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A New Approach for Controlling Low Boron Concentration in Nutrient Solutions

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Abstract: It has always been a problem for research workers to control low external boron concentrations in the nutrient solutions to study the boron deficiency effects on plant growth. Limitation of conventional solution culture techniques led to a range alternatives for the controlled study of plant nutrition including flowing culture, programmed nutrient addition and chelated-buffered nutrient solution. From a literature review it was found that a range of substances form chelates with boron including poly hydric alcohols like mannitol, sugars and phenolic compounds. However, none apart from hydrofluoric acid formed chelates with formation constant comparable to iron chelates like DTPA or EDDHA. Moreover, most chelating substances had deleterious side effects which reduced their suitability for use in water culture: many of the compounds are substrates for bacterial growth, some were toxic or harmful to handle, and others are toxic to plants. Current investigations center around the use of the Boron-specific resin, IRA 743 which strongly complexes H_3BO_3 on its N-methyl glucamine functional groups. The boron sorption capacity of the resin varies with the supply batch from 2.2 to 5.0 mg B/mL resin. Boron saturated resin maintains an equilibrium boron concentration in solution of 0.5 mg B/L when added at the rate of 2 mL of resin to 1L of boron free triple deionised water. Current investigations are to study loading and unloading techniques of resin with boron, lowering the boron concentration in solution when the exchange resin is used as a boron source and comparing the plant growth when the boron is supplied by two different ways ie. boron exchange resin and 0.1 mg boron solution.

Key words: Boron, deficiency, canola, hydroponics,

Introduction

Water culture has been used for experimental purposes for many decades, several criticism have been levelled at conventional water culture (Asher and Edwards, 1983). The primary drawback with conventional water culture is the unrealistically high nutrient concentrations to which plant roots are exposed. Generally the concentration in conventional solution culture exceed those normally found in soil solutions by one to three orders of magnitude, raising serious concerns over the relevance of the plant responses measured. For example, phosphorus, boron and manganese concentrations found in the commonly used Hoagland's formulation are toxic for some plants (Asher and Edwards, 1983). The reason for using high initial concentrations in conventional solution culture is to ensure adequate supply of nutrients through an experiment. This in turn is because nutrient solutions lack nutrient buffering capacity and have a limited volume.

The need to ensure that nutrient concentrations are realistic (ie. comparable to soil solution concentrations) has been recognised for some time and various strategies developed to cope with the problem of nutrient supply. Each procedure has its disadvantages. Large volume recirculating systems can maintain low, realistic solution concentrations but are very expensive to install and have been used in only a few laboratories (Asher and Edwards, 1983). Frequent replacement of solutions is used but is rather wasteful of time and chemical salts. Frequent incremental nutrient additions to pots known as "Programmed nutrient addition" depends on an accurate prior knowledge of plant growth rate (Asher & Blamey, 1987) and besides is unsuitable when the response of plants to solution concentration is the object of primary concern. An alternative approach to the twin problems of nutrient concentration and supply in solution cultures is to increase the nutrient buffering capacity of the solution so that it simulates a soil system. When combined with models of ionic specification in solutions, buffered nutrient solutions open up several new possibilities for water culture studies. Not only should it be possible to maintain realistic solution concentrations, and to estimate the capacity of nutrients in solution, but the system in principle should be relatively low cost and capable of use in most laboratories. For micronutrient, the buffered systems should also minimise the ever present concern of contamination (Bell *et al.*, 1991). Buffered nutrient solution systems have been developed for phosphorus and micronutrient including Fe, Mn, Zn, and Cu (Chaney *et al.*, 1989). However there are no published reports for boron chelate systems.

Boron forms stable cyclic anionic borate diesters with diol and polyol compounds (Loomis and Durst, 1992), suggesting that compounds with this configuration could be used as chelators for a boron chelator buffered solution. The most stable borate diesters are formed with cis-diols on a furanoid ring as in erythritan and methylmannofuranoside. Other compounds with the cis-diol configuration that form borate diesters include mannitol, dulcitol and sorbitol (Loomis and Durst, 1992). The only biological compounds with this configuration are apiose, which is common in cell walls of most plants, and ribose which is one of the sugars in nucleic acids. These compounds, and related ones such as polyethylglycol, are potential chelators for use in water culture to buffer boron activity in solution. Another possible buffer is the Boron-specific resin, IRA 743.

