

# Boron deficiency in maize

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**Abstract** Boron (B) deficiency depresses wheat, barley and triticale yield through male sterility. On the basis of field responses to B fertilization, maize (*Zea mays* L.) is affected by B deficiency in five continents. In a series of sand culture trials with maize subject to B0 (nil added B) and B20 (20  $\mu\text{M}$  added B) treatments, we described how B deficiency depressed maize grain yield while showing an imperceptible effect on vegetative dry weight. With manual application of pollen to the silk of each plant, B0 plants produced 0.4 grain ear<sup>-1</sup> compared with 410 grains ear<sup>-1</sup> in B20 plants. Symptoms of B deficiency was observed only in B0 plants, which exhibited symptoms of narrow white to transparent lengthwise streaks on leaves, multiple but small and abnormal ears with very short silk, small tassels with some

branches emerging dead, and small, shrivelled anthers devoid of pollen. Tassels, silk and pollen of B0 plants contained only 3–4 mg B kg<sup>-1</sup> DW compared with twice or more B in these reproductive tissues in B20 plants. A cross-fertilization experiment showed that, although the tassels and pollen were more affected, the silk was more sensitive to B deficiency. Pollen from B20 plants applied to B0 silk produced almost no grains, while pollen from B0 on B20 silk increased the number of grains to 37% of the 452 grains plant<sup>-1</sup> produced from B20 pollen on B20 silk. Therefore, the silk of the first ear may be targeted for precise diagnosis of B status at maize reproduction, for timely correction by foliar B application, and even for B-efficient genotype selection.

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## Introduction

Maize (*Zea mays* L.) is the most important crop by volume among all cereal grain crops, such as wheat and rice, which are widely grown throughout the world in subtropical and temperate agroclimatic regions (Fageria et al. 1991; Martin et al. 1976). Maize has been previously considered to have a relatively low boron (B) requirement compared with other cereals (Marten and Westermann 1991). However, based on field responses to B application, B

deficiency has been reported in maize across five continents (Bell and Dell 2008; Shorrocks 1997). For example, maize yield increases of 10% were reported in Rhodesia (now Zimbabwe), up to 26% in India (Shorrocks and Blaza 1973), more than 10% in Switzerland (cited by Mozafar 1987) and by 9% in China (Li and Liang 1997). Deficiency of B in field-grown maize was first observed in the 1960s in the United States (Shorrocks and Blaza 1973), and yield increases of more than 10% were observed in response to B application (Woodruff et al. 1987).

Knowing precisely where and how grain yield is adversely affected by nutrient limitations contributes to better management of nutrient deficiency in crops. In wheat, B deficiency depresses grain yield mainly through male sterility, causing grain set to fail (Rerkasem et al. 1993), without any apparent effect on vegetative growth including the somatic parts of the ear like the palea and lemma (Rerkasem and Jamjod 1997a). Moreover, grain set of wheat is closely correlated with B concentration in the ear (Rerkasem and Lordkaew 1992) and anthers (Rerkasem and Jamjod 2004). The identification of pollen and anther development in wheat and barley as most sensitive to B deficiency enabled better precision in diagnosis with tissue B analysis as well as correction of B deficiency with application of foliar B (Rerkasem et al. 2004). This led to effective screening of germplasm and breeding materials for B efficiency (Anantawiroon et al. 1997) and identification of B efficiency traits and relevant genes (Jamjod et al. 2004).

In B-deficient maize, poor grain-setting can result in barren cobs, and this was attributed by Vaughan (1977) to the silks being non-receptive. For an open pollinated crop with separated female and male inflorescences like maize, knowing precisely how B deficiency affects functions of the two reproductive organs and the rest of plant parts is useful for targeting the management of B deficiency through its sensitive diagnosis and timely application of foliar B. This understanding may also benefit the production of hybrid maize seed, with the primary concern being successful pollination and fertilization. Thus, the objectives of this set of experiments were to: (1) examine the effect of B deficiency on reproductive and vegetative development in maize, and (2) to examine the effect of B deficiency on morphology and function of the male (tassels, anthers, pollen) and female flowers (ear, silk).

## Materials and methods

### Plant materials and growth conditions

Four experiments were conducted at Chiang Mai University in Thailand from 2001 to 2006. A maize hybrid (NS72 developed at Nakhon Sawan Field Crop Research Center, Thailand) was grown in sand culture. The experiment was based on a randomized complete block (RCB) model with two levels of B treatments replicated three times each, with one separate set of pots for each of the three harvests (vegetative, anthesis and maturity). Seeds of maize were surface sterilized with Na-hypochlorite (5.7% available chlorine as chlorox: 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-lined aluminum tray until germination (approximately 2 days). Germinants were transplanted, 2 plants per container, to pots (0.30 m diameter and 0.30 m deep) filled with washed quartz river sand. The pots were supplied twice daily with 1 l of complete nutrient solution, adapted from Broughton and Dilworth (1971) and Mozafar (1989), with two levels of B, B0 and B20 (0 and 20  $\mu\text{M}$  B: B0 and B20 added to the nutrient solution). The complete nutrient solution consisted of  $\text{KNO}_3$ , 15,000  $\mu\text{M}$ ;  $\text{CaCl}_2$ , 1,000  $\mu\text{M}$ ;  $\text{Mg}(\text{SO}_4)_2 \cdot 7\text{H}_2\text{O}$ , 2,000  $\mu\text{M}$ ;  $\text{KH}_2\text{PO}_4$ , 1,000  $\mu\text{M}$ ; Fe-EDTA, 100  $\mu\text{M}$ ;  $\text{K}_2\text{SO}_4$ , 250  $\mu\text{M}$ ;  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 9  $\mu\text{M}$ ;  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.76  $\mu\text{M}$ ;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.31  $\mu\text{M}$ ;  $\text{CoSO}_4$ , 0.1  $\mu\text{M}$ ; and  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 0.1  $\mu\text{M}$ . All pots were flushed weekly with water to avoid accumulation of salts in the sand.

