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Effect of Calcium on the Alleviation of Boron Toxicity and Localization of Boron and Calcium in Cell Wall of Wheat

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Abstract

A greenhouse experiment involving four levels of B (0, 5, 10 and 20 mg B kg^{-1} as boric acid, H_3BO_3), and three levels of Ca (0, 100 and 200 mg Ca kg^{-1} calcium chloride, $CaCl_2$) were conducted on wheat plants (*Triticum aestivum* L. 'Gerek-79'). Boron toxicity symptoms strongly occurred at 10 and 20 mg B kg^{-1} levels with and without applied Ca. Without applied calcium, boron toxicity symptoms were also weakly expressed at 5 mg B kg^{-1} level. Growing wheat plants with application of high levels of Ca partially alleviated the toxicity symptoms. Shoot and root dry weight of plant was strongly depressed and decreased by applied boron; however, Ca applications reduced the inhibitory effect of B on the plant growth. Shoot and root boron concentrations and boron uptake were increased with increasing B application, but decreased with Ca applications. Controversially, applied calcium increased calcium concentrations and uptake and decreased boron concentrations and uptake. Most of total boron and calcium were localized in the cell wall of plant. While applied boron increased the concentration of B on the cell wall, it decreased the concentration of Ca on the cell wall. In addition, applied calcium increased the cell wall calcium concentration, whereas decreased the cell wall boron concentration. It was determined that boron toxicity could be alleviated by addition of calcium in plant growing media.

Keywords: wheat, alleviation boron toxicity, boron, calcium, cell wall

Introduction

Boron toxicity may be observed in semi-arid and arid regions, such as India, South Australia and Turkey. It is known that B toxicity may result in depressed vegetative growth; prominent necrotic spots with brownish leaf tips, chlorosis, and finally a substantial reduction in crop yield (Punchana *et al.*, 2004). There is a narrow margin between boron deficiency and toxicity in some plants; however, the risk of inducing toxicity should not be ignored.

While boron has been known for a long time as an essential micronutrient for the growth of plant (Warington, 1923), yet its widespread importance for field crops has been recognized recently. A distinctive function of boron in plant cell walls has been revealed recently (Matoh, 1997; O'Neill *et al.*, 2001). This role of boron on cell walls, however, is inadequate to explain all of the observed effects of boron deficiency or toxicity (Brown *et al.*, 2002).

Boron is important for metabolism and growth of higher plants in cell elongation and cell division, protein metabolisms, tissue differentiation, membrane permeability, pollen germination and pollen tube growth (Marschner, 1990).

Calcium, one of the essential minerals for the development and growth of plant, is a key element in most physiological and biochemical processes in plants. It is important for membrane permeability and stability, essential for selective ion uptake, required for cell wall (Endho *et al.*, 1971; Bangerth, 1979), cell elongation, cell division (Burstrom, 1968), pollen germination and growth of pollen tubes (Brewbaker and Kwack, 1963) having similar functions to boron. In the primary cell wall, cellulose microfibrils are linked together by cross-linking glycans, usually xyloglucan (XG) polymers but also glucoarabinoxylans in Poaceae (Gramineae) and other monocots (Willats *et al.*, 2001). These interlocked microfibrils are embedded in a matrix, in which pectin is the most abundant class of macromolecule.

The wheat plant makes up most important part of the daily dietary. That's why many scientists tend to work on the growth characteristics and techniques of wheat worldwide. Cereals are generally considered to be sensitive to moderate-to-high boron levels (Eaton, 1944). Wheat, being semi-tolerant for B toxicity (Gupta *et al.*, 1985), can tolerate boron up to 2 mg B kg^{-1}. Higher values may frequently cause toxicity symptoms such as chlorosis, necrosis and yield reduction of wheat plants.

The aim of this study was to determine the effects of calcium on the alleviation of boron toxicity and localization of boron and calcium in cell wall of wheat plants.

