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Effects of Boron Deficiency and Low Temperature on Wheat Sterility

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Abstract

Wheat exhibits sterility in many parts of subtropical and tropical Asia. Boron deficiency is believed to be the cause of sterility in many, but not all, cases. This creates the need for standards to allow B deficiency to be diagnosed and distinguished from other causes of sterility. This study was designed to investigate the responses of functional indicators of B requirements, namely leaf blade elongation rates and potassium leakage rates, to B supply (0 and 10 μM B) and night temperature (ambient: > 10°C and low: > 5°C). An objective of the study was to develop diagnostic and prognostic standardsfor B deficiency, based on the functional B requirements for leaf elongation and membrane permeability.

Although the interruption of B supply (–B) significantly decreased B concentrations in leaf blades and ears, it had no consistent effect on rates of leaf blade elongation or leaf K leakage in the two cultivars of wheat tested at either the vegetative or the reproductive stage. Low night temperature also had little effect. However, the grain set index (GSI) was significantly decreased by the –B treatment. Low night temperature had no effect on GSI in +B plants. In –B plants, ambient night temperature decreased GSI apparently by accelerating ear development, so that critical stages of grain set coincided more with the deficiency of B than in –B plants exposed to low night temperatures.

Boron (B) is an essential micronutrient for the growth of plants. Some grasses, such as wheat, have a much lower B requirement for normal growth than dicots (Dugger 1983). Nevertheless, B deficiency causing yield loss has been documented in wheat in southern and eastern Asian countries such as Bangladesh, China, Nepal, India and Thailand (Rerkasem et al. 1993).

Boron deficiency affects the growth and function of male or female reproductive organs of wheat, leading to the failure of fertilisation and grain setting (Rerkasem et al. 1993) and eventual yield loss. B deficiency causes the poor development of anthers and pollen and germination failure of pollen, owing to insufficient B supply in the pollen or in the stigma and style (Cheng and Rerkasem 1993).

Although there is an increasing understanding of the role of B deficiency in wheat sterility, the detection of B deficiency in wheat plants at the reproductive stage often allows no time for correction of the problem. Wheat vegetative growth is insensitive to B deficiency. There are usually no symptoms in the leaves and no significant growth reduction. Vegetative growth is therefore not a reliable diagnostic indicator of B deficiency. In addition, rate of dry matter accumulation responds to the declining B supply more slowly than leaf B concentrations (Kirk and Loneragan 1988). As a result, it is advisable to explore physiological responses of shoots to B deficiency for its early diagnosis.

Boron plays both structural and functional roles in plant cells (Loomis and Durst 1992; Parr and Loughman 1983). Boron is an essential component of cell wall structure (Loomis and Durst 1992) and B deficiency inhibits cell division and elongation (Hu and Brown 1994). Boron deficiency disturbs membrane integrity and increases membrane permeability (Cakmak et al. 1995; Parr and Loughman 1983).

Based on these functional roles of B in plant cells, it is presumed that leaf elongation and leaf K leakage are sensitive parameters by which to diagnose B deficiency in wheat plants. The response of leaf blade elongation rates to B deficiency has been successfully used to set a critical B concentration for the diagnosis of B deficiency in black gram (Noppakoonwong et al. 1993). In sunflower, leaf K leakage rates were closely correlated with leaf B concentrations

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(Cakmak et al. 1995).

Wheat production is increasing in the warmer areas of southern and eastern Asia, such as Bangladesh, Nepal, China and Thailand (Rerkasem et al. 1993). Field observation has shown that wheat plants grown in these areas might be exposed to low night temperature just before or during anthesis, enhancing the failure of grain set (Pant, pers. comm.).

Several reports appear in the literature linking low temperature damage in plants and B deficiency (Shorrocks 1991). These reports are limited to observations of increased damage to shoots by frost or low temperature when plant B status is low, and in some cases, alleviation of the injury with foliar B sprays. Experimental evidence that low temperatures increase sensitivity to B deficiency is limited to one brief report by Parr and Loughman (1983). Parr and Loughman examined the response of P uptake by *Zea mays* to decreasing temperature in the presence or absence of B in solution. In solutions supplied with B, the uptake of P declined with decreasing temperature. However, below 20°C there was a distinct inflection in the curve, which implied a temperature-dependent change in membrane conformation from a fluid to a gel. For plants grown in tropical and subtropical regions, low temperature even above freezing point might cause a change in membrane properties and enhance membrane permeability (Simon 1974). What had not been previously reported was the finding of Parr and Loughman (1983) that the critical temperature at which membrane properties changed, depressing P uptake, was 2°C higher in B-deficient solutions than in B-sufficient solutions. These limited results imply that plants with low or deficient B supply might be more sensitive to cold temperature damage to membranes of growing tissues than plants with sufficient B.

