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Effects of Boron Deficiency on Anther Development and Floret Fertility in Wheat (*Triticum aestivum* L. 'Wilgoyne')

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Boron (B) deficiency limits reproductive growth more than vegetative growth in cereals such as wheat. The purpose of the present study was to identify the critical stages of anther development of wheat ('Wilgoyne') during which B deficiency causes a significant and irreversible decrease of floret fertility in order to formulate timely measures for correcting or preventing this problem. Withdrawing B from the rooting medium for 3 d between premeiotic interphase through meiosis to late tetrad, limited anther elongation and resulted in the loss of pollen viability. The negative effects of B withdrawal on anther length suggest that the role of B in reproductive cell walls is similar to that in vegetative ones. The results indicated that as more florets reached meiosis within the period of B withdrawal, the lower was floret fertility and the number of grains set in a whole ear. For the whole ear, the critical period during which B deficiency causes maximal and irreversible damage to floret fertility was about 7 d, extending from the early emergence of the flag leaf to 2–3 d after its full emergence. The results suggest that there are two phases of pollen development sensitive to boron deficiency: the period from premeiotic interphase through meiosis to late tetrad was the most sensitive stage of microsporogenesis in wheat while the period from mitosis-I to II during which starch accumulation occurred in pollen grains was less sensitive.

Key words: Anther, boron (B) deficiency, floret fertility, Grain Set Index (GSI), pollen, wheat ('Wilgoyne').

INTRODUCTION

Wheat sterility is a widespread problem in rice-wheat farming systems of Asian countries, such as Bangladesh, Thailand, Nepal, China and India, leading to significant losses of grain yield (Rerkasem, 1996*a*). Many factors may contribute to the formation of sterile florets of wheat, including B deficiency (Rerkasem, 1996*a*, *b*), water deficit (Saini and Aspinall, 1981), high temperature or heat stress (Saini and Aspinall, 1982), and low temperature (chill/frost) (Huang *et al*., 1996; Subedi *et al*., 1998). In many cases, B deficiency is at least partially responsible for the induction of floret sterility and low grain set and its impact may be exacerbated by environmental factors (Rawson, 1996*a*; Rerkasem, 1996*a*). Wheat vegetative parts show no obvious leaf symptoms or growth reduction even when the new leaves have less than 2 mg B kg^{-1} dry matter, but the B requirement in anthers for successful grain set is 10 mg B kg^{-1} dry matter (Rerkasem, Lordkaew and Dell, 1997). The striking difference in the susceptibility to B deficiency between vegetative and reproductive growth makes the early detection and prediction of B deficiency difficult in the field. Therefore, identification of critical periods of anther and floret development would allow timely application of corrective B fertilizer treatments, as foliar B sprays, to reverse the effects of B deficiency on floret fertility.

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Boron plays essential roles in the structure and function of cell walls and cellular membranes (Cakmak and Römheld, 1997; Matoh, 1997). Although the direct roles of B in sporogenesis, pollen germination and pollen tube growth are yet to be confirmed (Dell and Huang, 1997), a role for B in pollen cell walls may be expected. Borate-rhamnogalacturonan-II complex was detected in the cell walls of pollen tubes (Matoh *et al*., 1998). In wheat, B deficiency causes poor anther and pollen development and low grain set (Cheng and Rerkasem, 1993). *In vitro* germination tests also showed that B was required for pollen germination and tube growth in wheat (Cheng and Rerkasem, 1993).

In the case of wheat, floret sterility induced by B deficiency is mainly caused by sterile pollen—male sterility (Cheng and Rerkasem, 1993; Rerkasem *et al*., 1997). Since B has limited phloem mobility in crops like wheat (Brown and Shelp, 1997), continuous B supply is required for healthy reproductive growth (anther, pollen and ovule development), in order to avoid the sterile impact of B deficiency on florets. However, the most sensitive stage of microsporogenesis of wheat in relation to B deficiency has yet to be identified. On the basis of studies on other environmental factors such as high temperature and water deficit, Rawson (1996*a*) suggested that meiosis of pollen mother cells would be the critical phase for B supply, but later evidence (Rawson, 1996*b*) only indicated a critical period of 1 week between the emergence of the flag leaf tip

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to the early emergence of ears. By contrast, Rerkasem *et al.* (1997) carried out a soil culture experiment with long-term low B supply and found that microsporogenesis proceeded normally up to the vacuolated young microspore stage, but pollen maturation failed, resulting in malformed pollen at anthesis.

