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Effect of Boron Deficiency on Some Physiological and Biochemical Aspects During the Developmental Stages of Wheat (*Triticum aestivum* L.) Plant

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Abstract: The results of experiment I showed that inhibition of root growth in wheat (*Triticum aestivum* L. Giza 157) plants transferred to boron-free medium coincided with a major decrease in the ascorbate concentration of root. Under low-boron concentration, in which root growth was partially inhibited, ascorbate concentration declined in proportion to growth rate. Furthermore, the decline in ascorbate concentration was not related to ascorbate free radical (AFR) and dehydroascorbate (DHA) as well as ascorbate oxidase in boron-deficient root. Boron deficiency caused a substantial decrease in leaf area, shoot and particularly root weights and as a consequence a strikingly higher shoot/root ratio compared to boron sufficient plants was obtained. In experiment II, it was found that the proportion of total boron partitioned in different organs was on average, leaves contained 70 % of the total boron content in the whole plant compared to 20 % in the roots and 10 % in the stems. One of the most important effects caused by boron deficiency was the decrease in the nitrate content and some cations such as magnesium, calcium and especially, potassium in the leaf, but nitrate content decreased in a higher proportion than these cations. Nitrate reductase (NR) and ATPase activity of boron-deficient plants was also declined and this decline did not occur in boron-sufficient plants. Moreover, boron deficient plants had much higher starch contents and an appreciable accumulation of hexoses and sucrose in the leaves than boron-sufficient ones. Soluble sugars might correct the osmotic imbalance elicited by the low content of nitrate and cations in plants subjected to boron deficiency. In experiment III, data showed that under boron deficiency, the number of grains was 18 per ear and sterility of competent florets was 96 % compared to 44 per ear and 46 % respectively under sufficient-boron plants.

Key words: Boron deficiency, *Triticum aestivum* L., nitrate reductase

Introduction

Boron is one of an essential micro nutrient for higher plants, which grow slowly and will not complete their life cycles without it, but its biochemical roles are uncertain. Boron is almost entirely absorbed from soil as undissociated boric acid, H_3BO_3 and it is not known to what extent the plant alters this ion. It may form strong complexes with oxygen atoms of vicinal hydroxyl groups present in sugars and polysaccharides, much boron probably exists in plants in such combination. However, it has been proposed that boron plays a role in the cell wall structure of higher plants forming complexes with pectic and galactouronic derivatives with a specific cis-diol configuration (Matoh *et al.*, 1993; Hu and Brown, 1994) and is involved in metabolic control through regulation of enzymatic reactions, membrane integrity and function, phenolic metabolism, translocation of sugar (via phloem), in synthesis of pyrimidine bases (RNA and DNA) and in synthesis of certain flavonoids (Blevins and Lukaszewski, 1998).

A direct effect of boron on ion uptake due to gradual hyper polarization of the plasma membrane in the root tips by boron was reported by Schon *et al.* (1990) and stimulated proton secretion (Goldbach *et al.*, 1990) as well as the activity of plasma membrane NADH oxidase (Barr *et al.*, 1993) and AFR oxidoreductase (Arrigoni *et al.*, 1981; Morre *et al.*, 1987) catalyzes the transfer of electrons to the AFR (ascorbic free radical) in the transmembrane NADH oxidase, thus, boron could be directly associated with cell growth.

Plants deficient in boron show a wide variety of symptoms, depending upon the species, such as cessation of root tip elongation probably caused by inhibited DNA synthesis, due to insufficient formation of the pyrimidines cytosine and thymine. Furthermore, boron deficiency has been identified as one of the most important factors causing sterility because it causes poor development of anthers and pollen and failure of pollen germination (Cheng and Rerkasem, 1993). Although the symptoms due to boron deficiency are rapid and clear, the primary physiological effect of boron remains unknown. In this study the link between boron and ascorbate content and the subsequent impact on growth was investigated. The effect of boron deficiency on some physiological and biochemical processes in wheat plants during seedlings stage was also studied. Also, on the number of florets, pollen viability, number of grains and ear fertility in the plants at mature stage.

Materials and Methods

Grains of wheat (*Triticum aestivum* L. Giza 157) were surface sterilized with 95% ethanol for 10 min followed by 0.1 % $HgCl_2$ for 2 min, then rinsed five times with distilled water and soaked in aerated distilled water for 24 h. Soaked seeds were divided into 3 groups and three experiments were carried out in this study. The experiment (I) was conducted in Plant Physiology Research Lab. In the Botany Department, while the experiments (II) and (III) were conducted during in the Botanic Garden, Faculty of Sciences, Alexandria University.

Experiment I

Plant culture and growth conditions: Soaked grains were spread over a ring of nickel chrome wire made to fit into the top of 1L growth jars and a circular piece of mosquito netting was stretched firmly on the wire ring as described by Abdel Aal (1984). The jars were filled with water just to the level of the netting where grains were left for 5 days. Seedlings were then transferred undisturbed while on their frame to other jars containing a nutrient solution constituted of 1mM KH_2PO_4, 2 mM $MgSO_4$, 5 mM $Ca(NO_3)_2$, 2 mM K_2SO_4 and 0.02 mM $FeSO_4$. The micro-nutrients consisted of 1.5 μM $ZnSO_4$, 2 μM $MnCl_2$, 0.16 μM $CuSO_4$, 0.12 μM Na_2MoO_4, and 10 μM H_3BO_3 (Bohnsack, 1991). All jars were aerated continuously and incubated in growth chamber under light condition of 400 μmol $m^{-2}S^{-1}$ at constant temperature 28 °C and 70% relative humidity. After 5 days roots were measured, then seedlings were placed in sponge support collars. Collars were then fitted into the tops of one liter glass bottles filled with treatments solutions containing 0,1, 2, 3, 4, 5, 20, 40, 60, 80 or 100 μM boric acid and placed in the growth chamber in conditions. After 24 h, the increase in the root length was measured.

