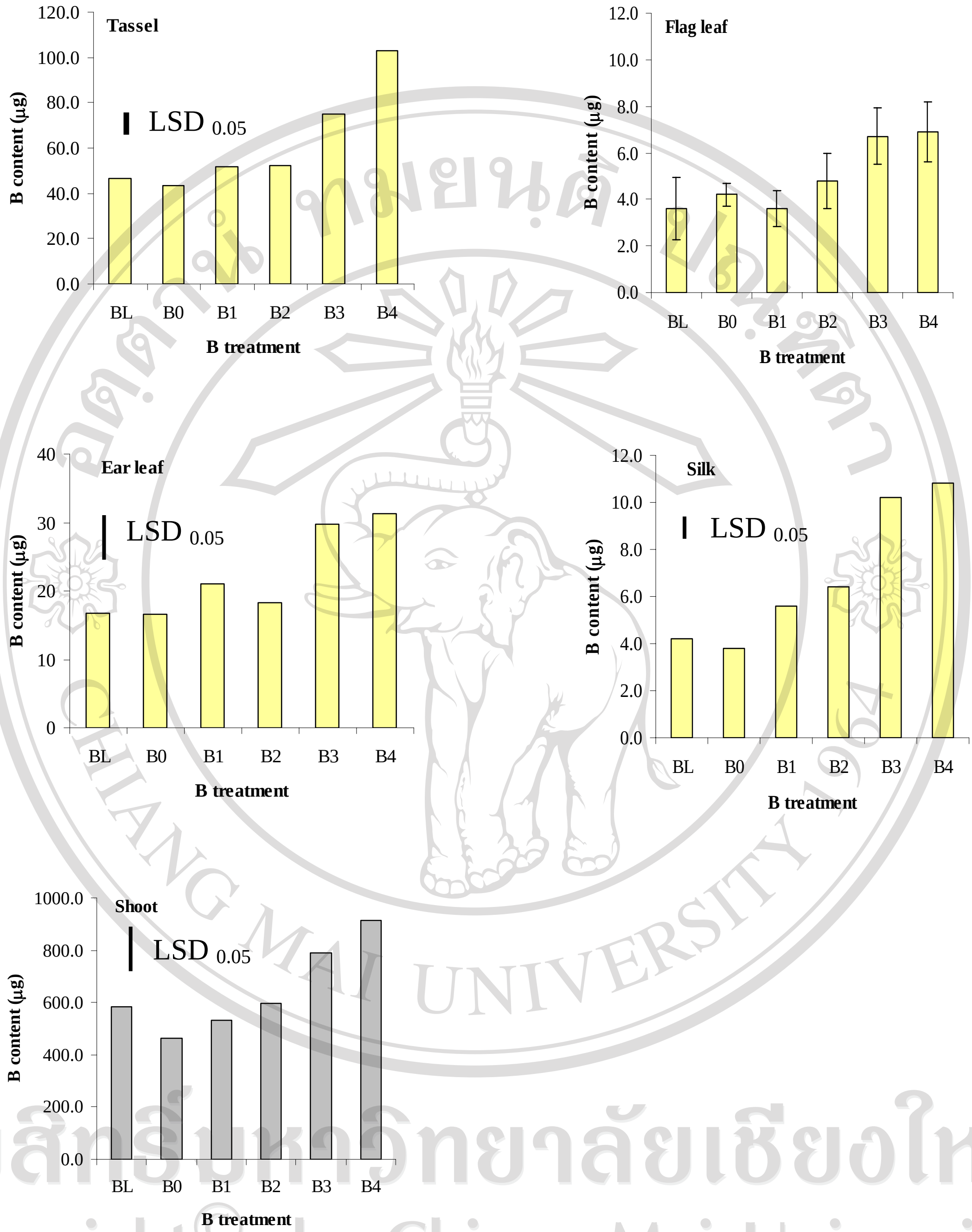

Figure 2.8 Boron content (µg B plant^{-1}) in various plant parts of maize at anthesis grown in the field with limed soil (BL : 1000 kg ha^{-1}) and boron treated at 0, 5, 10, 20 and 30 kg borax ha^{-1} (designated as B0, B1, B2, B3 and B4), respectively. Bars are mean of four replicates $\pm$ SE.

