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EFFECT OF BORON STRESS ON GROWTH, CARBOHYDRATE, PROTEIN AND PHENOL METABOLISM IN THE LEAVES AND ROOTS OF SUNFLOWER SEEDLINGS

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To evaluate the effect of boron on growth, carbohydrate, protein and phenol metabolism, seeds of sunflower (*Helianthus annus* L. var. sungold) were grown in solution culture with variable boron supply (0.0033, 0.033, 0.33, 3.3 and 33 mg B l^{-1}) under controlled conditions in green house. Seedlings grown under boron deficient (0.0033 and 0.033, mg B l^{-1}) and excess (3.3 and 33 mg B l^{-1}) supply showed growth retardation, decreased shoot and root length with decreased dry weight. Photosynthetic pigments in leaves were found to be decreased under boron stress. Altered carbohydrate, protein and phenol metabolism was observed in leaves and roots under deficiency and excess supply of boron.

Key words: Boron stress, carbohydrate, protein, phenols, sunflower seedlings.

Boron is an essential micronutrient for higher plants, but the range between deficient to toxic boron concentration is smaller than that for any other nutrient element. Boron can limit plant growth and yield if present below or above the threshold value. In soil, boron exists primarily as boric acid [$B(OH)_3$], which can be easily leached under high rainfall conditions leading to deficiencies in plants that grow there. Contrary to this, under low rainfall conditions, boron can not be sufficiently leached and therefore may accumulate to levels that become toxic to plant growth especially in arid and semiarid areas (Camacho-Cristobal *et al.* 2008).

Boron plays an important role in sugar transport, cell wall synthesis, lignification, cell wall structure membrane integrity, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid (IAA) and phenol metabolism (Blevins and Lukaszewaski 1998). The studies carried out so far were restricted to boron deficiency effects on aerial parts. The purpose of present study was to investigate the response of sunflower seedlings to boron deficiency as well as excess on carbohydrate, protein and phenol metabolism on leaves as well as roots.

MATERIALS AND METHODS

A solution culture experiment was conducted with the seedlings of sunflower (*Helianthus annus* var. Sungold). The seeds were first surface-sterilized with 5% (v/v) mercuric chloride solution and washed properly with deionised manesty still water (MSW) before germination. Sterile seeds were soaked in MSW in petridishes lined with three fold filter paper at 25^{0}C and after 48 hr, uniform seeds with 0.5-1.0 cm long radical, were transferred to a series of culture vessels containing appropriate nutrient solution filled to the rim. The culture vessels were polyethylene containers of 5”X5”X8” dimension with a top cover having 36 evenly spaced holes, each 4 mm in diameter. The radical passed through these holes into the nutrient solution.

The composition of nutrient solution used was a modification of Hoagland nutrient solution (Hoagland and Arnon 1950) with varying levels of boron supply ranging from deficiency to excess (0.0033, 0.033, 0.33, 3.3

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Table 1: Effect of boron supply on growth and dry matter yield in sunflower (*Helianthus annus* var. Sungold) seedlings grown in solution culture.

Growth parameters	Boron supply: mg B L^{-1}					LSD (P=0.05)
	0.0033	0.033	0.33	3.3	33	
Stem length (cm)	4.01	5.98	9.78	5.56	5.26	0.178
Root length (cm)	3.91	5.24	8.10	5.52	5.10	0.257
	Dry matter yield (g $seedling^{-1}$)					
Leaves	0.020	0.028	0.029	0.028	0.027	0.003
Stem	0.007	0.013	0.017	0.012	0.009	0.002
Tops	0.027	0.041	0.046	0.040	0.036	0.001
Root	0.004	0.008	0.013	0.007	0.003	0.001
Whole seedling	0.031	0.049	0.059	0.047	0.039	0.002
Stem/root	1.750	1.625	1.307	1.714	3.000	0.011

and 33 mg BL^{-1}). The entire experimental studies were conducted in green house under controlled conditions of light, humidity and temperature and nutrient solutions were changed every alternate day to maintain the desired level of nutrients.

The dry weight per seedling, stem and root length and stem/root ratio were measured after 7 days of treatment. Before taking the dry matter yield, seedlings were separated into appropriate parts- leaves, stem and roots. The dry matter yield was determined after drying the fresh material in a forced drought oven at 70^{o} C for 48 hours and was weighed accurately. Tissue boron concentration in oven dried leaves, shoot and roots was estimated colorimetrically by Azomethine-H method of Wolf (1971) after wet digestion. All the biochemical parameters were carried out in the first two leaves and roots of seedling which were harvested at 9 days growth, after receiving variable boron supply for 7 days.