Ascorbate (AA), ascorbate free radical (AFR) and dehydroascorbate (DHA) determination: The ascorbate and dehydroascorbate concentrations in the roots of wheat seedlings were measured according to Liso *et al.* (1984), described by Lukaszewski and

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Blevins (1996). The initial absorbance A_{265} was determined and ascorbate concentration was estimated by monitoring the decrease in ascorbate after the addition of 2 units of commercial ascorbic acid oxidase. After the oxidation of ascorbate was complete, ascorbate oxidase was inhibited with 10 mM sodium azide and DTT was added to the cuvettes to a final concentration of 2.5 mM following reduction with DTT (3-4 min at room temperature), the A_{265} was recorded again. DHA and AFR were determined from the difference between the final reading and the initial absorbance (Takahama and Oniki, 1994). Ascorbate concentration was expressed as μg of ascorbate per 100 mg fresh weight.

Ascorbic acid oxidase activity: Ascorbic acid oxidase activity was determined spectrophotometrically as described by Esaka *et al.* (1988) with minor modifications by Lukaszewski and Blevins (1996). Segments of root (1cm in length) were placed in 2 ml of incubation solution consisting of 10 mM citrate + 20 mM phosphate buffer, pH 6.2, 0.002% (w/v) m-phosphoric acid and variable concentrations of EDTA (0-5 mM). Following a 10 min pre incubation in a shaking water bath at 30 °C, ascorbate was added to the test solutions (100 μM final concentration) and incubation was continued for 30 min. At the end of this period, 500 μL samples of the incubation solution were mixed 1:1 (v/v) with 0.1% (w/v) m-phosphoric acid, centrifuged at 14000 g for 4 min and the A_{265} of the supernatant was measured. The amount of ascorbic acid oxidized in the reaction was quantified based on a standard curve and the activity of ascorbate oxidase was expressed as nmol of ascorbic acid oxidized per min.

Experiment II

Plant culture and growth conditions: Soaked grains were selected for uniformity in size and sown in plastic pots (10 grains per pot) filled with quartz sand which had been washed with 3% HCl overnight and then rinsed thoroughly with de-ionized distilled water. All pots on 16/12/2000 were maintained in a growth chamber for 7 days, at day and night temperature 30 ± 2 and 20 ± 1°C, respectively. Seedlings were grown with light/dark regimes of 16/8 h, with a light intensity of 400 mol m^{-2} s^{-1} (PAR) at the surface of the pots and a relative humidity of 70 %. Pots were irrigated each day with either a boron free nutrient solution (boron-deficient plants) containing 3 mM $Ca(NO_3)_2$, 2 mM KNO_3, 2 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 2 mM KH_2PO_4, 50 μM NaCl, 40 μM FeNa-EDTA, 10 μM $MnCl_2$, 2 μM $ZnSO_4$, 1 μM $CuSO_4$, 0.5 μM Na_2MoO_4 and 0.2 μM $CoCl_2$, or this nutrient solution plus 50 mM H_3BO_3 (boron-sufficient plants, unless stated otherwise). After three weeks, plants were irrigated with the nutrient solutions as described above but containing 12 mM nitrate [4 mM KNO_3 and 4 mM $Ca(NO_3)_2$] as the sole nitrogen source (Camacho-Cristobal and Gonzalez-Fontes, 1999). One week later, boron-sufficient and –deficient plants were transferred to 5l plastic containers filled with aerated 1/5 strength of nutrient solution (10μM boron, final concentration} and 1/5 strength boron free nutrient solution, respectively. In both treatments, nitrate was a sole nitrogen source (2.4 mM nitrate, final concentration).

Five-week-old wheat plants were harvested at 3 different times of day (after 8h in the dark; after 8h in light and after 16h from the beginning of light period). Selected plants were divided into shoots and roots and weighed for fresh weight and dried in a forced-air oven at 80 °C for 48 h and dry weight was obtained. Leaf area was determined using a moving belt electronic planimeter (Delta T Devices, Burwell, UK.).

Determination of boron, magnesium, calcium and potassium: Boron, magnesium, calcium and potassium cations were analyzed in the leaves according to the method of Camacho-Cristobal and Gonzalez-Fontes (1999) by grinding the leaves to a fine powder in a mortar pre-cooled with liquid nitrogen, weighed and transferred to porcelain crucibles and dried out at 100 °C until constant weight. Subsequently, dried samples were burnt to ashes at 500 °C for 6 h. Ashes, once at room temperature within a desiccator, were weighed and then dissolved with 0.1 M HCl. Boron was determined according to DeAndrade *et al.* (1988). Cations (Mg, Ca and K) were measured with an atomic absorption spectrometer (Perkin Elmer).

