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EFFECTS OF BORON STARVATION ON LIGNIN CONTENT AND MINERAL COMPOSITION OF N_2-FIXING SOYBEAN PLANTS (*GLYCINE MAX* L. MERR).

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ABSTRACT. In a greenhouse experiment, symbiotic system soybean *(Glycine max* L. merr)-*Bradyrhizobium japonicum* was grown as liquid culture without boron in the nutrient solution. The dry weight of nodules and leaves was reduced drastically in boron- starved plants. The lignin concentration in nodules and leaves was increased but decreased in the roots. That changes were accompanied by increased concentration of Ca and Mg in the nodules and decreased in the leaves of boron-starved plants. In the nodules of boron- starved plants were found increased concentration of soluble phenols.

All these changes suggested that negative effect of boron starvation depended not only by a factor but complex of factors, which finally negatively affected nitrogenase activity (measured as ARA) and nodulation of symbiotic soybean plants.

KEY WORDS: boron starvation, lignin, mineral nutrition, N_2-fixation.

INTRODUCTION

Boron (B) is a micronutrient required for normal development of higher plants. Brenchly and Thornton (1925) suggested the requirement of boron in symbiotic nitrogen fixation for first time. The role of boron in several physiological processes has been commonly implicated (Blevins and Lukaszewski, 1998). Yamagishi and Yamamoto (1994) reported the negative effect of boron removal from the nutrient solution affected markedly developing nodules in contrast to developed matured nodules.

The plant cell wall has strong requirement of boron in plants. Normal structure and function cell wall pectin depends of boron availability. Boron participates in formation of borate-dimeric rhamnogalacturonan II complex (Kobayashi et al., 1999).

The absence of boron in nutrient solution causes development of abnormal cell walls (Matoh et al., 2000).

The boron starvation has fount to affect significantly phenolic metabolism. Under condition of boron deficiency the plants accumulate high soluble phenols (Pfeffer et al., 1998; Cakmak et al., 1995). On the same time the phenyl- propanoid pathway known to deliver lignolic monomers for synthesis of plant cell wall also and can be affected by boron absence. An important step of the differentiation of plant cells is conversion of primary into second cell walls. From the other side, plant cell wall, especially those of roots plays an important role of ion exchanger contribution to development of Donan's and Water free spaces where the primary accumulation of ions from the solution occur. Thus the changes in cell wall properties due to altered component composition following boron starvation can be involved in the plant nutrition efficiency. There is not data about effects of boron starvation on lignification of symbiotic legumes and its relations with ion composition and nitrogen fixation of soybean plants, which inspire us to study this problem.

MATERIAL AND METHODS

Germinating seeds of soybean cv. Beeson were inoculated with bacterial suspension of 10^8 cells/ml of *Bradyrhizobium japonicum* strain 639 grown on YEM liquid medium. Plants were grown in green house with naturally lit and heated conditions as liquid culture in pots containing 1.2 L of nutrient solution with required macro- and microelements in which inorganic nitrogen /N/ was omitted (Yamadishi and Yamamoto, 1994). Boron was supplied as H_3B0_3 to control plants as 44μg B/L. In the experimental variants no boron/B/ was added in culture medium. The nutrient solution was renewed every 3 days. Six plants per variant were harvested 20 days after emergence. Soluble total phenols were assayed spectrophotometrically after extraction with 80% ethanol. Extractive free tissue was obtained and acetyl- bromide lignin was assayed according procedure described previously (Dean, 1997). Calcium, magnesium, sodium and potassium elements were determined by atomic absorption spectrometry (AAS- Karl Zeiss). The phosphorus was determined using automatic analytical system Contiflo (Hungary).

RESULTS

The total dry weight of B starved plants was reduced (Table 1). Significant reduction of dry weight and number was found also in the nodules (33% of control) and leaves (85.5% of control) of boron- starved plants (Table 2). In contrast roots dry weight of stressed plants slightly increased under boron deficiency.

Continuos boron starvation reduced strongly nodulation and nitrogenase activity (measured as ARA) of symbiotic soybean plants (Table 2). These changes were accompanied by lowering the total nitrogen concentration of nodules (Fig. 1). Continuous boron starvation of soybean plants showed also changes in the ion concentration accumulation patterns of nodules. Phosphorous, calcium and magnesium in nodules were markedly altered in comparison with control (Fig. 1, 2).

The concentrations of phosphorus and potassium of stressed plants increased in the leaves while the calcium and magnesium decreased under stress.

The boron nutrition plays an important role in regulation of metabolism of plant phenols. Imposition of boron starvation of symbiotically grown soybean plants was accompanied by elevation of tissue concentration of soluble phenols (Fig.3). The results showed increased concentrations of soluble phenols in nodules and roots but not in the leaves of boron starved plants. Insoluble phenols represented by acetyl bromide soluble lignin were also increased in nodules in contrast to the roots of boron- starved plants (Fig.3).