Materials and methods

Wheat (*Triticum aestivum* L., 'Gerek-79') was grown in plastic pots containing 2000 g air-dried soils in a greenhouse under natural light conditions. Some properties of the experimental soils taken from Aridisol great soil group were as follows; clay loam in texture (Bouyoucos, 1951), $CaCO_3$ 46 g kg^{-1} (Richards, 1954), pH 8.08, EC 0.102 dS cm^{-1} (both in 1:2.5 water extract), and organic matter 10.4 g kg^{-1} (Nelson and Sommers, 1982). The concentrations of NH_4OAc extractable potassium (K) and calcium (Ca) were 180 mg kg^{-1} and 5380 mg kg^{-1}, respectively. The $NaHCO_3$ extractable P was 8.76 mg kg^{-1} (Olsen *et al.* 1954) and NaOAc extractable B was 1.06 mg kg^{-1} (Wolf, 1971).

In the experiment, treatments consisted of four levels of boron (0, 5, 10 and 20 mg B kg^{-1} as H_3BO_3), and three levels of calcium (0, 100 and 200 mg Ca kg^{-1} as $CaCl_2$) and replicated four times in a completely randomized factorial experiment For basal fertilizers, 100 mg N kg^{-1} as NH_4NO_3 and 60 mg P_2O_5 kg^{-1} as triple super phosphate and 75 mg K_2O kg^{-1} as K_2SO_4 were applied to all pots before sowing.

Twelve wheat (*Triticum aestiv*um L. 'Gerek-79') seeds were sown into each pot. After emergence, the plants were thinned to eight at the 2 to 3 leaf stages. Abnormal traits and symptoms on the foliage were recorded throughout the growing period.

Sixty days after sowing, the vegetative growth were harvested and divided into shoot and root. Shoot and root samples were washed with boron free distilled water and nearly 2 g fresh leaf samples were taken for determination of boron and calcium concentrations on cell wall.

Determination of total boron and calcium concentrations of plants: The remaining shoot and root plant samples were dried at 65 °C and weighted. After grinding, plant samples were dry-ashed at 500 °C for total plant boron and calcium determination by using ICP-OES (Perkin Elmer Optima 2100 DV).

Determination of cell wall boron and calcium concentrations: Tips of leaves (nearly 2 cm length) was taken from each individual plant leaf in all pots. The cell wall was separated from the plants by the method described by Hu and Brown (1994). In this method, the samples of fresh plant leaf were homogenized with an ice-cold mortar and pestle in cold water. The homogenate was centrifuged at 1000 g for 10 min. The residue was washed three times with 10 volumes of 80 % ethanol and once with 10 volumes of methanol: chloroform mixture (1:1, v/v). Finally the precipitate was washed with 10 volumes of acetone. The samples were dried and ashed at 500 °C for cell wall boron and calcium determination by using ICP-OES (Perkin Elmer Optima 2100 DV).

The data obtained from different analyses and measurements were analyzed statistically by using Minitab package program (Minitab Release 10.51) and treatment means were compared using the MSTAT package program (Version 3.00) for Duncan's Multiple Range Test.

Results and discussions

Boron toxicity symptoms: Boron toxicity symptoms were generally observed at high levels of B treatments. The toxicity symptoms strongly occurred at 10 and 20 mg B kg^{-1} levels for with and without added Ca. On the other hand, boron toxicity symptom was slightly observed for treatment containing 5 mg B kg^{-1} of without added calcium. Application of higher (10 and 20 mg B kg^{-1}) levels of boron increased the sensitivity of plant for B and result in greater occurrence of leaf injury due to B toxicity. The boron toxicity symptoms occurred as a dark brown spots and lesions with chlorotic border in the oldest leaf of wheat. The leaf injury symptoms observed are in agreement with those previously reported by Bergmann (1992) and Oyewole and Aduayi (1992).

The toxic effect of B seen at both 10 and 20 mg B kg^{-1} treatments was alleviated by treatments of 100 and 200 mg Ca kg^{-1}. Taban *et al.* (1995) reported that B toxicity was determined when high level of boron (10 mg B kg^{-1}) was applied to the wheat plants, but this toxic effect was decreased when the growing medium was supplemented with Ca. The problem of boron toxicity was addressed by some investigators using the Ca application approach. This approach was based on some evidence that showed an interaction between B and Ca. Boron and calcium interaction was reported by Gupta (1979) and Taban *et al.* (1995) who found a negative correlation between B and Ca, with high Ca inducing B deficiency. According to them, when B was applied, Ca concentration was decreased, Ca was applied to the plant B concentration was decreased. The application of Ca could reduce the availability of B, resulting in decreased uptake of B (Gupta and Macleod, 1981; Taban *et al.*, 1995). This result might be explained that Ca which was localized in cell wall (Tab. 9) caused to uptake boron by plant. In the other words, localization of Ca in cell wall leads to decrease cell wall boron permeability.