Determination of carbohydrates content: The youngest fully-expanded leaf of one plant from each of five replicate pots were harvested 6 h after the start of the light period and cut into 0.5 cm lengths. Samples of 0.2-0.4 g fresh weight were extracted three times with 50ml 80% ethanol at 70 °C for 15 min. The volume of the extract was then reduced 3 to 4 ml by evaporation and cleared with ion exchange resins. Sucrose, fructose and glucose were determined by spectrophotometer according to the method of Guerrier and Patolia (1989). Starch was estimated in the residue according to Murata *et al.* (1968).

Determination of nitrate, amino acid and protein content: Quantitative determination of nitrate was carried out calorimetrically by the pheno-l disulphonic method described by Woolly *et al.* (1960). For protein quantification, frozen leaves were ground to fine powder, weighed, extracted with 10 volumes (v/w) of cold 10% (w/v) trichloroacetic acid and the mixture was allowed to stand for 15 min on ice. The suspension was centrifuged at 13000 g for 5 min, the supernatant discarded, the pellet was resuspended in 1 M NaOH and kept at room temperature for 30 min, then protein content was determined according to Bradford (1976) with bovine serum albumin as a standard. Amino acids were determined calorimetrically as described by Sugano *et al.* (1975). The colour developed was estimated at 570 nm. Leucine was used as a standard determination of nitrate reductase (NR) activity assay. Nitrate reductase enzyme was extracted and assayed according to Aslam *et al.* (1976) and Travis *et al.* (1970) respectively.

Determination of ATPase activity: Plasma membranes were prepared according to Hodges and Leonard (1974). The purified membrane pellets were diluted with gradient buffer (1 mM $MgSO_4$, 1 mM dithiothreitol, 1mM Tris-MES, pH 7.8) for ATPase assay. All steps were carried out at 4 °C. ATPase activity was measured by a modified procedure of Hodges and Leonard (1974) described by Watson *et al.* (1980). The activity was expressed in μmol Pi mg^{-1} protein h^{-1}.

Experiment III

Plant culture and growth conditions: Soaked grains of wheat on 20/12/2000 were sown and grown as described in experiment II. Pots in this experiment were maintained in greenhouse in Botanic Garden, Faculty of Science, Alexandria University. Pots were irrigated with nutrient solution as described in experiment II. Individual pots were irrigated with the nutrient solution, initially on alternate days but increasing to three times a day in the growing season. There were two boron treatments: B, in which boron was absent and B^+ in which 20μM of boron was supplied continuously from sowing to maturity. The plants were sampled at the following stages:

- GS-60, at complete emergence from flage leaf (74 days) and when pollen viability was assessed.
- GS-90, the final stage (96 days) in which grain set was measured. At each harvest, the whole plants were sampled and separated into roots, stems, leaves and ears.

Determination of pollen viability: Pollen grains from the plants were sampled at the beginning of anthesis (stage, GS-60, 74 days). Viability was determined by the reaction of grain with iodine as described by Cheng *et al.* (1992). Anthers from one floret of each of the basal, middle and apical zone of an ear were collected in 1ml iodine. One drop (0.05 ml) of pollen suspension was observed under microscope at x40 magnification and the total and live number of pollen grains counted.

Determination of ear fertility: The pattern of grain set within the ear was recorded at stage G-90 (96 days). The numbers of competent florets per ear and grains per competent floret were recorded. Sterility was calculated as the percentage of competent florets without grains (Subedi *et al.*, 1998).

Statistical analysis: The significance of differences between control and each treatment was evaluated by the t-test.

Results and Discussion

It has been proposed that boron is involved in different processes such as vegetative growth and tissue differentiation. In this study (experiment I) it was found that in the presence of boron, a maximum rate of root elongation was observed and maintained over the range 5 to 40 μM (Fig. 1A). By contrast, boron deficiency elicited an appreciable decrease in the root length. Thus, about 52 % inhibition in root elongation was obtained when boron concentration was lowered to 1 μM, whereas, in the absence of boron, root elongation was less than 12 % of control (Fig. 1B, insert), indicating that root elongation of wheat plants depended on boron concentration. Among the postulated roles of boron, many evidence favour boron involvement in cell wall and/or membrane structure and function which could be critical for cell growth (Shelp, 1993; Marschner, 1995). Accordingly, boron deficiency may disrupt cell division and cell elongation (Hu and Brown, 1994; Kobayashi *et al.*, 1996).

Furthermore, boron deficiency caused an appreciable decrease in the total biomass of shoots by 26% and this effect being even more pronounced in root biomass amounted to 51 %. As a consequence of a stronger effect on the root rather than on the shoot growth, a strikingly higher shoot/root ratio compared to boron sufficient seedlings was obtained (Table 1). The observed decrease in shoot biomass was closely related with the decrease in the area of new leaves produced after the imposition of the stress (Table 1).

The decreases in unit leaf area and consequently the reduction in photosynthetic performance had a negative impact on the production of assimilates and thus on the growth of the shoot. On the other hand, the decrease in the root biomass could be attributed to a marked inhibition of root elongation elicited by boron deficiency (Camacho-Cristobal and Gonzalez-Fontes, 1999). Therefore, shoot/root ratio and the root length elicited by boron were a characteristic of boron deficient plants when compared to boron sufficient ones (Dugger, 1983).