In previous studies, wheat plants were grown in sand culture and were subjected to lengthy treatments of B withdrawal (−B) from sowing to ear anthesis, or even to grain set (Cheng and Rerkasem, 1993; Rerkasem *et al.*, 1993; Rawson, 1996*b*). In these studies, it was difficult to separate the effects of B deficiency on floret fertility during the sensitive phase of floret development from those that occurred during other phases of growth and development. Also, sand culture does not allow precise control of B levels in the rooting media. There is usually an undefined period between withdrawing B supply and achieving the deficient levels of B in sand medium, as in the study by Rawson (1996*b*). Therefore, solution culture was used in the present study for the precise control of B supply to the plants.

The aim of the present study was to identify the critical stage of microsporogenesis of anthers and the critical phase of floret fertility, before which resupplying B may reverse or avoid floret sterility in wheat. As floret sterility induced by B deficiency is mainly related to sterile pollen (Cheng and Rerkasem, 1993), effects of B deficiency on pollen viability and anther elongation were examined. A brief episode of B withdrawal (3 d) was used to define the critical stage of microsporogenesis.

MATERIALS AND METHODS

Plant culture

The full-strength basal nutrient solution contained (μM): NH_4NO_3, 2000; KNO_3, 2800; $Ca(NO_3)_2$, 1600; $MgSO_4$, 1000; KH_2PO_4, 100; K_2HPO_4, 100; FeEDTA, 100; NaCl, 8; $ZnSO_4$, 2; $MnSO_4$, 2; $CuSO_4$, 0·5; and Na_2MoO_4, 0·08. Water used for making up all chemical and nutrient solutions was purified by passing through a column packed with B-specific resin (IRA-743, Sigma Chemical Co.). Analytical grade chemicals were used to make up the nutrient solutions.

Wheat ('Wilgoyne') seeds were germinated on paper towels moistened with 2 mM $CaSO_4$ at 25°C in the dark for 2 d. The germinated seedlings were then transferred into a tray containing 8 l of 1/3 strength nutrient solution in a glasshouse. After acclimatization, the seedlings were transferred into 5 l pots containing the full-strength nutrient solutions. Pots were placed randomly in temperature-controlled water baths with the water temperature set at 18–22°C and repositioned every alternate day.

To define the pollen developmental stages during which B withdrawal treatments were applied, anthers of the primary florets from the central four spikelets were dissected out daily from extra control (+B) plants. The anthers were squashed on a glass slide, stained with the DNA-specific fluorochrome DAPI (4′-6-Diamidino-2-phenylindole 2HCl, Sigma Lot 104F-0542), and examined under a UV-fluorescence microscope (Vergne, Delvallee and Dumas, 1987) to determine the pollen developmental stages of wheat (Bennett *et al.*, 1973). The spike reached terminal spikelet stage when plants had about six leaves. When the flag leaf (leaf 8) ligule had just fully emerged, the anthers of primary florets of the central spikelet had developed to stage 3 of premeiotic interphase/leptotene stage (just before starting meiosis) (see Bennett *et al.*, 1973), with a length of about 1 mm. The anther length at a particular sporogenesis stage is described in Table 2.

Boron determination

The plant samples were dried at 70°C to constant weight and digested with concentrated nitric acid at 140°C. Boron concentrations in the digest solutions were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Zarcinas, Cartwright and Spouncer, 1987).

Data analysis

The data were analysed by one-way analysis of variance and differences among mean values were determined by the Duncan's multiple range test ($P \leqslant 0{\cdot}05$) with the SPSS statistical package (SPSS, vs 6.0, SPSS Inc., 1993).

Experiment 1: Floret fertility response to B deficiency during different phases of floret development

The objective was to define the critical phase of ear development during which withdrawing B supply may cause the maximal loss of floret fertility, leading to low grain set in the whole ear.

There were eight pots of plants in each treatment. At the end of the −B treatment periods (Table 1), four pots of plants were resupplied with 10 μM B, and grown until the end of the experiment for the estimation of sterility in the ears of main stems (expressed as spikelet fertility and grain set index). The other four pots per treatment were harvested at the end of each −B treatment (see Table 1 for developmental stages of anthers) and the flag leaf (FL) and the leaf blades immediately older than the FL (FL-1) were sampled for the analysis of B concentrations.

Boron withdrawal treatments were applied for 5, 10, 17 and 25 d, respectively, commencing when the plants reached the terminal spikelet stage and had the sixth leaf blade fully expanded. At the end of each of these intervals, these plants were resupplied with 10 μM B, until the main stem ear reached anthesis. By the day of resupplying B, the anthers of the central primary florets of the main shoot had reached the stages given in Table 1.