The objective of the present study is to develop a boron chelator-buffered nutrient solution system for plant nutrition studies on boron. It has two main aims: firstly a range of possible boron chelators or substances which could release boron slowly into solution will be tested to evaluate their effectiveness in maintaining a range solution boron concentrations. Secondly, the boron buffered system will be used to study the growth of boron sensitive plants with conventional nutrient solution.

Materials and Methods

Plant culture: The full-strength basal nutrient solution used in this experiment contains macronutrients (μM): NH_4NO_3, 2000; KNO_3, 2800; $Ca(NO_3)_24H_2O$, 1600; $MgSO_4.7H_2O$, 1000; KH_2PO_4, 100; and K_2HPO_4, 100; and micronutrient (except for boron) (μM): $ZnSO_4.7H_2O$, 2; $MnSO_4.H_2O$, 2; $CuSO_45H_2O$, 0.5; $Na_2MoO_42H_2O$, 0.08; NaCl, 8; and FeEDTA, 40. Only analytical grade chemicals were used to make up the nutrient solution. Triple deionised water used through out the study and for making up the solutions, which was further purified and make free from boron by passing through the boron-specific resin column drop wise (Sigma Chemical Co., 1980) The macronutrient stock solutions were also purified with

boron-specific resin (IRA-743, Sigma Chemical Co.).

Canola plants were grown by water culture in a glasshouse. Canola seeds (cv Hyola 42) were germinated in paper towels moistened with 1.0 mM $Ca(NO_3)_2$ in the dark at 25 °C for 48 hours. Selected seedlings were transferred to 5-L plastic pots lined with polythene bags containing full strength of nutrient solution with exchange resin in cotton bags. The pots were randomly distributed in cooling tanks with constant temperature 18 °C. Solution pH was adjusted to 6.0±0.3 every other day with 4 per cent H_2SO_4 or 2 per cent NaOH (both were analytical grade chemicals). Nutrient solutions in all the pots were continuously aerated with filtered air through out the experiment. Nutrient solution changed after 7 days of interval with boron loaded exchange resin in case of experiment 1, while for experiment 2 it changed once after 10 days. The plants were allowed to grow for 20 days and the nutrient solution samples were collected after 5, 10 ,15 and 20 days from each pot for boron analysis.

The number of plants per pot was thinned to 8 on day 2 after transplanting. Four plants were harvested after 10 days of transplanting (harvest 1) and rest of the four plants were harvested after 20 days of transplanting (harvest 2). Each plant was divided into upper shoot (consisting of new growth of leaves and stems), lower shoot (consisting of seedling leaves which were present at the time of transplanting and lower stem) and root. Plants samples were dried at 70 °C to constant weight. The dried plant samples were finely ground and digested in concentrated nitric acid at 130 °C for boron determination by Inductively Coupled Plasma - atomic emission spectrometry (Zarcinas *et al.*, 1987).

Preparation of exchange resin:

Boron-specific resin (IRA-743, Sigma Chemical Co.) lot 127F 0546 was used for this study. Before loading with boron, the exchange resin was cleaned with the following procedure: A column of 500 mL was made with resin. One litre of boron free triple deionised water was passed through the column drop wise. After passing all the boron free TDI water through the column one litre of 10 per cent H_2SO_4 solution passed through the column drop wise. When all H_2SO_4 was drained out the column, 4% solution of NaOH passed through the column in the same way as H_2SO_4 solution. Final wash to the resin was given with two litre of boron free TDI water, which also allowed to pass through the column drop wise. After this procedure the exchange resin transferred to a cleaned (acid washed) plastic bottle.

For boron loading, 30 mL exchange resin shook 72 hours in one litre 100 per cent boron saturated solution on a mechanical shaker. After shaking the exchange resin transferred in a funnel having a filter paper and it washed with 100 mL boron free TDI water for removing the surface absorbed boron from the resin. Washing divided into three parts ie. 33 mL each time.