Effect of boron on the ascorbate concentration and ascorbate oxidase activity in the root: Since, the reduction in ascorbate concentration in response to insufficient boron indicates that boron may be involved in maintaining ascorbate levels in root meristems (Lukaszewski and Blevins, 1996), or in other words, there is a close positive correlation between root growth and ascorbate concentration in the root tissues. Accordingly, in this study (Exp. I) it was found that in seedlings supplied with sufficient boron, the concentration of ascorbate in the root was about 104 μg 100mg^{-1} fresh weight (Fig. 2). This result is in agreement with Mondy and Munshi (1993) who found an increase in ascorbate content following boron treatment of potatoes. In contrast, inhibition of root elongation of boron-deficient wheat seedlings was accompanied by a decline in ascorbate concentration to less than 4.8 μg 100 mg^{-1} fresh weight, in the roots. At low concentration of boron (1μM), ascorbate concentration was reduced to nearly 59 % of that in boron-sufficient (10μM) plants. The reduction in ascorbate concentration in response to boron-deficiency indicated that boron may be involved in maintaining ascorbate levels in root meristems and linking ascorbate with root elongation (Lukaszewski and Blevins, 1996).

Ascorbate oxidase enzyme catalyzes the oxidation of ascorbate to DHA via an AFR intermediate. In this connection, Lukaszewski and Blevins (1996) reported that the mechanism for boron-ascorbate interaction could be related to boron association with the ascorbate redox cycle and plasma membrane electron transport. Lin and Varner (1991) suggested that DHA might be involved in cell wall loosening and AFR was shown to enhance cell wall acidification and boron extrusion, as well as to stimulate cell elongation in onion root tips (Hidalgo *et al.*, 1991). However, in this study, the oxidized forms of ascorbate (AFR and DHA) showed no response to boron treatment (Fig. 3), since a little change was observed in the concentration of the oxidized form of ascorbate (DHA and AFR). The concentrations of total AFR and DHA in the root of wheat plant treated with 0,10,40 and 100 μM boron were 16.8, 17.5, 17.1 and 15.8 μg 100 mg^{-1} fresh weight respectively (Fig. 3), indicating that the decline in ascorbate concentration induced by boron-deficiency cannot be ascribed to an interference of boron with ascorbate oxidation, but could be attributed to accelerated catabolism, or more likely, to reduced ascorbate synthesis. Also, it was found that activity of ascorbate oxidase enzyme was changed very little with addition of boron up to 100 μM and inhibited in the absence of boron. (Fig. 3). This inhibition could alter the redox state of ascorbate and, therefore, growth (De-Cabo *et al.*, 1993; Gonzales-Reyes *et al.*, 1994).

Effect of boron deficiency on the ions contents in the leaves: The results of experiment II (Fig. 4) showed that leaves of wheat plants were the major sites for boron accumulation and concentrations, since the major changes provoked by boron-deficiency occurred in the leaf tissue. However, the concentration of boron in leaves was dependent not only on exposure time, but also on the boron concentration in the nutrient solution. Overall, as much as 70 % of the total boron content of the plant was accumulated in the leaves, compared to 20 % in the roots and 10% in the stem (Fig. 4). This difference may be due to a more efficient transport of boron by transpiration through the leaves.

Furthermore, plants supplied with sufficient boron had significantly higher content of K^+, among the cations measured in boron-sufficient plants than those grown without adding boron (Table 2). These increases may represent a compensation to maintain the electrical and chemical balance of the cell, since Ca^{2+}, Mg^{2+} and $K^+$are cations that may substitute for each other in case of lack or excess of one of them. (Lyenger and Reddy, 1993). In contrast, leaves under boron-deficiency conditions showed significant decreases for K^+, Mg^{2+} and Ca^{2+} (Table 2) indicating that boron has an important role in the maintenance of membrane integrity and its absence causes a loss of selectivity and other problems in the plasmatic membrane, which allow the decrease of the essential ions such as phosphate, nitrate, potassium and in low rate calcium and magnesium in the cell. Also these cations were decreased significantly during the course of light period (Table 2) particularly K^+ content.

Table 1: Effect of boron-sufficient (+B) and –deficient (-B) plants on the leaf area; total biomass of shoot and root and shoot/root ratio of wheat seedlings. Each value is mean ± SE (n = 5)

	Treatments	
Parameters	+B	- B
Leaf area (cm^2)	20.40 ± 1.40	14.500 ± 0.90
Shoot mg dry wt. seedling^{-1}	1.20 ± 0.24	0.888 ± 0.12
Root mg dry wt. seedling^{-1}	0.84 ± 0.08	0.428 ± 0.10
Shoot/Root ratio	1.43 ± 0.11	2.070 ± 0.21

In addition, leaf nitrate content in the presence of boron was declined slightly (not significantly) during light periods and increased in the dark period. Whereas, in absence of boron, NO_3^- content was decreased significantly (Fig. 5). This decrease could simply result from a lower root efficiency for NO_3 uptake elicited by boron deficiency, along with the diminishment in root mass.