Shoot and root dry weight of wheat plants: Applied boron with and without Ca decreased the dry weight in shoot and root of the wheat plants (Tab. 1, 2). Moreover, the inhibitory effect of boron on the shoot and root growth was reduced by the presence of calcium. Without Ca application, the highest growth reduction in dry weight occurred at 20 mg kg^{-1} and 10 mg kg^{-1} treatment followed. The reduction of shoot and root dry weight at 10 and 20 mg kg^{-1} boron applications were 56.7 % and 78.7 % respectively in shoot, 61.3 % and 81.1 % respectively in root, compared to the control. The shoot and root growth reduction at 20 mg B kg^{-1} was limited by the application of 100 and 200 mg kg^{-1} Ca to 54.6 % and 45.7 %, respectively in shoot, 67.2 % and 56.3 % respectively in root, compared to the control treatment (Tab. 1, 2). Calcium application increased the dry weight of plants. These increases occurred

Tab. 1. The effect of calcium on the shoot dry weight (g pot^{-1}) of wheat plants grown with increasing levels of boron

B treatments, mg kg^{-1}	Ca treatments, mg kg^{-1}		
	0	100	200
0	4.32±0.9 aA	4.67±0.8 bA	4.91±0.8 cA
5	3.90±0.7 aA	4.17±0.5 bB	4.17±0.7 bA
10	1.87±0.4 aB	2.65±0.4 bC	2.95±0.3 cB
20	0.92±0.5 aC	2.12±0.2 bD	2.61±0.4 cC
B		**	
Ca		**	
BxCa int.		**	

** p <0.01. Means followed by the same letter are not significantly different (Duncan's multiple-range test, p<0.05). Small letters for each row, capital letters for each column.

at higher amounts (10 and 20 mg B kg^{-1}) of applied boron (Tab. 1). The toxic effect of boron was observed to be alleviated by calcium application, particularly at 10 and 20 mg B kg^{-1} levels, suggesting the negative impact of Ca on B availability, in agreement of the statement of by Gupta (1972) and Taban *et al.* (1995). Ca counteracted the toxic effect of B, and result in higher dry weight of B treated plants. These results agreed with Chatterjee *et al.* (1987) and Taban *et al.* (1995).

Total boron and calcium concentrations and uptake of wheat plants: Increasing the amount of boron and calcium applied showed varied effects on total B and Ca concentrations and their uptake by plants. Total shoot and root boron concentration (Tab. 3, 4) and shoot boron uptake (Tab. 5) were increased by B application, being similar to the results of Paull et al. (1988), Nable (1988) and Taban et al. (1995). The increase in B was greater in the absence of Ca than in the presence of it (Tab. 3). Impeding effect of calcium on the boron uptake could be explained as B uptake decreased by Ca that was associated with pectic constituents of cell walls of tomato leaves (Yamauchi et al., 1986). Calcium applications increased total shoot calcium concentrations and decreased total shoot and root boron concentrations of wheat plants. Similar results were reported by Gupta (1972), Oyewole and Aduayi (1992) and Taban et al. (1995). Applied B caused to accumulate B in

Tab. 2. The effect of calcium on the root dry weight (g pot^{-1}) of wheat plants grown with increasing levels of boron

B treatments, mg kg^{-1}	Ca treatments, mg kg^{-1}		
	0	100	200
0	2.12±0.9 aA	2.50±0.8 bA	3.23±0.8 cA
5	2.06±0.7 aA	2.05±0.5 bB	2.47±0.7 bA
10	0.82±0.4 aB	1.02±0.4 bC	1.60±0.3 cB
20	0.40±0.5 aC	0.82±0.2 bD	1.42±0.4 cC
B		**	
Ca		**	
BxCa int.		**	

** p <0.01. Means followed by the same letter are not significantly different (Duncan's multiple-range test, p<0.05). Small letters for each row, capital letters for each column.