When all the water drained out from the resin, 0.04, 0.2, 1 and 5 mL of resin transferred to acid washed cotton bags (as treatment T1, T2, T3, T4 respectively) for glasshouse experiment 1. While for glasshouse experiment 2, 5 mL boron loaded resin mixed separately with 0.5, 1.0, 2.0 and 4 mL fresh resin (same batch) in acid washed cotton bags (as treatment T1, T2, T3, T4 respectively). For T5, 0.1 mg (9.2 μM) boron concentration was maintained with H_3BO_3 solution in case of both the experiments. The purpose of the above division of boron loaded resin and the addition of fresh resin to boron-loaded resin was to have different boron concentrations in nutrient solutions.

Data analysis: Both the experiments replicated four times. The results are analysed by standard analysis of variance techniques by using accessible general linear modeling package (Gagnon *et al.*, 1984). Significant main effects were separate with Fisher's Protected LSD Test at $P \leq 0.05$.

Results

Initial work with exchange resin: From a literature study it was found that a range of substances including Mannitol, Sorbitol, Glycerol, Ethane-1, 2-Diol, D Tartaric Acid, Catechol, Sulphonic acid, Gallic acid, Pyrogallol, D - Dulcitol form stable chelates with boron. Although these substances form chelates with boron, they could not be used for further research because of their detrimental/side effects.

For buffering the boron concentration in the solution culture boron exchange resin "Amberlite IRA - 743" was selected for further studies.

A series of following laboratory experiments were conducted to study how the exchange resin can be used to equilibrate the boron concentration in the nutrient solution:

- Absorption of boron by the exchange resin
- Absorption of boron with the passage of time
- Release of boron from the loaded exchange resin

The absorption of boron by the exchange resin: The purpose of the experiment was to observe the absorption of boron by the different batches of resin. For this experiment, two lots of exchange resin were selected: 127 F 0546 and 23 F 005 and heir boron absorption capacities are 2.162 and 5 mg/mL, respectively (Sigma Chemical Co., 1980). Solutions having boron concentrations of 5, 10, 20, 30, 40, 60, 80, 100, 200, 400, and 500 mg/L were prepared. After adding 2 mL (wet weight 2 grams or dry weight 0.6 gram) of exchange resin in each solution they were shaken on a mechanical shaker for 72 hours. The samples were analysed for boron by Inductively Coupled Plasma - Atomic Emission Spectrometry (Brown & Hu, 1993) (Table 1).

Table 1: Boron absorbed by two different lots of exchange resin

Boron added (mg/L)	Boron Absorbed (mg/g)	
	Lot 127 F 0546	Lot 23 F 005
5	2.26	5.00
10	2.27	5.06
20	2.27	5.11
30	2.27	5.23
40	2.27	5.23
60	2.27	5.23
80	2.29	5.30
100	2.31	5.35
200	2.31	5.35
400	2.31	5.35
500	2.31	5.35

Table 2: Effect of time on boron (mg/g) adsorbed by two lots of resin (Amberlite IRA-743) form the solutions of 0.925 and 9.25 mM B. Data are presented as mean ±S.E., n=2 replicates

Time (h)	Lot 1		Lot 2	
	0.925 mMB	9.25mMB	0.925 mMB	9.25mMB
0.25	0.32±0.05	0.33±0.08	0.72±0.05	0.73±0.08
1	0.90±0.07	0.92±0.07	2.00±0.17	2.06±0.15
6	1.90±0.11	2.01±0.16	4.20±0.34	4.27±0.44
24	2.21±0.33	2.24±0.24	4.84±0.46	4.92±0.63
48	2.26±0.21	2.29±0.15	5.02±0.71	5.12±0.52
72	2.27±0.18	2.31±0.16	5.02±0.55	5.12±0.41
96	2.27±0.22	2.31±0.24	5.02±0.48	5.12±0.43

The results of the experiments indicate that in 5 mg B/L solution the absorption of boron is the same as described by the manufacturer

Table 3: Boron eluted and retained by the exchange resin after 40ml increments of boron free water were passed through the resin Data are presented as mean ± S.E., n=2 replicates