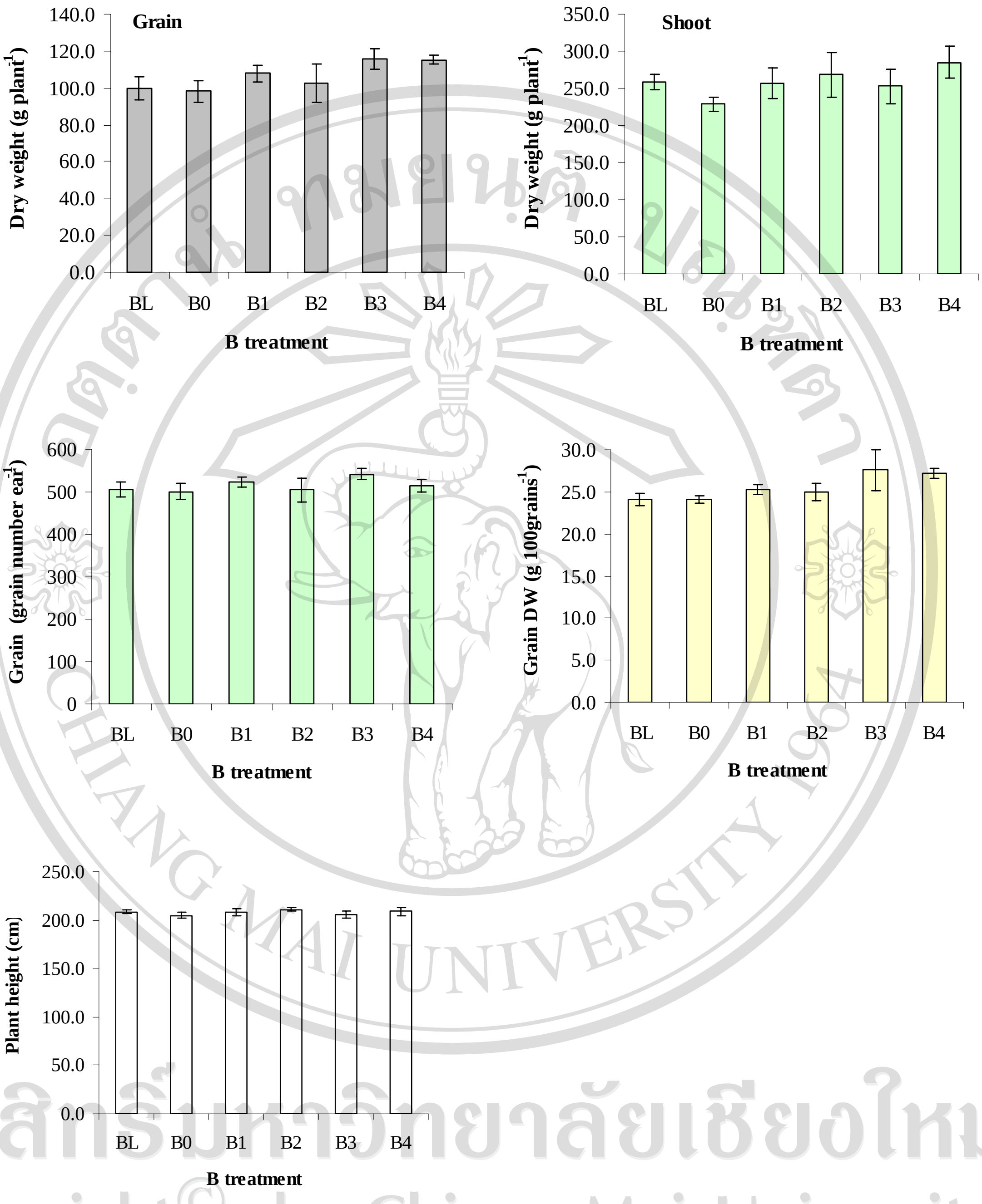

Figure 2.9 Grain yield and yield components of maize grown in the field with limed soil (BL: 1000 kg ha^{-1}) and boron treated at 0, 5, 10, 20 and 30 kg borax ha^{-1} (designated as B0, B1, B2, B3 and B4), respectively. Bars are mean of four replicates $\pm$ SE.

3.3.2 Experiment 2: Response of maize to two B levels in sand culture

At vegetative growth, there was no differences in total shoot dry weight or plant parts between B treatment (Figure 2.1). The dry weight of shoot per root ratio was similar between B levels was 1.8 in the absence of B (B0) and 1.9 in supplied B at 20 μM B (B20). There was highly a significant between B treatment in their effect on B concentration in above ground *i.e.* leaves and shoot but not in root. The concentration of B in plant parts increased with added B but not in root (Table 2.1). For example, B concentration in leaves (YEB-1, YEB and YEB+1) was about 5 mg B kg^{-1} of B0 and increased to about 7 mg B kg^{-1} in added B plants.

