Plant and Soil **233:** 31–45, 2001.

A critical comparison of the external and internal boron requirements for contrasting species in boron-buffered solution culture

A. Asad[1,*], R. W. Bell[1] & B. Dell[2]

[1]*School of Environmental Science, Division of Science and Engineering, Murdoch University, Western Australia 6150;* [2]*School of Biological Sciences and Biotechnology, Division of Science and Engineering, Murdoch University, Western Australia 6150;* **Corresponding author. Current Address: School of Land and Food Sciences, The University of Queensland, St. Lucia, Brisbane, Queensland, Australia 4072. Fax: +61-7-3365-1177; E-mail: a.asad@mailbox.uq.edu.au*

Received 28 March 2000. Accepted in revised form 31 January 2001.

Key words: boron, deficiency, dicot, marri, monocot, sunflower, transport, uptake, wheat

Abstract

Despite reports that boron (B) requirements differ among plant species there is a shortage of critical evidence to demonstrate unequivocally whether species differ in internal or external B requirements or both. The present research was conducted to establish the external and internal B requirements of three contrasting species, a woody dicot (marri), an herbaceous dicot (sunflower) and a monocot (wheat) using B-buffered solution culture. Boron-buffered solution culture provided satisfactory control of external B concentrations ranging from 0.04 to 30 μM throughout the 20- (sunflower and wheat) or 40-day (marri) growth period. At low external B concentrations ($\leq$ 0.13 μM), the growth of marri and sunflower was severely depressed but by contrast the vegetative growth of wheat plants was satisfactory and free of B deficiency symptoms. Marri and sunflower plants achieved total maximum shoot growth at $\geq$1.2 μM B in solutions while wheat plants did so at $\geq$ 0.6 μM B. The critical B concentrations (mg kg^{-1} dry matter) in the youngest open leaf blades of marri, sunflower and wheat plants were 17.9, 19.7 and 1.2 on 20, 10 and 10 days after transplanting (DAT), respectively. Lower internal and external B requirements of wheat were matched by a lower uptake rate of B compared to marri and sunflower.

Introduction

Boron is an essential micronutrient for the normal growth of monocots, dicots, conifers and ferns (Bould et al., 1984; Shelp, 1993), but B requirements are reported to differ among these groups and also among plant species within these groups. For example, in wheat, a B concentration of 3 mg kg^{-1} dry matter was reported to be adequate during vegetative stage (Marten and Westermann, 1991; Reuter et al., 1997). By contrast, during reproductive growth, pollen appears to require 8–10 mg kg^{-1} in order to avoid grain yield losses from sterility (Rerkasem et al., 1997). Among the reproductive parts of wheat, the B requirement of the male reproductive organs for pollen germination was greater than that for female gamete development (Rerkasem et al., 1997). By contrast, it is reported that the internal B requirements of dicots are generally higher than monocots such as wheat both at vegetative and reproductive stages of growth (Gupta, 1993). Recently, Bell (1997) reported that leaf B concentrations of $<$1 mg B kg^{-1} dry matter in monocots such as wheat compared to $<$10 mg B kg^{-1} dry matter in dicots are generally associated with B deficiency symptoms.

Sunflower is one of the crops most sensitive to B deficiency and it has poor vegetative growth and seed set when the growth medium has a limited B supply (Blamey et al., 1987, 1997). In sunflower, B deficiency symptoms first become evident on the younger leaves which have a bronze colour and become hardened, malformed and necrotic (Blamey et al., 1997). Sun-

flower roots are also sensitive to B deficiency as they stop their growth within 6 h after the removal of B from the growth medium (Dugger, 1983).

Various Eucalyptus tree species also have high B requirements. The internal B requirements for eucalyptus leaves are reported as 12 mg B kg^{-1} dry matter (Dell et al., 1995). Poor stem growth and malformed leaves in plantations of *Eucalyptus globulus*, *Eucalyptus grandis* and *Eucalyptus urophylla* were reported because of B deficiency (Dell and Malajczuk, 1994). Other common B deficiency symptoms in eucalyptus include change in pigmentation in the young leaves, malformed developing leaves with missing sectors at the margin or within the blade, and yellowing of leaves which can extend from the leaf margin interveinally over the whole blade (Dell et al., 1995). Bangash and Gardiner (1985) also reported that the absence of B from nutrient solutions resulted in apical bud-dieback in *E. pauciflora* and *E. viminalis*.

Low internal B requirements in cereals compared to dicots (Loomis and Durst, 1992) have been attributed to differences in their cell wall characteristics (Yamauchi, 1971). However, previously, Tanaka (1967) reported that the roots of monocots had a lower capacity to absorb B than the roots of dicots. His findings of low absorption rate of B by monocots are consistent with their low internal B requirements. The cell wall pectin concentration of a plant species may also control the B uptake rates.

Although previous studies have reported differential uptake of B by different plant species, inadequate control of low B concentrations in the growth medium may lead to incorrect conclusions about the internal and external B requirements in plants. Boron-buffered solution culture system provided satisfactory control of external B concentrations in nutrient solutions for short-term studies (Asad et al., 1997a) with canola and seemed an ideal medium in which to compare the internal and external B requirements of contrasting species.

The objectives of the present research were to compare the external and internal B requirements of three plant species (marri, sunflower and wheat) and their uptake and distribution of B in B-buffered solution culture. Further the benefits of B-buffered solution culture system for different plant species will also be considered.

Material and methods

General procedures for cleaning the B specific resin have been described by Asad et al. (1997a). For this experiment, when all B-free triple deionized (TDI) water had drained from the cleaned resin, a constant amount of wet resin (24 g) was loaded with 1, 2, 4, 8, 16, 32 and 100% of its B saturation capacity by shaking the resin for 72 h in 1 l of TDI water containing 0.5, 1.0, 2.0, 4.2, 8.3, 16.6 and 51.8 mg B (H_3BO_3), respectively. After loading the resin with B in the above solutions, B-loaded resin was rinsed with B-free double deionized water (Asad et al., 1997a). One gram of wet resin was transferred to an acid washed cotton bag for each 5-l pot. The B sorption capacity of this batch of resin was 2.16 mg B per g wet resin (Sigma, 1980).

The full-strength basal nutrient solution used in this experiment 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; $ZnSO_4$, 2; $MnSO_4$, 2; $CuSO_4$, 0.5; Na_2MoO_4, 0.08; NaCl, 8; and FeEDTA, 40. During the growth period of plants, the nutrient solutions were not changed. Instead, programmed nutrient addition was used (Asher and Blamey, 1987) to repeatedly add small amounts of all nutrients except B into each pot. The amount of salts needed was calculated from the dry weight increment of extra plants harvested at 4–5-day intervals. Optimum shoot nutrient concentrations for sunflower and wheat plants were derived from Weir and Cresswell (1994) and for marri from Dell et al. (1995). Analytical grade chemicals were used to make up the nutrient solutions. Triple deionized water was used throughout the study and for making up the solutions, and was further purified to remove B by passing drop wise through a column containing B-specific resin. The macronutrient stock solutions were prepared with B-free water and further purified by bubbling these solutions with B-specific resin for 24 h.