### Experiment 1: morphological and physiological effects of boron deficiency

Boron deficiency symptoms were observed as they developed throughout the experiment in 2002. Plants were harvested during vegetative (the YEB: youngest emerged leaf blade); YEB+1: next leaf older than the YEB; YEB-1: next leaf younger than the YEB; remainders) and reproductive growth. At the silking stage, florets were collected from the central part of the main tassel axis and the central part of the lateral tassel branches. One hundred florets were separated into anthers and bracts (lemma and palea) and measured for dry weight and B concentration. Plant samples were dried at 75°C to constant weight, then B was analyzed

by dry ashing (ash samples at 500°C for 8 h) followed by the azomethine-H method determination (Lohse 1982). Ears of the plants in the 3rd set of the pots were pollinated by manually collected pollen and direct application to the silk during anthesis. At maturity, grain and straw dry weight were determined; yield and yield components were recorded (e.g., the number of grain per ear and grain weight).

#### Experiment 2: effect of B deficiency on anatomy and morphology of the anther, pollen and silk

The objective of this experiment was to examine the effect of B deficiency on anatomy and morphology of male and female flowers of maize. Plant samples were produced in November 2002 to March 2003. Plants were grown in B0 and B20 as in experiment 1 and harvested during reproductive growth. For light microscopy, the maize tassels and ears were collected at anthesis and fixed in 3% glutaraldehyde in 0.025 M phosphate buffer, pH 7, at 4°C overnight, and then washed several times with 0.025 M phosphate buffer and stored at 4°C. To prepare for microscopy, the samples were 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 h 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°C overnight. Sections (2.5 µm thick) were cut (3 anthers for each treatment) using glass knives (25×6.4 mm) on a Sorvall-microtome and stained. Images of representative areas were captured with an Olympus DP70 digital camera attached to an Olympus BX51 compound microscope. Two staining procedures were carried out. Firstly, for the 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, allowed to dry at room temperature and mounted in DPX (bibutyl phthalate xylene mix). Secondly, an Iodine (KI/I<sub>2</sub>)

staining solution was used: pollen (from the different floret positions) were examined for starch accumulation with KI/I<sub>2</sub> staining solution (1 g iodine and 2 g KI in 100 ml water), then observed under the microscope at ×10–40 magnification (approximately 200–400 pollen grains per sample).

Silks were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer pH 7.0 with vacuum infiltration for 15–20 min. and left in the fixative for 24 h, 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 specimens were dried in a Critical Point Dryer with liquid CO<sub>2</sub>. The dried specimens were mounted on the SEM specimen holder (stub) with carbon tabs (Pro Sci Tech) and then sputter-coated with gold. The specimens were examined by a SEM (Philips XL20, Bio-transmission electron microscope) at 80 kv.

#### Experiment 3: pollen viability

This experiment was conducted from November 2005 to February 2006. Seeds of cv. NS72 were grown in sand culture with B0 and B20 as in experiment 1. There were ten pots per treatment and two plants per pot, with two complete sets of pots. With plants in the first set of pots, tassels were collected during pollen shedding between 0900 and 1130 hours from the central axis. Fresh pollen, taken from anthers just emerged from the upper spikelets of each main tassel, were dropped onto a germination medium in Petri dishes containing 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. Pollen grains were scored in each of five sectors on each Petri dish (approximately 300–1,200 pollen grains/sample). With plants in the second set of pots, whole tassels were collected for measuring the number of florets, dimension of anthers and estimating the number of pollen grains. Starch reserves in the pollen grain of 3–4 anthers of each plant was determined by releasing pollen from each anther into 0.5 ml KI/I<sub>2</sub> solution, and observed under a microscope at ×35 magnifica-

tion after 5 min. Negative staining with iodine indicated absence of starch deposits, indicating infertility, while positive staining with iodine to a blue-black color indicated normal pollen that could be viable. The number of pollen grains per anther and percentage of infertile and possibly viable pollen were estimated. For each tassel, the number of florets, anthers and the number of branches were recorded.

#### Experiment 4: manual cross-pollination between B0 and B20 maize plants

This experiment was conducted to determine the response of the male and the female flower to low B. Plants were grown from November 2001 to March 2002 in sand culture with B0 and B20 as in experiment 1. Ears and tassels were bagged before flowering. At anthesis, cross-pollination was done by applying: (1) B0-pollens on the silks of B0 and B20 plants, and (2) B20-pollens on the silks of B0 and B20 plants, respectively. Freshly shedding pollen grains (from 6 plants, 3 pots) were collected on white paper (A4 size) during 0900 to 1130 hours and immediately applied to the silks. Each manual pollination was done at least three times to ensure a sufficient supply of pollen. Plants were separated into tassel, flag leaf, ear leaf, silk, young ear, husk, shank, shoot and root for measuring dry weight and B determination. Grain weight and grain number were determined.

**Table 1** Dry weight, B concentration and boron content in parts of maize (cv. NS72) grown in sand culture with (B20) and without added B (B0) at vegetative growth (5-leaf stage) (Experiment 1)

B level ( $\mu\text{M}$ )	YEB-1	YEB	YEB+1	Shoot	Root	Shoot:root
Dry weight ( $\text{g plant}^{-1}$ )						
0 (B0)	0.440	0.287	0.203	2.775	1.013	2.7
20 (B20)	0.540	0.391	0.250	3.690	1.280	2.9
<i>F</i> test	NS	NS	NS	NS	NS	NS
B concentration ( $\text{mg B kg}^{-1}$ DW)						
0 (B0)	3.8	4.7	5.1	4.5	6.1	
20 (B20)	16.6	18.1	17.9	16.8	10.0	
<i>F</i> test	***	**	***	***	**	
$\text{LSD}_{0.05}$	1.8	5.0	1.8	2.0	1.8	
B content ( $\mu\text{g plant}^{-1}$ )						
0 (B0)	1.7	1.3	1.0	12.5	6.2	3.0
20 (B20)	8.9	7.2	4.5	62.2	12.9	5.5
<i>F</i> test	**	**	**	**	**	**
$\text{LSD}_{0.05}$	2.8	3.0	1.4	22.2	4.1	1.3

NS Not significant

\*\*, \*\*\*Significant at  $P < 0.01$  and  $0.001$ , respectively

#### Statistical analysis

Where possible, the data were subjected to analysis of variance (ANOVA) using commercial software (Statistix V. 8; Analytical Software). Significantly different means were separated at the 0.05 probability level by LSD.