In 1994, at Tonglu in Zhejiang Province, China, we observed oilseed rape plants showing symptoms of frost damage to the leaves. This was in late March, some 10 to 14 days after snowfalls that remained on the ground for two days. It was only in plots without B fertiliser that frost damage occurred; plants treated with B fertiliser at sowing were free of the symptoms. Thus it appears that leaf tissue of oilseed rape was more sensitive to frost damage when low in B. The converse conclusion, that low temperature increases internal B requirements, has not been demonstrated.

Interesting as these observations are, their relationship to B deficiency and to internal B requirements is not clear. Episodes of low temperature could be the cause of site-to-site and year-to-year variation in internal B requirements of wheat, and therefore in grain set. Further studies to establish a meaningful causal link between low temperature damage and plant B status would be particularly useful. Controlled environment studies would appear to be necessary to establish such a link. Field demonstration of the significance of a low temperature effect is also necessary.

The objectives of the present study were to examine effects of B deficiency and low night temperature on leaf blade elongation and leaf K leakage, and to establish a relationship between these responses and leaf B concentrations. On the basis of this relationship, the study aimed at estimating diagnostic standards for B deficiency in wheat vegetative growth, and prognostic standards for predicting the sterility of florets and grain set failure in wheat plants during the reproductive stage. It was hypothesised that low night temperature might exacerbate B deficiency in wheat plants subjected to low B supply. This paper reports the responses of leaf blade elongation rates, leaf K leakage, B concentrations, spikelet fertility and grain set index to B deficiency and low night temperature for wheat plants at vegetative and reproductive stages.

Materials and Methods

Plant culture

Wheat plants were grown in solution culture in a glasshouse. The full-strength basal nutrient solution used initially contained the following chemicals. Concentrations in parentheses are µmol/L: NH_4NO_3 (2000), KNO_3 (2800), $Ca(NO_3)_2 \cdot 4H_2O$ (1600), $MgSO_4 \cdot 7H_2O$ (1000), KH_2PO_4 (100), and K_2HPO_4 (100), FeEDTA (100), NaCl (8), $ZnSO_4 \cdot 7H_2O$ (2), $MnSO_4 \cdot H_2O$ (2), $CuSO_4 \cdot 5H_2O$ (0.5) and $Na_2MoO_4 \cdot 2H_2O$ (0.08). Water used for making up the solutions and the macronutrient stock solutions were purified with B-specific resin (IRA-743, Sigma Chemical Co.). Only analytical grade chemicals were used to make up the solutions. The initial pH in the solution was about 6. The pH was buffered at 7.0 to 7.5 by adding 0.1 g $CaCO_3$ to each pot of solution.

The programmed nutrient addition technique (Asher and Edwards 1983) was used to supplement all nutrients during plant growth, except for B during the B treatment period. The following nutrient concentrations were assumed to be adequate in whole shoots: 4.5% N, 0.4% P, 3.0% K, 0.3% Ca, 0.3% S, 0.2% Mg, 50 mg/kg Fe, 20 mg/kg Mn, 20 mg/kg Zn, 5 mg/kg B, 3 mg/kg Cu and 0.5 mg/kg Mo. The following model was used to predict plant dry matter at a given day:

$$Y = A \times \log_{10}(A) + e^{(RGR \times T)}$$

where Y is the predicted dry weight per plant, A is the existing dry weight (g per plant), RGR is relative growth rate (g/g/day), and T is the number of days. The amounts of nutrients and B needed were calculated by the 'Nutradd' software (Asher and Blamey 1987). The RGR was determined by sequentially harvesting plants in extra pots of the +B treatment.

Experiment 1—Reproductive stage

This experiment was designed to investigate the responses of the permeability of leaf cell membranes (measured as K leakage) and leaf blade elongation in plants at the reproductive stage to the experimental treatments, and to attempt to correlate the responses with pollen viability and grain set index.

Wheat seeds (*Triticum aestivum* cv. SW 41, from Thailand) were soaked in aerated $CaSO_4$ solution (2 mM) for four days in the dark at room temperature (10°–16°C). The germinated seedlings were then transplanted into a plastic tray containing 8 L of one-third strength basal nutrient solution (with 5 μM B) and placed in the glasshouse. After five days in the trays, the seedlings were transplanted into pots containing 5 L of full strength nutrient solution with 5 μM B. Nine seedlings, with about 1½ leaves each, were planted into each pot. The pots were randomly positioned in a temperature-controlled water bath set at 18 ± 1°C. The pots were repositioned every two days to minimise positional effects on growth.

At 13 days after transfer (DAT), plants were thinned to eight per pot. Dry weights of the thinned plants were recorded at transfer and at 13 DAT, and relative growth rates were calculated using the Nutradd software (Asher and Blamey 1987). Calculated amounts of nutrients were added to the solutions on 15, 22, 44 and 64 DAT. The healthy appearance of the plants in the B-sufficient pots indicated that the nutrients added were adequate for plant growth.

