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Susceptibility of periwinkle (*Catharanthus roseus* L. var. nirmal) to boron deficiency

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Abstract: To check the susceptibility of periwinkle (*Catharanthus roseus* L. var. nirmal) to boron deficiency, a sand culture experiment was conducted in glass house and enzymic changes in leaves was studied. Plants of periwinkle were grown at 0.066 (deficient) and 0.33 (control) mg B L^{-1} supplies till maturity. 36 and 48 days after treatment leaves were examined for concentration of photosynthetic pigments and activities of PPO, SOD, CAT, POD, APX and GR enzymes. Plants subjected to boron deficiency showed growth retardation and reduced biomass. Reduction in photosynthetic pigments (chl a, b and carotenoids) was observed in leaves of periwinkle plants subjected to boron deficiency. Expressed on fresh weight and protein basis, increased relative activities of PPO, SOD. POD, APX and GR and decreased relative activity of CAT compared to control, was observed.

Key words: Boron deficiency, periwinkle, photosynthetic pigments, enzymes

Introduction

Boron is an essential micronutrient required for healthy plant growth and development. Boron is present in soil solution in several different forms-BO_2^-, $B_4O_7^-$, BO_3, $H_2BO_3^-$ and [B (OH) $_4$] and is found mostly in the topsoil. Dry weather reduces moisture in the topsoil and boron uptake by the plant, causing boron deficiency. Even high rainfall areas witness leaching out of borosilicate from the soil, which leads to boron deficiency. Boron deficiency is a world wide nutritional problem in agricultural production which has been reported in over 80 countries including India and 132 crops (Shorrocks, 1997).

Boron plays an important role in different process such as vegetative growth, tissue differentiation, metabolic control through regulation of enzymatic reactions, membrane integrity and function, phenolic metabolism, sugar translocation and nucleic acid synthesis (Blevins and Lukaszewski, 1998).

Periwinkle (*Catharanthus roseus* L. var. nirmal), a native of Madagascar belonging to family apocyanacae is a medicinally important crop because of its anticancerous properties. In the present study we evaluated the susceptibility of periwinkle to boron deficiency and for this we studied the changes in plant biomass, stomatal morphology, concentration of photosynthetic pigments and activity of enzymes- polyphenol oxidase (PPO, EC 1.14.18.1), superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.4.2).

Materials and Methods

Seeds of the periwinkle (*Catharanthus roseus* L. var. nirmal) were surface sterilized by soaking in 5% (v/v) mercuric chloride for 5 min followed by thorough washing with deionised manesty still water (MSW). Sterile seeds were soaked in MSW at 25^{0}C and after 48 hr, uniform seeds were sown at the uniform depth of 0.5 cm in polyethylene pots containing purified sand (Sharma, 1996). Entire study was conducted in glass house under controlled conditions.

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 $NiSO_4$ with supply of boron deficient (0.066 mg B L^{-1}) and optimum (0.33 mg B L^{-1}). All the biochemical analysis was carried out in triplicates with a completely randomized design at two stages of treatment (36d and 48d).

To study the stomatal morphology the peeling of leaves were taken off with the help of forceps. Chlorophyll (Chl) and carotenoids (Car) in leaves were extracted in 80% acetone by the method of Lichtenthaler (1987). PPO was assayed by the method described by Shenshi and Noguchi (1975). The assay mixture for polyphenol oxidase contained 0.1 M phosphate buffer pH 6.5 and suitably diluted enzyme extract. The reaction was initiated by the addition of 0.01 M DL-DOPA (3, 4 –dihydroxy 1- phenol alanine). The reaction was allowed to proceed for 30 minutes at 30^{o}C and 0.25 M lead acetate was added to stop the reaction. Optical density of the supernatant was measured at 470 nm.

The activitiy of enzymes catalase (CAT) and peroxidase (POX) were assayed in the fresh leaf tissue extracts (10%) prepared in glass distilled water. Catalase was assayed by an adaptation of the permanganate titration method described by Pandey *et al*., (2009). Peroxidase was assayed by addition of suitable enzyme extract to a reaction mixture containing 0.1 M phosphate buffer pH 6.0, 0.01% H_2O_2 and 0.5% p-phenylene diamine. Reaction was stopped by adding 4 N H_2SO_4. The colour intensity was read at 485 nm (Pandey *et al*., 2009).