#### Results

##### Experiment 1: morphological and physiological effects of boron deficiency

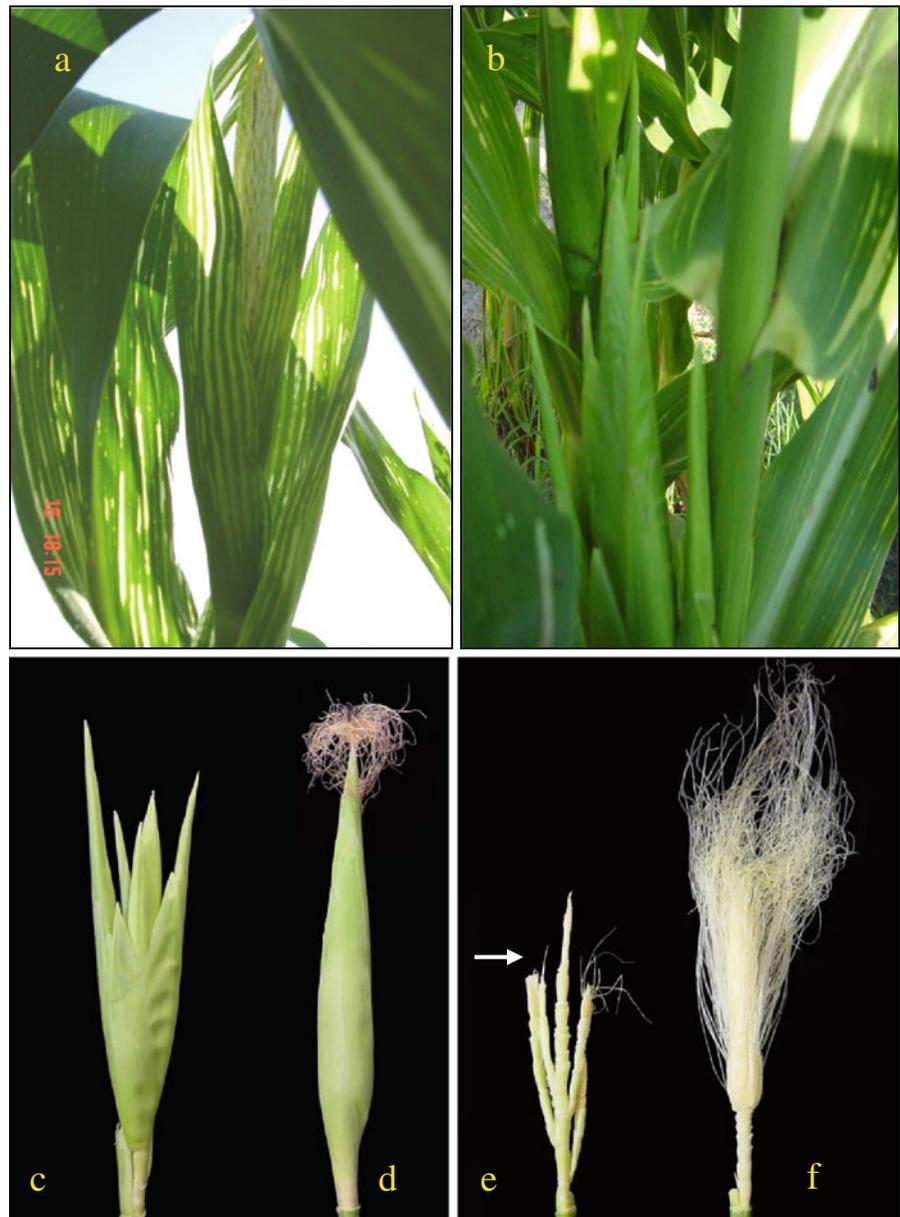
At the vegetative stage (5-leaf stage: Harvest 1), plants growing in B0 and B20 were clearly distinguishable by the B concentration and contents in their tissues (Table 1). However, there was no corresponding significant effect of B on dry weight of whole or some parts of the plant or the root:shoot ratio. The B concentration in leaves and shoot in B20 were about four times those in B0, and in the roots it was 1.6 times. No visible symptoms were present in any part of the plant at this stage.

As the maize plants entered the reproductive stage, with emergence of tassel and ears, symptoms began to be observed in B0 but not in B20 plants. These included small white spots on the upper leaves that later joined together to become narrow white stripes along the length of the leaves. Subsequently, by the time of anthesis, the stripes had widened, lengthened

and thinned to become papery, translucent streaks giving most of the upper leaves a stripy appearance (Fig. 1a). Some B0 plants produced multiple ears (Fig. 1b, c), and removal of the husks revealed an appearance of a tassel with very few short silk or silk completely absent (Fig. 1e) instead of the normal ears in B20 plants (Fig. 1f). In most cases, the silk in B0 plants did not emerge from the husk. The differences in the appearance of the reproductive tissues were

reflected in tassel and silk dry weights, and the number and length of silk at anthesis, which were all significantly lower in B0 than in B20 (Table 2). The tassels in B0 were visibly smaller (Fig. 2a) than in B20 plants, and some had dead, papery dry white branches. Anthers in B0 ranged from being small, shrivelled and devoid of pollen (Fig. 2b) to thin anthers with some pollen (Fig. 2c); B20 plants had anthers of normal appearance (Fig. 2d). Based on the

**Fig. 1** Symptoms of B deficiency of maize (cv. NS72) grown in sand culture without added B at anthesis showing: white stripes or transparent streaks on leaf lamina (a), multiple ears (b, c) and short silks (e: *arrow*, removed husk) compared with normal ear of B20 (d, f: ear after removal of husk)



**Table 2** Dry weight of tassel and silk, the number of silk per ear, silk length and B concentration in tassel, silk and pollen of maize (cv. NS72) grown in sand culture with (B20) and without added B (B0) at anthesis (75 days after germination) (Experiment 1)

B level ( $\mu\text{M}$ )	Dry weight ( $\text{g plant}^{-1}$ )		Silk thread		B concentration ( $\text{mg B kg}^{-1}$ DW)		
	Tassel	Silk	Number ear $^{-1}$	Length (cm)	Tassel	Silk	Pollen
0 (B0)	3.7	0.52	118	5.3	3.9	4.4	4.4
20 (B20)	8.8	0.93	420	13.2	8.1	11.3	9.0
<i>F</i> test	*	***	*	**	**	***	**
LSD <sub>0.05</sub>	1.6	0.1	234	2.7	2	1.8	0.8