Moreover, the decrease in the content of cations due to boron deficiency would cause an osmotic imbalance that might be

Table 2: Change in leaf boron, magnesium, calcium and potassium contents of boron sufficient (+B) and –deficient (-B) plants. Boron and cations content were measured in the primary leaf. Values are means ± SE (n = 5)

		Time of harvest		
Cations	Treatments	8h dark	8h light	16h light
Boron	-B	4.2 ± 0.16	3.80 ± 0.30	3.40 ± 0.22
μmol (g dw)$^{-1}$	+B	0.7 ± 0.04	0.57 ± 0.03	0.53 ± 0.03
Magnesium	-B	0.6 ± 0.02	0.36 ± 0.01	0.24 ± 0.01
mmol (g dw)$^{-1}$	+B	0.3 ± 0.01	0.42 ± 0.02	0.40 ± 0.01
Calcium	-B	1.6 ± 0.13	0.75 ± 0.05	0.84 ± 0.02
mmol (g dw)$^{-1}$	+B	0.5 ± 0.03	0.67 ± 0.05	0.47 ± 0.04
Potassium	-B	2.1 ± 0.61	2.40 ± 0.28	1.80 ± 0.21
mmol (g dw)$^{-1}$	+B	0.6 ± 0.04	0.67 ± 0.03	0.54 ± 0.08

Table 3: Changes in amino acid, protein, and carbohydrates contents of boron-sufficient (+B) and –deficient (-B) wheat plants. Each value is the mean ± SE (n = 5). Plants were harvested in the dark and light at the indicated time

	Time					
	8 h dark		8 h light		16 h light	
Parameters	- B	+ B	- B	+ B	- B	+ B
Amino acid (μmol g^{-1} fwt)	8.6 ± 0.7	8.3 ± 0.2	12.6 ± 0.8	11.6 ± 2.1	10.5 ± 1.21	2.10 ± 1.6
Protein (mg g^{-1} fwt)	19.8 ± 1.6	14.8 ± 1.1	15.9 ± 1.1	15.4 ± 1.5	13.9 ± 2.2	16.40 ± 2.2
Glucose (μmol g^{-1} fwt)	6.9 ± 0.4	1.2 ± 0.20	2.4 ± 1.8	2.4 ± 0.3	18.7 ± 1.3	1.80 ± 0.1
Fructose (μmol g^{-1} fwt)	3.2 ± 0.1	0.2 ± 0.01	6.1 ± 0.8	0.4 ± 0.01	9.6 ± 0.8	0.42 ± 0.02
Sucrose (μmol g^{-1} fwt)	3.4 ± 0.2	1.1 ± 0.08	4.0 ± 0.1	2.1 ± 0.1	6.9 ± 0.5	2.60 ± 0.2
Starch (mg g^{-1} fwt)	135 ± 8.4	8.3 ± 0.40	142 ± 9.9	16.8 ± 1.6	204.0 ± 11.4	40.80 ± 4.5

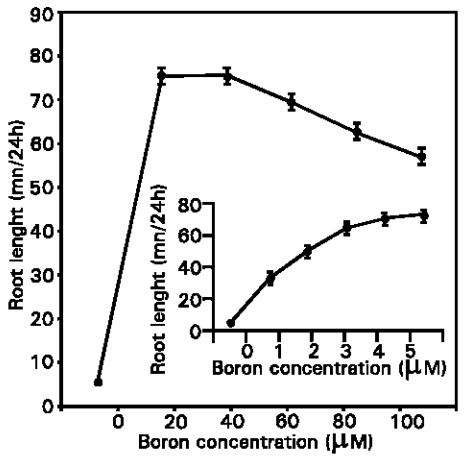

Fig. 1: Effect of boron concentrations on root length of wheat plant after 24 h of treatment. Values are ± SE (n = 5)

corrected by accumulation of osmo-regulators such as hexoses and sucrose (Table 3).

Effect of boron on the amino acid, protein and carbohydrate contents in the leaf: The results (Table 3) showed that amino acids content in leaves rose during the first 8 h after illumination and then remained more or less constant. Also, it was found that there were no substantial differences in the amino acid contents between plants grown in presence or absence of boron. Furthermore, data show that in plants treated with boron, leaf protein content increased during light period and decreased in the dark period. In contrast, it decreased significantly under light condition and increased in the dark in boron-deficient plants. The decrease is frequent in several severe stress conditions as a consequence of altered N-metabolism and reduced rates of protein synthesis (Rabe, 1993). The significant decrease observed in protein content in boron-deficient plants could result from the lower rate of photosynthesis which induces shortage in energy molecules and C-chain molecules to accept the NH_4^+. In contrast,

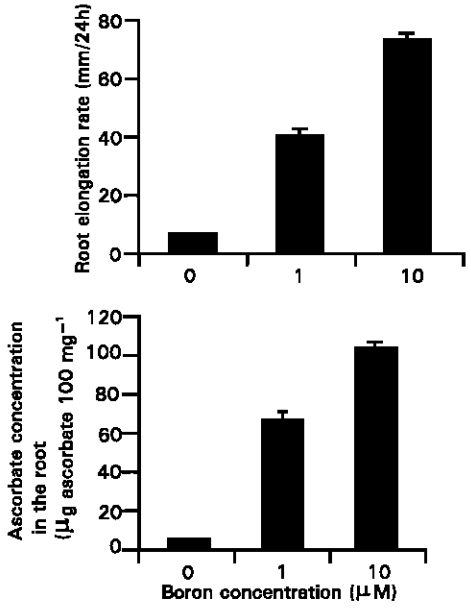

Fig. 2: Effect of boron concentration on root elongation and ascorbate concentration in the root of wheat plants after 24 h of treatment. Values are means ± SE (n = 5)

the reverse was true in plants treated with boron (Table 3). Moreover, a decline in leaf protein content of boron deficient plants during light period was consistent with the concomitant decrease in NR activity.