At 31 DAT (about 8th leaf stage), the B and night temperature treatments were begun using two sets of four replicates in each treatment. The B treatments were 0 μM (–B) and 10 μM (+B) H_3BO_3. The night temperature treatments were ambient glasshouse temperature (> 10°C) and low temperature (> 5°C). The detailed glasshouse environmental conditions are shown in Table 1. A recorded example of the low night temperature profile is shown in Figure 1. During the treatment, the low night temperature averaged 9.2°C (max. 13.7° – min. 5.7°). Low temperatures were imposed by transferring pots every evening to a temperature-controlled room. The room was programmed so that temperatures decreased progressively from ambient at 5:30 p.m. to a minimum of 6°C at 6 a.m. Plants were transferred out of the cold room each day at 7 a.m. to the glasshouse. During the day both sets of plants experienced the same environmental conditions. The plants were exposed to the B and night temperature treatments for 18 days and then B was resupplied to the –B plants until grain set.

The length of the ninth leaf of a main stem was measured daily over seven consecutive days in three plants per replicate from its emergence to its maximum length for the determination of elongation rate. From one set of the plants, the youngest emerging blades (YEB) and the blades immediately older than YEB (YEB + 1) were sampled from the main stems of two plants per replicate for the determination of B concentrations at 1, 3, 7, 12 and 18 days after the start of treatment. At 12 and 18 days after the start of treatment, ears of the main stems were also harvested for the determination of B concentrations. Plant growth stage at each harvest is given in Table 2.

For the determination of leaf K leakage in YEB of the main stem, two plants per replicate were sampled from the other set of plants. The leaves were cut off at the base of the emerged blades. As B deficiency affects growing tissues more than mature ones, the basal part of the YEB was considered to be the most sensitive to B deficiency. The leaf segment up to 5 cm from the base was cut into 1 cm strips. After being washed in three changes of triple-deionised (TDI) water, these leaf strips were placed in a plastic container with 10 mL TDI water and gently shaken for two hours (10 a.m. to 12 noon) at glasshouse temperature in

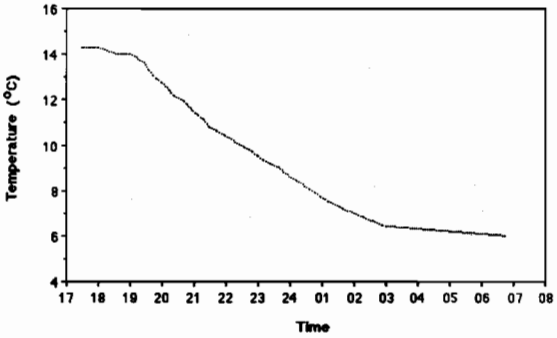

Figure 1. A example of a night temperature (°C) profile in the cold room used to expose wheat plants to low temperature. The temperature was set to decrease gradually between 5:30 p.m. and 6 a.m. the next day from ambient temperature down to 6°C.

the light (Navari-Izzo et al. 1989). The solutions were sampled for the determination of K leaked out of the leaf strips. The leaf strips were frozen at –20°C and thawed at room temperature for total membrane disruption in the leaf cells. The thawed leaf samples were incubated in 20 mL TDI water overnight and K concentrations in the incubation solutions were determined by inductively coupled plasma (ICP) spectrometry.

Ears of the main stems were harvested for the determination of grain set index 52 days after the start of treatment, when full grains were observed in ears of the +B plants.

Experiment 2—Vegetative stage

Wheat cultivar Wilgoyne was used to investigate effects of B supply and low night temperature on responses of leaf blade elongation, leaf K leakage and leaf B concentrations at the vegetative stage for the diagnosis of B deficiency. Wheat seedlings were prepared as described for Experiment 1. Wheat seeds were soaked in the dark at room temperature (about 18°–25°C) in aerated $CaSO_4$ solution for three days until germination. Uniformly germinated seedlings were transplanted into a plastic tray with 8 L of one-third strength nutrient solution. After being acclimatised in the glasshouse for six days, ten randomly selected plants were transferred into each plastic pot, which contained 5 L of complete, full-strength nutrient solution.