Tab. 3. The effect of calcium on total boron concentration (mg kg-1) in shoot of wheat plants grown with increasing levels of boron

B treatments, mg kg^{-1}	Ca treatments, mg kg^{-1}		
	0	100	200
0	41.2±2.3 aA	34.2±1.8 bA	26.6±3.1 cA
5	92.6±2.8 aB	59.3±3.5 bB	32.3±3.8 cB
10	181.3±5.5 aC	119.2±5.8 bC	104.7±4.6 cC
20	320.5±8.3 aD	198.8±6.4 bD	135.6±5.7 cD
B		**	
Ca		**	
BxCa int.		**	

** p <0.01. Means followed by the same letter are not significantly different (Duncan's multiple-range test, p<0.05). Small letters for each row, capital letters for each column.

shoot. In really, boron uptake was increased by applied B and was decreased by applied Ca (Tab. 5). Application of Ca resulted in increasing the total shoot calcium concentration and uptake, whereas B caused a decrease both of them (Tab. 6, 7).Cell wall boron and calcium concentration of wheat plants: Applications of boron and calcium affected the cell wall boron and calcium concentration of the plants, both B and Ca were accumulated in the cell wall. Amount of accumulation depended on applied levels of each element. The more B applied the more B accumulated in the cell wall, whilst the more Ca applied the less B accumulated(Tab. 8). The effect of Ca on cell wall was same as B (Tab. 9). Boron plays a fundamental role has an important position in the physical structure of expanding wall was found to be high. Several authors have demonstrated that a significant proportion of the boron in cell walls is associated with pectins (Yamaouchi et al., 1986; Bassil et al., 2004).

One of structural element in plant cell wall is Ca. Calcium bounded with pectin molecules and localized in the cell wall as Ca-pectate complexes which may act to stabilize boron in cell wall (Yamaouchi et al., 1986, Cleland et al., 1990).

Tab. 4. The effect of calcium on total boron concentration (mg kg^{-1}) in root of wheat plants grown with increasing levels of boron

B treatments, mg kg^{-1}	Ca treatments, mg kg^{-1}		
	0	100	200
0	36.7 ±2.8 aA	22.3±1.8 bA	21.2±3.1 cA
5	66.5±3.2 aB	41.1±3.5 bB	19.2±3.8 cA
10	139.5±5.1 aC	93.4±5.8 bC	83.3±4.6 cB
20	225.7±10.3 aD	133.8±6.4 bD	105.4±5.7 cC
B		**	
Ca		**	
BxCa int.		**	

** p <0.01. Means followed by the same letter are not significantly different (Duncan's multiple-range test, p<0.05). Small letters for each row, capital letters for each column.

Tab. 5. The effect of calcium on total boron uptake (μg dw plant^{-1}) in shoot of wheat plants grown with increasing levels of boron

B treatments, mg kg^{-1}	Ca treatments, mg kg^{-1}		
	0	100	200
0	178.0±8.3 aA	159.7±6.8 bA	130.6±5.1 cA
5	361.1±10.8 aB	247.3±9.5 bB	134.7±8.8 cB
10	339.0±12.5 aC	315.9±11.8 bC	308.9±7.6 cC
20	294.9±15.3 aD	421.5±16.4 bD	353.9±12.7 cD
B		**	
Ca		**	
BxCa int.		**	

** p <0.01. Means followed by the same letter are not significantly different (Duncan's multiple-range test, p<0.05). Small letters for each row, capital letters for each column.

Tab. 6. The effect of calcium on total calcium concentration (g kg^{-1}) in shoot of wheat plants grown with increasing levels of boron

B treatments, mg kg^{-1}	Ca treatments, mg kg^{-1}		
	0	100	200
0	1.67±0.3 aA	3.08±0.9 bA	3.54±0.6 cA
5	1.53±0.8 aB	2.82±0.6 bB	3.13±0.4 cB
10	1.47±0.2 aC	2.56±0.5 bC	2.76±0.7 cC
20	1.17±0.3 aD	2.35±0.2 bD	2.45±0.3 cD
B		**	
Ca		**	
BxCa int.		**	

** p <0.01. Means followed by the same letter are not significantly different (Duncan's multiple-range test, p<0.05). Small letters for each row, capital letters for each column.