The growth conditions in the glasshouse during the experimental period were: mean air temperature 20°C (range: 16·0–25·2°C); mean relative humidity 57% (range: 44–65%); mean photosynthetic photon flux (PPF) 758 μmol m^{-2} s^{-1} (natural sunlight in the glasshouse from 1000–1500 h). pH in the nutrient solution was initially adjusted to about 6 and buffered at 7·0–7·5 by adding 0·1 g $CaCO_3$ to each pot of solution as required. Nutrients were

TABLE 1. *Boron deficiency treatments imposed during spike development of the main shoot of wheat 'Wilgoyne'*

Boron treatment	Duration of −B (d)	Spike developmental stages during −B treatment
+B	0	
−B	5	TSS to white anther (premeiotic stage in pollen mother cells)
−B	10	TSS to early green anther (early meiosis)
−B	17	TSS to green anther (mitosis-I)
−B	25	TSS to anthesis (mature pollen)

The control plants were supplied with adequate B (+B, 10 μM B) throughout the entire reproductive growth until grain set. The −B (0 μM B) treatments were applied at the terminal spikelet stage (TSS) until the four stages described in the table. The stages of anther and pollen development were derived from examination of the primary florets of the central four spikelets of the main shoot (expt 1).

supplied to the plants by programmed nutrient addition as detailed in Huang *et al.* (1996).

The distribution of floret fertility within each ear of the main shoot and the first tiller (tiller 1) was mapped from the apex of the ear (spikelet 1) to the base, according to Rawson (1996*b*). Ears were harvested before grains turned hard and were counted for the number of florets and grains per spikelet. Spikelet fertility was defined as the ratio of the number of grains to the number of competent florets in each spikelet. A competent floret was defined at a floret with the potential to set a grain. According to Bagga and Rawson (1977), a floret was judged as competent if its lemma length was not significantly shorter than that of fertile florets in +B treatments which were used as the standard for competence. At anthesis, competent florets had mature styles curved outwards and stigmatic branches spreading wide. A floret containing a grain was considered fertile and scored 1, and an infertile floret (gauged large enough to have a grain, but empty) was scored 0 (Bagga and Rawson, 1977).

Wheat spike fertility is an overall assessment of floret fertility of an ear, which is quantified as the percentage of fertile florets (with grain) in the total number of competent florets of a spike. The CMU and LAC grain set indices (GSI) are most commonly used for assessing floret fertility (Rerkasem, 1996*a*). The CMU-GSI is defined as the percentage of grain bearing florets in the two basal florets of ten central spikelets, and the LAC fertility index is defined as the percentage of grain bearing florets in the total number of competent florets of the whole ear. Both the CMU method (Rerkasem, Saunders and Dell, 1989) and the LAC method (Sthapit, 1988) were used to calculate the grain set index for the assessment of spike fertility:

CMU GSI (%) = (C/20) × 100, where C is the number of grains per primary and secondary floret of ten central spikelets.

LAC GSI (%) = B × 100/A, where A is the number of competent florets per spike, and B is the number of grains per spike.

Experiment 2: Critical stages of microsporogenesis

On the basis of the results of expt 1, expt 2 was conducted to define the critical stage of microsporogenesis at which withdrawing B supply impairs the formation of viable pollen.

Plant culture was the same as described above, except that solution pH was maintained with 2–5 mM MES buffer to 5·8–6·5, and B-loaded resin was used to maintain adequate B supply to plants. The number of plants per pot was thinned to two when the main shoot ears reached the terminal spikelet stage. The growth conditions in the glasshouse were: mean air temperature 24°C (range: 19–32°C); mean relative humidity 54% (range: 66–33%); mean PPF 823 (491–1151) μmol m^{-2} s^{-1} (natural sunlight in the glasshouse from 1000–1500 h).

There were two B treatments: 10 and 0 μM B. Four pots of control plants were supplied with 10 μM B throughout the experiment. From the terminal spikelet stage to anthesis, B concentrations in the nutrient solutions were maintained within the range 10–15 μM B (determined by ICP-AES), with the B-loaded B-specific resin method (Huang, Bell and Dell, 1999). To minimize B contamination in the B0 treatments, 2 g cleaned B-specific resin contained in a nylon mesh bag was added to the nutrient solution of each pot, to remove any B possibly present in the solution phase. Other nutrients (except for B) were supplied to the plants by programmed nutrient addition as detailed in Huang *et al.* (1996).

