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SHORT COMMUNICATION

BORON-STRESS INDUCED CHANGES IN WATER STATUS AND STOMATAL MORPHOLOGY IN *ZEA MAYS* L. AND *CATHARANTHUS ROSEUS* L.

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Boron-stress induced changes in water status and stomatal morphology were studied in maize (*Zea mays* L. var. 32-A09) and periwinkle (*Catharanthus roseus* L. var. Nirmal) plants. Compared to boron sufficient plants (0.33 mg B l^{-1} supply), boron deficient (0.033 mg B l^{-1} supply) and toxic (3.3 mg B l^{-1} supply) plants showed accumulation of proline. The leaf water status measured as specific and relative water content was significantly increased with decreased water potential (ψ) in both crops under boron deficiency as well as toxicity. At deficient and toxic supply of boron, both plants showed reduced stomatal size and increase in stomatal index. Stomatal opening was also affected under boron deficiency and toxicity, more so in former in both plants.

***Key words*: Boron deficiency and toxicity, maize, periwinkle, proline, stomatal morphology, water status.**

Boron is an essential micronutrient required for growth and development of vascular plants (Loomis and Durst 1992). The property of boron to form diester bonds with diol groups of polysaccharides and occurrence of a large proportion of boron in cell walls (Loomis and Durst 1992) is suggestive of a role of boron in cell walls. The borate cross linking of the cell wall RG-II (rhamnogalacturon-II) provides the structural organization to the cell walls needed for turgor driven growth of plant cell (O' Neill *et al*. 2001).

The role of boron in membrane integrity and membrane functions such as formation and maintenance of membrane potentials and membrane permeability has been well established (Blevins and Lukaszewski 1998).

Stomata play an important role in the regulation of gas exchange, photosynthesis and transpiration in flowering plants and are distributed throughout the epidermis. Sharma *et al.* (1984) showed that boron deprivation of cabbage plants led to a decrease in the stomatal opening associated with increase in diffusive resistance and decrease in transpiration rate. Similar observations were made for cauliflower (Sharma and Sharma 1987) and mustard (Sharma and Ramachandra 1990). Boron deficient plants also show enhanced accumulation of proline (Sharma *et al*. 1984) which is a typical feature of water stressed plants.

Plants are known to vary in their boron requirement. It is reported that the internal boron requirements of dicots are generally higher than monocots, both at vegetative and reproductive stage of growth (Gupta 1993). The present study was carried out to assess the effect of boron-stress (deficiency and excess) on water status, stomatal opening as well as proline accumulation in plants. Earlier reports were based only on dicot plants subjected to boron deficiency. Hence, it was proposed to carry out a comparative study of the effect of boron deficiency as well as toxicity on the water relations and

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stomatal morphology of two different types of crops maize (a monocot) and periwinkle (a dicot) belonging to different families, poaceae and apocynaceae respectively.

Plants of maize (*Zea mays* L. var. 32-A09) and periwinkle (*Catharanthus roseus* L. var. Nirmal) were raised, from seeds sown in polyethylene pots containing purified sand. The seeds were surface-sterilized with 5% (v/v) mercuric chloride solution and were washed thoroughly before sowing. The composition of nutrient solution used for growing the plants was: 4 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 0.1 mM Fe EDTA, 10 µM $MnSO_4$, 1 µM $CuSO_4$, 1 µM $ZnSO_4$, 0.1 µM Na_2MoO_4, 0.1 µM NaCl, 0.1 µM $CoSO_4$ and 0.1 µM $NiSO_4$. Boron was supplied as 0.033, (deficient) 0.33 (control) and 3.3 (toxic) mg l^{-1}, to assess the effect of boron-stress on fresh and dry weight, concentration of proline, relative water content of leaves and stomatal morphology. The above parameters were studied at 48 days of treatment when deficiency as well as toxicity effects of boron had become apparent in both maize and periwinkle.

For taking the fresh weight plants were separated into appropriate parts- root, stem and leaves and then weighed. After drying these fresh materials in a forced drought oven at 70 °C for 48 hours, the oven-dried plant samples were transferred to a desiccator, and when cool, weighed accurately to determine the dry matter yield. To make a comparative study of the effect of boron stress on yield of the two plants, the relative fresh and dry weight was calculated by considering weight of control (plants receiving 0.33 mg B l^{-1} supply) plants as 100%.