The treatments of B supply and low night temperature were applied to the plants at 26 DAT. Boron treatments were 0 (–B) and 10 (+B) μM and the temperature treatments were ambient glasshouse temperature (16°–20°C) and low temperature (5°–15°C). In the –B treatment, B-specific resin (6 g air-dry per pot) was placed in

Table 1. Environmental conditions in the glasshouse during the treatment periods of experiment 1 and 2

		Air T (°C)	Water T (°C)	RH %	Daily average light intensity (μmol/m²/s)	Total radiation (MJ/m²/day)
Experiment 1 (SW 41)						
Day	Mean	21.5	18.3	69	268	6.61
	Maximum	25.2	21.1	83	760	9.41
	Minimum	15.5	16.2	59	64	1.54
Night	Mean	17.3	16.9	80		
	Maximum	21.3	17.9	84		
	Minimum	15.8	16.9	75		
Experiment 2 (Wilgoyne)						
Day	Mean	21.9	19.6	73	291	7.14
	Maximum	25.2	23.0	85	758	8.65
	Minimum	16.0	16.5	64	198	5.28
Night	Mean	17.9	17.5	81		
	Maximum	21.5	18.7	84		
	Minimum	15.8	16.9	75		

the solution to minimise B concentrations in solution. Each treatment was replicated four times. The detailed glasshouse environmental conditions are shown in Table 1. A recorded example of night temperature profile in the cold room is shown in Figure 1. During the treatment, the low night temperature averaged 10°C (max. 14.2° – min. 7.1°) in the cold room.

Potassium (K) leakage was measured in the youngest emerging blades. Two plants per pot were sampled for YEB from the main stems of one set of plants at 0, 3, 6, 9 and 12 days after the start of treatment. The measurements were conducted in the same way as in experiment 1.

The length of the seventh leaf of a main stem was measured daily over seven consecutive days in three plants per replicate from their initial emergence to their maximum length for the determination of their elongation rate.

In the other set of plants, the YEB and YEB + 1 blades were sampled from the main stems of two plants per replicate for the determination of B concentrations at 0, 3, 6, 9 and 12 days after the start of treatment. Plant growth stage at each harvest date is given in Table 2.

Boron determination

The 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 ICP spectrometry (Zarcinas et al. 1987).

Data analysis

Two-way analysis of variance was applied to the data to detect the effects of B supply and night temperature treatments on leaf blade elongation rates, leaf K leakage and B concentrations.

Results

Leaf blade elongation and K leakage—Experiment 1

At the start of treatments, B concentrations in leaf 8 were relatively low, at 3 to 5 mg/kg (Tables 3a, 3b). Subsequently, in +B solutions, leaf B concentrations in plants at ambient night temperatures increased to 9 mg/kg at 12 days after the start of treatment (DAS) and then declined markedly at 18 DAS to 3 mg/kg. In +B plants at low night temperature, B concentrations in leaf 8

Table 2. Plant growth stages at each harvest in experiments 1 and 2

Harvest	Date	Day	Plant stage
Experiment 1 (SW 41—reproductive stage)			
	9 June	0	8th leaf emerging
Harvest 1	10 June	1	8th leaf emerging, 7th leaf fully emerged
Harvest 2	12 June	3	8th leaf fully emerged, 9th (flag) leaf emerging
Harvest 3	16 June	7	Flag leaf half emerged
Harvest 4	21 June	12	Flag leaf ligule emerged, ear size ≈ 4 cm
Harvest 5	27 June	18	Late booting in Ta plants
(B resupply)			Mid booting in Tl plants
	6 July	27	Anthesis in Ta plants
	12 July	33	Anthesis in Tl plants
Harvest 6	31 July	52	Late milky to soft dough. Main stem grain full
Experiment 2 (Wilgoyne—vegetative stage)			
Harvest 1	23 June	0	6th leaf $^1/_3$–$^1/_2$ emerged
Harvest 2	26 June	3	6th leaf fully emerged, 7th leaf $^1/_4$–$^1/_3$ emerged
Harvest 3	29 June	6	7th leaf $^4/_5$ emerged
Harvest 4	2 July	9	7th leaf fully emerged, 8th leaf emerging
Harvest 5	5 July	12	8th leaf $^1/_2$ emerged

Ta = ambient night temperature, Tl = low night temperature

ranged from 4 to 8 mg/kg over the period 3–12 DAS and, as in plants at ambient night temperature, dropped at 18 DAS to 3 mg/kg.

In –B treatments, B concentrations in leaf 8 declined strongly between 1 and 3 DAS and, by 7 DAS, had declined to 2 to 3 mg/kg, where they remained.

In leaf 9, which emerged at 7 DAS, B concentrations in –B plants were 1 to 1.5 mg/kg at emergence and remained there. By contrast, B concentrations in leaf 9 of +B plants were 3 to 7 mg/kg.

At 12 and 18 DAS, low temperature increased B concentrations in the ears of +B plants, and decreased them in –B plants.

No obvious symptoms of B deficiency or injury caused by low temperature were observed in the –B plants or low-temperature-treated plants.

Although the removal of B supply decreased B concentrations to as low as 1 mg/kg dry matter, it did not induce any significant increase in K leakage out of the newly emerged leaf blades (Table 4). The effects of low temperature on leaf K leakage were not consistent over time.