To determine the critical stage of microsporogenesis at which B withdrawal causes pollen sterility, four groups of plants with four pots each, were transferred into 0 μM B treatment (B0) for 3 d during specified intervals of microsporogenesis between premeiosis of pollen mother cells to pollen mitosis-II (Table 2). At the end of each 3 d period, 10 μM B buffered with B-loaded B-specific resin was resupplied to the plants. The B0 treatment periods coincided with the pollen developmental stages detailed in Table 2. These stages were identified for the anthers of primary florets of the central four spikelets of the main shoot ear by the method described by Vergne *et al.* (1987).

Before transfer into the 0 μM B nutrient solutions, roots from each pot were flushed with triple deionized (TDI) water purified with B-specific resin and soaked for 15 mins in each of three changes of 5 l TDI water containing 2 mM $Ca(NO_3)_2$. Four pots of plants in each treatment were used for pollen viability testing and a pot of plants from the control and S2B0 (see Table 2) treatments was used to measure anther length.

The anthers from primary florets of the central four spikelets of the main shoot ears were sampled from the control and S2B0 plants by harvesting one pot of plants every day from the end of the 3 d B0 period onwards for

TABLE 2. *The sporogenesis stages of the primary anthers of the central four spikelets of the main shoot ear and the timing of boron withdrawal treatments (expt 2)*

Day	Anther length (mm)	B0 treatment	Pollen development of primary florets	Pollen development of tertiary florets
0	0·8	S1B0 B withdrawal	Pollen mother cells (the 3rd archesporial cell cycle)	1st archesporial cycle
1				
2				
3	1·6	B resupply	Early meiosis	Premeiotic interphase
3	1·6	S2B0 B withdrawal	Early meiosis	Premeiotic interphase
4				
5				
6	2·4–2·6	B resupply	Young vacuolated microspores	Late tetrad
6	2·4–2·6	S3B0 B withdrawal	Young vacuolated microspores	Late tetrad
7				
8				
9	3·5–3·7	B resupply	Mitosis-I completed, some starch granules present	Young vacuolated microspores (just before mitosis-I)
9	3·5–3·7	S4B0 B withdrawal	Mitosis-I completed, some starch granules present	Young vacuolated microspores (just before mitosis-I)
10				
11				
12	4·0	B resupply	Mitosis-II completed, sperm cells elongated, abundant starch granules, anthesis imminent	Active mitosis-II

Anthesis of primary florets occurred on day 9 after meiosis.

TABLE 3. *Effects of withdrawing B supply (−B) on B concentrations (mg B kg^{-1} dry matter) of the flag leaf (FL) blades and the blades immediately older than FL (FL-1) of wheat ('Wilgoyne') plants at 5 to 25 d after formation of the terminal spikelet*

	Days after terminal spikelet initiation†			
Treatment*	5	10	17	25
		FL‡		
− B	1·89 ± 0·34	0·70 ± 0·10	0·48 ± 0·04	0·82 ± 0·04
+ B	4·67 ± 0·13	4·48 ± 0·16	4·53 ± 0·41	9·29 ± 0·51
		FL-1		
− B	1·87 ± 0·05	1·16 ± 0·05	1·09 ± 0·05	1·64 ± 0·02
+ B	4·46 ± 0·14	8·14 ± 0·25	2·80 ± 0·03	3·99 ± 0·46

Values are means of four replicates, with corresponding standard errors (expt 1).
* Treatments: −B = 0 μM, +B = 10 μM; † Boron treatments were imposed at the terminal spikelet stage. ‡ The flag leaf was 30% emerged by the time of the first harvest (6 d after B deficiency), 70–80% emerged at day 10 and fully emerged at day 17.

5 consecutive days. Anther length was measured under a dissecting microscope with a calibrated micro-ruler in the eye lens.

At anthesis, pollen from the tertiary (third) florets of the central four spikelets of the main shoot was collected for viability tests with the fluorochromatic (FCR) method (Heslop-Harrison, Heslop-Harrison and Shivanna, 1984). Due to events beyond our control, we were unable to collect pollen from the primary and secondary florets. The anther developmental stages of the tertiary florets were 30–48 h behind the primary florets (Bennett *et al.* 1973). Pollen developmental stages of the tertiary florets at which 0 μM B was imposed are detailed in Table 2.