Leaf proline was estimated colorimetrically at 520 nm by method of Bates *et al*. (1973). Water status of leaf tissue was measured in terms of relative water content (RWC), by method of Barrs and Weatherley (1962), in leaf discs. The RWC was determined by using the formula: RWC= [(FW-DW)/ (TW-DW)] X 100; where FW= fresh weight, DW=dry weight and TW= turgid weight. Water saturation deficit (WSD) was given by subtracting the RWC values from 100 to find out the maximum amount of water by which the tissue was deficient. Specific water content in leaves expressed as g g^{-1} dry weight was calculated by using formula: (FW–DW)/(DW). Water potential (ψ) of leaves was measured by dew point hygrometric method using L-44 leaf chambers with Wescor microvolt meter model HR-33T.

The topmost fully expanded leaf from main shoot was considered for stomatal studies. Peelings of leaf epidermal layers were taken. The number of stomata and epidermal cells was counted in microscopic fields. Changes in stomatal frequency were expressed as stomatal index using formula: SI= [No. of stomata/No. of stomata + No. of epidermal cells] X 100. Measurements of stomatal characters such as length and width were carried out using ocular and stage micrometers.

The data were statistically analyzed (ANOVA) for significance (LSD at P=0.05). All measurements were made on samples drawn in triplicates.

Fresh and dry matter yield in both plants decreased under deficiency as well as excess of boron, more so the former (Table 1). The decrease in relative biomass, i.e. relative fresh weight and dry weight under boron stress was more in the dicot plant periwinkle than in maize plants. This is suggestive of higher requirement of boron for the dicot than for the monocot plant and is in consonance with similar results obtained in many crop plants (Sharma *et al.* 1993, Gupta 1993, Bell *et al*. 1997).

Severe retardation in growth and visual deficiency effects such as stem thickening and death of apical growing points were obtained in both plants raised at deficient level of boron supply (0.033 mg B l^{-1}). Some of the effects of boron deficiency in maize recorded were appearance of white translucent streaks in the middle of the leaf lamina, reduced leaf area, failure of young emerging leaf to unroll, short and thick internodes and cessation of apical growth (Fig. 1A).

The deficiency symptoms of boron observed in periwinkle include reduction in growth, leaf area and chlorosis. Shortening of internodes, as observed in maize were also observed in periwinkle. However, thickening and curling of leaves, terminal chlorosis, downward cupping of young emerging leaves and death of growing

Table 1. Effect of deficient and excess level of boron supply on the fresh and dry matter yield of maize (*Zea mays* var. 32-A09) and periwinkle (*Catharanthus roseus* L. var. Nirmal) at 48 days of treatment.

Plant	Plant parts	Boron supply (mg l^{-1})			LSD (P=0.05)
		0.033	0.33	3.3	
		Fresh matter yield (g $plant^{-1}$)			
Maize	Leaves	16.064 (50.40)	31.870 (100)	18.078 (56.72)	1.36
	Stem	41.128 (72.72)	56.558 (100)	45.805 (80.99)	3.21
	Root	10.296 (54.65)	18.840 (100)	13.653 (72.47)	2.01
Periwinkle	Leaves	5.140 (19.15)	26.845 (100)	17.115 (63.75)	9.17
	Stem	2.560 (18.28)	14.005 (100)	9.870 (70.47)	3.71
	Root	1.475 (31.12)	4.740 (100)	3.720 (78.48)	0.819
		Dry matter yield (g $plant^{-1}$)			
Maize	Leaves	2.172 (34.11)	6.367 (100)	4.813 (75.59)	1.04
	Stem	2.940 (58.94)	4.988 (100)	4.060 (81.39)	0.911
	Root	1.495 (73.83)	2.025 (100)	1.954 (96.49)	0.639
Periwinkle	Leaves	0.696 (22.32)	3.118 (100)	2.078 (66.65)	0.986
	Stem	0.498 (31.74)	1.569 (100)	1.129 (71.96)	0.354
	Root	0.214 (26.82)	0.798 (100)	0.589 (73.81)	0.178

Figures in paranthesis indicate relative values compared to control

tips of stem were symptoms typical to periwinkle (Fig. 1B).