Seeds of marri (*Corymbia calophylla*) were allowed to germinate in water. Seeds started to germinate after 12 days continuous bubbling in aerated TDI water renewed every 24 h. After germination, the seedlings were transferred to 0.25 strength basal nutrient solution. Thirty-four days after germination, six seedlings were transferred to 5-l plastic pots lined with polythene bags containing full strength basal solutions with B-specific resin in acid washed cotton bags. Sunflower (*Helianthus annuus* cv Hysun 25) and wheat (*Triticum aestivum* cv BT-Schomburgk) were germin-

ated by wrapping them in paper towels moistened with 1000 μM Ca $(NO_3)_2$ in the dark at 25°C for 48 h. Ten selected seedlings of these plants were transferred to 5-l plastic pots lined with polythene bags containing full strength nutrient solution with B-specific resin in acid-washed cotton bags. The pots were randomly distributed in water baths maintained at 18, 20 and 22°C for wheat, marri and sunflower, respectively. Solution pH was adjusted to 6.0±0.2 every day with 4% H_2SO_4 or 2% NaOH (both were analytical grade chemicals). Nutrient solutions in all the pots were continuously aerated with filtered air. For B analysis in nutrient solutions, 250-ml samples were collected on Days 0, 20 and 40 from pots containing marri plants, while they were collected on Days 0, 10 and 20 for sunflower and wheat pots. Boron concentrations in solutions were determined by inductively coupled plasma atomic emission spectrophotometry (ICP-AES) after concentrating B using the B-specific resin as reported by Asad et al. (1997b).

Harvest procedure

The number of plants per pot was thinned to four in the case of marri and eight for sunflower and wheat, 2 days after transplanting (DAT). Plants were harvested at two stages. The first harvest was 20 DAT in case of marri and 10 DAT in the case of sunflower and wheat. The second harvest was when marri plants were 40 DAT and sunflower and wheat plants were 20 DAT. Plants were separated into leaf blades, petioles, stems and roots for marri and sunflower. Wheat plants were separated into leaf blades, sheath, remainder of shoot and roots. Plant samples were dried at 70°C to constant weight. Dry weights of the plants were recorded at each harvest. The dried plant samples were finely milled and digested in concentrated nitric acid at 130°C for B determination by ICP-AES (Zarcinas et al., 1987). The critical internal values of B in the youngest opened leaves (YOL), YOL+1, YOL+2 and YOL+3 were determined at 90–95% of maximum shoot dry matter when modeled with the Mitscherlich equation (Ware et al., 1982). These values are reported for harvest 1, as they were more or less the same for harvest 2.

Mean rates of nutrient absorption were calculated using the formula of Williams (1948) and the root efficiency was calculated according to Edwards and Asher (1974). The relative transport rates, defined as the movement of ions from root to top of plants from harvest 1 to 2 were calculated using the formula of Loneragan and Snowball (1969).

Data analysis

All treatments were arranged in a randomized complete block design with four replicates for each harvest time. The results were analysed by standard analysis of variance techniques (Gagnon et al., 1984). Significant treatment effects were separated with Fisher's protected LSD Test at $p \leq 0.05$. Analysis of variance of B concentrations in nutrient solution and plants was based on log transformed data because of non-normal distributions of the treatment variances (Gomez et al., 1994).

Results

On Day 0, B concentration increased from 0.03 to 28.3 μM B in the nutrient solutions with increasing the loading of B on resin from 1 to 100% of full B saturation (data not shown). There was no significant change of B concentrations in nutrient solutions from Days 0 to 20 (marri) and Days 0 to 10 (sunflower and wheat) (data not shown). In the case of marri, B concentrations decreased from Day 0 to 40, when the resin was loaded at 16 and 32% of its full saturation (with B concentration 1.13 and 1.3 μM, respectively, on Day 0). Similarly in pots containing sunflower, solution B concentration declined where the resin was loaded to 16% of its full saturation capacity (with B concentration 1.13 μM on Day 0). Thus, the B-specific resin did not fully maintain the B equilibrium in 16 and 32% of full B saturation of resin in the case of marri and 16% of full saturation of resin in the case of sunflower. But from the results of this experiment it was clear that B-specific resin continued to supply B over time to these plant species as their dry weight and B content increased 3–5-fold from Harvest 1 to 2 (Tables 1 and 2). Thus, the B specific resin generally buffered the B concentrations in nutrient solutions for 0–20 days with the fast-growing sunflower and wheat and from 0 to 40 days for the smaller marri.

Marri

After 20 days growth in B-buffered nutrient solutions (Harvest 1), plants at ≤0.08 μM mean B, had small cotyledons and had produced no new leaves apart from those present at transplanting. Roots of these plants were malformed. Some plants at 0.13 μM B produced

Table 1. Dry weight (mg/plant) of plants after 10 (H1) and 20 (H2) days in case of sunflower and wheat, and 20 (H1) and 40 (H2) days in the case of marri in solutions treated with boron loaded resin: values are means of four replicates

	Mean boron concentrations (μM)	Leaf blades		Petioles		Stems		Roots	
		H1	H2	H1	H2	H1	H2	H1	H2
Marri	0.05	—[a]	—	—	—	8	11	16	42
	0.08	—	—	—	—	9	15	37	78
	0.13	45	156	2	6	10	47	54	171
	0.36	59	221	3	14	14	80	107	275
	0.91	77	577	6	20	22	205	114	595
	1.24	133	663	10	22	26	289	159	619
	28.6	173	924	12	34	34	338	184	1048
Sunflower	0.04	–	–	–	–	7	10	38	134
	0.05	–	–	–	–	13	19	57	177
	0.16	19	98	4	7	20	315	138	214
	0.36	25	133	6	12	30	296	155	212
	0.97	94	391	9	22	92	553	154	861
	1.20	150	676	18	36	129	814	181	1573
	26.4	273	883	19	49	146	981	217	2200
Wheat[b]	0.04	223	503	9	18	75	56	175	726
	0.07	255	670	10	23	94	113	200	904
	0.16	259	714	11	24	113	124	175	946
	0.36	266	750	10	25	120	122	185	961
	1.23	290	805	11	33	121	148	183	1139
	1.40	295	822	12	48	119	231	186	1065
	28.3	296	836	14	56	133	301	195	1282
LSD									
Boron Levels (B)		32**	90**	2**	7**	13**	68**	33**	200**
Species (S)		21**	60**	2**	4**	10**	45**	22**	132**
B×S		57**	160**	5**	13**	28**	120**	60**	351**

**ANOVA, *significant at ($p \leq 0.05$).
[a]Plants did not produce these parts
[b]In the case of wheat, the petiole category represents leaf sheaths

young leaves but their size was stunted compared to plants at higher B levels ($\geq$ 1.2 μM). The shape of leaves in plants at 0.36 and 0.91 μM B was more rounded than elongated. Plants at $\geq$ 1.24 μM B in solution had healthy growth of young shoot and the shape of their leaves were normal. Roots of these plants were large and healthy as compared to plants at $\leq$ 1.24 μM B. After 40 days of growth (Harvest 2), plants at 0.05–0.08 μM B in solution maintained the growth of their cotyledons but still did not produce any new young leaves. Cotyledons of these plants were desiccated after 40 days growth and their roots remained short. Plants in 0.13 μM B solutions produced some young leaves, and whilst the leaves were a healthy looking, but the size of leaves and tap roots of these plants were miniature. In 0.36 μM B solutions, plants had small rounded leaves and slim roots as compared to plants of 0.91 μM B solutions, which had healthy leaves and roots. Plants grown at $\geq$ 1.2 μM B had good growth throughout the experimental period.

At 20 DAT, maximum dry weight of leaves, petioles, stems and roots was found at $\geq$ 1.2 μM external B. At 40 DAT, the maximum dry weight of leaf blades and petioles was found at 28.6 μM B and at $\geq$ 1.2 μM B for the rest of the plant parts (Table 1). Dry weight of leaf blades, petioles, stems and roots was similarly depressed in plants at 0.13 and 0.36 μM B. At $\leq$ 0.08 μM external B concentration, the dry weight of

Table 2. Boron concentrations (mg/kg) in various parts of plants after 10 (H1) and 20 (H2) days, in the case of sunflower and wheat, and 20 (H1) and 40 (H2) days in the case of marri in solutions treated with boron loaded resin: values are means of four replications.