Tab. 7. The effect of calcium on total calcium uptake (mg dw plant^{-1}) in shoot of wheat plants grown with increasing levels of boron

B treatments, mg kg^{-1}	Ca treatments, mg kg^{-1}		
	0	100	200
0	7.21±0.01 aA	14.38±0.09 bA	17.38±0.09 cA
5	5.97±0.07 aB	11.76±0.08 bB	13.05±0.06 cB
10	2.75±0.03 aC	6.78±0.03 bC	8.14±0.05 cC
20	1.08±0.02 aD	4.98±0.02 bD	6.39±0.03 cD
B		**	
Ca		**	
BxCa int.		**	

** p <0.01. Means followed by the same letter are not significantly different (Duncan's multiple-range test, p<0.05). Small letters for each row, capital letters for each column.

Both boron and calcium play a significant role in cell wall structure, by cross linking molecules by covalent bonds; if one of these ions accumulates in the cell wall more than the other, the accumulated ion inhibits the generation of the other resulting in interception of toxic effect due to over-deposition. The hypothesis of Ca alleviated B toxicity could be explained as Ca accumulation in cell wall at high levels of applied Ca such as 100 and 200 mg Ca kg-1, and the depression of B uptake of the cell (Tab. 6).

Tab. 8. The effect of calcium on cell wall boron concentration (mg kg^{-1}) in shoot of wheat plants grown with increasing levels of boron

B treatments, mg kg^{-1}	Ca treatments, mg kg^{-1}		
	0	100	200
0	20.3±0.5 aA	19.7±0.6 bA	12.8±0.9 cA
5	43.9±1.2 aB	29.3±1.5 bB	19.6±1.2 cB
10	121.3±3.5 aC	99.2±2.8 bC	74.7±2.6 cC
20	224.6±5.3 aD	168.8±4.4 bD	115.6±3.7 cD
B		**	
Ca		**	
BxCa int.		**	

** p <0.01. Means followed by the same letter are not significantly different (Duncan's multiple-range test, p<0.05). Small letters for each row, capital letters for each column.

Tab. 9. The effect of calcium on cell wall calcium concentration (g kg^{-1}) in shoot of wheat plants grown with increasing levels of boron

B treatments, mg kg^{-1}	Ca treatments, mg kg^{-1}		
	0	100	200
0	1.27±0.6 aA	2.15±0.2 bD	2.95±0.3 cA
5	0.95±0.5 aB	1.63±0.7 bA	2.58±0.6 cB
10	0.82±0.4 aC	1.35±0.8 bB	2.21±0.8 cC
20	0.63±0.2 aD	1.16±0.5 bC	1.95±0.7 cD
B		**	
Ca		**	
BxCa int.		**	

** p <0.01
Means followed by the same letter are not significantly different (Duncan's multiple-range test, p<0.05). Small letters for each row, capital letters for each column.

Conclusions

It could be stated that increasing the amount of applied Ca decreased the B accumulation of plants. B toxicity may be categorized as a risk for arid and semi-arid regions, not a serious threat or not to be often observed. More efforts seem to be performed to find solutions to overcome the effects of B toxicity on the plant growth. It could be proposed in the light of the present experiments, application of Ca to growing medium might be one of the answers to B toxicity problems.

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References

Bangerth, F. (1979). Calcium related physiological disorders of plants. Annu. Rev. Phytopath. 17:97-122.

Bassil, E., H. Hu, and P. H. Brown (2004). Use of phenylboronic acids to investigate boron function in plants. Possible role of boron in transvacuolar cytoplasmic strands and cell-to-wall adhesion. Plant Physiol. 136:3383-3395.

Bergmann, W. (1992). Nutritional disorders of plants. Development, visual and analytical diagnosis. Gustav Fisher Verlag Jena, New York, USA.

Bouyoucos, G. J. (1951). A recalibration of hidrometer for making mechanical analysis of soil. Agron. J. 43: 434-437.

Brewbaker, J. L., and B. H. Kwack (1963). The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Botany. 50:859-865.

Brown, P. H., N. Bellaloui, M. A. Wimmer, E. S. Bassil, J. Ruiz, H. Hu, H. Pfeffer, F. Dannel, and V. Romheld (2002). Boron in plant biology. Plant Biol. 4:205-223.

Burstrom, H. G. (1968). Calcium and plant growth. Biol. Rev. 43:287-316.