The effects of –B and low temperature on leaf blade elongation rates of the ninth leaf were generally not significant (Table 5).

Leaf blade elongation and K leakage—Experiment 2

Boron concentrations in the YEB were decreased only by the –B treatment, not by low temperature. There was no interaction between –B and low temperature on B concentrations in the leaves (Table 6).

The treatments of –B and low night temperature generally caused no response in K leakage from YEB, except that –B significantly increased K leakage from YEB at ambient temperature compared with low temperature at 6 DAS (Table 7). The elongation rates of YEB were not consistently affected by the B or temperature treatments (Table 8). A significant decrease in leaf blade elongation rates caused by low temperature was observed at 2–3 DAS, but the reverse applied at 4–6 DAS. The young leaves became flaccid when returned to the glasshouse after being exposed to low night temperature.

Spikelet fertility and grain set index

The interruption of B supply significantly decreased spikelet fertility and grain set index (GSI) in SW 41 plants (Table 9, Fig. 2). There was slower ear development in the low night temperature treatment than at ambient night temperature. The low temperature treatment had no effect on spikelet fertility or GSI of plants with B, but increased them in plants without B. In comparison, the numbers of grain and florets in each spikelet of both –B and +B plants was increased by the low temperature treatment (Fig. 2). These effects of B and night temperature treatments occurred mostly in the central florets (spikelets 3–17).

Discussion

The present study aimed at exploring sensitive physiological responses of wheat to B deficiency and their relationship to B concentrations in shoot parts. However, the relationship between rates of leaf blade elongation and leaf K leakage and concentrations of B in leaf blades and ears could not be established, owing to the lack of response of these two variables to B withdrawal (–B) and low night temperature.

The results indicate that the growth of vegetative parts requires very low internal B supply at both the vegetative and reproductive stages. A previous study with SW 41 (Rerkasem et al. 1993) showed that withdrawing B supply for 12 days also had little effect on the length of YEB. For wheat plants during the vegetative stage, a B concentration of 3 mg/kg dry matter or greater was adequate for growth (Reuter and Robinson 1986). In the present study, –B treatment decreased B concentrations in the YEB to around 1 mg/kg dry matter in the plants at vegetative (Wilgoyne) and reproductive (SW 41) stages, but did not have any effect on rates of leaf blade elongation and leaf K leakage from the YEB. In contrast, dicot crops have much higher B requirement for their growth and much higher critical B concentrations than the adequate B concentrations for wheat (Reuter and Robinson 1986). For example, critical B concentrations in the youngest open leaves (similar to the YEB in wheat plants) of canola plants are as high as 10 to 14 mg/kg dry matter (Huang and Bell 1994). The low internal B requirement of wheat plants gives rise to the practical difficulty of establishing the changes in leaf blade elongation and leaf K leakage rates of YEB in response to the narrow range of leaf B concentrations from deficiency to adequacy.

Table 3a. Boron concentrations (mg/kg dry matter) in the eighth and ninth leaves of SW 41 wheat for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the reproductive stage. The values are means of four replicates, followed by standard deviation in parentheses

Treatment		1 DAST	3 DAST	7 DAST	12 DAST	18 DAST
Eighth leaf	–B, Tl	3.45 (0.84)	2.82 (0.78)	1.88 (0.50)	2.44 (0.24)	1.92 (0.34)
	–B, Ta	3.76 (1.31)	2.39 (0.26)	2.18 (0.25)	3.13 (0.86)	1.88 (0.56)
	+B, Tl	5.10 (0.42)	4.55 (0.25)	4.05 (0.70)	7.64 (1.04)	3.42 (0.18)
	+B, Ta	3.67 (0.28)	6.38 (0.68)	7.09 (0.47)	9.27 (0.95)	3.30 (0.82)
Ninth leaf	–B, Tl		ND	0.93 (0.65)	1.38 (0.74)	1.08 (0.20)
	–B, Ta			1.37 (0.55)	1.18 (0.84)	1.55 (0.42)
	+B, Tl			3.35 (0.51)	5.67 (0.84)	5.93 (0.69)
	+B, Ta			5.22 (1.37)	5.35 (0.17)	6.94 (1.40)
Ear	–B, Tl		ND		3.13 (1.51)	1.14 (0.53)
	–B, Ta				6.81 (1.12)	2.72 (1.80)
	+B, Tl				7.50 (1.32)	5.48 (0.81)
	+B, Ta				6.28 (0.48)	3.12 (0.24)

–B = 0 µM boron added, +B = 10 µM B added, Tl = low night temperature, Ta = ambient night temperature. The treatments of B supply and night temperature started at 31 days after transplanting when the eighth leaf was emerging. The eighth leaf was the youngest emerged blade at the start of treatment. The ninth leaf was the flag leaf. ND = not determined.