	Mean boron concentrations (μM)	Leaf blades		Petioles		Stems		Roots	
		H1	H2	H1	H2	H1	H2	H1	H2
Marri	0.05	–[a]	–	–	–	2.3c	5.2d	1.3c	2.1d
	0.08	–	–	–	–	4.6c	8.1d	1.9c	2.0d
	0.13	5.2d	9.0d	5.5a	7.6b	8.2c	10.1c	4.1c	5.1cd
	0.36	10.7d	14.8c	7.5a	12.3a	9.0c	11.7c	4.4b	4.7c
	0.91	11.2c	17.7b	8.9a	12.9a	10.4b	15.8b	6.1b	5.5b
	1.24	16.7b	19.9ab	9.6a	13.6a	12.4b	17.2b	7.1a	5.9b
	28.55	29.2a	26.3a	10.5a	14.8a	18.2a	23.8a	13.4a	13.3a
Sunflower	0.04	–	–	–	–	4.9d	5.7d	5.9c	6.9d
	0.05	–	–	–	–	5.2cd	6.3e	6.5c	7.3d
	0.16	4.2d	8.3d	4.6b	8.5c	9.6c	13.8c	8.8b	11.0d
	0.36	5.6d	13.4c	4.6b	14.1b	11.3cd	16.4bc	10.6b	11.1d
	0.97	11.6c	19.0b	4.8b	17.0b	13.1b	18.6b	10.7b	13.3c
	1.20	24.6b	21.7b	5.0a	17.0b	15.5b	18.6b	12.3ab	12.7b
	26.44	32.1a	30.9a	6.9a	22.0a	21.0a	25.7a	18.4a	20.3a
Wheat[b]	0.04	0.5e	0.7d	0.3c	0.6e	0.7d	0.6d	1.1a	0.3c
	0.07	0.7de	1.0c	0.8c	1.3d	1.3bc	1.0c	1.0a	0.5bc
	0.16	0.7de	1.3c	1.8b	1.6cd	1.0cd	1.1c	1.3a	0.5bc
	0.36	0.9cd	1.3c	2.1b	2.2c	1.3bc	1.3c	1.1a	0.72bc
	1.23	1.3bc	2.1b	2.2b	3.4b	1.6b	1.9b	1.2a	0.7ab
	1.40	1.7b	1.9b	2.8b	5.2a	1.8b	2.1b	1.8a	1.9ab
	28.33	6.6a	8.1a	4.9a	5.9a	4.2a	3.2a	3.7a	3.9a

[a] Plants did not produce these parts.
[b] In the case the of wheat, the petiole category represents leaf sheaths.
* Analysis of variance of boron concentrations in plants was based on log transformed scale. In a column, means followed by a common letter are not significantly different at $p < 0.05$.

stems and roots was strongly depressed as compared to adequate B levels.

Boron concentrations in different plant parts increased with increasing B concentrations in nutrient solutions (Table 2). For example, in leaf blades on 20 DAT, B concentration (mg kg^{-1}) was 11.2, 16.7 and 29.2, and on 40 DAT it was 17.7, 19.9 and 26.3 in plants of 0.91, 1.24 and 28.6 μM B solutions, respectively. The sharp increase in B concentration in leaf blades from 20 to 40 DAT in plants at $\leq$ 0.91 μM B solutions was also found in stems and petioles (Table 2). However, there was no major difference in B concentrations of roots from one harvest to the other at various external B levels. In most plant parts, maximum B concentrations were found in the plants of 28.5 μM B solutions. Minimum B concentrations were found in stems and roots at $\leq$ 0.08 μM external B concentrations.

The critical B concentrations of youngest opened leaves (YOL), YOL+1, YOL+2 and YOL+3 of marri, associated with 90% of maximum shoot growth were 17.9, 16.1, 17.6 and 19.6 mg B kg^{-1}, respectively (Fig. 1).

Sunflower

Plants at $\leq$ 0.05 μM mean B concentration produced only cotyledons and their roots were short and slim. Plants at 0.16 and 0.36 μM B produced some new leaves in addition to those present at the time of transplanting and their roots were healthy but these plants were short at this stage as compared to plants at higher B levels. Plants in 0.97 μM B solutions though producing the same parts as those of higher B levels were

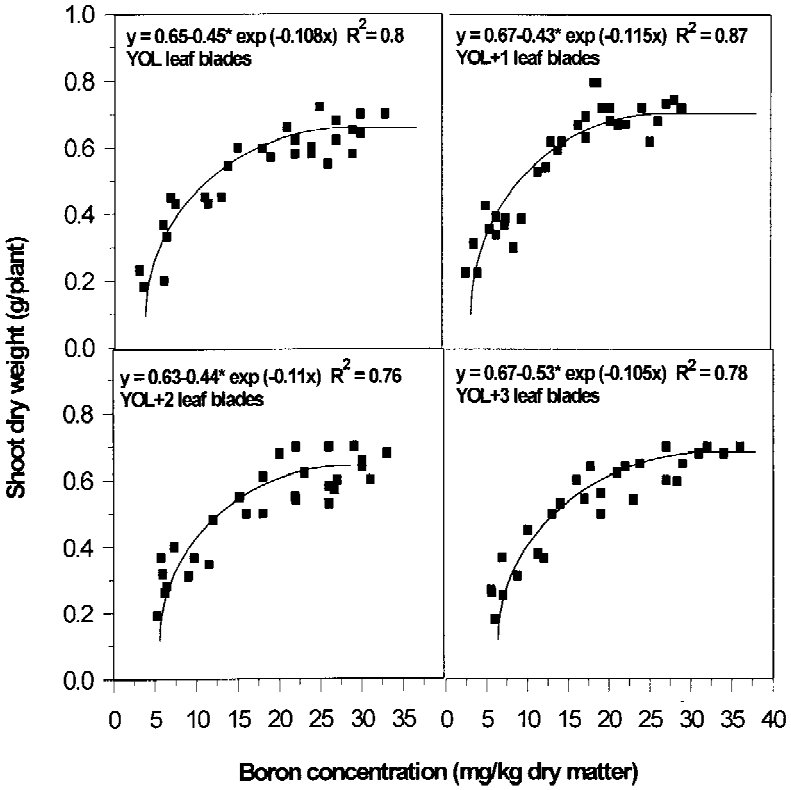

Figure 1. The relationship between boron concentrations (mg/kg dry matter) in blades of YOL, YOL+1, YOL+2 and YOL+3 and shoot dry weight (g/plant) of 20-day-old marri in boron buffered culture solutions.

nevertheless smaller in size. There were no B deficiency symptoms found in shoot and root of plants at ≥ 1.2 μM external B concentration at 10 DAT.

Like marri, sunflower plants grown in low B solutions (≤ 1.2 μM B) did not produce all the plant parts. Dry weight of different plant parts was significantly ($p \leq 0.05$) depressed in plants of < 1.2 μM B solutions (Table 1). Apart from stems and roots on 10 DAT, the dry weights of all plant parts at both harvests were significantly ($p \leq 0.05$) higher in plants at 26.4 μM B solutions. Markedly ($p \leq 0.05$) less dry weight in all plant parts was found in plants of ≤ 0.05 μM B solutions.

Boron concentrations in different plant parts increased with increasing the external B concentration. Significantly ($p \leq 0.05$) higher B concentrations (mg kg^{-1}) were found in parts of plants grown with 26.4 μM B except in petioles and roots on 10 DAT where the B concentrations were unchanged at ≥ 1.2 μm B in solution (Table 2). Once again like marri, B concentration in petioles and stems of sunflower increased while in leaf blades it was decreased from Days 10 to 20 at maximum external B concentrations (Table 2). However, B concentration in sunflower roots was nearly the same from one harvest to the other at various external B levels.

The critical B concentrations associated with 90% of maximum shoot growth of sunflower were 19.7, 18.0, 20.1 and 19.9 mg B kg^{-1} for YOL, YOL+1, YOL+2 and YOL+3, respectively (Fig. 2).