Boron treatment did not affect on the dry weight of baby corn and root at anthesis stage (tassel emergence). There was affected of B on the dry weight of tassel and silk (Table 2.7). Without added B, the dry weight of tassel and silk decreased from 5.81 and 1.48 g $plant^{-1}$ to 3.71 and 0.94 g $plant^{-1}$. This corresponded to the concentration of B in tassel and silk in which decreased from 8.1 and 15.4 mg B kg^{-1} to about 4mg B kg^{-1}. The absence of B also affected on the concentration of B in all plant parts but not root, for example, flag leaf and ear leaf B decreased from 9.8 to 5.1 mg B kg^{-1}. The content of B was increased with supplied B but not in root. Under B deficiency, among differed plant parts, the tassel greatest B content was about 14 μg B $plant^{-1}$ whereas silks contained only 3.8 μg B $plant^{-1}$. The B content of shoot per root ratio was not affected by B treatment.

The symptom of B deficiency, was appeared during reproductive stage at early tassel emergence, the primary symptom were small white spot and become to short white strips in upper leaf (Figure 2.11). Severely B deficiency symptom expressed during tassel emergence (Figure 2.12, a and c) until pollen shedding time resulted in transparent streaks on the upper leaf (Figure 2.12, c) and small tassel (Figure 2.13, c). In B0 tassel showed dead anthers and thin anthers (Figure 2.14, a and b) whereas B20-anthers were healthy (Figure 2.4, c). Moreover, some B0 plants produced more ear with short silks or no silk (Figure 2.13, a).

There was highly significantly between B levels on grain yield and the number of grain per ear but not the dry weight of cob, straw and root. B deficient plant decreased grain dry weight significantly to 0.3 g $plant^{-1}$ compared with 69.2 g $plant^{-1}$ of the added B plants (B20) (Table 2.8). Differences in grain yield between B treatment corresponded to the number of grain per ear. Under B0 deficiency, grain number decreased from 395 to 0.3 grains ear^{-1}or no grain.