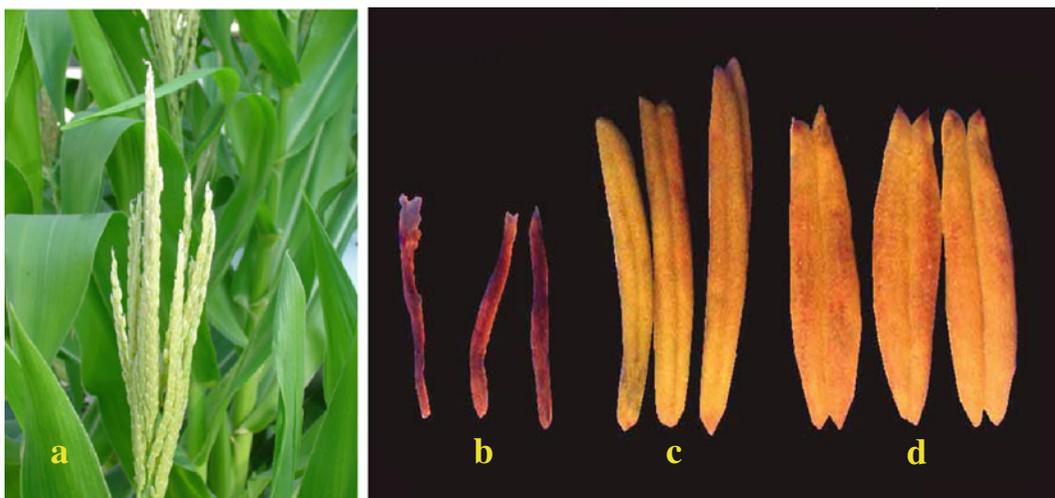
\*, \*\*, \*\*\*Significant at  $P < 0.05$ , 0.01 and 0.001, respectively

100 floret samples, the average weight of florets and anthers in B0 were only about half those in B20 on the lateral tassel branches, although the difference was not detectable in florets on the main tassel axis (Table 3). Boron concentration was about the same in the anthers and bracts, and did not differ between those from the central tassel and its branches. The B concentration in the anthers and bracts of B0 plants were about half those in B20 plants (Table 3).

At maturity there was a highly significant effect of B supply on grain yield and the number of grain per ear, which was much more pronounced than the imperceptible effect on dry weight of straw and roots and plant height (Table 4). The maize plants in B0 had an average of 0.4 grain ear $^{-1}$  compared with 410 grains ear $^{-1}$  in B20, resulting in grain yields of 0.5 g plant $^{-1}$  in B0 and 72.3 g plant $^{-1}$  in B20.

Experiment 2: effect of B deficiency on anatomy and morphology of the anther, pollen and silk

The SEM images of young ears at anthesis revealed differences in silk formation and appearance between B0 and B20 plants (Fig. 3). Silk at the tip of the young ear were visibly thinner in B0 than in B20 plants. By contrast, silk located in the middle and basal parts of the ear did not differ between B0 and B20 plants; both had uniformly abundant silk. In the male flower, a cross-section of the anther showed shrivelled and empty pollen grains lacking starch deposits or cytoplasm in B0 plants compared with normal rounded pollen containing numerous PAS positive starch deposits (Fig. 4) and a thickened anther wall (endothecium) in B20 plants. In the case of the pollen, there were major differences due to the position of the florets on the spikelet. The maize



**Fig. 2** Male inflorescences of maize (cv. NS72) plants grown without (B0) added B: small tassel (a), shrivelled anthers (b) and narrow thin anthers (c), compared with normal anthers from B20 (d)

**Table 3** Dry weight and B concentration in anther and bract of maize (cv. NS72) from 100 florets on the main axis and branch tassel at time of silk emergence (75 days after germination) grown in sand culture with (B20) and without added B (B0) (Experiment 1)

B level ( $\mu\text{M}$ )	Dry weight (g)				B concentration ( $\text{mg B kg}^{-1}$ DW)			
	Anther		Bract <sup>a</sup>		Anther		Bract <sup>a</sup>	
	Main	Branch	Main	Branch	Main	Branch	Main	Branch
0 (B0)	0.24	0.10	0.12	0.08	2.9	3.5	3.4	3.6
20 (B20)	0.28	0.18	0.14	0.14	6.4	7.4	5.2	5.2
<i>F</i> test	NS	*	NS	*	**	**	***	**
LSD <sub>0.05</sub>		0.06		0.04	1.4	2.3	0.6	0.6

NS Not significant

\*, \*\*, \*\*\*Significant at  $P < 0.05$ , 0.01 and 0.001, respectively

<sup>a</sup> Lemma + palea

staminate spikelet is composed of two florets, the terminal (F1) and the basal (F2). Iodine staining found more sterile pollen without starch deposits in B0 anthers than in anthers of B20 plants, but the effect varied with the position of the floret on the spikelet (Table 5; Fig. 5). In B20, there were very few pollen grains that were not stained with iodine, where as the percentage of pollen that were judged sterile by the negative iodine staining in B0 was 100% in the basal floret (F2) and 45% in the terminal floret (F1).

### Experiment 3: pollen viability

This experiment confirmed that an external supply of B was indeed essential for maize pollen germination. Only 5.5% of the pollen from B0 plants germinated in vitro without B added to the medium, and adding B to the germinating medium increased germination to 25.5% (Table 6). Germination of pollen from B20 plants was only 19.3% without B added to the germinating medium, and 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 plants, 11% for silk and 61% for tassel. The lower dry weight of tassel in B0 was associated with fewer pollen grains per anther. There were 1,386 pollen grains per anther in B0, compared with 3,000 in B20 plants (Table 7). Three-quarters of the B0 pollen showed absence of starch deposits by not staining with iodine, while numerous starch deposits were present in 81% of the pollen from B20 plants. However, there was no significant difference in the number of florets per plant, tassel length, and the number of tassel branches between B0 and B20 plants. The much lower silk dry weight in B0 was also associated with much fewer silk threads per ear, only 30 in B0 and 406 in B20. The dry weights of the young ear and husk, however, were not significantly different between B0 and B20 plants. Except for the silk and tassel, there was no dry weight difference between B0 and B20 plants in shoot, root, and other plant parts (Table 8).