Wheat

Ten days after transplanting (Harvest 1), no visual differences resembling B deficiency were observed in wheat shoots or roots grown at various levels of B. Previously, Snowball and Robson (1983) also reported that wheat plants grew quite normally for considerable time without added B. However, mild interveinal chlorosis resembling iron deficiency (Hansen et al., 1996) was observed in the leaves of plants grown at 0.04 μM B 2 days after transplanting. These symptoms disappeared four days after transplanting probably because of increased phytosiderophore release by roots and increased iron uptake by wheat plants.

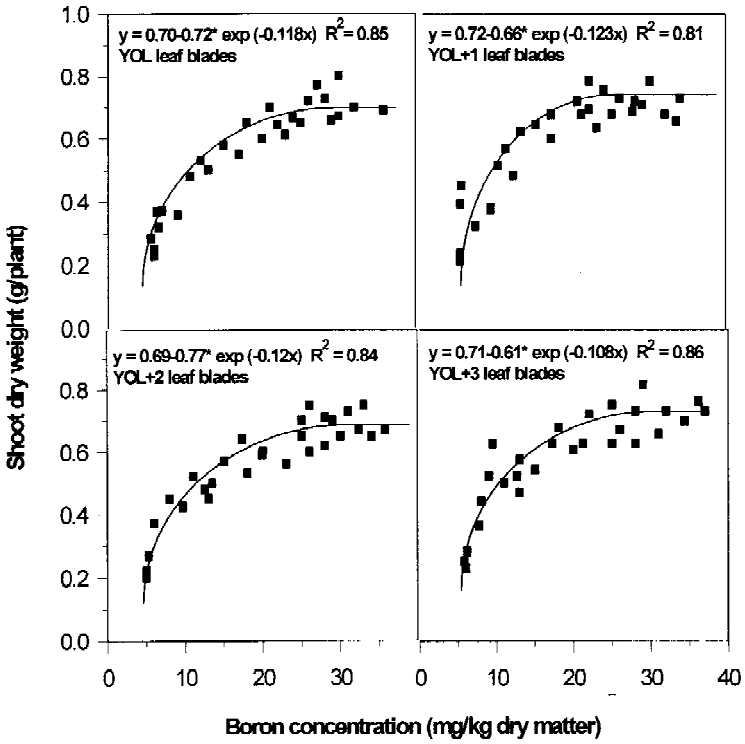

Figure 2. The relationship between boron concentrations (mg/kg dry matter) in blades of YOL, YOL+1, YOL+2 and YOL+3 and shoot dry weight (g/plant) of 10-day-old sunflower plants in boron-buffered culture solutions.

Twenty days after transplanting (Harvest 2), wheat plants at all B levels looked similar to each other, except the plants in 0.04 μM B solutions. At 0.04 μM B, plants had slightly reduced size compared to higher B levels but they continued their growth without any B deficiency symptoms on 20 DAT. However, their roots showed significant B deficiency symptoms, as they were short. Plants grown in solutions at 0.07 and 0.16 μM B, also had reduced root size. Plants at $\geq$ 1.23 μM B had no B deficiency symptoms.

In plants at 0.04 to 1.23 μM B, dry weight of leaf blades on 10 DAT was increased by increasing solution B (Table 1). Maximum dry weight of sheath and stalk on 10 DAT was found at $\geq$ 0.07 and $\geq$ 0.16 μM B, respectively. However, dry weight of wheat roots was not increased with increasing the B concentration in solution on 10 DAT.

At 20 DAT, dry weight of leaf blades increased with B concentration in nutrient solution to 0.16 μM external B and beyond this B level, there was no difference found in the dry weight of leaf blades. However, maximum dry weight of sheath and stem was obtained in 1.4 μM B on 20 DAT as compared to 0.16 μM B on 10 DAT. By contrast with its lack of response at 10 DAT, dry weight of wheat root responded towards increasing B concentrations in nutrient solution at 20 DAT.

Internal B concentration in various parts of wheat increased with B concentration in nutrient solutions. Maximum B concentration was found in the leaf blades of wheat as compared to other plant parts (Table 2). At 10 DAT, leaf blades, sheath and stem at 28.3 μM external B had maximum B concentrations (6.6, 4.9 and 4.2 mg B kg^{-1} dry weight, respectively).

At 20 DAT, leaf blade and stem of wheat plants maintained the maximum B concentration (8.1 and 3.2 mg B kg^{-1} dry weight, respectively) in 28.3 μM B solutions. Maximum B concentration in leaf sheath and roots was found in $\geq$ 1.4 and 1.23 μM B solution, respectively, on 20 DAT (Table 2).

The critical B concentrations of youngest opened leaves (YOL), YOL+1, YOL+2 and YOL+3 of wheat, associated with 90% of maximum shoot growth were 1.2, 1.3, 2.1 and 2.1 mg B kg^{-1}, respectively (Fig. 3).

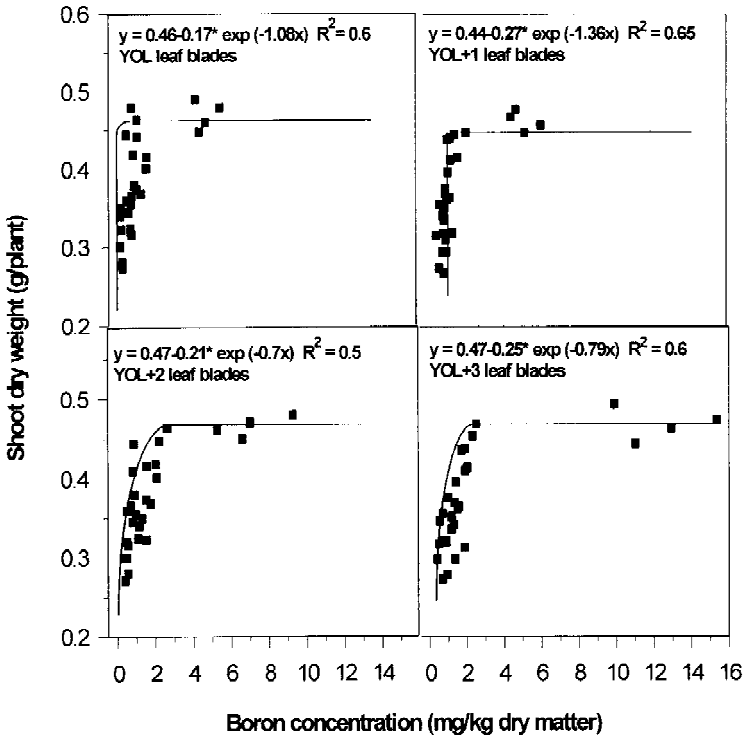

Figure 3. The relationship between boron concentrations (mg/kg dry matter) in blades of YOL, YOL+1, YOL+2 and YOL+3 and shoot dry weight (g/plant) of 10-day-old wheat plants in boron-buffered culture solutions.

Boron absorption and root efficiency

Relative uptake rate of B by all three plant species increased with increasing B concentration in nutrient solutions (Table 3). Whilst relative B uptake rates in wheat also increased with increasing solution B, rates at each solution B levels were only 3–7% of those in marri and sunflower. The rates of B absorption (μmol g^{-1} root dry weight day^{-1}) at which maximum or near maximum plant dry weight was obtained were 0.96, 0.94 and 0.05 in marri, sunflower and wheat, respectively. Root efficiency (Edwards and Asher, 1974) declined progressively in all species as solution B concentration increased from 0.05 to 28.5 μM (Table 3). Maximum root efficiency (4.0×100) was found in sunflower at 0.13 μM external B concentration. Relative transport rate to tops also increased with increasing the B concentrations in nutrient solutions. Maximum relative transport rates (μmol g^{-1} root dry weight day^{-1}) were 0.84, 0.84 and 0.04 for marri, sunflower and wheat, respectively. Again relative transport rates from roots to tops for wheat were low when compared with marri and sunflower.