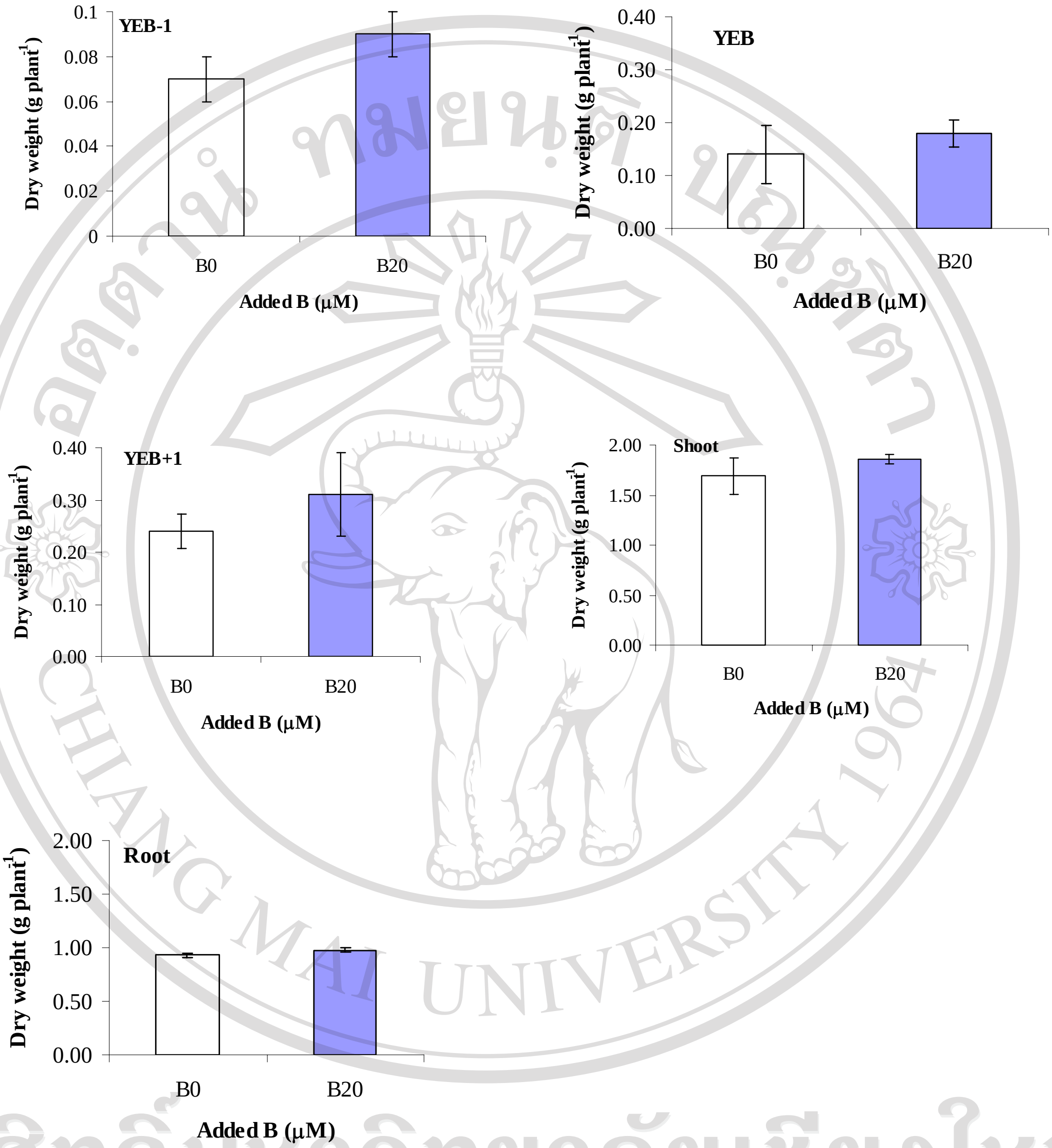

Figure 2.10 Dry weight (g plant^{-1}) in various plant parts of maize at 5-leaf stage grown in sand culture with and without added B. Bars are mean of three replicates ± SE.

Table 2.1 Boron concentration (mg B kg^{-1}) and B content (μg) in various plant parts of maize at 5-leaf stage grown in sand culture with and without added B.

Added B (μM)	Plant parts					shoot:root
	YEB-1	YEB	YEB+1	Total shoot	Root	
			B concentration			
0	4.9	4.5	5.0	4.7	6.6	
20	7.0	7.2	6.5	7.2	6.5	
F-test	**	**	**	**	NS	
$LSD_{0.05}$	2.5	1.5	1.4	1.4		
			B content			
0	0.33	0.6	1.2	7.8	5.8	1.4
20	0.67	1.3	2.0	14.5	6.4	2.3
F-test	*	***	**	***	NS	**
$LSD_{0.05}$	0.23	0.17	0.41	1.7		0.47

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

Table 2.2 Dry weight (g plant^{-1}), B concentration (mg B kg^{-1}) and B content (μg) in various plant parts of maize grown in sand culture with and without added B at anthesis stage.

Added B (μM)	Plant parts							
	tassel	silk	ear leaf	flag leaf	baby corn	shoot	root	Shoot: root
				Dry weight (g plant^{-1})				
0	3.71	0.94	2.53	0.70	0.10	67.43	23.78	2.8
20	5.81	1.48	2.48	0.84	0.16	72.22	22.10	3.1
F-test	*	**	NS	NS	NS	NS	NS	NS
LSD 0.05	0.82	0.35						
				B concentration (mg B kg^{-1})				
0	3.9	4.1	5.1	5.1	6.7	4.6	7.4	
20	8.1	15.4	9.8	9.8	13.7	9.4	9.0	
F-test	**	***	*	*	*	*	NS	
LSD 0.05	0.9	7.8	3.3	0.6	4.0	0.7		
				B content (μg plant^{-1})				
0	14.1	3.8	13.0	3.0	0.7	255.8	175.7	1.5
20	47.2	22.7	24.2	8.1	2.2	572.8	198.6	3.0
F-test	*	***	*	*	*	*	NS	NS
LSD 0.05	21.5	2.4	9.7	3.7	1.4	144.4		

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

Figure 2.11 Symptom of B deficiency of maize grown in sand culture without added B at anthesis.

Figure 2.12 Symptom of B deficiency of maize: white strips or transparent streaks on leaf lamina (c) at anthesis grown in sand culture without added B (a:B0) compared with added B (b:normal plants).