Boron toxicity (3.3 mg Bl^{-1}) also retarded the growth in both plants. The toxicity symptoms observed in maize plant were yellowing of veins and irregular white patches in leaves (Fig. 1C). In periwinkle toxicity effects appeared as apical and marginal chlorosis in older leaves (Fig. 1D).

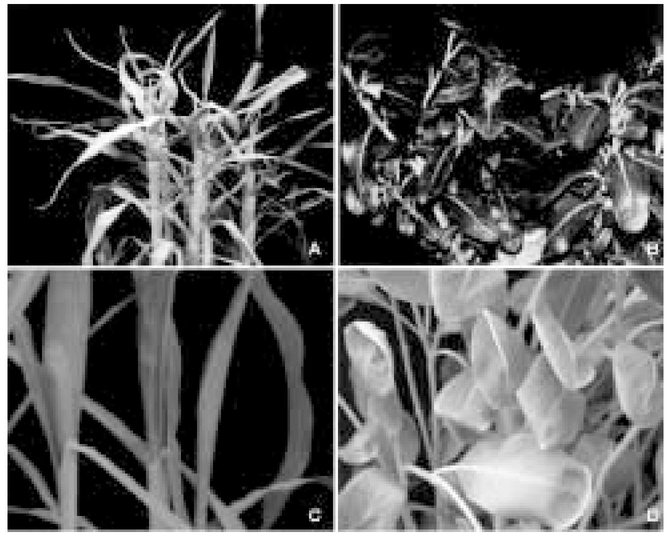

Fig. 1. (A-D) Plates showing boron deficiency (A, B) and toxicity (C, D) symptoms in maize (A, C) and periwinkle (B, D) respectively.

Plants of maize and periwinkle grown at deficient and excess boron supply showed higher frequency of stomata as indicated by greater stomatal index (SI) values (Table 2). Deka Boruah *et al.* (2008) have recently shown that the increase in the number of stomata on a leaf was proportional to degree of water loss. An increase in stomatal frequency in these plants under deficiency and toxic boron supply is probably due to water stress condition or reduced total water content in leaves as compared to control. The increase in SI was more in maize than in periwinkle under deficient and toxic boron supply. Studies have shown that leaves of plants raised in drought condition have smaller and numerous stomata (Hetherington and Woodward, 2003). The water status of plants is known to be directly related to stomatal behavior and stomatal frequency and size are important in this regard. Stomata size decreased in response to boron stress and the decrease in size of stomata in maize was more pronounced than in periwinkle. Stomatal responses to environmental changes are reflected in stomatal opening and closing. The stomata of boron deficient plants (maize and periwinkle) exhibited shrinkage in guard cells and failed to open (Fig. 2A & D). The stomatal opening in boron toxic (Fig. 2C & F) plants was less than the control plants (Fig. 2B & E) in both maize and periwinkle. Shrinkage in guard cells was observed in maize plants only under toxic boron supply.

Table 2. Effect of deficient and excess level of boron supply on stomatal characters in maize (*Zea mays* L. var. 32-A09) and periwinkle (*Catharanthus roseus* L. var. Nirmal) at 48 days of treatment.

Plant	B supply (mg l^{-1})			LSD (P= 0.05)
	0.033	0.33	3.3	
Length of stomata (µm)				
Maize	30.86	42.16	41.09	2.91
Periwinkle	24.75	31.35	28.05	3.29
Width of stomata (µm)				
Maize	8.25	16.5	8.25	0.87
Periwinkle	16.5	19.8	19.3	1.53
No. of stomata mm^{-2}				
Maize	31	17	23	4.64
Periwinkle	32	30	36	3.51
No. of epidermal cells mm^{-2}				
Maize	47	38	41	5.82
Periwinkle	41	40	47	4.64
Stomatal index (%)				
Maize	39.70	30.90	35.90	2.89
Periwinkle	43.83	42.85	43.37	1.40