During the experiment, most of the B added to pots remained in resin and smaller proportions were taken up by plants or were present in nutrient solutions (Table 4). Results of this experiment demonstrated that the absolute rate of absorption of B by marri plants exceeded the rate of release of B from resin, causing a decline in B concentration in solution at 16 and 32% of full B saturation between 20 and 40 DAT (Table 4). Daily uptake rate of B (μg pot^{-1} day^{-1}) by marri was 0.42 and 0.30 from 0 to 20 and 20 to 40 DAT in solutions having 16% of full B-saturated resin, respectively (from the calculations of Table 4). From solutions of 32% of full B saturated resin, the daily uptake rate of B (μg pot^{-1} day^{-1}) was 0.57 and 0.35 from 0 to 20 and 20 to 40 DAT, respectively (from the calculations of Table 4). Again in case of sunflower, at 16% of full B-saturated resin, B concentration declined significantly ($p \leq 0.05$) from Harvest 1 to 2. This is because B contents in the solution reduced 56.2% from Harvest 1 to 2 (Table 4). Daily uptake of B (μg pot^{-1} day^{-1}) by sunflower in 16% of full B saturation resin was 1.14 and 0.81 from 0 to 10 and 10 to 20 DAT, respectively (from the calculations of Table 4). However, at $\geq$ 32% of full B-saturated resin solutions, adequate B was present in nutrient solutions, therefore B concentration remained constant from 10 to 20 DAT (Data not shown). The total absorption of B by wheat

Table 3. Effect of mean boron concentration in solution on boron absorption and root efficiency in boron absorption by plants grown for 10–20 days in the case of sunflower and wheat and 20–40 days in the case of marri: values are means of four replicates.

Mean boron conc. in nutrient solutions (μM)	Relative uptake rate (μmol g^{-1} root dry wt. day^{-1})			Root efficiency[a] ($\times$100)			Relative transport (μmol g^{-1} root dry wt. day^{-1})		
	Marri	Sunflower	Wheat	Marri	Sunflower	Wheat	Marri	Sunflower	Wheat
0.05	–[b]	–	0.02	–	–	0.39	–	–	0.01
0.08	–	–	0.02	–	–	0.23	–	–	0.01
0.13	0.43	0.64	0.02	3.24	4.01	0.11	0.27	0.53	0.01
0.36	0.51	0.69	0.03	1.44	1.93	0.08	0.44	0.59	0.02
0.91	0.71	0.74	0.05	0.78	0.77	0.04	0.61	0.68	0.04
1.24	0.96	0.90	0.06	0.78	0.75	0.04	0.83	0.77	0.05
28.5	1.02	0.94	0.07	0.04	0.04	0.01	0.84	0.84	0.05
LSD ($p \leq 0.05$)	0.03	0.02	0.15	0.93	0.05	0.19	0.01	0.01	0.01

[a]Root efficiency defined as the ratio of the rate of nutrient absorption (μmol g^{-1} root dry weight day^{-1}) to the external nutrient concentration (μM) (Edwards and Asher 1974).
[b]Plants did not produce upper shoot.

Table 4. Comparison between amount of boron loaded in resin, B taken up by plants of three species and amount of B in nutrient solution.

Species	B loaded on B-specific resin as % of its satu. capacity	Boron contents (μg/pot) loaded on 1 g wet resin on Day 0	Total boron absorbed by plants (μg/pot)		Boron contents (μg/pot) in solution at	
			H1	H2	H1	H2
Marri	1	20	0.04	0.15	3.21	2.31
	2	40	0.11	0.28	4.61	3.95
	4	80	0.55	2.80	7.57	5.95
	8	170	1.25	5.67	23.03	14.42
	16	350	1.85	16.98	59.46	27.03
	32	700	3.77	22.12	76.53	43.24
	100	2162	8.25	46.80	1638	1459
Sunflower	1	20	0.26	0.98	2.12	1.46
	2	40	0.44	1.41	3.04	2.39
	4	80	1.50	7.57	9.51	8.11
	8	170	2.15	9.18	21.98	16.22
	16	350	3.98	29.53	61.25	34.41
	32	700	7.99	50.38	73.83	50.44
	100	2162	15.96	98.23	1477	1279
Wheat	1	20	0.36	0.25	3.26	2.29
	2	40	0.51	0.56	4.15	3.42
	4	80	0.54	1.58	8.97	7.93
	8	170	0.62	1.88	18.92	18.74
	16	350	0.82	2.88	64.86	63.06
	32	700	1.08	4.32	81.08	68.50
	100	2162	3.30	13.06	1567	1495

H1 = 10 DAT in case of sunflower and wheat and 20 DAT for marri;
H2 = 20 DAT in case of sunflower and wheat and 40 DAT for marri.

plants was low compared to marri and sunflower plants (Table 4). Boron concentrations in nutrient solutions of wheat plants remained unchanged from Day 0 to 20 (Data not shown).

Shoot: root ratio for dry matter and B content

The ratio of shoot dry matter:root dry matter of wheat plants increased as external B concentrations increased from 0.03 to 28.3 μM on 10 DAT and remained the same on 20 DAT (Table 5). The ratio of shoot dry matter:root dry matter of sunflower plants increased with increasing the external B concentrations on 10 DAT. In case of marri plants at 0.15 μM B, shoot:root ratio was close to maximum because low B severely limited root growth (Table 5). Shoot:root ratio of marri increased slightly from 20 to 40 DAT under all B treatments. Shoot:root ratio of dry matter in sunflower increased over time at low B levels ($\leq$ 0.37 μM B) and declined in plants at $\geq$ 1.13 μM B.

The ratio of B contents in shoot:root increased with increasing the B concentrations in nutrient solutions at Harvest 1 in case of all the three plant species marri, sunflower and wheat (Table 5). The ratio of B contents in shoot:root also increased from Harvest 1 to 2 in marri as compared to sunflower and wheat plants (Table 5).

Among the three plant species, total shoot and root dry weight of wheat plants was markedly less responsive to solution B than sunflower and marri (Fig. 4). The total dry weight of shoot and root of marri and sunflower increased with increasing B concentration in nutrient solutions at both Harvest 1 and 2.

Discussion

Boron concentrations in nutrient solutions generally remained constant in B treatments during the 20- or 40-day period from transplanting to the second harvest of all three plant species. In nutrient solutions containing wheat, the B concentrations were best buffered presumably because the B uptake demand was lowest in this species. Thus B-specific resin generally controlled the B concentrations in nutrient solutions and these results are in agreement with those of Asad et al. (1997b).

The present study has demonstrated that plant species can differ in both external and internal B requirements. In comparison to wheat, a monocot, the two dicots sunflower and marri had higher external and internal B requirements. Marri and sunflower were unable to produce new leaves in nutrient solutions containing $\leq$ 0.15 μM B. Thus whilst plants maintained functions in existing tissue they could not produce new tissue, suggesting that the B requirement for maintaining plant growth was low compared to that for cell expansion as suggested by Hu and Brown (1994). By contrast in non-renewed solutions minus B treatments typically cause rapid apical tissue abortion and plant death (Blamey et al., 1978).

The maximum vegetative growth of marri and sunflower was obtained at $\geq$ 1.2 μM external B concentrations (Table 1). However, present research found that 90% of maximum vegetative growth of wheat is achievable with minimum external B concentration of 0.6 μM, from Harvests 1-2. By comparison, Chapman et al. (1997) obtained 80% of maximum wheat yield at 0.16 μM B in flowing solution culture. The current findings and those of Chapman et al. (1997) suggest that dicots have higher external B requirements as compared to cereals at vegetative growth stage.