For assaying SOD, APX and GR, fresh leaves were homogenized with 150 mM potassium phosphate buffer pH 7.0

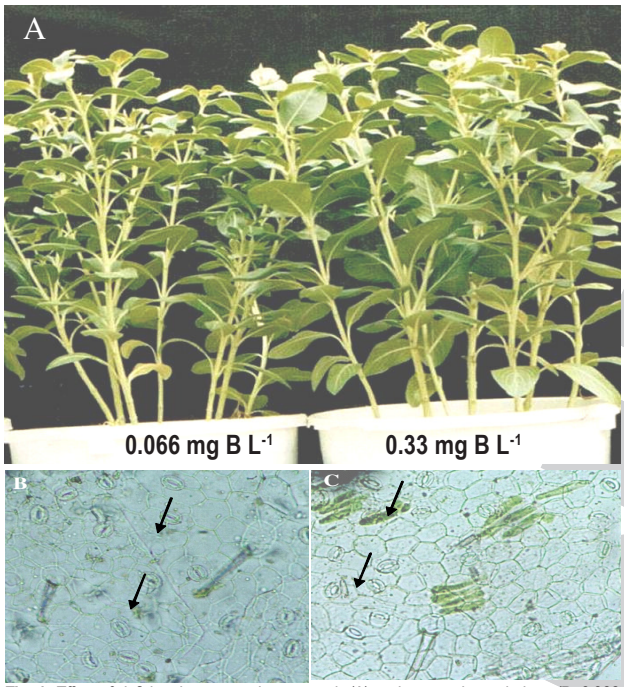

Fig. 1: Effect of deficient boron supply on growth (A) and stomatal morphology (B=0.066 mg B L^{-1}; C=0.33 mg B L^{-1} supply). Arrows indicating partially opened stomata (B) at deficient boron supply and fully opened stomata (C) at sufficient boron supply

containing 1 mM EDTA and 2% PVP and all the enzymic reactions were carried out as earlier described by Pandey *et al*., (2009).

To make a comparative study of the effect of boron deficiency and optimum supply on plants of periwinkle, all the parameters have been presented as relative values considering control (plants receiving 0.33 mg B L^{-1} supply) as 100% in bar diagrams.

Results and Discussion

Growth differences were shown by plants after 25 days of boron supply. Plants treated with 0.066 mg B L^{-1} supply showed depression in growth as compared to plants receiving supply of 0.33 mg B L^{-1} (Fig. 1). The deficiency symptoms in periwinkle included growth retardation, reduced leaf area and chlorosis with shortening of internodes and stem thickening. The deficiency symptoms first appeared in young leaves as reduced and curled lamina. Chlorotic areas developed in younger leaves. Boron deficiency symptoms first appeared in the younger leaves, suggested the immobility of boron via phloem tissues (Gupta, 1993; Sharma, 2006). Significantly reduced stomatal size and partial closure of stomata and slight shrinkage in guard cells was observed at deficient B supply (0.066 mg B L^{-1}) compared to control (Fig. 1), suggested the involvement of boron in stomatal