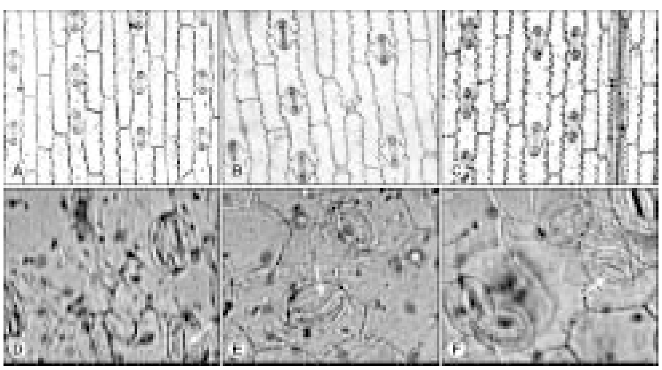

Fig. 2. (A-F). Photomicrographs of stomata of maize (A, B, C) and periwinkle (D, E, F) at 0.033 (A, D), 0.33 (B, E) and 3.3 (C, F) mg B l^{-1} supply. Arrows indicate stomatal opening. (x10)

The stomatal characters were more affected under deficient than toxic supply of boron in both plants (Table 2).

A decrease in total water content and high specific water content was observed in both plants under boron stress (Table 3). Leaves of plants of maize and periwinkle receiving deficient and toxic boron supply showed increased relative water content with decreased water potential. Thus, plants receiving deficient and excess boron supply had less water saturation deficit (WSD) than control plants (Table 3). The increased RWC in plants subjected to deficient and toxic boron supply is accordance to Sharma *et al.* (1984), and Sharma and Ramachandra (1990) and is probably due to increase in apoplastic bound water.

The accumulation of proline occurred both under deficient and toxic boron supply in both plants. In maize accumulation was slightly more at deficient boron supply whereas in periwinkle accumulation was more under boron toxicity (Table 3). Enhanced accumulation of proline under boron stress as observed in present study, is in accord with the observations of Sharma *et al.* (1984), who reported increased proline content in the

Table 3. Effect of deficient and excess level of boron supply on water status in leaves of maize (*Zea mays* L. var. 32-A09) and periwinkle (*Catharanthus roseus* L. var. Nirmal) at 48 days of treatment.

Plant	B supply (mg l^{-1})			LSD (P= 0.05)
	0.033	0.33	3.3	
Proline (µmol g^{-1} fw)				
Maize	0.98	0.76	0.97	0.18
Periwinkle	0.41	0.30	0.48	0.04
Total water (g $plant^{-1}$)				
Maize	13.89	18.00	12.27	2.56
Periwinkle	4.37	22.32	14.45	0.94
Specific water content (gg^{-1} dw)				
Maize	5.75	4.67	7.50	1.04
Periwinkle	6.25	4.81	5.75	0.48
Water potential (ψ) (bars)				
Maize	-1.88	-1.34	-2.45	0.13
Periwinkle	-3.12	-1.75	-2.98	0.46
Relative water content (RWC) (%)				
Maize	85.18	80.06	85.78	2.34
Periwinkle	86.20	79.30	85.70	1.40
Water saturation deficit (WSD) (%)				
Maize	14.82	19.94	14.22	2.34
Periwinkle	13.80	20.70	14.30	1.40

leaves of cabbage plants subjected to boron deficiency. Eraslan *et al*. (2007) also observed increased accumulation of proline content in tomato and pepper plants grown under boron toxicity. Our results were contradictory to those of Karabal *et al*. (2003) and Papadakis *et al*. (2004) who found no significant changes in proline concentration under excess boron.

The observed decrease in water potential and significantly high accumulation of proline in the leaves of both plants are suggestive of water stress in plants receiving boron deficient and excess supply. These water stressed plants had high tissue water content in leaves probably due to high concentration of hydrophilic colloids or accumulation of their precursors such as soluble nitrogenous fractions, sugars and starch, which lead to an increase in bound water by lowering of matric potential in the leaves as indicated earlier by Sharma *et al*. (1984) and Sharma and Ramachandra (1990). The study also suggested that both deficiency as well as toxicity of boron causes water stress and altered stomatal characters (length, width and stomatal index) in plants. An optimum quantity of boron is required for stomatal opening probably by regulating the opening of stomata via regulating the osmoticum of guard cells or via accumulation of ABA or K^+ ions. The exact mechanism through which boron regulates the stomatal opening is still not known.

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