In both boron-sufficient and –deficient plants, sucrose and starch contents increased throughout the light period, as a consequence of photosynthesis and decreased during the dark period as a consequence of mobilization and respiration, although the levels

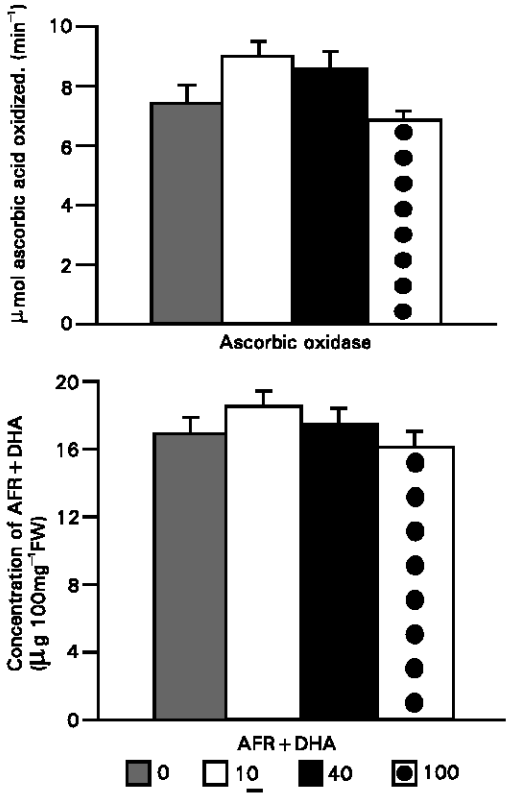

Fig. 3: Effect of boron concentrations (µM) on the activity of ascorbic oxidase and total concentration of AFR+ DHA in the root wheat plant. Values are mean ± SE (n = 5)

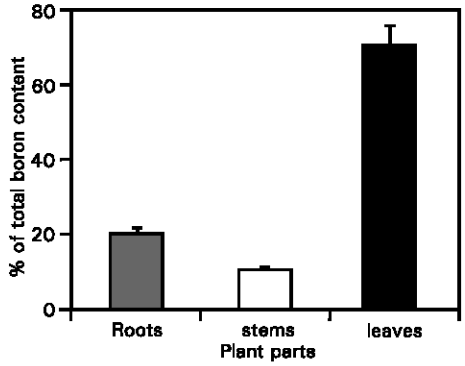

Fig. 4: Percentage of total boron content portioned among different parts of wheat plant after 4 week. Values are mean ± SE (n = 5)

of these carbohydrates in boron-deficient plants were much higher (Table 3). The contents of glucose and fructose were decreasing after 8 h illumination as a consequence of their consumption in glycolysis and Krebs cycle in order to obtain assimilatory power and carbon skeletons needed in the primary metabolism. However, boron-deficiency caused a continuous accumulation of hexoses in the leaves.

Effect of boron on nitrate reductase (NR), and H^+-ATPase activities: There was a significant trend of NR activity with boron treatment, the highest activity being measured in the leaf of boron sufficient plants after 6 h illumination and then decreased, but still

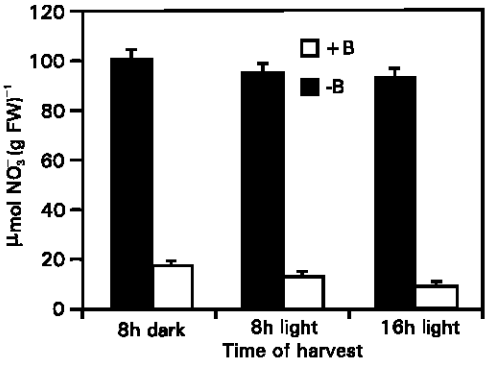

Fig. 5: Changes in the leaf nitrate content of boron- sufficient (+B) and - different (-B) wheat plant. Values are mean are means ± SE (n = 5)

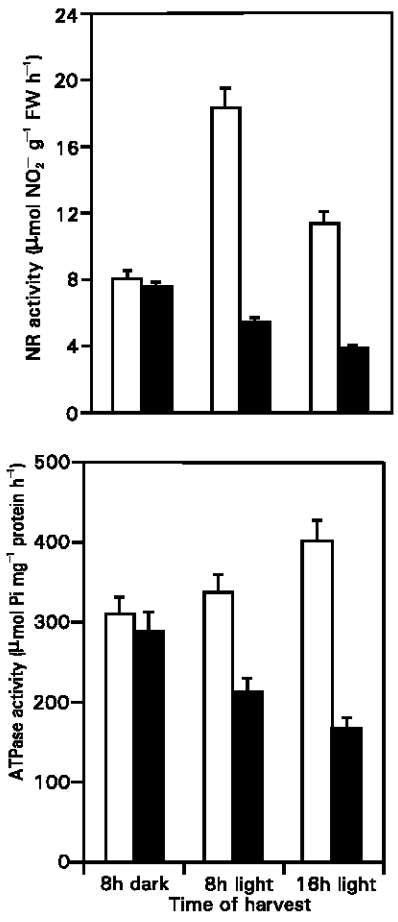

Fig. 6: Changes in NR and ATPase activity in leaves are mean SE(n = 5) +B -B

higher than the activity under boron deficiency, where the NR this activity in boron-deficient plants to values similar to those from boron-sufficient plants (Fig. 6). These results suggest that light combined with boron deficiency represents an environmental condition particularly negative for NR activity (Camach-Cristobal and Gonzalez-Fontes, 1999). An explanation might be that light and boron deficiency work synergistically in accelerating the degradation of NR transcript and /or protein.