The youngest expanding leaf blades present on all shoots (including all tillers) were sampled at the end of the 3 d B0 treatment period, in the control, S2B0 and S3B0 plants. Leaf B concentrations were determined to confirm the effect of B withdrawal from nutrient solution on the B status of new leaves. Due to the limited number of plants available in each treatment, only one plant per treatment per day was sampled for the analysis of B in the youngest expanding leaves.

RESULTS

Experiment 1

Withdrawing B from the nutrient solution decreased B concentration in the FL and FL-1 leaves to less than 1–2 mg B kg^{-1} dry matter after 5 d of −B treatment, without causing the appearance of any leaf symptoms even after 25 d (Table 3).

Increasing the duration of B withdrawal beyond 5 d decreased the number of competent florets and grain set, and therefore overall floret fertility declined (Table 4). At the premeiotic stage (5 d after the terminal spikelet), withdrawing B had no significant effect on the number of competent florets and grains or floret fertility in the main shoots and tiller 1. The negative effect of B withdrawal during floret development became irreversible only after 10 d of treatment when anther development reached early meiosis (see Table 1 for treatment duration). In particular, when the duration of B deficiency spanned from the terminal spikelet stage of mitosis-I (17 d of −B), spike fertility was severely depressed (Table 4). In both the main shoots and tiller 1, prolonged B deficiency (25 d of −B) severely affected floret development and thus their fertility; ears had no competent floret or grain in either the main shoot or tiller 1 (Table 4).

After 17 d of −B, the grain and floret numbers of tiller 1 and floret fertility were depressed but to a smaller extent than in the main shoot ear (Table 4, Fig. 1). The floret development of tiller 1 lagged 2–3 d behind that of the main shoot of the same plant.

Experiment 2

A significant decrease in anther length was detected in the S2B0 plants, but not in other treatments, 2 d after the end of the B withdrawal period (Fig. 2). From the third day of B resupply onwards, the S2B0 anther elongation rate partially recovered, but its length remained shorter than the control.

Pollen viability was most severely affected by the 3 d B withdrawal treatment during the stages of premeiotic interphase and late tetrad (S2B0 plants) (Fig. 3). The S2B0 plants had the lowest pollen viability, compared to the control and the other B0 treatments. In comparison, the percentage of viable pollen of the S4B0 was lower than that of the control, S1B0 and S3B0 plants, and the latter two did not differ from the control.

At the end of each 3 d period, B concentrations in the youngest emerging leaf blades (YEB) of the B0 plants decreased to 2 mg B kg^{-1} dry matter or below, in comparison to 4·7 mg B kg^{-1} dry matter in the control YEB (Table 5).

Symptoms of B deficiency were observed in the roots and anthers but not in leaves at the end of the 3 d B0 treatment. At the end of B0 treatment, roots labelled prior to the B treatments were sampled for measurement of length by floating them in deionized water in a flat tray. At the end of the 3 d period of each B0 treatment, the distance between the main root tip and the youngest visible lateral root tip was reduced compared to the control. By visual observation of roots of +B and −B treatments, the length of the newly initiated lateral roots was generally shorter in the B0 plants than the control. Anthers of the S2B0 plants differed in appearance from the control, S1B0 and S3B0 plants. S2B0 anthers were terminally tapered, pale green and the surface had a matt finish, in contrast to the brilliant shiny appearance of anthers from the control, S1B0 and S3B0.

DISCUSSION

The present results indicate a short critical period of microsporogenesis during which B deficiency retards anther development and lowers pollen viability. Withdrawing B

TABLE 4. *Effects of withdrawing B (−B) for 5, 10, 17 and 25 d on the number of grain per ear, the number of florets per ear, and grain set index (GSI) of the main shoot and tiller 1 of wheat 'Wilgoyne'*

	Ear components		GSI (floret fertility)*	
Days of −B	Grains per ear	Florets per ear	(LAC%)	(CMU%)
Main shoot				
0 (control)	52·2 ± 2·2^{A}	57·8 ± 0·6^{A}	90·5 ± 2·3^{A}	98·0 ± 0·0^{A}
5	53·0 ± 1·3^{A}	58·0 ± 2·0^{A}	92·0 ± 1·7^{A}	99·3 ± 0·5^{A}
10	36·5 ± 4·7^{B}	48·5 ± 3·0^{B}	72·8 ± 7·6^{B}	60·0 ± 11^{A}
17†	2·25 ± 0·75^{C}	2·25 ± 0·75^{C}	ND	2·75 ± 1·03^{C}
25‡	ND	ND	ND	ND
Tiller 1				
0 (control)	42·5 ± 1·6^{A}	46·5 ± 1·2^{A}	91·5 ± 0·65^{A}	94·0 ± 4·08^{A}
5	48·3 ± 1·3^{A}	50·0 ± 1·6^{A}	96·3 ± 0·48^{A}	98·8 ± 0·75^{A}
10	37·3 ± 1·7^{B}	43·8 ± 1·6^{A}	84·5 ± 3·28^{B}	82·6 ± 5·1^{B}
17†	19·3 ± 4·8^{C}	27·3 ± 6·0^{B}	69·7 ± 7·42^{C}	30·0 ± 6·9^{C}
25‡	ND	ND	ND	ND