In B0, the concentration of B in the pollen was 2.8 mg B kg<sup>-1</sup> DW and in the silk was 4.3 mg B kg<sup>-1</sup>

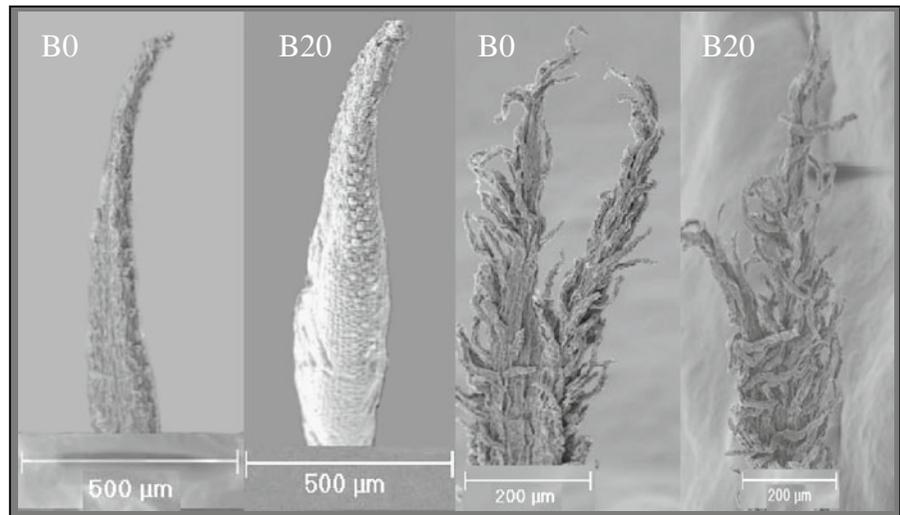
**Table 4** Grain number per ear, dry weight of grain, shoot and root and plant height of maize (cv. NS72) grown in sand culture with (B20) and without added B (B0) (Experiment 1)

Added B ( $\mu\text{M}$ )	Grain no. ear <sup>-1</sup>	Dry weight ( $\text{g plant}^{-1}$ )			Plant height (cm)
		Grain	Shoot	Root	
0 (B0)	0.4	0.5	129.6	32.2	210.3
20 (B20)	410.0	72.3	133.4	23.6	214.0
<i>F</i> test	***	***	NS	NS	NS
LSD <sub>0.05</sub>	72	11.7			

NS Not significant

\*\*\*Significant at  $P < 0.001$

**Fig. 3** SEM images of maize (cv. NS72) showing a silk from the upper (*far left* B0, *middle left* B20) and lower part (*middle right* B0, *far right* B20) of young ear of maize (cv. NS72) at anthesis stage



DW, which were only about one-third of the B concentration in these tissues in B20 plants (Table 8). 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 the young ear, husk and shank were also significantly lower in B0 than B20 plants, though much less pronounced. The root B concentration did not differ significantly.

Experiment 4: manual cross-pollination between B0 and B20 maize plants

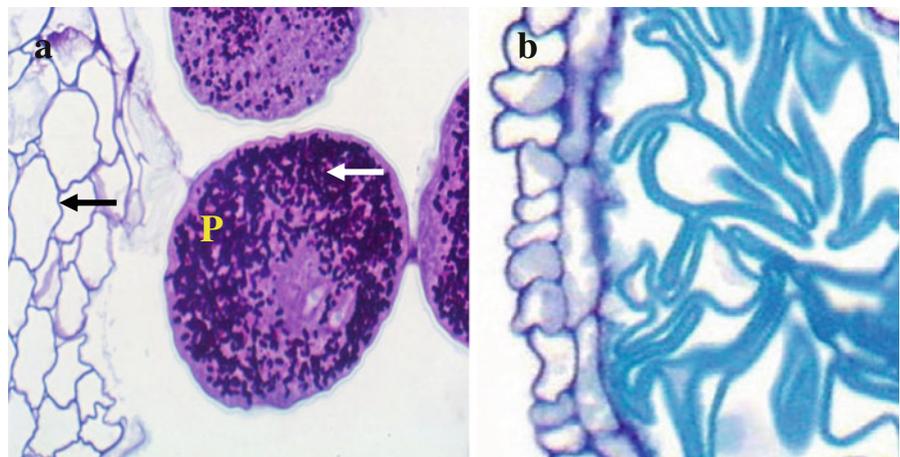
By reciprocal crosses, the B20 pollen applied to the B20 silk produced 452 grains plant<sup>-1</sup> (Table 9). When B20 silk was pollinated with B0 pollen, 169 grains

plant<sup>-1</sup> were produced, but applying B20 pollen to B0 silk produced only 2 grains plant<sup>-1</sup>, which was almost the same as when B0 pollen was applied to B0 silk (Fig. 6).

## Discussion

In many crops (from almond, avocado, barley and hazelnut to peach, triticale and wheat), reproductive growth and grain or fruit yield are more responsive to B than vegetative growth (e.g., see Hanson and Breen 1985; Kamali and Childers 1970; Li et al. 1978; Nyomora et al. 1997; Rerkasem and Loneragan 1994; Robberstse et al. 1990; Shrestha et al. 1987; Smith et al. 1997; Wongmo et al. 2004). The same has been

**Fig. 4** Cross-section of an anther stained with PAS to indicate the accumulation of starch (*white arrow*, positive staining with PAS reagent) in pollen (*P*) grains and the endothecium of the anther wall (*black arrow*) of B20 maize (cv. NS72) plant (**a**) compared with the shrivelled and empty pollen without starch deposit in an anther of B0 plant (**b**)



**Table 5** Pollen sterility (% negative staining with iodine) of maize (cv. NS72) grown in sand culture with (B20) and without added B (B0) (Experiment 3)

B level ( $\mu\text{M}$ )	Floret <sup>a</sup>		
	F1 (terminal)		F2 (basal)
0 (B0)	44.7 b		100.0 a
20 (B20)	1.0 c		2.9 c
<i>F</i> test	B***	F***	B × F ***
LSD <sub>0.05</sub>	4.8	2.7	3.9

Numbers followed by different letters indicated significant differences by LSD ( $P < 0.05$ )

\*\*\*Significant at  $P < 0.001$

<sup>a</sup> About three staminate florets were taken from the central part of the main tassel axis at pollen shedding. Each spikelet has two florets, F1 the terminal floret and F2 the basal floret