With the development of B-buffered solution culture system it is also possible to determine the critical external and internal B concentrations in different plant species. Critical external B concentrations of three plant species determined in B-buffered solution culture system were 1.2, 1.2 and 0.6 μM in case of marri, sunflower and wheat, respectively (data not shown). The critical external B concentration for 22-day-old canola was 0.5–0.6 μM in B-buffered solution culture (Asad et al., 1997b). In flowing solution culture experiments, 3 μM B was routinely maintained for optimal growth of a variety of plant species (Alva et al., 1986; Asher and Loneragan, 1967; Bell et al., 1989) as compared to 46 μM in conventional solution culture experiments (Asher, 1977; Hoagland and Arnon, 1950). From these findings it may be concluded that B-buffered solution culture system maintained B concentrations at low B concentrations comparable to those of soil solutions (Nable et al., 1997) as B concentration reported in soil solution ranges from as low as 1 to as high as 1000 μM (Aitken et al., 1987; Romero and Aguilar, 1986).

The critical internal B concentration is determined by a relationship between shoot dry matter and B concentration in a plant part (Bell, 1997). In conventional solution cultures where there is a significant decline in external B supply over time this approach generates critical concentrations that may not reflect the functional B requirement (Bell, 1997). However, Bell (1997) concluded that the establishment of a critical

Table 5. Effect of loading B-specific resin to increasing percentages of B-saturation on shoot: root ratio for dry matter and for boron contents in marri, sunflower and wheat: values are means of four replicates.

Species	B Conc. on Day '0' (μM)	Shoot:root ratio			
		Dry weight		B Contents	
		Day 10	Day 20*	Day 10	Day 20*
Marri	0.04	–[b]	–	–	–
	0.07	–	–	–	–
	0.15	1.05	1.22	1.47	2.21
	0.37	0.71	1.15	1.65	3.39
	1.13	0.93	1.35	1.66	4.19
	1.30	1.07	1.57	2.35	5.06
	28.3	1.19	1.24	2.35	2.36
Sunflower	0.04	–	–	–	–
	0.06	–	–	–	–
	0.15	0.31	1.96	0.24	2.21
	0.37	0.39	2.08	0.31	2.89
	1.13	1.27	1.12	1.42	1.58
	1.30	1.64	0.97	2.59	1.52
	28.3	2.02	0.87	3.00	1.20
Wheat	0.03	1.76	0.79	0.87	1.82
	0.06	1.80	0.89	1.55	1.80
	0.15	2.18	0.91	1.38	2.33
	0.37	2.14	0.93	2.05	1.72
	1.13	2.31	0.87	2.71	2.62
	1.30	2.29	1.03	2.24	1.14
	28.3	2.27	0.93	3.57	1.61
LSD ($p \leq 0.05$)	0.4	0.4	0.7	1.4	

*Marri was harvested at day 20 and 40.
[b]Plants did not produce upper shoots.

concentration from the relationship between shoot dry matter and leaf B concentration can still be a valid approach for establishing a critical value for B deficiency provided precautions are in place to prevent a rapid decline in B supply during plant growth. Boron-buffered solution culture provides such a system where external B concentrations can be controlled. The selection of plant parts for plant analysis can significantly influence the critical nutrient concentrations (Reuter and Robinson, 1997), since the distribution and concentration of a nutrient in different plant parts varies with nutrient mobility and physiological age of the plant part (Marschner, 1995). For B analysis we consider young and immature leaves, as they are more sensitive to the decreasing supply of phloem-immobile nutrient. However, critical B concentrations for plant analysis have been mostly estimated by correlating shoot dry matter with B concentrations in whole shoots, composites of young and mature leaves or recently matured leaves in agricultural crops and pasture species (Rashid et al., 1994; Reuter and Robinson, 1997). For current studies we estimated the critical B concentrations of youngest opened leaves (YOL), YOL+1, YOL+2 and YOL+3.

The critical B concentrations of youngest opened leaves (YOL), YOL+1, YOL+2 and YOL+3 of marri, associated with 90% of maximum shoot growth were 17.9, 16.1, 17.6 and 19.6 mg B kg^{-1}, respectively (Fig. 1). Recently, Boardman et al. (1997) reported 13–30 mg B kg^{-1} as adequate B in the youngest mature leaf at juvenile growth stage of various species of eucalyptus. Our values are in the range of previous

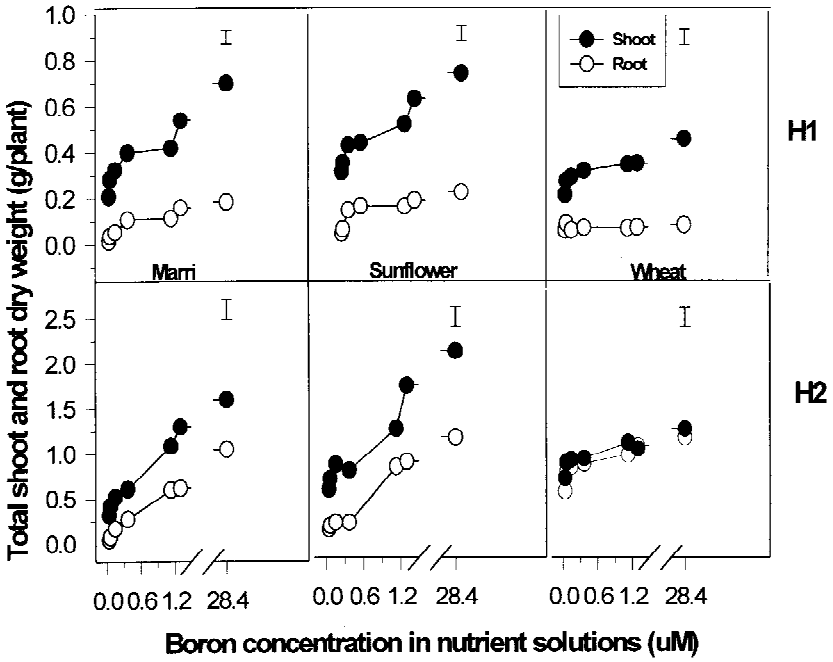

Figure 4. Dry weight of total shoot and root of marri, sunflower and wheat in nutrient solutions treated with B-specific resin loaded to different percentages of boron saturation. Vertical bars indicate LSD ($p \leq 0.05$) of the seven treatments for both shoot and root.

findings with considering the difference between the types of plant samples used.

For YOL, YOL+1, YOL+2 and YOL+3 of sunflower, the critical B concentrations associated with 90% of maximum shoot growth were 19.7, 18.0, 20.1 and 19.9 mg B kg^{-1}, respectively (Fig. 2). Previous studies of Stoyanov (1985; cited in Blamey et al., 1987) reported critical B concentrations of 26 mg kg^{-1}in top mature leaves of sunflower. Our values of critical B concentrations in various young leaves for sunflower plants grown in B-buffered solution culture were lower but not by a large margin. Thus, the present estimates are in reasonable agreement with those of previous findings considering the difference between the types of plant samples used. Recently, Cakmak et al. (1995) reported that for preventing potassium leakage from young expanding leaves, sunflower needs 16–17 mg B kg^{-1} which also supports the present estimates. The reason for this inconsistency of present and previous estimates of internal B concentrations may also be related to the fact that highest external B concentrations used in this study was 28.6 μM which considered not to be enough to produce the maximum shoot dry weight under the given conditions. In other studies the external B concentrations were used range from 50 to 250 μM for sunflower (Cakmak et al., 1995; Dannel et al., 1997).

