

## **EFFECTS OF BORON STARVATION ON THE APOPLASTIC AND TOTAL SOLUTE CONCENTRATIONS INFLUENCING NODULE GROWTH AND ACETYLENE REDUCTION RATE**

*Gr. T. Zehirov\**, *G. I. Georgiev*

*Acad. M. Popov Institute of Plant Physiology, Sofia 1113, Bulgaria*

**Summary.** The effect of boron (B) deficiency on cell permeability and connected with that, changes in soluble sugars, amino acids and ureides partitioning between root and nodule apoplast and symplast of N<sub>2</sub> fixing soybean plants grown in water culture were studied. Exposure of a transient 10 -day B deficiency stress was found to inhibit nodule number and to increase nodule dry weight. The formation of larger nodules in B deficient plants was found to coincide with the deterioration of solute exchange between root and nodule apoplast and symplast. These results are discussed as induced by B deficiency in cell wall and membrane permeability changes.

**Key words:** Boron deficiency, symplast, apoplast, nitrogen fixation, soybean

### **Introduction**

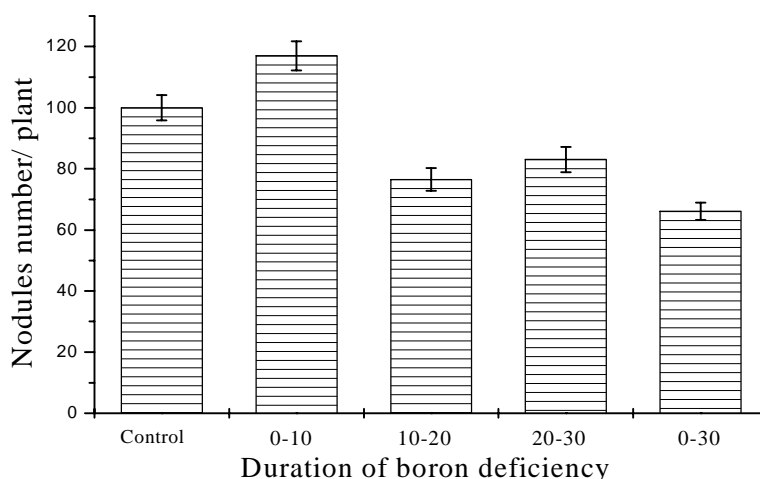
The main effects of boron (B) deficiency stress on nodulation and N<sub>2</sub> fixation in legumes are usually connected with changes in cell wall structure and permeability, transport of carbohydrates and synthesis of phenols (Zehirov and Georgiev, 2001; Pfeffer et al., 1998; Lewis, 1989). The last function of B, which is poorly understood, was found to relate to the altered exchange of some specific flavonoids, known to participate in the processes of recognition of symbiotic N<sub>2</sub> fixing partners (Zehirov and Georgiev, 2002a, 2002b; Phillips et al., 1994 ). Boron starvation inhibits the development of the cell wall and this effect is related directly to the altered exchange of molecules between partners. Boron deficiency leads to formation of abnormal nodules (containing no bacteroids in the infected area) (Bolanos et al., 1996). Most of data about B deficiency effects come from a prolonged period of stress (more than 30 days

\* Corresponding author, e-mail: grig@obzor.bio21.bas.bg

of growth). However, short-term of B deficiency has been investigated less and is more interesting as during such a period of B stress the large changes in some phytohormone levels has been found (Blevins and Lukaszewski, 1998). This probably relates to changes in cell wall formation. As the effects of boron deficiency are connected mainly with changes in cell wall structure and permeability, it was interesting to elucidate how the metabolite exchange between nodules and root may affect nodule formation and function. This was the aim of this study.

### Material and methods

Germinating seeds of soybean cv. Beesson were inoculated with a bacterial suspension of  $10^8$  cells/ml of *Bradyrhizobium japonicum* strain 639 cultured on liquid YEM medium. Plants were grown in a greenhouse as liquid cultures in pots containing 1.2 l of nutrient solution with the required macro- and microelements and free of nitrogen, established for the growth of  $N_2$  fixing legumes (Yamagishi and Yamamoto, 1994, Zehirov and Georgiev, 2000). Boron was supplied as  $44 \mu\text{g B/L H}_3\text{BO}_3$  for control plants. In the experimental treatments boron (B) was omitted from the medium for 20–30 DAP of the growth period. Plants were grown in a naturally lit and heated greenhouse (day  $+30^\circ\text{C}$ , night  $+20^\circ\text{C}$ ). Nutrient solutions were aerated daily and renewed every 3 days during growth. Plants were harvested for assay at the end of the stress period (30 DAP). The acetylene reduction rates of the freshly harvested detached nodules were analyzed by gas-chromatography (“Perkin-Elmer” 104 supplied with a flame-ionization detector) (Hardy et al, 1968, Georgiev et al, 1996). Soluble sugars in plant