Chatterjee, C., P. Sinha, N. Nautical, S. C. Agarwala, and C. P. Sharma (1987). Metabolic changeses associated with boron-calcium interaction in maize. Soil Sci. Plant Nutr. 33:607-617.

Cleland, R. E., S. S. Virk, D. Taylor, and T. Bjorkman (1990). Calcium, cell walls and growth. In Leonard, R.T., Hepler, P.K.: Calcium in plant growth and development. Am.Soc. Plant Physiol. Symp. 4:9-16.

Eaton, F. M. (1944). Deficiency, toxicity and accumulation of boron in plants. J. Agric. Res. 69:237-277.

Endho, M., K. Ohira, and A. Fujiwara (1971). A role of calcium as a component of cell wall of radish. J. Sci. Soil Manure. 42:390-394.

Gupta, U. C. (1972). Interaction effects of boron and lime on barley. Soil Sci. Soc. Amer. Proc. 36:332-334.

Gupta, U. C. (1979). Boron nutrition of crops. Adv. Agron. 31:273-307.

Gupta, U. C., and J. A. Macleod (1981). Plant and soil boron as influenced by soil pH and calcium sources on podzol soils. Soil Sci. 131:20-25.

Gupta, U. C., Y. W. Jame, C. A. Campbell, A. J. Leyshon, and W. Micholaichuk (1985). Boron toxicity and deficiency. Canadian J. Soil Sci. 65:381-409.

Hu, H., and P. H. Brown (1994). Localization 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.

Marschner, H. (1990). Mineral Nutrition of Higher Plants. Academic Press, London.

Matoh, T. (1997). Boron in plant cell walls. Plant Soil 193:59-70.

Nable, R. O. (1988). Resistance to boron toxicity amongst several barley and wheat cultivars: A preliminary examination of the resistance mechanism. Plant Soil. 112:45-52.

Nelson, D. W., and L. Sommers (1982). Total carbon, organic carbon and organic matter, p. 539-579. In: A.L. Page (Eds) Methods of Soil Analysis, Part 2. American Society of Agronomy. Madison, Wisconsin, USA.

Olsen, S. R., C. V. Cole, F.S. Watanabe, and L. A. Dean (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. of Agric. Circ. 939.

O'Neill, M. A., S. Eberhard, P. Albersheim, A. G. Darvill (2001). Requirement of borate cross-linking of cell wall rhamnogalacturonan II for arabidopsis growth. Science. 294:846-849.

Oyewole, O. I., and E. A. Aduayi (1992). Evaluation of the growth and quality of the "Ife Plum" tomato as affected by boron and calcium fertilization. J. Plant Nutrition. 15:199-209.

Paull, J. G., B. Cartwright, and A. J. Rathjen (1988). Responses of wheat and barley genotypes to toxic concentrations of soil boron. Euphytica. 39:137-144.

Punchana, S., S. Jamjod, and B. Rerkasem (2004). Response to boron toxicity in boron efficient and inefficient wheat genotypes. 4th International Crop Science Congress. http://www.cropscience.org.au/icsc2004/poster/2/5/5/899_punchanas.htm

Richards, L. A. (1954). Diagnosis and Improvement of Saline and Alkali Soil. United States Department of Agriculture, Agricultural Handbook 60, Washington.

Taban, S., M. Alpaslan, C. Kutuk, A. Inal, and I. Erdal (1995). Relationship between boron and calcium on wheat (Triticum aestivum L.). 9th International Symposium of CIEC, "Soil fertility and fertilization management-bridge between science, industry and practice", September 25-30, Kuşadası-Turkey. pp, 85-90.

Warington, K. (1923). The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. 37:629-672.

Willats, W. G. T., L. McCartney, L. Mackie, and J. P. Knox (2001). Pectin: cell biology and prospects for functional analysis. Plant Mol. Biol. 47:9-27.

Wolf, B. (1971). The determination of boron in soil extracts, plant materials, composts, manures, water and nutrient solutions. Soil Sci. and Plant Analysis 2:363-374.

Yamaouchi, M., T. Hara, and Y. Sonoda (1986). Distribution of calcium and boron in the pectin fraction of tomato leaf cell wall. Plant Cell Physiol. 27:729-732.