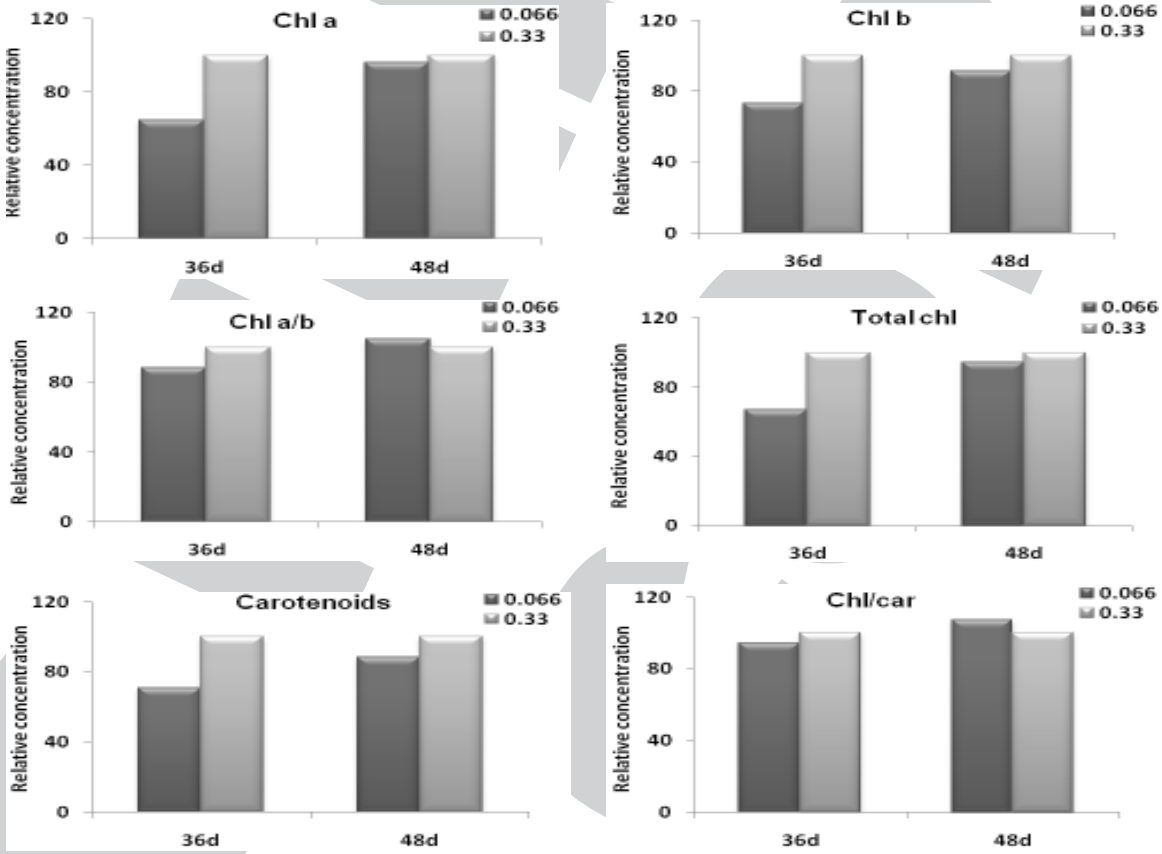

Fig. 2: Effect of boron deficiency on the relative concentration of photosynthetic pigments in leaves of periwinkle plants

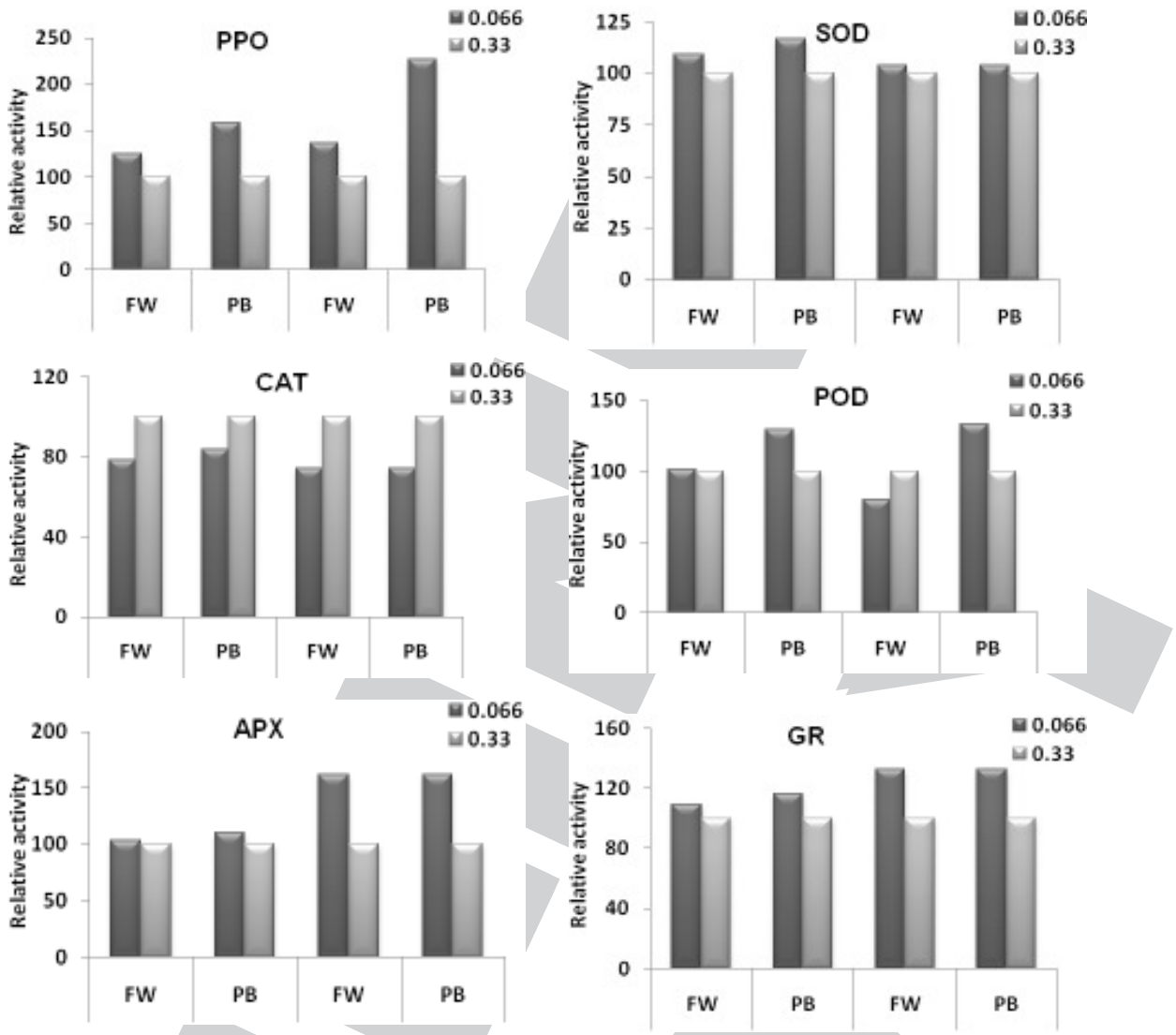

Fig. 3: Effect of boron deficiency on the relative activity of PPO, SOD, CAT, POD, APX and GR in leaves of periwinkle plants