Values are means of four replicates per treatment ± s.e. Values for the same parameter labelled with the same letter are not significantly different ($P \leqslant 0{\cdot}05$) (expt 1).

* The grain set index (GSI) was calculated by the CMU and LAC methods as defined in Materials and Methods; † the ears only had a small number of grains and competent florets; ‡ ears in this treatment were very thin and had no grain or competent florets; ND, not determined as there were no competent florets.

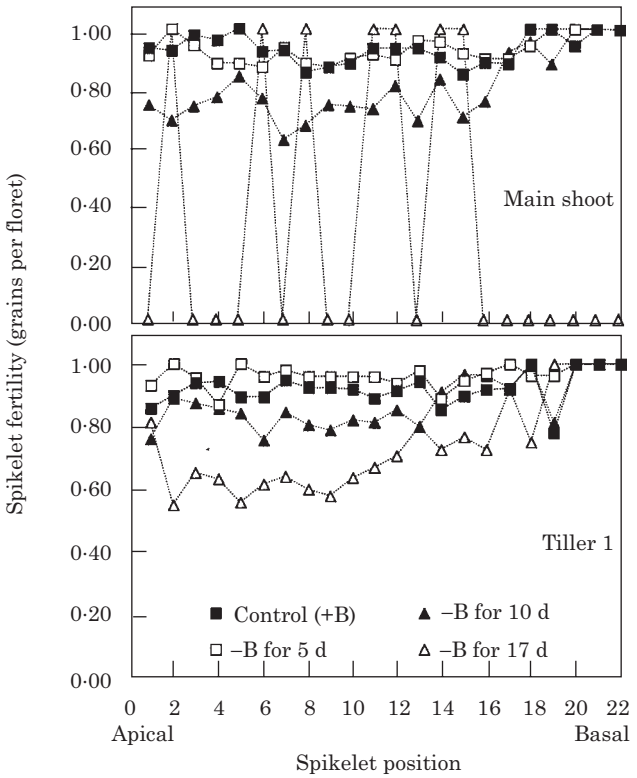

FIG. 1. Spikelet fertility (number of grains per floret) of the main shoot and tiller 1 in wheat plants subjected to B withdrawal treatments during reproductive development from early terminal spikelet stage to late booting (ear emergence). Values are means of four replicates per treatment. The treatment of −B for 25 d was excluded from the results as its ears were very thin, with no grain or competent florets (expt 1).

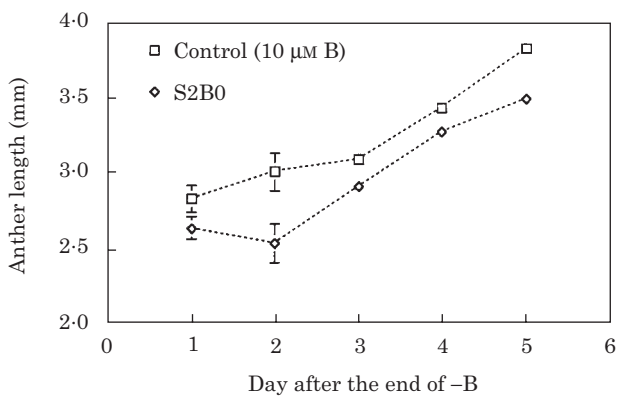

FIG. 2. Effects of B withdrawal (−B) for 3 d on anther length (mm) of the primary florets of the central four spikelets of the main spike of wheat plants. At the end of the 3 d −B treatment, 10 μM B was resupplied to the plants. S2B0, B withdrawal between early meiosis and young microspore stages (see Table 2). The values are averages of at least four anthers from two plants on each day, with standard error bars where they exceed the size of the symbols (expt 2).