Table 3b. Statistical summary of effects of B supply and temperature treatments on B concentrations in the eighth and ninth leaves and the ears in wheat plants (cv. SW 41) for selected days after starting the treatment (DAST). The values are mean squares. Levels of significance are * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Source of variation		df	1 DAST	3 DAST	7 DAST	12 DAST	18 DAST
Eighth leaf	B supply	1	1.9	32.7***	50.0***	128.6***	8.5***
	Temperature	1	1.0	1.9*	11.2***	5.3*	0.0
	Interaction	1	2.4*	5.1**	7.5***	0.9	0.0
	Residual	12	0.5	0.3	0.3	0.7	0.3
Ninth leaf	B supply	1			39.3***	71.6***	104.8***
	Temperature	1			5.3*	0.3	2.2
	Interaction	1			2.1	0.0	0.3
	Residual	12			0.7	0.5	0.7
Ear	B supply	1				13.7**	22.5***
	Temperature	1				5.6	0.6
	Interaction	1				22.2**	14.5**
	Residual	12				1.4	1.1

Table 4. Potassium leakage (µg K^+ per g fresh weight over 2 hours) out of the youngest emerged leaf blades of wheat (cv. SW 41) for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the reproductive stage. The values are means of four replicates, followed by standard deviation in the parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

	Treatment	1 DAST	3 DAST	7 DAST	12 DAST	18 DAST
	–B, Tl	227 (38)	230 (24)	246 (49)	187 (12)	170 (15)
	–B, Ta	185 (18)	295 (53)	194 (64)	189 (23)	206 (22)
	+B, Tl	199 (22)	236 (17)	329 (90)	177 (25)	205 (34)
	+B, Ta	159 (24)	254 (15)	210 (26)	164 (17)	174 (13)
Source of variation	df					
B supply	1	2948	1246	9555	1228	9
Temperature	1	6765 **	6988 *	29223 *	138	17
Interaction	1	2	2266	4523	204	4506 *
Residual	12	720	989	3837	397	518

–B = 0 µM boron added, +B = 10 µM B added, Tl = low night temperature, Ta = ambient night temperature. The treatments of B supply and night temperature started 31 days after transplanting when the eighth leaf was emerging. At 1 and 3 DAST, the youngest emerging blades (YEB) were the eighth leaves; at 7, 12 and 18 DAST, the YEB were the ninth leaves.

Table 5. Elongation rate (cm/day) of the ninth leaf blade of wheat (cv. SW 41) for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at reproductive stage. The values are means of four replicates, followed by standard deviation in the parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

	Treatment	5–6 DAST	6–7 DAST	7–8 DAST	8–10 DAST
	–B, Tl	4.4 (0.47)	3.9 (0.32)	3.7 (0.40)	6.6 (1.09)
	–B, Ta	5.3 (0.14)	4.5 (0.27)	4.0 (0.92)	5.2 (0.96)
	+B, Tl	4.4 (0.32)	4.1 (0.52)	3.6 (0.24)	6.0 (0.49)
	+B, Ta	5.8 (1.34)	4.2 (0.35)	3.9 (0.30)	5.8 (0.39)
Source of variation	df				
B supply	1	0.23	0.03	0.02	0.0
Temperature	1	5.57 **	0.50	0.36	2.9
Interaction NS					

Table 6. Boron concentrations (mg/kg dry matter) in the youngest emerged blades of wheat (cv. Wilgoyne) for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the vegetative stage. The values are means of four replicates, followed by standard deviation in parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

	Treatment	0 DAST	3 DAST	6 DAST	9 DAST	12 DAST
	–B, Tl	2.05 (0.19)	0.93 (0.17)	0.54 (0.14)	0.85 (0.23)	0.89 (0.61)
	–B, Ta	2.05 (0.19)	1.74 (0.18)	0.68 (0.34)	1.01 (0.47)	1.26 (0.44)
	+B, Tl	2.63 (0.21)	3.05 (0.43)	3.55 (0.31)	4.33 (0.39)	4.07 (0.43)
	+B, Ta	2.63 (0.21)	3.60 (0.70)	4.14 (0.65)	5.45 (1.06)	4.36 (0.14)
Source of variation	df					
B supply	1	1.2***	13.5***	35.8***	57.2***	36.4***
Temperature	1	0.0	1.6*	0.4	1.6	0.4
Interaction NS						

–B = 0 µM boron added, +B = 10 µM B added, Tl = low night temperature, Ta = ambient night temperature. The treatments of B supply and night temperature started 26 days after transplanting, when the seventh leaves were emerging.