The critical B concentrations in the leaf blades of wheat ranged from 1.2 to 2.1 mg kg^{-1} dry matter (Fig. 3). The critical B concentration in whole shoot of wheat was 2.6 mg kg^{-1} dry matter (data not shown). These values are comparable to those of Reuter et al. (1997), who reported that for wheat plants during vegetative stage, a B concentration of 3 mg kg^{-1} dry matter was considered to be adequate. In the present study, we found 1.2 mg B kg^{-1}dry matter as the critical B level in YOL of wheat. For the youngest emerged leaf blade of wheat, a tentative critical B concentration reported by Huang et al. (1996) was 1 mg B kg^{-1}dry matter in solution culture medium, where B was applied by programmed nutrient addition (Asher and Edwards, 1983). Results of the present experiment are comparable with those of previous studies, where the B concentrations were controlled by other means.

The previous studies of Shelp et al. (1995) reported that the distribution of absorbed B takes place within plants to sink organs that do not readily transpire. In the present experiment, we found that marri and sunflower plants in nutrient solution with < 0.08 μM B did not die and maintained growth and leaf functions despite severe B deficiency (based on daily observa-

tion of the plants in the glasshouse). Similar findings were also found in canola (Asad et al., 1999) where canola plants did not die at $\leq$ 0.41 μM B and continued to grow despite severe B deficiency. Indeed relative growth rate of marri, sunflower (in the present experiment; Data not shown) and canola (Asad et al., 1999) was 1 to 7 g 100 g^{-1} day^{-1}, after 40, 20 and 55 days of growth with minimum external B concentrations ranging from 0.04 to 0.07 μM. These findings may suggest that low rates of B uptake maintain metabolic function in existing tissues and the continued carbon fixation allows continued dry matter accumulation. Recent studies of Oertli (1993) and Shelp et al. (1995) found that B is redistributed from stems to other parts of plants under B-deficient conditions. However, redistribution of B via the phloem if it did occur at low external B concentration was not sufficient to support the new tissue growth in the present and previous studies of Asad et al., (1999).

Boron distribution in various plant parts was different in three plant species. Boron concentrations in various plant parts of dicots increased with increased B supply in nutrient solutions (data not show, calculated from Table 3). However, at low external B concentration, B uptake occurred at a rate exceeding the increase in B supply in solution, whereas at high external B concentrations the opposite occurred. Similar findings are also reported by Shelp (1988) that tissue B levels increased with increasing B in nutrient solution in broccoli. Blamey et al. (1997) also reported an increase in B concentration in the youngest mature leaf blades of sunflower with increase in B supply from 0 to 800 mg pot^{-1}. The present investigations also found an increase in B concentrations in marri, sunflower and wheat with increase in external B concentration in nutrient solutions (Table 2).

The relative uptake rates (μmole g^{-1} root dry wt. day^{-1}) of all three plant species increased with increasing the B concentrations in nutrient solutions. The relative uptake rate of B by marri and sunflower was 15- and 13-fold higher than wheat at 28.3 μM external B concentrations (Table 3). These findings supported the view that relative uptake rate of B by dicots is significantly higher than cereal as previously suggested by Chapman et al. (1997). The relative uptake rate of summer-grown canola was 2-, 2.2- and 31- fold higher than marri, sunflower and wheat when plants were 20–22 days old (Table 3; Asad et al., 1999). As compared to sunflower and marri, the relative uptake rate of B was higher in canola but its internal demand for B (8–10 mg B kg^{-1} dry weight; Huang et al., 1996) was lower (17.9–19.6 and 19.7–19.9 mg B kg^{-1} dry matter, respectively, Figs. 1 and 2). Canola has a high shoot:root ratio and this may explain why a high relative B uptake rate is required to satisfy B demands of the plant despite its lower internal and external B requirements than the other dicots (Table 5; Asad et al., 1999). By contrast, in wheat, relative uptake rate of B was low because of both low internal demand for B and low B absorption capacity (Tanaka, 1967).

In summary, the present results confirm that B-buffered solution culture system can be used to study plant B nutrition in short term studies of 10–20 days before the solution must be replaced. The present research demonstrated that external and internal B requirements are different in different plant species and supported the previous suggestions that dicots have higher B requirements than cereals because both internal and external B requirements were higher. Marri and sunflower survived but did not produce new leaves at $\leq$ 0.08 μM B, whereas wheat achieved $>$ 70% of maximum growth. The three plant species were different in their internal and external B requirements. The critical B concentration of YOL of marri (20 DAT), sunflower (10 DAT) and wheat (10 DAT) was 17.9, 19.7 and 1.2 mg kg^{-1} dry matter at 1.2, 1.2 and 0.6 μM external B solutions, respectively.

Acknowledgment

The senior author is grateful to Murdoch University for a Postgraduate Research Scholarship Award and for research support under its Special Research Grants Scheme.

References

Aitken R L, Jeffrey A J and Compton B L 1987 Evaluation of selected extractants for boron in some Queensland soils. Aust. J. Soil Res. 25, 263–273.

Alva A K, Edwards D g, Asher C J and Blamey FPC 1986 Relationships between root length of soybean and calculated activities of aluminium monomers in nutrient solution. Soil Sci. Soc. Am. J. 50, 959–962.

Asad A, Bell R W, Dell B and Huang L 1997a Development of a boron buffered solution culture system for studying plant boron nutrition. Plant Soil 188, 21–32.

Asad A, Bell R W, Dell B and Huang L 1997b External boron requirements for canola (*Brassica napus* L.) in boron buffered solution culture. Ann. Bot. 80, 65–73.

Asad A, Bell R W and Dell B 2000 Uptake and distribution of boron in canola (*Brassica napus* L.) at vegetative and early flowering

stages using boron buffered solution culture. Comm. Soil Sci. Plant Anal. 31 (11–14), 2233–2249

Asher C J 1977 Natural and synthetic culture media for spermatophytes. *In* CRC Handbook Series in Nutrition and Food Section G: Diets, Culture Media, Food Supplements. Ed. m Rechcigl. pp. 575-609. CRC Press. Boca Raton, FL. Volume 3.

Asher C J and Blamey F P C 1987 Experimental control of plant nutrient status using programmed nutrient addition. J. Plant Nutr. 10, 1371–1380.

Asher C J and Edwards DG 1983 Modern solution culture techniques. *In*: Encyclopedia of Plant Physiology, New Series. Eds A Lãuchli and RL Bieleski. pp. 94–119. Springer, Berlin.

Asher C J and Loneragan J F 1967 Response of plants to phosphate concentration in solution culture. I. Growth and phosphorus content. Soil Sci. 103, 225–233.

Bangash S H and Gardiner B N 1985 Dieback of trees. Pak. J. For. 35 (1): 21–32.

Bell R W 1997 Diagnosis and prediction of boron deficiency for plant production. *In* Boron in Soil and Plants. Reviews. Eds B Dell, P H Brown and R W Bell. pp. 149–168. Kluwer Academic Publishers. Dordrecht, The Netherlands.

Bell R W, McLay L, Plaskett D, Dell B and Loneragan J F 1989 Germination and vigour of black gram (*Vigna mungo* L. Hepper) seed from plants grown with and without boron. Aust. J. Agric. Res. 40: 273–279.

Blamey F P C, Mould D and Chapman J 1978 Critical boron concentrations in plant tissues of two sunflower cultivars. Agron. J. 71: 243–247.

Blamey F P C, Edwards D G and Asher C J 1987 Boron deficiency. *In* Nutritional Disorders of Sunflower. Department of Agriculture, University of Queensland. pp. 1–54.

Blamey F P C, Asher C J and Edwards D g 1997 Boron toxicity in sunflower. *In* Boron in Soils and Plants. Proceedings. Eds RW Bell and B Rerkasem. pp. 145–150. Kluwer Academic Publisher. Dordrecht, The Netherlands.