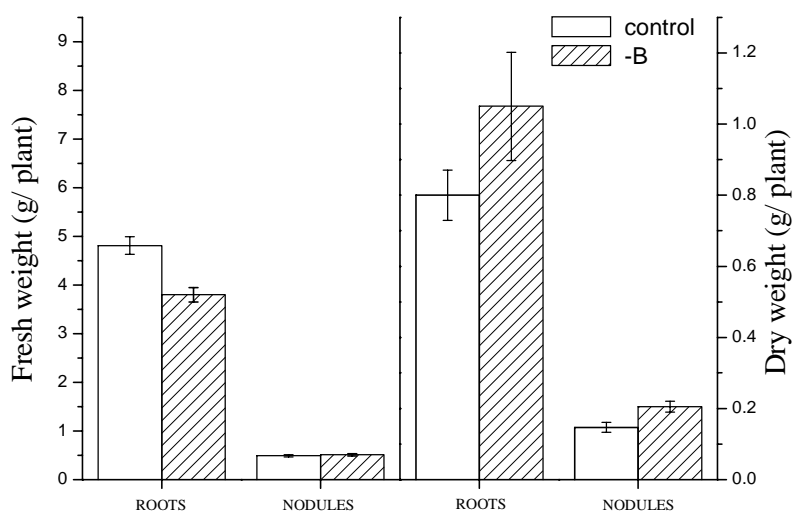


**Fig. 1.** Influence of 10 days boron deficiency, imposed at different periods of growth on soybean plants, on the nodulation rate.

samples were analyzed by the phenol-sulphuric method (Dubois et al., 1956), free amino acids and amides were determined with the ninhydrin reagent (Yemm and Cocking, 1955) ureides (Trijebels and Vogels, 1966). Extractive free tissue (EFT) and Klason and acetyl bromide lignins were determined (Dean, 1995). Cellulose, hemicellulose and pectins were analysed by titrimetric methods (Pochinok, 1976). Injury index was estimated from the formula:  $I(\%) = 1 - (1 - T_1/T_2)/(1 - C_1/C_2) \times 100$ , where  $T_1$  and  $T_2$  are the first and second (after autoclaving) measurement of conductivity of the solutions in which the treated samples were immersed and  $C_1$  and  $C_2$  are the respective values for the controls (Premachandra et al., 1992). Apoplastic concentrations of studied metabolites were analyzed after infiltration studies of samples with 50% methanol for 10 min, 4°C. The analysis of exudates from free space or “apoplast” was done with the recovered rinses as water solutions (Streeter and Salminen, 1993). The symplast extracts of nodules and roots were obtained from the rest of the rinsed sample after the re-extraction with 80% ethanol.

## Results and Discussion

Short term boron deficiency strongly influences not only the growth but the number of soybean nodules as well ( Figs. 1, 2 ). After a 10 day stress period the fresh weight decreased but at the same time, the dry weight of nodules increased. The changes of the growth of nodules coincided with a decrease in their nitrogen fixing activity (Table 1). Essential for the normal development and functioning of plant nodules is



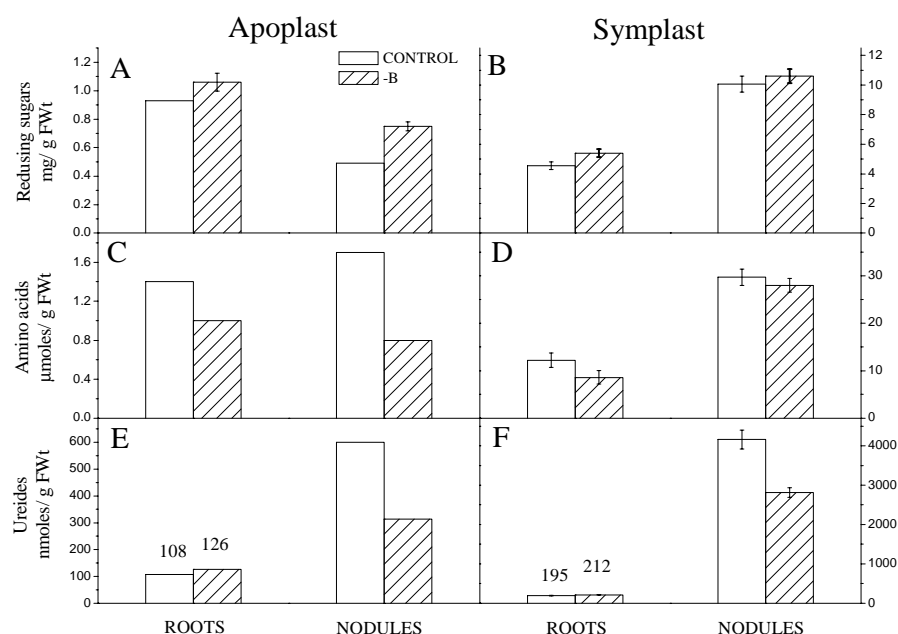
**Fig. 2.** Fresh and dry weight of roots and nodules of soybean plants subjected to 10 days B-deficiency.