The plasmalemma H^+-ATPase plays an essential role in NO_3 uptake,

since it is responsible for the maintenance of the electrochemical gradient for transport of NO_3 and protons inward (Tsay *et al.*, 1993). The activity of H^+-ATPase was measured in the roots of wheat plants grown under boron deficiency. It was found that H^+-ATPase activity was higher in the roots of wheat plants grown under sufficient boron and declined under deficiency in boron (Fig. 6).

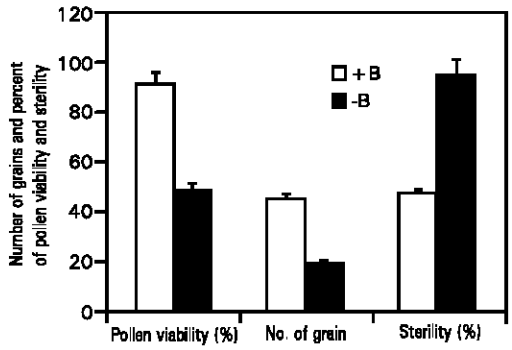

Fig. 7: Effect of boron-sufficiency (+B) and -deficiency (⁻) on the number of grain per ear and % of pollen viability and sterility in wheat plants. Values are mean ± SE (n = 5)

Effect of boron on the pollen viability and ear fertility: Data obtained from experiment III showed that pollen viability was severely affected by boron deficiency, where pollen grains were very small and did not stain with iodine (Fig. 7). The reduction in the number of grains and sterility per ear due to boron deficiency amounted to 60 and 52% respectively of boron-sufficient plants indicating that boron is an essential element for growth and productivity of wheat plant.

References

Abdel Aal, A.E., 1984. Effect of nitrogen starvation on some metabolic processes in barley seedlings. Ph.D. Thesis. Faculty of Science, Alexandria University, Alexandria, Egypt.

Arrigoni, O., S. Dipierro and G. Borraccino, 1981. Ascorbater free radical reductase, a key enzyme of the ascorbic acid system. FEBS Lett., 125: 242-244.

Aslam, M., A. Oaks and R.C. Huffaker, 1976. Effect of light and glucose on the induction of nitrate reductase and on the distribution of nitrate in etiolated barley leaves. Pl. Physiol., 58: 588-594.

Barr, R., M. Bottger and F.L. Crane, 1993. The effect of boron on plasma membrane electron transport and associated proton secretion by cultured carrot cells. Biochem. Mol. Biol. Int., 31: 31-39.

Blevins, D.G. and Lukaszewski, 1998. Boron in plant structure and function. Annu. Rev. Plant Physiol. Plant Mol. Biol., 49: 481-500.

Bohnsack, C.W., 1991. Investigating the boron requirement of plants. Am. Bio. Teach., 53: 486-488.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Camacho-Cristobal, J.J. and A. Gonzalez-Fontes, 1999. Boron deficiency causes a drastic decrease in nitrate content and nitrate reductase activity and increases the content of carbohydrates in leaves from tobacco plants. Planta, 209: 528-534.

Cheng, C.H. and B. Rerkasem, 1993. Effects of boron on pollen viability in wheat. Plant Soil, 155/156: 313-315.

Cheng, C.H., J.A. McComb and B. Rerkasem, 1992. Techniques of study the anthers in wheat. In Boron Deficiency in Wheat. Eds CE Mann and Rerkasem. pp: 32-33. Wheat Special Report No. 11. Mexico D.F. CIMMYI.

DeAndrade, J.C., M. Ferreira, N. Baccan and O.C. Bataglia, 1988. Spectrophotometric determination of boron in plants using monosegmented continuous flow analysis. Analyst., 113: 289-293.

De Cabo, R.C, J.A. Gonzales-Reyes and P. Navas, 1993. The onset of cell proliferation is stimulated by ascorbate free radical in onion root primordia. Biol. Cell, 77: 231-233.

Dugger, W.M., 1983. Boron in plant metabolism. In Lauchli A, Bieleski, R.L. (eds) Encyclopedia of plant physiology, NS, Vol. 15B. Springer, Berlin, pp: 626-650.

Esaka, M., J. Imagi, K. Suzuli and K. Kubota, 1988. Formation of ascorbate oxidase in cultured pumokin cells. Plant Cell Physiol., 29: 231-235.

Goldbach, H.E., D. Hartmann and T. Rotzer, 1990. Boron is required for the ferricyanide-induced proton release by auxins in suspension cultured cells of *Daucus carota* and *Lycopersicon esculentum*. Pl. Physiol., 80:114-118.

Gonzales-Reyes, J.A., F.J. Alkain, J.A. Caler, A. Serrano, F. Cordoba and P. Navas, 1994. Relationship between apoplastic ascorbate regeneration and the stimulation of root growth in *Allium cepa* L. Pl. Sci., 100: 23-29.