morphology (Sharma and Sharma, 1987; Sharma and Ramachandra, 1990).

Compared to control, concentration of photosynthetic pigments, chl a, chl b and carotenoids decreased under boron deficiency while the ratio of chl a/b and chl/car decreased at earlier treatment stage and increased at later treatment stage of boron. The decreased photosynthetic pigments concentration under boron deficiency is in consonance with earlier results (Han *et al*., 2009; Pandey *et al*., 2009). Boron deficiency induced changes in chloroplast structure might be the cause of decreased concentration of chl a and b. Ratio of chl a/b decreased at earlier stage suggested that chl a is more affected than chl b, but at later stage of boron treatment a reverse trend was observed which suggested that under severe deficiency chl b is more affected than chl a (Fig. 2).

Carotenoids protect the chloroplast from photooxidative damage. The concentration of carotenoid decreased under boron deficiency. The decreased chl/car ratio at earlier stage might be an adaptive feature to protect chloroplast from photooxidative damage (Yamamoto and Bassi, 1996), but at later stage the ratio of chl/car increased which suggested the more damage of carotenoids under severity of boron deficiency (Fig. 2).

Increased activity of PPO enzyme was observed both at fresh weight basis and at protein basis in leaves of boron deficient plants (Fig. 3). This was earlier reported by several other workers (Pfeffer *et al*., 1998; Camacho-Cristobal *et al*., 2002). PPO enzyme catalyses the oxidation of phenolic compounds into quinones. The increased PPO activity might be the cause of excess production of quinones which caused oxidative damage in plants subjected to boron deficiency. Although it has been proposed that the loss of

membrane integrity under boron deficiency may be due to accumulated phenolics and their oxidation products (Cakmak and Römheld, 1997), it has been demonstrated that resupply of boron to deficient leaves doesnot recover plasmamembrane integrity throughout complexing phenols or inhibiting PPO activity (Pfeffer *et al*., 1998; Ruiz *et al*., 1999; Cara *et al*., 2002).

Activity of SOD, which constitutes the first line of defense against the reactive oxygen species (ROS) (Alscher *et al*., 2002) was found to be increased and is in consonance with the results of other workers (Garcia-Gonzales *et al.,* 1988; Cakmak and Römheld, 1997). Decreased relative activity of CAT was observed both on fresh weight and protein basis at both the stages of treatment. The CAT, a H_2O_2 scavenging enzyme with relatively low affinity for H_2O_2 showed decreased activity in periwinkle under B deficiency. This is in accordance with the findings of Agarwala *et al.,* (1981), Liu and Yang *et al*., (2000), Han *et al*., (2009) (Fig. 3).

The activity of POD was also increased both on fresh weight and protein basis but there was slight decrease in activity at fresh weight basis at the later stage of boron treatment (Fig. 3). The specific activity of non-specific peroxidase (POD) a group of enzyme with Fe as a co-factor and concerned with different activities like detoxification of H_2O_2 and lignification of cell walls showed increased activity. The observed increase in the activity of POD due to boron deficiency has also been reported by Shive and Barnett (1973) and Agarwala *et al.,* (1978, 1991).

Compared to control, relative activity of enzymes expressed on fresh weight and protein basis- APX and GR increased under boron deficiency condition at both the stages of treatment but the increase was more pronounced at later stage (Fig. 3). Like CAT and POD, APX is an important antioxidant enzyme involved in scavenging of H_2O_2. The specific activity of APX increased in periwinkle under deficiency of boron, is consonance with the result of Han *et al*., (2009).

Glutathione reductase (GR) is another important antioxidant enzyme concerned with reduction of oxidized glutathione in the chloroplast and cytosol and regeneration of ascorbic acid found to be significantly enhanced under B deficiency and is consonance with the result of Han *et al*., (2009) and contradictory with the findings of Cakmak and Römheld, (1997).

From the present study it has been concluded that the periwinkle is sensitive to boron nutrition and experiences a growth retardation, altered stomatal morphology and changes in enzymic metabolism under boron deficiency.

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