**Table 1.** Influence of boron deficiency on cell wall components of nodulated soybean roots.

	Cellulose, %	Hemi-cellulose, %	Water soluble pectin, %	Uronic acids, %
control	19.23±0.85	4.75±0.21	0.53±0.024	2.74±0.114
-B	21.27±1.12	16.32±0.72	0.27±0.041	3.54±0.154
	Klason lignin, g/100g EFT*	Acetyl- bromide lignin, g/100g EFT	Injury index, %	ARA, $\mu\text{MC}_2\text{H}_4/\text{g FW/h}$
control	29.1±1.24	18.80±0.94	4.2±0.21	12.46±0.431
-B	15.7±0.87	8.85±0.42	6.8±0.38	5.80±0.214

\*EFT – extractive free tissues. Data are means of 3 replicates  $\pm$ SD

the correct exchange of nitrogen and carbon carrying metabolites between host and nodules during growth. The influence of boron deficiency on cell permeability may be the main reason for that negative growth effect on soybean nodules (Yamagishi and Yamamoto, 1994). The analysis of cell wall components show significant changes in the quantity of cellulose, hemicellulose, pectins and lignins of soybean root cell walls (Table 1). The quantities of uronic acids were increased (129.2% of control), but, at the same time, the water soluble pectin ( neutral ) were decreased (50.90% of control). Lignins also showed some changes. The fractions of insoluble “Klason” and acid soluble (acetyl bromide) lignins were decreased. In contrast to these data, the quantities of cell wall cellulose and hemicellulose were found to increase. The altered cell wall of B deficient roots correlated with the increased cell permeability as shown by the injury index data (Table 1). Boron is involved in the processes of cell differentiation and its absence can influence cell volume (Hu and Brown, 1994). As result of this, some effect on the symplast/apoplast ratio of plant tissue may be suggested. The main volume of the cell free space is considered to be the cell wall and vacuole (Stree-ter and Salminen, 1993). Thus, the changes in the cell wall under B deficiency can influence these cell compartments. At the same time, it is known that Boron can affect the transport of sugars (Loomis and Durst, 1992). Our results show that the root apoplast and symplast contents of reducing sugars in the surviving 10 days B deficiency plants were increased (Fig. 3). These changes can be due, partly, to the altered permeability of cell wall and membranes after the B starvation which can influence the exchange rate of sugars between roots and nodules. On the other hand, this may be the result of a lowered content of boron- sugar complexes which is known to participate in the transport of sugars from the shoot to the root (Loomis and Durst, 1992). At the same time, the content of free amino acids and amides decreased in both the symplast and apoplast of roots and nodules (Fig. 3), but there was a difference between nodule and root apoplast content of amino acids and amides. On the contrary, the ureides of



**Fig. 3.** Contents of some metabolites in the roots and nodule apoplast and symplast of soybean plants subjected to 10 days B-deficiency. A, C, E – apoplast content; B, D and F – symplast content of studied metabolites;

the root apoplast and symplast were even increased in the stressed plants, which while the ureides of stressed nodule apoplast and symplast were decreased. The accumulation of ureides and reducing sugars in the root apoplast of stressed plants may be result of increased resistance to metabolite flow entering the vascular transport system of plants. These deteriorations can be predicted from the data of changed lignin content and structure of B deficient roots. It is known that lignins take part in the formation of xylem and metaxylem elements of vascular system of plants and every alteration in its content and structure can influence the size and permeability of these plant parts. Thus the boron participation in these processes cannot be excluded. The altered rate of transpiration of B deficient plants can be used as other evidence for such a possibility. The leaves of plants under boron deficiency showed some decrease in the transpiration rate ( $85.2 \text{ mg H}_2\text{O/m}^2/\text{s}$  of stressed plants against  $90.9 \text{ mg H}_2\text{O m}^2/\text{s}$  of the control). The decreased water transport in plants may be caused by changes in hydraulic resistance between cell elements due to altered cell wall and vascular permeability of B stressed plants. On the other hand, the increase of sugar content in the apoplast of roots may contribute to the decrease in the osmotic potential of this plant compartment under B deficiency. This may be another reason for the increase of resistance of metabolite flow between plant organs under stress. The increase in the concentration of sugars in the underground parts of plants may influence in some way the dry matter

accumulation which was found under B deficiency. Under such circumstances despite of the negative effect of boron deficiency on the growth some increase of nodule and root weight was found. However, despite the increased organ weight, the nitrogen fixing activity of such nodules was strongly inhibited, which goes to show that such an alteration was not normal evidence of growth.