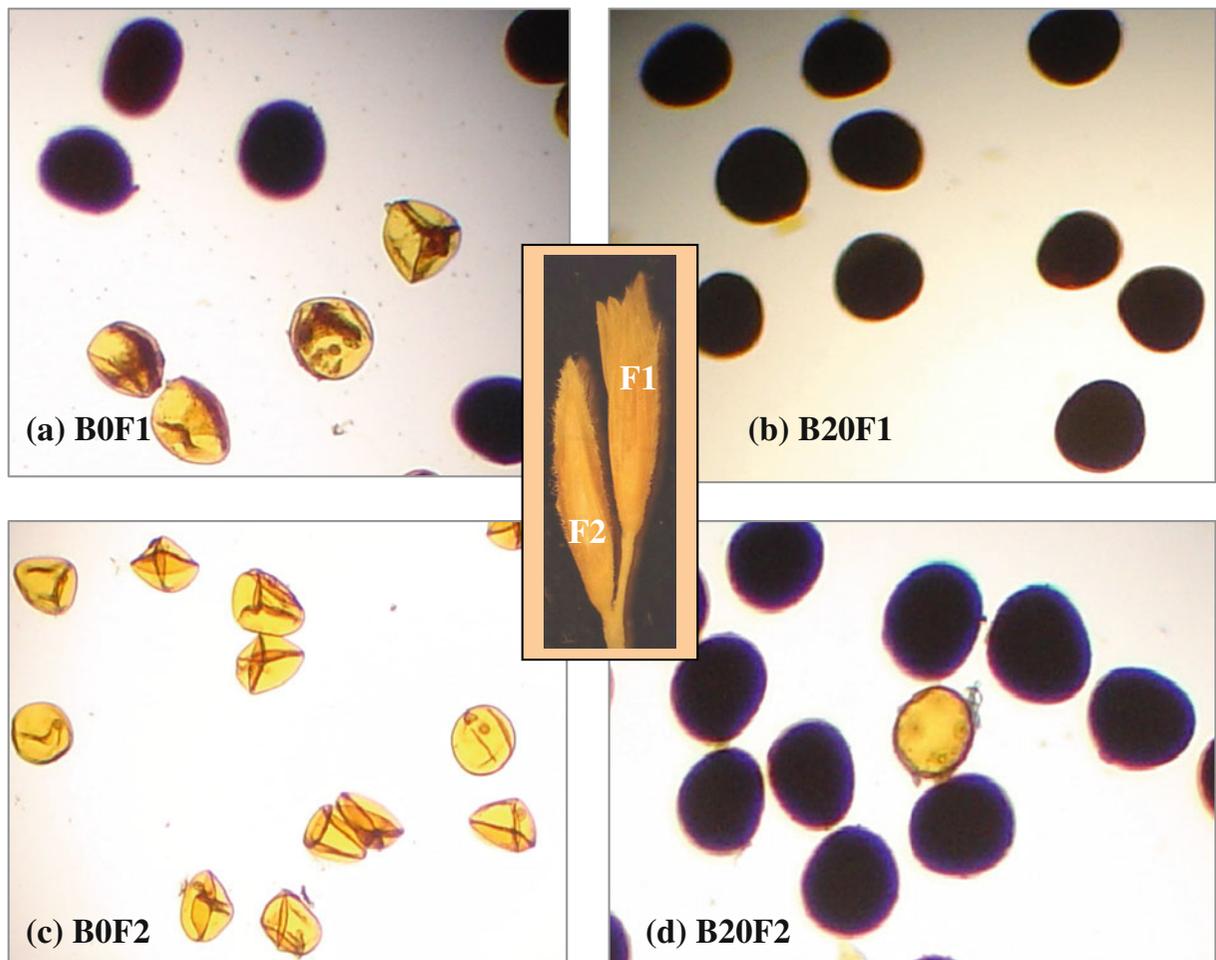
shown here for maize, but with two major differences in relative sensitivity to B deficiency of the female and male flowers, and apparent B requirement in different tissues.

There are numerous reports in the literature of the effect of B deficiency on plant reproductive growth and development. Many of these focus on development and function of the staminate flower, especially the pollen. ‘Male sterility’ is used to describe the state of B-deficient plants at anthesis in barley (Simojoki 1972), rice (Garg et al. 1979) and wheat (Li et al. 1978), in which the pollen appear misshapen and empty of storage starch. Boron deficiency has been reported to cause impaired ovule and flower development in oilseed rape (Xu et al. 2002), but at a level of deficiency that was so severe that growth of the whole plant was affected. It has sometimes been suggested that the anther and pollen may be more sensitive to B deficiency than the pistil (Brown et al. 2002; Dell and Huang 1997), but comparative studies of the effect of B deficiency on different organs and stages of growth of the same plant are rare. While a single pollen grain may or may not be more sensitive to B deficiency than a single pistil, this study has shown that in a maize plant it was the pistil, both in its development and function, which was most sensitive to B deficiency, followed by the stamen, with vegetative growth being the least sensitive. The effect of B deficiency on vegetative dry weights of maize was imperceptible even when reproductive growth was clearly depressed. Evidence of greater

sensitivity to B deficiency of the pistil flower than the staminate flower of maize was found in both their development and function. On the same B0 plant, effects of B deficiency on the maize tassel, anthers and pollen were milder than those on the pistil. Extreme B deficiency caused malformation of the whole maize ear, to resemble a tassel instead of an ear (Fig. 1b, c, e). The unisexuality of the maize florets is established by a process of elimination of the pistil in the tassel as described by the function of *tasselseed* genes (Calderon-Urrea and Dellaporta 1999). It remains to be explored if the staminate florets in the B-deficient maize ear were still suppressed and how B deficiency triggers development of the branching tassel-like organ where the ear should be. On those ears that appeared normal, fertilization was precluded in those ova with styles (silk threads) that failed to develop or were too short to exert beyond the ear husk. With external B requirement for pollen germination established here in vitro, and in vivo in wheat (Cheng and Rerkasem 1993), the silk B could have also been limiting grain set.