Vol. of B-free water eluted (ml)	Boron concentration in eluent (μM)	Boron in resin (mg/6 g resin)
40	60±5.3	12.3±0.2
80	57±3.2	11.7±0.2
120	56±3.0	11.1±0.2
160	64±6.5	10.4±0.1
200	62±5.0	9.7±0.1
240	61±2.1	9.0±0.2
280	61±2.8	8.4±0.5
320	60±4.2	7.7±0.3
360	58±2.1	7.2±0.3
400	56±3.5	6.5±0.7
440	54±4.5	6.0±0.5
480	52±1.5	5.4±0.3
520	51±3.5	4.8±0.1
560	48±4.5	4.3±0.2
600	46±2.2	3.8±0.1
640	43±4.6	3.3±0.2
680	42±5.2	2.9±0.1
720	40±3.0	2.4±0.3
760	38±2.1	2.0±0.2
800	36±3.2	1.6±0.3
840	35±1.6	1.5±0.1
880	35±1.5	0.9±0.04

of the exchange resin (Sigma Chemical Co., 1980) Absorption of boron by both the lots of resin was slightly higher from the higher boron concentration solutions. Increased boron adsorption may represent the surface absorption of boron by resin. This excess amount of boron can easily be removed by simple washing of the resin with boron free triple deionized.

Rate of boron absorption by exchange resin : The purpose of the experiment was to study the time during which the exchange resin absorbs the maximum boron. For this experiment, 2 mL (wet weight 2 grams or dry weight 0.6 gram) resin of two different lots (127 F 0546 and 23 F 005 with boron absorption capacity of 2.162 and 5 mg B/mL resin) were shaken in the solutions of 10 mg and 100 mg B/L for 96 hours on a mechanical shaker. Solution samples were collected after 15 minutes, 1, 6, 24, 48, 72, and 96 hours (Table 2).

These results indicate that both the batches of exchange resin adsorbs most of boron during the first six hours of contact. Adsorbed boron reached a maximum at 24 hours. Slight changes are observed in absorption between 24 to 96 hours of shaking. Absorption was slightly higher with both batches of exchange resin, where they were loaded in 100 mg B/L solution because of surface absorption (Table 2).

Table 4: Boron concentration in nutrient solution. Glass house experiment 1. Data are presented as means ±S.E., n=4 replicates.

Treatments Solution (μM)	Boron Concentration in			
B-specific resin (g/5L solution)	5 Days	10 Days	15 Days	20 Days
0.04	4±0.5	3.6±0.4	4.0±0.2	3.1±0.3
0.20	14±1.0	11.0±0.5	13.0±0.6	10.0±0.6
1.0	34±5.0	29.0±4.0	35.0±3.0	29.0±2.0
5.0	95±4.0	87.0±8.0	102.0±10	92.0±10
Control	9±0.1	8.0±0.2	8.3±0.3	7.5±0.2

Release of boron from the loaded exchange resin (column study): The purpose of this experiment was to study the release characters of absorbed boron from the loaded exchange resin. For this experiment, exchange resin of lot no. 127 F 0546 was selected (with boron absorption capacity 2.162 mg B/mL resin). A column of 6 mL (wet weight 6 grams or dry weight 1.83 grams) was made

Table 5: Dry weight of plants, after 10 days and 20 days growth in solutions treated with boron loaded resin or conventional nutrient solution (9.2μMB) as control. Glasshouse experiment 1. Values are means of four replications

Treatments	Upper shoot		Lower shoot		Roots	
	10 days	20 days	10 days	20 days	10 days	20 days
	Dry weight (grams)					
0.04 g resin	0.15	3.0	0.12	0.26	0.02	0.27
0.2 g resin	0.17	3.3	0.13	0.30	0.02	0.30
1.0 g resin	0.18	3.5	0.15	0.37	0.03	0.32
5.0 g resin	0.18	3.7	0.14	0.32	0.03	0.31
Control*	0.17	3.3	0.18	0.32	0.03	0.35
LSD (0.05)	0.15	ns	0.025	ns	ns	ns

* No resin

Table 6: Boron concentration in upper and lower shoots and roots of plants, glass house experiment 1, grown in solutions treated with boron loaded exchange resin or in conventional nutrient solution (9.2 μMB) as control. Values are means of four replications

Treatments	Upper shoot		Lower shoot		Roots	
	10 days	20 days	10 days	20 days	10 days	20 days
	Dry weight (grams)					
0.04 g resin	21	23	31	26	18	16
0.2 g resin	30	35	38	32	18	19
1.0 g resin	38	38	49	46	21	22
5.0 g resin	43	40	64	60	24	25
Control*	28	34	36	36	25	18
LSD (0.05)	7	ns	3	4	ns	2

*No resin

Table 7: Boron concentration in nutrient solution. Glass house experiment 2. Data are presented as mean ± S.E., n = 4 replicates