Chlorophyll (chl) and carotenoids (car) in leaves were extracted in 80% acetone by the method of Lichtenthaler (1987). For the estimation of sugar, starch and phenols, plant

Table 2: Effect of boron supply on tissue concentration of boron in sunflower (*Helianthus annus* var. Sungold) seedlings grown in solution culture.

Plant parts	Boron supply: mg B L^{-1}					LSD (P=0.05)
	0.0033	0.033	0.33	3.3	33	
	Boron concentration: µg g^{-}_{1} dry wt.					
Leaves	21.43	28.45	38.63	69.58	83.13	2.53
Stem	36.38	38.33	40.58	50.65	98.90	1.49
Root	32.53	39.45	47.05	55.68	63.40	3.43

Table 3: Effect of boron supply on photosynthetic pigments in sunflower (*Helianthus annus* var. Sungold) seedlings grown in solution culture.

Parameters	Boron supply: mg B L^{-1}					LSD (P=0.05)
	0.0033	0.033	0.33	3.3	33	
	mg g^{-1} fresh weight					
Chlorophyll a	0.157	0.210	0.237	0.195	0.151	0.005
Chlorophyll b	0.128	0.169	0.184	0.155	0.139	0.010
Total chl	0.285	0.379	0.421	0.350	0.290	0.015
Chl a/b	1.227	1.243	1.288	1.258	1.086	0.012
Carotenoids	0.167	0.175	0.190	0.174	0.168	0.009
Chl/car	1.707	2.166	2.216	2.011	1.726	0.017

material was fixed in boiling 80% ethanol in the proportion of 1:10. Sugars were determined colorimetrically by the method of Nelson (1944). Starch was estimated by the method of Montgomery (1957). Total phenols were determined in alcohol soluble fractions by the method of Swain and Hillis (1959).

Soluble protein in enzyme extract was measured by Coomassie Brilliant Blue G250 protein binding method of Bradford (1976) using bovine serum albumin as standard. Ribonuclease (EC 3.1.1.22) was assayed by the method of Tuve and Anfinson (1960). Polyphenol oxidase (EC 1.14.18.1) was assayed by the method of Shenshi and Noguchi (1975).

Standard analyses of variance (ANOVA) were used to assess the significance of treatment means. Differences between treatments means were compared using LSD at the 0.05 probability level.

RESULTS AND DISCUSSION

Optimum growth was observed in seedlings receiving 0.33 mg B L^{-1} supply which was treated as control. There was a marked decrease in dry weight of seedlings under boron stress (0.0033, 3.3 and 33 mg BL^{-1}) (Table 1). This is in consonance with similar results obtained in many crops (Bell 1997). We observed root growth inhibition in boron stressed seedlings and also an increase in adventitious rooting in seedlings grown with 0.33 mg BL^{-1} boron supply. Josten and Kutschera (1999) also suggested that optimum boron is required for the development of adventitious roots in sunflower cuttings. Increased stem/root ratio under boron stress suggested the effect of boron was more on root growth than shoot growth (Table 1).

Boron concentration and in its distribution in different plant organs is known to vary in different plants. Tissue concentration of boron in stem, leaves and roots of sunflower seedling was increased with increase in boron supply from 0.0033 to 33 mg BL^{-1}. The concentration of boron in seedling ranged from 21.43 to 98.90 µg B g^{-1}dry weight (Table 2). Such a wide variation in boron concentration among different plant species and their cultivars have been observed by Bellaloui and Brown (1998).

Chlorophyll concentration in leaves decreased under deficiency and excess of boron (Table 3). This observation is in agreement with El-Shintinawy (1999) and Keles *et al.* (2004). A decrease in chlorophyll concentration in case of boron deficiency might be due to changes in chloroplast structure and in case of excess boron supply it might be due to inhibited biosynthesis of

Table 4. Effect of boron supply on carbohydrate metabolism in sunflower (*Helianthus annus* var. Sungold) seedlings grown in solution culture.

Plant part	Boron supply: mg B L^{-1}					LSD (P=0.05)
	0.0033	0.033	0.33	3.3	33	
	Reducing sugars: % fresh weight					
Leaves	0.085	0.098	0.072	0.073	0.084	0.003
Roots	0.066	0.072	0.079	0.074	0.073	0.002
	Non-reducing sugars: % fresh weight					
Leaves	0.136	0.097	0.081	0.114	0.121	0.004
Roots	0.091	0.098	0.113	0.109	0.104	0.005
	Total sugars: % fresh weight					
Leaves	0.221	0.195	0.153	0.187	0.205	0.007
Roots	0.157	0.170	0.192	0.183	0.177	0.006
	Starch: % fresh weight					
Leaves	0.059	0.044	0.017	0.027	0.052	0.005
Roots	0.024	0.030	0.117	0.033	0.020	0.003

precursors of chlorophyll (Mortvedt *et al.* 1991). The chla/b was decreased under boron stress (Table 3) and indicated that chl a concentration is more affected than chl b, indicating a preferential degradation of chl a over chl b, probably because chl a being in close association with light harvesting reaction centres (LHC) is liable to be destroyed to a larger extent (Yamamoto and Bassi 1996).