Table 7. Potassium leakage (µg K^+ per g fresh weight over 2 hours) out of the youngest emerged leaf blades of wheat (cv. Wilgoyne) for selected days after starting the treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the reproductive stage. The values are means of four replicates, followed by standard deviation in parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

	Treatment	0 DAST	3 DAST	6 DAST	9 DAST	12 DAST
	–B, Tl	249 (81)	277 (147)	218 (35)	335 (206)	270 (75)
	–B, Ta	249 (81)	334 (273)	412 (70)	268 (57)	273 (58)
	+B, Tl	240 (31)	346 (157)	260 (70)	257 (80)	274 (63)
	+B, Ta	240 (31)	429 (316)	224 (27)	309 (71)	251 (26)
Source of variation	df					
B supply	1	306	26732	21462 *	1388	342
Temperature	1	0	19600	24806 *	189	420
Interaction	1	0	729	52900 **	14221	676
Residual	12	3794	55491	2962	14273	3401

–B = 0 µM boron added, +B = 10 µM B added, Tl = low night temperature, Ta = ambient night temperature. The treatments of B supply and night temperature started 26 days after transplanting, when the seventh leaf was emerging.

Table 8. Elongation rate (cm/day) of the seventh leaf blade of wheat (cv. Wilgoyne) for selected days after starting treatment (DAST). Plants were subjected to a factorial combination of temperature and B supply treatments at the vegetative stage. The values are means of four replicates, followed by standard deviation in parentheses. Mean squares are shown together with levels of significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

	Treatment	1–2 DAST	2–3 DAST	3–4 DAST	4–5 DAST	5–6 DAST
	–B, Tl	4.8 (1.45)	4.3 (0.96)	3.9 (0.40)	8.5 (0.28)	3.5 (0.64)
	–B, Ta	5.4 (0.45)	4.5 (0.69)	5.2 (0.80)	7.9 (0.40)	2.7 (0.32)
	+B, Tl	5.6 (1.03)	3.7 (0.46)	4.8 (0.60)	9.2 (0.45)	4.0 (0.33)
	+B, Ta	6.1 (1.99)	5.2 (0.30)	5.0 (0.34)	8.4 (0.62)	2.4 (1.07)
Source of variation	df					
B supply	1	2.4	0.0	0.4	1.4*	0.0
Temperature	1	1.3	2.7*	2.2*	1.9**	6.0**
Interaction NS						

Table 9. Effects of B supply and night temperature treatments for 18 days on the grain set index in wheat plants (cv. SW 41). The values are means of four replicates, followed by standard errors in parentheses

Treatment	CMU %	LAC %
–B, Tl	52.6 (18.2)	53.5 (14.4)
–B, Ta	6.8 (4.6)	19.3 (5.6)
+B, Tl	94.4 (3.0)	81.4 (4.8)
+B, Ta	95.2 (2.5)	80.4 (9.0)

–B = 0 µM boron added, +B = 10 µM B added, Tl = low night temperature, Ta = ambient night temperature. CMU % is defined as the percentage of grain-set florets out of the central 20 florets of an ear (the method devised and used at Chiang Mai University); LAC % is defined as the percentage of grain-set florets out of the total number of florets of an ear (the method used by Lumle Agricultural Research Centre).

In contrast to the responses of leaf blades, spikelet fertility and grain set index were significantly decreased by the –B treatment in the present study. A similar result was also observed in the study by Rerkasem et al. (1993). These effects of B deficiency are attributed to poor pollen viability at low B supply and possibly to low B in the stigma and style of the floret (Cheng and Rerkasem 1993). The depression in grain set index in plants at low night temperature was associated with leaf B concentrations of 1 to 2 mg/kg and with ear concentrations of 1 mg/kg. In contrast, in plants at ambient night temperature, similar concentrations of leaf B were associated with substantially lower grain set index. Moreover, B concentrations in the ears at 12 and 18 DAT were actually higher in the plants at ambient temperatures than those at low night temperature, whereas grain set index was markedly lower. However, clearly, ear B concentrations of more than 3 mg/kg were adequate for grain set whereas concentrations of less than this at 18 DAT were associated with marked decreases in grain set index.

Low night temperature did not enhance the effects of the –B treatment on leaf blade elongation and K leakage from YEB in plants at vegetative and reproductive stages in the present experiment. It significantly delayed the development of plants at the reproductive stage, however, resulting in about six days' delay in anthesis of the main stems. As a result, the +B plants at low night temperature had fewer florets per spikelet than those at ambient night temperature.