Treatments (gram resin /5L solution)	Boron concentration in solution (μM)			
	5 Days	10 Days	15 Days	20 Days
4.0 g B-free + 5 g B loaded resir	33 ±3	30 ±2	35 ±4	31 ±4
2.0 g B-free + 5 g B loaded resir	41 ±3	39 ±4	40 ±2	38 ±7
1.0 g B-free + 5 g B loaded resir	75 ±1	73 ±2	77 ±4	75 ±6
0.5 g B-free + 5 g B loaded resir	82 ±3	82 ±3	84 ±4	74 ±8
Control*	9 ±2	8 ±4	9 ±2	8 ±1

* No resin

Table 8: Dry Weight of plants, after 10 days and 20 days growth in solutions treated with increasing amounts of boron green resin added with 5 g of boron loaded resin or conventional nutrient solution (9.2 μMB) as control. Glasshouse experiment 2. Values are means of four replication.

Treatments B loaded resin mixed with fresh	Upper shoot		Lower shoot		Roots	
	10 days	20 days	10 days	20 days	10 days	20 days
	Dry weight (grams)					
0.5 g resin	0.11	3.0	0.08	0.26	0.04	0.3
1.0 g resin	0.12	3.0	0.08	0.25	0.05	0.32
2.0 g resin	0.12	3.0	0.08	0.27	0.04	0.32
4.0 g resin	0.15	3.0	0.08	0.28	0.05	0.33
Control*	0.05	1.0	0.04	0.16	0.03	0.25
LSD (0.05)	0.02	0.52	0.01	0.034	0.01	0.025

* No resin

Table 9: Boron concentration in upper and lower shoots and roots of dry plant matter, glasshouse experiment 2, grown in boron loaded exchange resin or in conventional nutrient solution (9.2 μMB) as control. Values are means of four replications

Treatments B loaded resin mixed with fresh	Upper shoot		Lower shoot		Roots	
	10 days	20 days	10 days	20 days	10 days	20 days
	Dry weight (grams)					
4.0 g resin	33	31	45	35	17	17
2.0 g resin	30	36	42	40	23	18
1.0 g resin	34	35	55	54	19	22
0.5 g resin	40	40	66	65	20	22
Control*	30	35	36	36	22	18
LSD (0.05)	ns	6	4	5	2.27	ns

* No resin

Table 10: Elemental composition of nutrient solution with and without boron specific resin at 2 g/l. Data are presented as mean ± S.E., n = 3 replicates

Elements	No Resin		With Resin	
	Day 1	Day 10	Day 5	Day 10
	Concentrations (mg/L)			
Nitrogen	107.0 ± 1.0	108.0 ± 1	114.0 ± 1	124.0 ± 2.0
Phosphorus	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
Potassium	70.0 ± 2.0	72.0 ± 1	73.0 ± 1	74.0 ± 2.0
Sulfur	34.0 ± 1.0	34.0 ± 1	36.0 ± 2	38.0 ± 2.0
Magnesium	25.0 ± 0.1	27.0 ± 1	27.0 ± 2	28.0 ± 2.0
Calcium	65.0 ± 2.0	65.0 ± 2	67.0 ± 3	68.0 ± 3.0
Boron	-	-	0.8 ± 0	0.9 ± 0.1
Copper	0.03 ± 0	0.03 ± 0	0.03 ± 0	0.03 ± 0
Zinc	0.14 ± 0	0.15 ± 0	0.14 ± 0	0.14 ± 0
Iron	1.3 ± 0.1	1.1 ± 0.1	1.0 ± 0.3	1.0 ± 0.2
Manganese	0.12 ± 0	0.12 ± 0	0.1 ± 0	0.12 ± 0
Sodium	2.5 ± 0.1	2.6 ± 0	2.7 ± 0.2	2.6 ± 0.2

with boron loaded exchange resin. Boron free triple deionised water was passed through the column drop wise at the flow rate of 0.5 mL per minute. Samples were collected at the interval of 40 mL, which analysed on ICP for boron determinations (Table 3).

Results of the experiment indicate that release of boron from the loaded exchange resin is quite low and boron-holding capacity of the exchange resin is high because even after passing 880 mL boron free triple deionised water, it contained 0.9 mg boron out of 12.97 mg, which was loaded on it.