On individual maize plants, while dry weight of each tassel and the anthers in it and average number of pollen per anther were clearly depressed by B deficiency, there was significant variation within each tassel, with more anthers that appeared normal and more potentially viable pollen on the main tassel axis than in the lateral branches, and in terminal than basal florets in each spikelet. Limited accessibility to the vascular bundle was proposed as the reason for greater sensitivity to B deficiency of reproductive organs (Dell and Huang 1997; Hanson and Breen 1985). On the basis of individual maize plants, results of manual cross-pollination, in which the B0 silk was found to be more limiting to grain set than B0 pollen, provide the ultimate proof of greater sensitivity to B deficiency of the maize pistil than its stamen.

From the evidence of B concentration in different tissues, there was no indication that the pistil required more B than the anthers and pollen, with the ear leaf, flag leaf, tassel, anthers and silk from B0 that were affected differently by B deficiency all averaging 3–5 mg B kg<sup>-1</sup> dry weight. Neither were the smaller florets and anthers from the lateral branches and those from the main tassel axis, that exhibited different effects of B deficiency on the same plant, distinguishable by their B concentrations. This is different from wheat, in which the functional requirement for B was



**Fig. 5** Appearance of pollen of maize (cv. NS72) stained with iodine taken from the upper (*F1*) (a and b) and lower (*F2*) (c and d) florets of each spikelet from the main tassel axis at anthesis. Plant

were grown in sand culture without B (*B0*) (a and c) and with added B (*B20*) (b and d). Normal pollen with starch deposits are black

**Table 6** Pollen germination (%) in media with and without added B of maize (cv. NS72) grown in sand culture with (*B20*) and without added B (*B0*) (Experiment 3)

Pollen source	Pollen germination in media (%)	
	Without added B	With 0.01% $H_3BO_3$ <sup>a</sup>
<i>B0</i> plants	5.5 c	25.5 b
<i>B20</i> plants	19.3 b	97.5 a
<i>F</i> test (plant B × medium B)	***	
LSD <sub>0.05</sub>	6.3	

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

\*\*\*Significant at  $P < 0.001$

<sup>a</sup> 1.6 mM B

**Table 7** Effects of B deficiency on reproductive growth in maize (cv. NS72) grown in sand culture with (*B20*) and without added B (*B0*) (Experiment 3)

Plant response	Added B ( $\mu M$ )		LSD <sub>0.05</sub>
	0	20	
Tassel length (cm)	39	44	NS
Number of floret plant <sup>-1</sup>	1109	1,208	NS
Number of branch of tassel plant <sup>-1</sup>	18	18	NS
Number of pollen anther <sup>-1</sup>	1386	2,999	1,268*
Starch filled pollen (%)	24	81	27**
Number of silk thread ear <sup>-1</sup>	30	406	125*

NS Nnot significant

\*, \*\*Significant at  $P < 0.05$  and 0.01, respectively

**Table 8** Effects of B deficiency on dry weight, boron concentration and boron content in various plant parts and shoot: root dry weight of maize (cv. NS72) grown in sand culture with (B20) and without added B (B0) at pollen shedding (Experiment 3)

Plant part	Added B ( $\mu\text{M}$ )			LSD <sub>0.05</sub>			Added B ( $\mu\text{M}$ )			LSD <sub>0.05</sub>		
	0		20	0		20	0		20	0		20
	Dry weight (g plant <sup>-1</sup> )			Boron concentration (mg B kg <sup>-1</sup> DW)			Boron content ( $\mu\text{g}$ B plant <sup>-1</sup> )					
Pollen	No data	No data		2.8	7.3	1.2***	No data	No data				
Tassel	2.8	4.6	1.0**	4.7	18.0	3.0***	13.2	82.7	27.2***			
Flag leaf	0.4	0.5	NS	5.1	35.8	6.3***	2.0	16.0	5.1***			
Ear leaf	3.5	3.6	NS	5.3	28.5	5.7***	18.6	101.9	239.0***			
Silk	0.1	0.9	0.2***	4.3	17.7	3.0***	0.4	15.7	3.4***			
Young ear (pre-anthesis)	2.9	2.7	NS	3.8	6.7	1.2***	10.9	18.1	NS			
Husk	14.4	20.8	NS	3.1	5.1	1.1***	44.7	106.0	43.3*			
Shank	3.0	3.8	NS	4.1	5.7	0.8**	12.3	21.5	NS			
Shoot	167.5	183.8	NS	4.2	16.9	0.8**	703.5	3,106.1	789.0***			
Root	24.7	30.2	NS	4.5	5.6	NS	113.3	169.1	NS			
Shoot:root ratio DW	6.7	6.3	NS									

NS Not significant

\*, \*\*, \*\*\*Significant at  $P < 0.05$ , 0.01 and 0.001, respectively

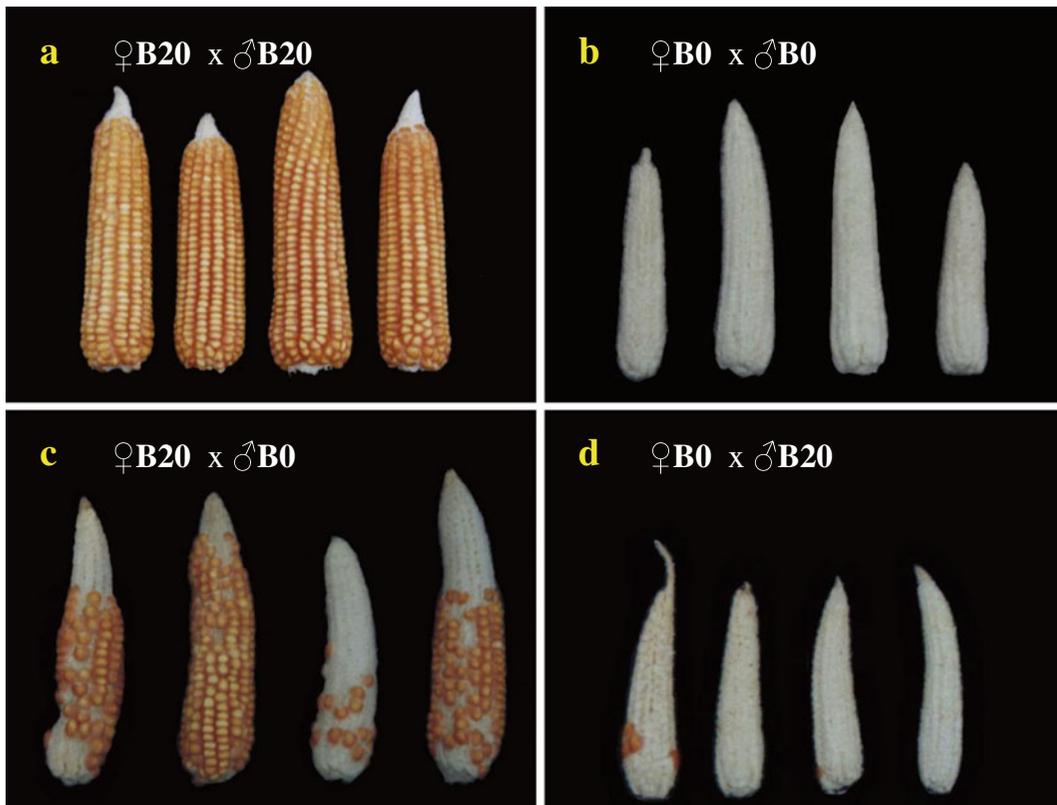
shown to be highest in the anthers, followed by the pistil, and lowest in the leaves and somatic part of the ear (Rerkasem and Jamjod 1997b). However, on the B-deficient plant, the effect on the female flower is more severe than on the male flower such as maize producing abnormal ears. In the visible normal morphology of the deficient ear, the silks were much shorter or much fewer in numbers, and appeared to be thinner and also collapsed internally under the microscope. With cross-pollination experiments, these B-deficient silks may not function properly and grain setting may still fail when healthy pollen is applied to them because healthy pollen requires sufficient external B supply to germinate fully. These symptoms