## References

- Blevins, D., K. Lukaszewski, 1998. Boron in plant structure and function. *Ann. Rev. of Plant Physiol., Pl. Mol. Biol.*, 49, 481–500.
- Bolanos, L., N. Brewin, I. Bonilla, 1996. Effects of boron on *Rhizobium*- Legume cell-surface interactions and nodule development. *Plant Physiol.*, 110, 1249–1256.
- Dean, J., 1995. In: *Methods of Plant Biochem. And Mol. Biol.*, EdW. Dashek, CRC Press, Lignin analysis, 199–216.
- Dubois, M., K. Gilles, J. Hamilton, P. Roberts, F. Smith, 1956. Colorimetric assay for sugars and related compounds in plant tissue. *Anal. Chem.*, 23–24.
- Georgiev, G., B. Atanasov, K. Kalmuckov, E. Alexandrova, A. Uzunova, 1996. Assimilates and nutrients partitioning and efficiency of nitrogen fixation of black locust (*R. pseudoacacia* L.) grown in excess of Cu in soil. *Bulg. J. Plant Physiol.*, 22(2-3), 40–55.
- Hardy, R. F. W., D. Holstain, E. Jackson, R. Burris, 1968. The acetylene-ethylene assay for nitrogen fixation: lab and field assay for nitrogen evaluation. *Plant Physiol.*, 43, 1185–1207.
- Hu, H, P. Brown, 1994. Localization of boron in the cell wall and its association with pectin. Evidence of a structural role of boron in the cell wall. *Plant Physiol.*, 105, 681–689.
- Lewis, D., 1980. Boron lignification and the origin of vascular plants: a unified hypothesis. *New Phytol.*, 84, 209–229.
- Loomis, W. D, R.W. Durst, 1992. Chemistry and biology of boron. *Biofactors*. 3, 229–39.
- Pfeffer, H., F. Dannel, V. Romheld, 1998. Are the connections between phenol metabolism, ascorbate metabolism and membrane integrity in leaves of boron-deficient sunflower plants? *Physiol. Plant.*, 104, 479–485.
- Philips, D., F. Dakora, E. Sande, C. Joseph, J. Zon, 1994. Synthesis, release and transmission of alfalfa signals to rhizobial symbionts. *Plant Soil*, 161, 69–80.
- Pochinok, H. N., 1976. In: *Methods of Plant Biochem.*, Ed. A. S. Okanenko. *Naukova dumka*.
- Premachandra, G., H. Saneoca, K. Fujita, S. Ogata, 1992. Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum. *J. Exp. Bot.*, 43(257), 1569–1576.
- Streeter, J. S., S. Salminen, 1993. Alterations in apoplastic and total solute concentrations in soybean plants. *J. Exp. Bot.*, 44(261), 821–828.

- Triebels, F., G. Vogels, 1966. Degradation of allantoin by *Pseudomonas acidovorans*. *Biochim. Biophys. Acta*, 113, 292–301.
- Yamagishi, M., Y. Yamamoto, 1994. Effect of boron on nodule development and symbiotic nitrogen fixation in soybean plants. *Soil Sci. Plant Nutr.*, 40(2), 265–274
- Yemm, E., E. Cocking, 1955. The determination of amino acids with ninhydrin. *Analyst*, 80, 209–213.
- Zehirov, G., G. Georgiev, 2001. Alteration in cell permeability, apoplastic and tissue compartmentation of solutes in the root and nodules of boron deficient N<sub>2</sub> fixing soybean plants. *Compt. Rend. Acad. Bulg. Sci.*, 54(1), 71–74.
- Zehirov, G., G. Georgiev, 2002(a). Growth, chemotaxis and nodulation to host roots of *Bradyrhizobium japonicum* 639 in response to the effect of root exudates released from boron deficient soybean plants. *Compt. Rend. Acad. Bulg. Sci.*, 55(4), 87–92.
- Zehirov, G., G. Georgiev, 2002(b). Host-specificity of attachment of *Bradyrhizobium japonicum* 639 cells to the roots of boron deficient soybean (*Glycine Max L. Merr.*) plants related to root cell wall structure. *Compt. Rend. Acad. Bulg. Sci.*, 55(5), 65–68.