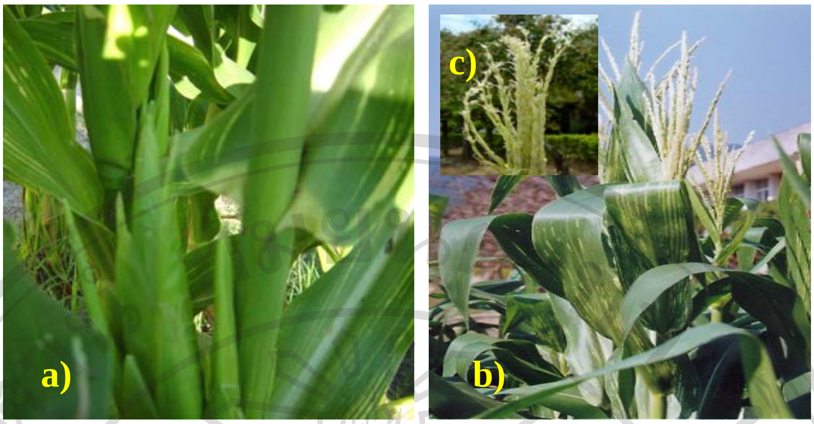

Figure 2.13 Boron deficiency symptom of NS72: multiple ears (a) and small tassel (b) at anthesis grown in sand culture.

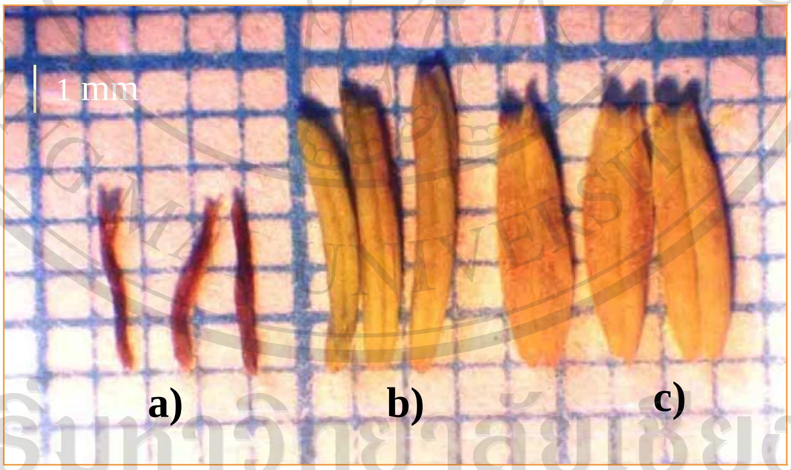

Figure 2.14 Anthers from B-deficient plant (B0): dead anthers (a)thin anthers (b) and normal plant (B20): normal anthers

Table 2.3 Yield, yield component, plant height and dry weight of shoot and root of maize grown in sand culture with and without added B.

Added B (μM)	Grain no ear^{-1}	Dry weight (g plant^{-1}) Grain	Cob	Shoot	Root	Plant height (cm)
0	0.3	0.3	7.6	129.6	32.2	210.3
20	395	69.2	17.4	133.4	23.6	214.0
F-test	***	**	NS	NS	NS	NS
LSD $_{0.05}$	57	30.3				

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

2.4 Discussion

The results of experiment 1 showed B treatment had no effect on plant growth. The addition of B resulted in increasing of B concentration and B content in some plant parts of maize. Soil-applied B was resulted increased in the dry weight of tassel, maximum tassel DW was 11.5 g plant^{-1} at the highest of B application (30 kg borax ha^{-1}: B4) whereas without B application was about 9 g plant^{-1}. The yield from this experiment indicated that grain yield was not affected by soil B treatment. The concentration of B in the ear leaf ranging from 4.1 to 6.6 mg B kg^{-1} at anthesis was adequate for plant growth in which corresponded with an adequate range of 4 to 26 mg B kg^{-1} was reported by Touchton and Boswell (1975).

By contrast, plants grown in sand, B had more effect in grain yield in which the addition of B produced 72 g plant^{-1} whereas in B-deficient plants almost no set grain. This effects corresponded to the number of grain per ear by decreasing from 395 of B20-plant to very few grains ear^{-1}or no grain at B0. As reproductive stage (tassel emergence), B in silk was about 4 mg B kg^{-1} nearly equal the concentration of B in both soil and sand experiment. In B-deficient plants produced less silks or not emerged out from the husk (Figure 2.13, a). Moreover, the symptom of B deficiency showed abnormal anthers, including thin and dead anthers at pollen shedding time. It is indicated that B deficient plant may not produced enough pollen to fertilization and set grain during fertilization time.

In conclusion, B deficiency depressed reproductive development in maize more than vegetative growth, B concentration in the silk and tassel looking to be the key limiting process. In sand culture, B deficiency decreases grain set when decreasing of pollen viability because of anther death, thin or blast and decrease the amount of pollen shedding. Moreover, In B-deficiency plant inability to produce and/or adequate amount of silk to receive pollen for fertilization. Thus, further work need to conducting to evaluate the effects of low B on the male and female flower.