After conducting the above basic studies with exchange resin, the system has been tested on oil seed rape crop (canola, *Brassica napus* L. cultivar Hyola 42). The oil seed rape crop was selected for this study because of its sensitivity to boron deficiency as compared to other plant species.

B) To study the plant growth in boron buffered nutrient solution culture system

Glasshouse experiment 1: Mean boron concentrations in nutrient solutions increased from 3.7, 12, 31.5 and 94 μM B with increasing amounts of B-saturated resin from 0.04, 0.2, 1 and 5 g (Table 4). However, dry weight of plants growing on these solutions did not

respond to changes in solution boron concentration induced by the resin treatments (Table 5) and yields were the same as the control solutions with 9.2 μM B.

In lower shoots, boron concentrations increased at harvests 1 and 2 with increasing amounts of resin ($P \leq 0.05$) but were unchanged in the upper shoots, and roots (Cutting, 1971). At 9.2 μM B, the control treatment, boron concentrations in the lower shoots but not in upper shoots or roots were lower than with the highest addition of boron saturated resin. Mean boron concentrations in shoots were always above 20 mg B/kg dry weight and in roots above 15 mg B/kg dry weight.

Glasshouse experiment 2: Increasing amounts of boron free resin decreased the solution boron concentration provided by the 5 g of boron saturated resin from 80.5 to 31.5 μM B (Table 7). Solution boron concentration was nevertheless still much higher with 4 g of boron free resin mixed with 5 g of boron-saturated resin than with the lowest addition of boron-saturated resin in glasshouse experiment 1. As in glasshouse experiment 1, the dry weight of upper shoots, lower shoots and roots at both harvests were unaffected by the change in solution boron concentrations induced by resin treatment (Table 8). Plants growing in control treatments of 9.2 μM B had significantly ($P \leq 0.05$) less dry weight of upper shoots, lower shoots and roots at both harvest 1 and harvest 2 than those in solutions supplied with resin (Table 8).

Increasing amounts of boron free resin depressed boron concentrations in lower shoots but not in upper shoots and roots (Table 9). Even with the addition of increasing amounts of boron free resin, mean boron concentrations in shoots were never below 30 mg B/kg dry weight (Table 9). Boron concentrations in plant material of the control treatments (9.2 μM B) were comparable to those with the lowest concentrations in plants grown in solutions with boron free resin. This clearly indicates that the plants received more or less the same amount of boron from the resin and 9.2 μM B solutions and plant growth in control solutions (Table 8) were depressed for some reason other than the boron supply.

Discussion

The boron specific resin appeared to be very promising as a solution buffer to regulate boron concentration in solution. Laboratory experiments on the boron specific resin indicate that maximum absorption of boron by the resin ranged from 2 to 5 mg B/g resin. The maximum boron adsorption values varied between batches of resin and were very similar to those indicated by the manufacturer (Sigma Chemical Co., 1980). Most of the adsorption of boron by the resin took place during the first 6 to 24 hours of shaking (Table 2). The results of laboratory experiment confirmed that the adsorption of boron by resin is strong (Table 3) and the release of boron from the resin is a slow process.

Since solution boron concentrations of 92.5 μM B are relatively high for most plants (Keren & Bingham, 1985), two approaches were developed to lower the solution boron concentration: decreasing the amount of boron saturated resin from 1 g/L to 0.008 g/L, decreased solution boron concentration from 92.5 to 3.7 μM B. At about 0.04 g of boron saturated resin per litre, boron concentrations were maintained at 9.2 μM B, which is commonly used in solution culture for adequate boron supply (Kirk and Loneragan, 1988; Bell *et al.*, 1990). Thus relatively small amounts of boron saturated resin were sufficient to raise solution boron concentrations to levels which are adequate for most plants, and to maintain those concentrations for up to 10 days. Further testing is required to determine the maximum length of time for which buffering of boron concentrations in solution can be achieved with the relatively small amount of resin.

At the lowest amount of boron saturated resin added, the solution contained 3.7 μM B which was still adequate for growth. Not only was plant dry weight equivalent to that of plants in solutions containing $\geq$13.8 μM B, but boron concentrations in shoots were more than adequate with > 20 mg B/kg (Huang *et al.*, unpublished data). Indeed, 3.7 μM B was also equivalent to soil solution boron concentrations in boron adequate soils (Loneragan, 1975).