Blamey F P C, Edwards D g and Asher C J 1987 Boron deficiency. *In* Nutritional Disorders of Sunflower. Department of Agriculture, University of Queensland. pp. 1–54.

Bould C, Hewitt E J, Needham P 1984 Diagnosis of Mineral Disorder in Plants. Vol. 1. Principles. pp. 68-69. CIP Chemical Publishing, New York.

Broadman R, Cromer R N, Lambert m J and Webb m J 1997 Forest Plantation (Chapter 10). *In* Plant Analysis — An Interpretation Manual. Eds DJ Reuter and JB Robinson. pp. 505–566. Melbourne, Australia .

Brown P H and Hu H 1997 Does boron play only a structural role in the growing tissues of higher plants. *In* Plant Nutrition for Sustainable Food Production and Environment. Eds T Ando, K Fujita, T Mae, H Matsumoto, S Mori and J Sekiya. pp. 63–67. The XIII International Plant Nutrition Colloquium. Proceedings. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Cakmak I, Kurz H and Marschner H 1995 Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. Physiol. Plant 95, 11–18.

Chapman V J, Edwards D G, Blamey F P C and Asher C J 1997 Challenging the dogma of a narrow supply range between deficiency and toxicity of boron. *In* Boron in Soil and Plants. Proceedings. Eds. RW Bell and B Rerkasem. pp. 151–155. Kluwer Academic Publisher. Dordrecht, The Netherlands.

Dannel F, Pfeffer H and Rõmheld V 1997 Effect of pH and boron concentration in the nutrient solution on translocation of boron in the xylem of sunflower. *In* Boron in Soil and Plants. Proceedings. Eds. RW Bell and B Rerkasem. pp. 183-186. Kluwer Academic Publisher. Dordrecht, The Netherlands.

Dell B and Malajczuk N 1994 Boron deficiency in eucalyptus plantations in China. Can. J. For. Res. 24, 2409–2416.

Dell B, Malajczuk N and Grove T S 1995 Nutrient disorders in plantation eucalyptus. ACIAR Monograph No. 31. The Australian Centre for International Agricultural Research. Canberra, Australia.

Dugger W M 1983 Boron in plant metabolism. *In* Encyclopedia of Plant Physiology, Inorganic Plant Nutrition. Eds A Lãuchli and RL Bieleski. pp. 626-650. Springer, New York.

Edwards D G and Asher C J 1974 The significance of solution flow rate in flowing solution culture experiments. Plant Soil 41, 161–175.

Gagnon J, Roth J, Finzer B, Hofmann R, Haycock K, Simpson J and Feldman D 1984 The Accessible General Linear Modelling Package. Abacus Concept, Berkeley, CA, USA.

Gomez K A Bartolome V I, Calinga R T and Cosico A B 1994 IRRISTAT. Version 3.1. Biometrics Unit. International Rice Research Institute. Manila, Philippines.

Gupta U C 1993 Factors affecting boron uptake by plants. *In*: Boron and its Role in Crop Production. pp. 87-104. CRC Press, FL, USA.

Hansen N C, Jolley V D, Berg W A, Hodges M E and Krenzer E G 1996 Phytosiderophore release related to susceptibility of wheat to iron deficiency. Crop Sci. 36: 1473–1476.

Hoagland D R and Arnon D I 1950 The water culture method for growing plants without soil. Calif. Agric. Exp. Stn. College of Agriculture, University of California, Berkeley. pp. 1–32.

Hu H and Brown P H 1994 Localisation of boron in cell walls of squash and tobacco and its association with pectin — evidence for a structural role of boron in the cell wall. Plant Physiol. 105, 681–689.

Huang L, Ye Z Q and Bell R W 1996 The importance of sampling young leaves for the diagnosis of boron deficiency in canola (*Brassica napus* L.). Plant Soil. 183, 187–198.

Loneragan J F and Snowball K 1969 Rate of calcium absorption by plant roots and its relation to growth. Aust. J. Agric. Res. 20: 479–490.

Loomis W D and Durst R W 1992 Chemistry and biology of boron. Bio-Factors 3: 229–239.

Marschner H 1995 Mineral Nutrition of Higher Plants. 2nd Edition. Academic Press, New York.

Martens J M and Westermann D T 1991 Fertiliser applications for correcting micronutrient deficiencies. *In* Micronutrients in Agriculture, 2nd Edition. Eds. JJ Mordtvedt, PM Giordano and WL Lindsay. pp. 549–592. Soil Sci. Soc. Am., Madison, WI.

Oertli J J 1993 The mobility of boron in plants. Plant Soil 155/156, 301–304.

Nable R O, Banuelos g S and Paull J g 1997 Boron toxicity. *In* Boron in Soils and Plants: Reviews. Eds. B Dell, PH Brown and RW Bell. pp 181–198. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Rashid A, Buthio N and Rafique E 1994 Diagnosing zinc deficiency in rapeseed and mustard by seed analysis. Commun. Soil Sci. Plant Anal. 25, 3405–3412.

Rerkasem B, Lordkaew S and Dell B 1997 Boron requirement for reproductive development in wheat. *In* Plant Nutrition for Sustainable Food Production and Environment. Eds T Ando, K Fujita, T Mae, H Matsumoto, S Mori and J Sekiya. pp. 153–156. The XIII International Plant Nutrition Colloquium. Proceedings. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Reuter D J and Robinson J B 1997 Plant Analysis — An Interpretation Manual. Melbourne, Australia.

Reuter D J, Edwards D g and Wilhelm N S 1997 Temperate and Tropical Crops (Chapter 5). *In* Plant Analysis — An interpretation manual. Eds. DJ Reuter and JB Robinson. pp. 236–252. CSIRO, Melbourne, Australia.

Romero L and Aguilar A 1986 The availability and absorption of boron in soil–plant systems. Agrochimica 30, 335–350.

Shelp B J 1988 Boron mobility and nutrition in broccoli (*Brassica oleracea* var. italica). Ann. Bot. 61, 83–91.

Shelp B J 1993 Physiology and biochemistry of boron in plants. *In* Boron and its Role in Crop Production. Ed UC Gupta. pp. 53-85. CRC Press, FL, USA.

Shelp B J, Marentes E, Kitheka A m and Vivekanandan P 1995 Boron mobility in plants. Physiol. Plant. 94, 356–361.

Sigma Chemical Co 1980 Technical Bulletin Fluid Process Chemicals. P.O. Box 14508. St Louis, MO 63178, USA. pp. 1–3.

Snowball K and Robson A D 1983 Visual deficiency symptoms in *Triticum aestivum* cv Gamenya. Symptoms of Nutrient Deficiencies: Subterranean Clover and Wheat. pp. 67-69. Department of Soil Science and Plant Nutrition. Institute of Agriculture. University of Western Australia, Western Australia.

Tanaka H 1967 Boron absorption by excised sunflower root. Soil Sci. Plant Nutr. 13, 77–78.

Ware G O, Ohki K and Moon L L 1982 The Mitscherlich plant growth model for determining critical nutrient deficiency levels. Agron. J. 74: 84–91.

Weir R G and Cresswell G C 1994 Plant Nutrient Disorders 4. Pasture and Field Crops. pp 103-124. Melbourne, Australia. Inkata Press.

Williams R F 1948 The effects of phosphorus supply on rates of intake of phosphorus and nitrogen and upon certain aspects of phosphorus metabolism in graminaceous plants. Aust. J. Sci. Res. Ser. 1, 333–336.

Yamauchi M 1971 The role of boron in higher plants. Relationship between boron and calcium on the pectin substances in plants. J. Sci. Soil Nat. 42, 207–213.

Zarcinas B A, Cartwright B and Spouncer L 1987 Nitric acid digestion and multielement analysis of plant material by inductively coupled plasma spectrometry. Commun. Soil Sci. Plant Anal. 18, 131–146.

Section editor: A.C. Borstlap