**Table 9** Number of grains obtained by manual cross pollination of pollen and silk from maize plants (cv. NS72) grown in sand culture with (B20) and without (B0) added B (Experiment 4)

Cross-pollination		Grain number (plant <sup>-1</sup> )	Grain set (%)
Silk <sup>a</sup>	Pollen <sup>a</sup>		
B20	B20	452.0	100.0
B20	B0	169.0	37.4
B0	B20	2.0	0.4
B0	B0	0.0	0.0

<sup>a</sup> Silk or pollen of plants grown with (B20) and without (B0) B added to the nutrient solution

of B deficiency were associated with silk that contained 4 mg B kg<sup>-1</sup> DW whereas in normal function it was 11–18 mg B kg<sup>-1</sup> DW, while normal function for pollen contained about 7 mg B kg<sup>-1</sup> DW. This is in contrast to the early report that a minimum about 3 mg B kg<sup>-1</sup> DW was necessary for growth and normal function in all parts of maize and the extended silks (Vaughan 1977). Moreover, the silk B had about 9 mg B kg<sup>-1</sup> DW in which appeared the symptoms of B-toxicity in the vegetative growth, but this not found in this experiment. Several factors have been suggested that may contribute to differential impact of low external B supply on different organs and stages of plant reproductive development (Dell and Huang 1997). One of these, which is unlikely to explain the greater sensitivity of the pistil in maize, is relative sink size, as the dry weight of fully developed maize ear plus silk with sufficient B was only four-fifths of the dry weight of the tassel. By the time of pollen shedding, more B was taken up in the ear than in the tassel, 76% more in B0 and 41% more in B20. Three-quarters to four-fifths of the B taken up into the ear, however, ended up in the ear-covering husks. The fewer silk threads, their shorter length and lower dry weight in B0 plants all suggested an adverse effect of B deficiency on silk development. That pollination with B0 pollen on B20 was only 37% as successful as B20 pollen on



**Fig. 6** Effects of B on grain set by reciprocal cross in maize (cv. NS72). **a** B20♀ x B20♂; **b** B0♀ x B0♂; **c** B20♀ x B0♂ and **d** B0♀ x B20♂. Female (♀) and male (♂) is silk and pollen, respectively

B20 silk could be attributed to effects of B deficiency on pollen viability and silk function. However, since B deficiency can sometimes produce a branching maize female inflorescence that looks more like a tassel than an ear, the possibility that B deficiency may have also affected development of the ovary cannot be ruled out.

The variation in the effect of B deficiency within each maize plant and within each tassel found here, on the other hand, agrees with the suggestion that B supply to the reproductive organs is influenced by their architectural position (Hanson and Breen 1985), B delivery via the transpiration stream (Marschner 1995), and possible competition for B among different tissues. In individual maize plants, the tassel has been shown to be a much higher priority sink than the ear in the partitioning of limited supply of photosynthate (Edmeades and Daynard 1979). On a plant, those organs more sensitive to B deficiency differed from those with more moderate effects (i.e., silk vs tassel; lateral tassel branches vs the main axis; basal vs

terminal florets of the staminate spikelet) by occupying somewhat secondary positions related to the growth axes and thus vascular bundles. While the tassel develops at the shoot apex of the maize plant, and the terminal floret from the main growth axis of the staminate spikelet, both the ear and the basal floret of the staminate spikelet develop from secondary branching of their respective growth axes (Bonnet 1966). In wheat and barley, the position of the stamen and pistil is reversed: the B-sensitive stamens branch off the main growth axis of each floret, while the less sensitive pistil occupies the central position.

The relative sensitivities of the pistil and stamen have some important implications on the management of those crops that are at risk from B deficiency. Where the stamen is more sensitive, irreversible damage to the pollen had already been caused by B deficiency by the time of anthesis. Pollen viability in wheat can be almost halved by B deprivation for only 5 days during the young microspore (green anther) stage (Nachiangmai et al. 2004). This partly explains

why foliar B application at anthesis is only partially effective in overcoming B deficiency in wheat (Rerkasem and Jamjod 2004). In maize, in contrast, the function of B-deficient silk can be expected to be enhanced much more significantly by B application directly onto the silk during anthesis. The potential for improving fertilization and seed set should be further investigated in the production of hybrid maize seed as well as the grain crop where B supply is marginal. The greater sensitivity to B deficiency of the pistil also means that B analysis of the ear and the silk should give the most direct and precise diagnosis for B deficiency in maize. As many crop species exhibit a wide range of genotypic variation in B efficiency (Rerkasem and Jamjod 1997b), it should be useful to explore this among maize genotypes, with B delivery to the growing ear and silk as the primary criterion.

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