supply for 3 d during meiosis caused a maximum decrease in pollen viability and limited anther elongation. The extent of floret fertility and grain set reduction of the whole ear is closely related to the number of florets reaching meiosis within the period of B withdrawal from the nutrient

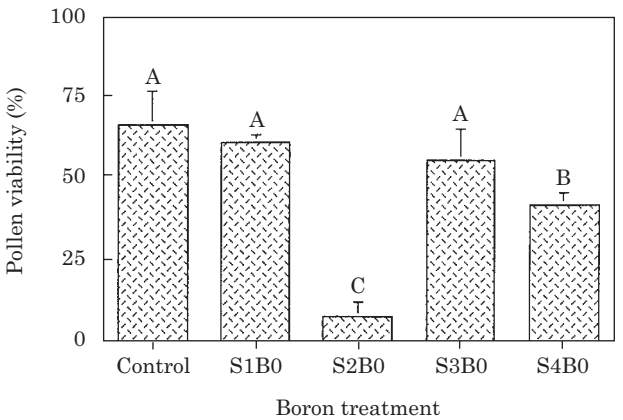

FIG. 3. Responses of pollen viability to B withdrawal during the sporogenesis stages of the first archesporial cycle—premeiotic stage (S1B0), premeiotic stage—late tetrad (S2B0), late tetrad—young vacuolated microspores (just before mitosis I) (S3B0), young vacuolated microspores (just before mitosis I)—active mitosis-II (S4B0). Pollen was sampled from the tertiary florets of the central four spikelets of the main spike. Pollen viability was assessed by the fluorochromatic (FCR) test. Values are means of four replicates, with a corresponding standard error (expt 2). Columns labelled with different letters were significantly different ($P \leqslant 0{\cdot}05$).

TABLE 5. *Effects of B withdrawal from nutrient solution on B concentration (mg kg^{-1} dry matter) in the youngest emerging blades (YEB) of wheat plants subjected to the −B treatment for 3 d at two stages of microsporogenesis: early meiosis to young microspore (S2B0) and young microspore to mitosis-I (S3B0)*

	Treatment		
Day after B resupply	Control (10 μM B)	S2B0	S3B0
0	4·7	2·1	1·6
1	5·7	4·7	2·9
2	7·1	3·4	3·4
3	4·8	4·7	ND

ND, Not determined.

At the end of the 3 d period, 10 μM B was resupplied to the plants (d 0). The YEB leaves were sampled from one representative pot of plants in each treatment and were pooled from all tillers with YEB present at the time of sampling and therefore no standard errors are presented (expt 2).

solution. As a result, the number of competent florets and floret fertility of the whole ear were most affected when the period of B withdrawal coincided with the period from emergence of flag leaf to 3 d after its full emergence. From the response of floret fertility in the main shoot and tiller 1 to the varying length of B withdrawal treatments, it is deduced that this critical period of whole ear fertility lasted about 7 d.

During reproductive growth, a brief episode (3 d) of B withdrawal during the premeiotic interphase to late tetrad resulted in the formation of defective pollen. Rawson (1996*a*) raised a similar hypothesis that meiosis is the most sensitive stage of microsporogenesis to B deficiency.

However, physiological and biochemical mechanisms by which B deficiency disturbs microsporogenesis in wheat were not elucidated. By contrast, Rerkasem *et al.* (1997) found that at low soil B supply, microsporogenesis proceeded normally at least until the vacuolated young microspore stage, but at anthesis pollen was sterile. However, the exact period when pollen development was impaired was not identified by these authors because anthers were not sampled frequently. In expt 2, pollen viability was partially depressed in S4B0 plants. These findings suggest that there may be two phases of pollen development during with B deficiency is likely to impair pollen viability: (1) meiosis to young microspore; and (2) mitosis-I to pollen maturation (starch accumulation). During the first phase, impaired formation of pollen cell walls and cell expansion may be the primary consequence of B deficiency. This hypothesis may be partially supported by the restricted anther elongation after B withdrawal. Nevertheless, B withdrawal may also have decreased cell division in the early growth of anthers, leading to the reduced number of cells compared to +B anthers. Therefore, the decreased number of cells per anther and restricted cell dimensions may both have contributed to the shorter anther length in S2B0 treatment. During the second phase, B deficiency may partially impair the delivery of carbohydrate necessary for starch accumulation from mitosis-I to anthesis; this will be investigated in further research.