The results suggest that the timing of B supply to the florets is crucial for the development of the reproductive parts and eventual fertilisation. There was a higher spikelet fertility and grain set index in the –B plants at low night temperature than those at ambient temperature. A part of the developmental process of ear and pollen in the –B plants at low night temperature might have coincided

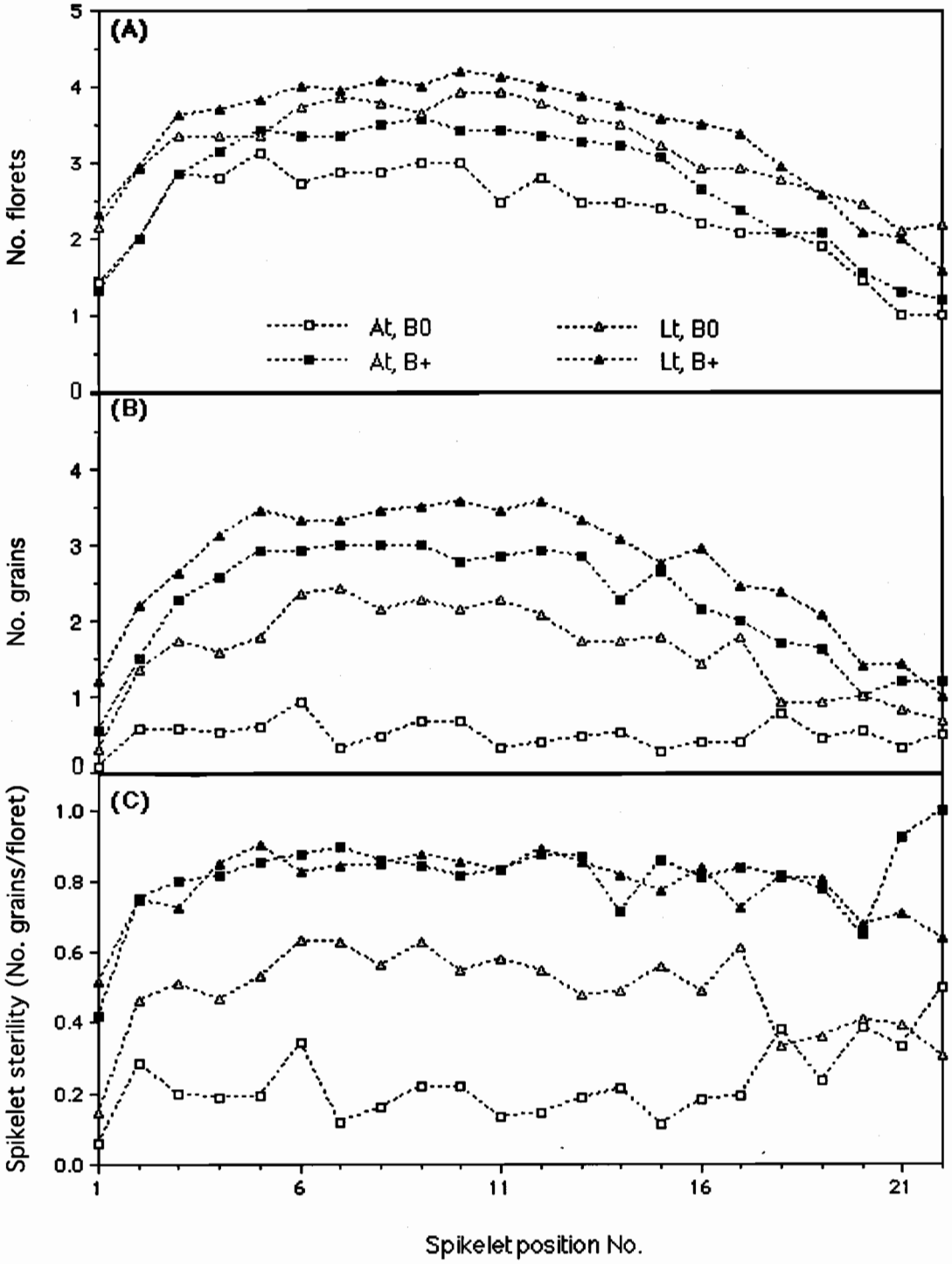

Figure 2. Effects of B supply and night temperature treatments on (A) number of florets per spikelet, (B) number of grain per spikelet and (C) spikelet fertility (number of grains per floret) in wheat plants (cv. SW 41) grown in the glasshouse. Tl = low night temperature; Ta = ambient night temperature, B0 = 0 µM B added, B+ = 10 µM B added.

with the resupply of B at the end of the treatment period (18 days after starting treatment). It is important to identify the critical stage of reproductive development when B deficiency causes irreversible damage to the fertility of florets. This knowledge can provide a basis for the correct timing of foliar B fertilisation to minimise the probability of grain set failure in wheat.

In conclusion, the results from the present study further confirmed the low internal B requirement for the vegetative growth of wheat plants. There was a distinct difference in sensitivity to B withdrawal between vegetative and reproductive parts of the same plant. This difference could be caused by the differences in external or internal B requirements, or both.

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