Guerrier, G. and J.S. Patolia, 1989. Intere, principles et problems poses lors de l'obtention de plantes daptees au NaCl. Revue de Cytologie et de Biologie Vegetales (English Abstract), 12: 3-15.

Hidalgo, A., G. Garcia-Herdugo, J.A. Conzales-Reyes, D.J. Morre and P. Navas, 1991. Ascorbate free radical stimulates onion root growth by increasing cell elongation, Pl. Physiol., 20: 267-275.

Hodges, T.K. and R.T. Leonard, 1974. Purification of a plasma membrane-bound adenosine triphosphatase from plant roots, 392-406 in S.P. Colowick and N.O. Kaplan. eds. Methods in Enzymology. Vol. 33. Academic Press. N.Y.

Hu, H. and P.H. Brown, 1994. Localization of boron in cell walls of squash and tobacco and it's association with pectin. Evidence for a structural role of boron in the cell wall. Pl. Physiol., 105: 681-689.

Hu, H., P.H. Brown and J.M. Labavitch, 1996. Species variability in boron requirement is correlated with cell wall pectin. J. Exp. Bot., 47: 227-232.

Johnson, C.M. and A. Ulrich, 1959. Analytical methods for use in plant sciences. Bull. Calif. Agric. Exp. St. No. 799.

Kobayashi, M., T. Matoh and J. Azuma, 1996. Two chains of rhamnoga-lacturonan II are cross-linked by borate-diol ester bonds in higher plants cell walls. Pl. Physiol., 110: 1017-1020.

Lin, L.S. and J. E. Varner, 1991. Expression of ascorbic acid oxidase in zucchini squash (*Cucurbita pepo* L). Pl. Physiol., 96: 159-165.

Liso, R., G. Calabrese, M.B. Bitoni and O. Arrigoni, 1984 Relationship between ascorbic acid and cell division. Exp. Cell Res., 150: 314-320.

Lukaszewski, K.M. and D.G. Blevins, 1996. Root growth inhibition in boron-deficient or aluminum stressed squash may be a result of impaired ascorbate metabolism. Pl. Physiol., 112: 1135-1140.

Lyenger, E.R. and M.P. Reddy, 1993. Crop response to salt stress: see water application and prospects. In Hand book of Plant and Crop Stress. Ed. M. Pessaraki., pp: 183-201. Marcel Dekker Inc. New York.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, ed Academic Press, San Diego, CA., pp: 379-396.

Matoh, T., K.I. Ishigaki, O. Kaori and J.I. Azuma, 1993. Isolation and characterization of a boron-polysaccharide complex from radish roots. Pl. Cell Physiol., 34: 639-642.

Mondy, N.I. and C.B. Munshi, 1993. Effect of boron on enzymatic discoloration and phenolic and ascorbic acid content of potatoes, J. Agric. Food Chem., 41: 554-556.

Morre, D.J., F.L. Crane, I.L. Sun and P. Navas, 1987. The role of ascorbate in biomembrane energetics. Ann. NY. Acad. Sci., 498: 153-171.

Murata, T., T. Akazawa and F. Shikiko, 1968. Enzymic mechanism of starch breakdown in germinating rice seeds. Pl. Physiol., 43: 1899-1909.

Rabe, E., 1993. Altered nitrogen metabolism under environmental stress conditions. In Handbook of Plant and Crop Stress. Ed. M Pessarakli. pp: 261-276. Marcel Dekker Inc., New York.

Schon, M.K., A. Novacky and D.G. Blevins, 1990. Boron in relation to membrane function in higher plants. J. Exp. Bot., 28: 831-841.

Shelp, B.,1993. Physiology and biochemistry of boron in plants. In UC Gupta, ed, Boron and its Role in Crop Production. CRC Press, Boca Raton, FL, pp: 53-85.

Subedi, K.D., P.J. Gregory, R.J. Summerfield and M.J. Gooding, 1998. Cold temperatures and boron deficiency caused grain set failure in spring wheat (*Triticum aestivum* L.). Field Crops Res., 57: 277-288.

Sugano, N., T. Tanaka, E. Yamamoto and A. Nishi, 1975. Phenylalanine ammonialyase in carrot cells in suspension cultures. Phytochem., 14: 2435-2440.

Takahama, U. and T. Oniki, 1994. The association of ascorbate and ascorbate oxidase in the apoplast with IAA-enhanced elongation of epicotyls from *Vigna angularisd*. Pl. Cell Physiol., 35: 257-266.

Travis, R.L., R.C. Huffaker and J.L. Key, 1970. Light-induced development of polyribosomes and the induction of nitrate reductase in corn leaves. Pl. Physiol., 46: 800-806.

Tsay, Y.F., J.I. Schroeder, K.A. Feldmann and N.M. Crawford, 1993. The herbicide sensitivity gener CHLI of Arabidopsis encodes a nitrate-inducible nitrate transporter Cell, 72: 705-713.

Varner, J.E.,1995. Foreword: 101 reasons to learn more plant biochemistry. Pl. Cell, 7:795-796.

Watson, M.C., P.G. Bortles and K.C. Hamilton, 1980. Action of several herbicides and tween 20 on oat (*Avena sativa*) membranes. Weed Sci., 28: 122-127.

Woolly, J.T., G.P. Hick and R.H. Hageman, 1960. Rapid determination of nitrate and nitrite in plant material. J. Agric. Food Chem., 8: 482-484.