Boron acts as an essential ingredient in the formation of cell walls through the borate-diester bonding with rhamnogalacturonan II (RG-II) (Matoh, 1997) and B deficiency inhibits cell expansion (Hu and Brown, 1994). Recent immunocytochemical evidence revealed the presence of the borate-RG-II complex in cell walls of lily pollen tubes (Matoh *et al.*, 1998), but, so far, pollen grains have not been studied during their development. Boron deficiency may alter the deposition of callose, cellulose and pectate in cell walls of anther and pollen cells and immunocytochemical investigation may help to reveal effects of low B on these processes.

Starch accumulation in anther and pollen cells may warrant particular attention. During the period of pollen mitosis-I and II, the anthers are sinks for carbohydrates as starch accumulates in pollen grains. The inhibition of carbohydrate transport is suggested to be a secondary effect of B deficiency in leaves (Marschner, 1995) and the flag leaf is the main contributor of assimilates to the developing ear after the leaf emerges fully (Rawson and Hofstra, 1969). The decreased sugar transport to reproductive tissue has been suggested as a trigger of carbohydrate metabolism disorder which leads to the failure of pollen development (Saini, 1997). Lalonde, Beebe and Saini (1997) found that anthers of water-stressed wheat plants generally lacked starch. Hence, the impairment of carbohydrate metabolism in the anther may have also contributed to the failure of pollen maturation in B-deficient wheat ears.

The critical phase for floret fertility of the whole ear lasted for about 7 d from the early emergence of the flag leaf to 2–3 d after its full emergence. Rawson (1996*b*) also found that adequate B was required for developing fertile florets during a similar period. There are time differences in anther/pollen development among the primary, secondary and tertiary florets of the same spikelet and among the upper, central and basal spikelets (Bennett *et al.*, 1973; Rawson, 1996*b*). By calculation, in an ear with 20 spikelets, the overall developmental difference among the florets from tip to base may equal about 3 d. By about 3 d after the full emergence of the flag leaf, the advanced florets of the middle of the ear would have reached the pollen mitosis-I stage and the less advanced florets of the acropetal and basal spikelets would have completed meiosis. As premeiotic interphase/meiosis is the major critical period of microsporogenesis for B-deficiency-induced male sterility, the more florets in an ear that developed to the meiosis stage within the period of B withdrawal, the higher the floret sterility. This is further supported by floret fertility in tiller-1 being relatively less affected by B deficiency than that in the main shoot. When B deficiency persisted for 10 d, floret fertility was decreased by 30–40% in the main shoot ear, but only 20% in the tiller-1 ear. Anthesis of the tiller-1 ear was about 2 d delayed relative to that of the main shoot.

In wheat, the B requirement for reproductive growth is higher than for vegetative growth (Rerkasem *et al.*, 1997). A fertile floret of wheat requires >8 mg B kg^{-1} dry matter in anthers and 5–6 mg B kg^{-1} dry matter in carpels (Rerkasem and Lordkaew, 1996; Rerkasem *et al.*, 1997). Anthers and carpels account for about 2% of the ear total dry weight, but hold 6–7% and 4–5% of the total B in the ear, respectively (Rerkasem and Lordkaew, 1996). Therefore, florets are relatively strong sinks for B during their development, compared to other floral parts. The B deficiency treatments in the present study may have decreased the B supply to the florets, given that the B concentrations in flag leaf blades declined to <1 mg B kg^{-1} dry matter. Long periods of B withdrawal (e.g. 17, 25 d) may have also affected female fertility of wheat florets, contributing to the low grain set.

Under field conditions, other environmental factors such as water deficit may affect microsporogenesis alone or in combination with B deficiency. Water deficit may either lower B availability in soil or depress B uptake capacity of plants (Huang, Wang and Bell, 1997). During meiosis, water deficit disrupts microsporogenesis of wheat (Dorion, Lalonde and Saini, 1996; Lalonde *et al.*, 1997). Effects included degeneration of meiocytes, loss of orientation of the reproductive cells, and abnormal vacuolation of tapetal cells (Lalonde *et al.*, 1997). Later pollen abortion induced by water deficit was attributed to the disrupted carbohydrate metabolism within anther cells (Dorion *et al.*, 1996; Saini, 1997). Under mediterranean-type climates, water deficit in the soil usually co-exists with high air temperature, which decreases B movement in the soil and B availability to plant roots. In contrast, in the subtropics, high temperature often occurs in conjunction with high air humidity, which limits leaf transpiration and, therefore, plant B uptake. Therefore, the occurrence of these unfavourable environmental factors during pollen meiosis should also be considered when assessing the impact of B deficiency on pollen development in the field (Rawson, 1996*a*).

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