The decrease in chlorophyll concentration could also be due to photooxidative damage of chloroplast caused by decreased carotenoid concentration under boron stress. The decrease in chl/car ratio (Table 3) might be an adaptive

Table 5: Effect of boron supply on protein metabolism in sunflower (*Helianthus annus* var. Sungold) seedlings grown in solution culture.

Plant part	Boron supply: mg BL^{-1}					LSD (P=0.05)
	0.0033	0.033	0.33	3.3	33	
	Protein: mg protein 100 mg^{-1} fresh weight					
Leaves	0.885	0.918	2.050	1.430	1.212	0.019
Roots	0.387	0.457	0.811	0.610	0.579	0.021
	Ribonuclease: Units mg^{-1} protein					
Leaves	0.153	0.141	0.119	0.132	0.136	0.010
Roots	0.049	0.045	0.039	0.041	0.043	0.002

Table 6: Effect of boron supply on phenol metabolism in sunflower (*Helianthus annus* var. Sungold) seedlings grown in solution culture.

Plant part	Boron supply: mg BL^{-1}					LSD (P=0.05)
	0.0033	0.033	0.33	3.3	33	
	Total phenols: µg 100 mg^{-1} fresh weight					
Leaves	24.05	20.83	17.36	21.19	24.73	2.24
Roots	34.28	30.62	21.94	32.93	34.71	1.19
	Polyphenol oxidase: Units mg^{-1} protein					
Leaves	0.078	0.069	0.013	0.072	0.084	0.179
Roots	0.012	0.008	0.001	0.016	0.018	0.003

response against photooxidative damage in plants facing stress (Yamamoto and Bassi 1996).

Boron has been suggested to play an important role in carbohydrate metabolism. It readily forms complexes with sugars which are more readily translocated through cellular membrane than non borated sugar molecules (Cakmak and Römheld 1997). Accumulation of sugars both reducing and non-reducing and starch under deficiency and excess of boron was observed in leaves of sunflower (Table 4). This is similar to the earlier findings of Agarwala *et al.* (1991). However in roots, there was decrease in concentration of sugars both reducing and non-reducing and starch under deficiency and excess of boron. In case of low boron supply, there might be inhibition in the formation of borate-sugar complex, which would result in poor translocation of carbohydrates and would lead to their accumulation in the leaves. On the other hand decreased concentration of sugars and starch in roots might be due to poor translocation from source (leaves) which caused root growth inhibition and a resultant decrease in demand for reduced carbon (carbohydrates) by these tissues.

The increase in RNase activity and decreased protein content in boron stressed seedlings as observed in the present study (Table 5) is in agreement with reports of Agarwala *et al.* (1991) and Sharma *et al.* (1993) and might be the cause of inhibited protein synthesis and cell division (Dave and Kannan 1980) resulting in inhibition in growth rate.

In the present study we observed enhanced accumulation of phenols in leaves as well as roots (Table 6). Accumulation of phenolic compounds in boron stressed tissues is a critical step in the initiation of the impairment of several cellular functions (Camacho- Cristobal *et al.* 2008). Enhanced accumulation of phenolics could result from increased activity of enzymes involved in phenyl propanoid biosynthesis, such as polphenol oxidase as observed in present study (Table 6). Enhanced accumulation of phenolics has also been suggested to elevate the endogenous levels of IAA to toxic limits. It was initially proposed that boron regulate the IAA concentrations to an optimum which was not inhibitory to root growth (Jarvis *et al.* 1984). Observations from the study suggested that the inhibition of root elongation by boron stress is mediated by increased phenolic concentrations which may alter the IAA concentration in tissues to values which are detrimental for root growth.

From the present study it has been

concluded that boron deficiency as well as excess altered the metabolism of carbohydrate, protein and phenols. Under boron stress both leaves as well as roots showed protein inhibition and accumulation of phenols while in case of carbohydrate metabolism there was accumulation of sugars and starch in leaves but decreased concentration of sugars and starch in roots. In leaves accumulation of sugars and starch was found to be more under deficiency than excess supply of boron. It was also observed that protein inhibition was more in leaves than roots both under deficiency and excess of boron. Phenol accumulation was observed under deficient and excess supply of boron both in leaves and roots, but compared to leaves, the accumulation was found to be more in roots. We concluded that the observed decrease in protein and carbohydrate fraction and excessive accumulation of phenols in the roots might be the cause of more damage of roots than leaves under boron stress.

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