As an alternative to decreasing the amount of boron saturated resin added to the solution, which inevitably decreases its long term boron buffering capacity, the present study showed that mixing increasing amounts of boron free resin to the boron saturated resin decreased solution boron concentration. Mixing 4 g of boron free resin with 5 g of boron saturated resin decreased the equilibrium solution boron concentration from 83 to 33 μM B. Higher ratios of boron free : boron saturated resin would undoubtedly further decrease solution boron concentrations.

In glass house experiment 1, plant growth in resin treated solutions and in solutions supplied with H_3BO_3 at 9.2 μM B were similar ($P = 0.05$) at both harvest 1 and 2. The boron concentrations in plant material were also similar for the two sets of plants (Cutting, 1971) providing further evidence that the uptake of boron by plants was similar whether boron was supplied by the resin or by H_3BO_3. This indicates that plants had no difficulty in obtaining boron from solutions equilibrated with boron specific resin and there was no detrimental effect of the resin on plant growth.

In glass house experiment 2, boron concentrations in plant material, were similar in plants growing in resin treated and control solutions at 9.2 μM B solution. That the lower shoots of plants at 80.5 and 75 μM B/L (Table 9) contained higher concentrations of boron can be attributed to their higher average boron concentration in nutrient solution than for those in control solutions. Again it is quite clear that absorption of boron from solutions treated with boron specific resin and those in control solutions 9.2 μM B, were a direct response to solution boron concentration. By contrast with shoot boron concentrations, biomass in the resin treated solutions was significantly greater than those in the control solutions with 9.2 μM B and both sets of plants had lower dry weight than the equivalent plants in experiment 1. Since the solutions were changed once after 10 days in experiment 2, and once every 7 days in experiment 1, the lower plant growth in experiment 2 may be due to lower basal nutrient supply. However, lower basal nutrient supply does not immediately explain the superior growth of plants in the resin treated solutions.

In order to resolve the cause of poor growth of control plants in experiment 2, a small laboratory experiment was carried out to determine which nutrients were released by the resin. Two sets of the same nutrient solution as used for glass house experiments 1 and 2 were prepared. In one set, 2 g of boron loaded resin was added while in other set no resin was added. These solutions were aerated as in the glasshouse experiments but no plants were grown in them. The solution samples were collected after 0 and 10 days in the case where no resin was added and after 5 and 10 days where resin was added. Apart from boron and nitrogen, the concentrations of all the other nutrient elements were more or less the same with or without resin in solution (Table 10). The resin released up to 20 mg nitrogen per gram resin. Acid digestion of resin confirmed that it contained 24 mg N per gram of resin but negligible or undetectable amounts of the remaining elements reported in Table 10.

Thus in glass house experiment 2, the release of 20 mg N per gram of resin, in resin treated solutions, significantly ($P \leq 0.05$) increased the growth of oil seed rape plants, which have high internal requirements for nitrogen (Hocking, 1993). In glass house experiment 1, no response to nitrogen release from the resin was

found because the nutrient solution was renewed after 7 days, so all the plants already received adequate nitrogen. Thus in solutions containing a low supply of nitrogen, nitrogen supplied by the resin may stimulate plant growth for reasons unrelated to boron supply. To reconfirm the results, another glasshouse experiment was conducted (data not shown) in which plants were suppled with 1/3rd, full and triple strength basal nutrient solutions. Plant growth of resin treated solutions were similar to those of control (9.2 μM B) solutions only in case of triple strength basal nutrient solutions. Triple strength basal nutrient solutions also increased leaf nitrogen concentrations in canola to values above the critical nitrogen level for deficiency (Hocking, 1993). Thus, both shoot growth and leaf nitrogen responses to increasing the nutrient concentrations in the basal solution used in the present studies suggest that the basal solution was deficient in nitrogen for canola. In such a solution, the nitrogen released by the boron specific resin and increased frequency of solution replacement were sufficient causes to explain the stimulation in plant growth in resin treated solutions in experiments 1 and 2.

The present research demonstrated that boron specific resin can be used to buffer boron in solution cultures for plant growth for periods of up to 10 days. Different concentrations of boron in the nutrient solution can be established with the resin either by changing the amount of boron loaded exchange resin added to solutions or by mixing boron free resin with boron loaded resin.

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