DISCUSSION

The negative effect of boron deficiency on nitrogen fixation and nodulation of soybean plants during growth was initially reported by Yamagishi and Yamamoto (1994). They found that developing nodules were more susceptible to boron deficiency than the matured nodules. The boron participates mainly in regulation of pectin structure in cell walls. Now it is well established that boron was bound to the apiosyl residues promoting dimerization of rahmnogalacturonan II (O'Neill et al. 2001). There is not enough information about the effect of boron nutrition on the other components of plant cell walls. As showed Yamagushi and Yamamoto (1994) boron starvation can cause significant changes in ion composition of soybean plants. Further Carpena et al., (2000) has been studied in more details interaction between calcium and boron ions. The authors found that the boron starvation had more affect on boron content in the nodules, while the calcium was in the roots of pea plants. On the other side, Eklund and Eliasson. (1990) established that higher calcium supply led to increased concentration of calcium in the cells which provoke activation in the synthesis of non-cellulose polysaccharides and lignin. Correspondingly, our results showed higher concentration of calcium in nodules and low in roots of symbiotically soybean plants. In confidence with the other results our experiments confirmed that the accumulation of excessive calcium correlates with higher lignin content found in the root nodules of soybean. The concentration of calcium in the roots of boron-starved plants was low after boron starvation and followed by a decrease of lignin concentration. Yamauchi et al. (1986) showed inhibition in translocation of Ca and induction of abnormal calcium metabolism changes under boron starvation. This deterioration possibly affected translocation not only of calcium but magnesium which also is known to participate in plant cell wall structure regulation by forming Mg pectates. As a result of these changes the dry weight, number and functioning of nodules were drastically reduced. These alterations in nodule and root cell wall may contribute to explain the inhibited nitrogenase activity detected under boron starvation, since cell wall properties affect oxygen diffusion barrier of functioning nodules. Finally the significant negative effect of boron starvation is most likely to be caused by complex factors but mainly is a result of deterioration of cell wall structure and mineral composition.

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Table 1. *Effect of boron starvation on dry weight of symbiotic soybean plants. [a]Mean ± standard error of six plants.*

Treatments	Dry weight (g)				
	Leaf	Stem	Root	Nodule	Whole plant
Control	3.8± 0.12[a]	2.0± 0.09	4.53± 0.21	0.48± 0.02	10.81
B starved plants	3.21± 0.10	1.59± 0.08	4.58± 0.18	0.16±0.02	9.54

Table 2. *Effect of boron starvation on nodulation and acetylene reduction of soybean plants. [a]Mean ± standard error of six plants.*

Treatments	Nodule number/ plant	Nodule dry weight (mg)	Acetylene reduction assay µM C_2H_4 g^{-1} FW h^{-1}
Control	60.1 ± 5.2[a]	8.0± 0.21	7.78 ± 0.41
B starved plants	34.3 ± 5.4	4.7 ± 0.18	2.27 ± 0.27

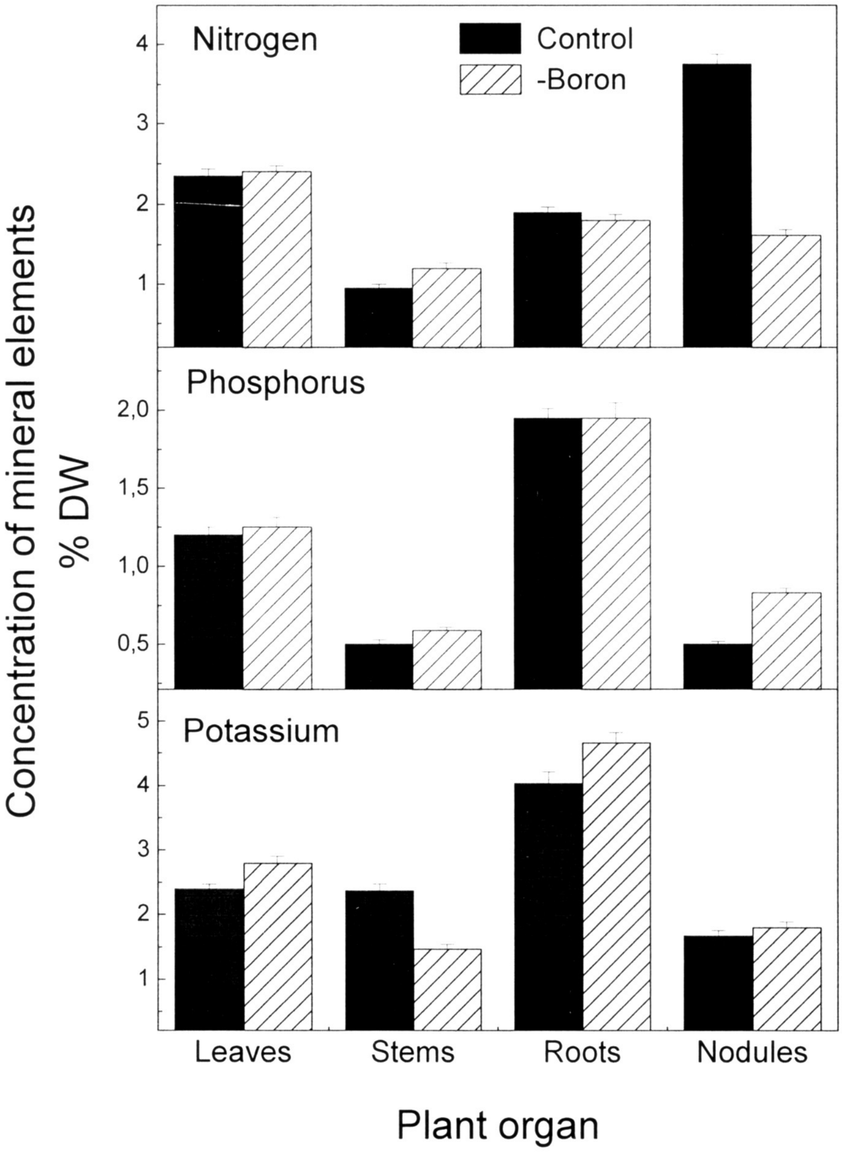

Figure 1. *Influence of boron starvation on N, P and K concentrations in plant organs of symbiotic soybean plants. Bar represent SE*

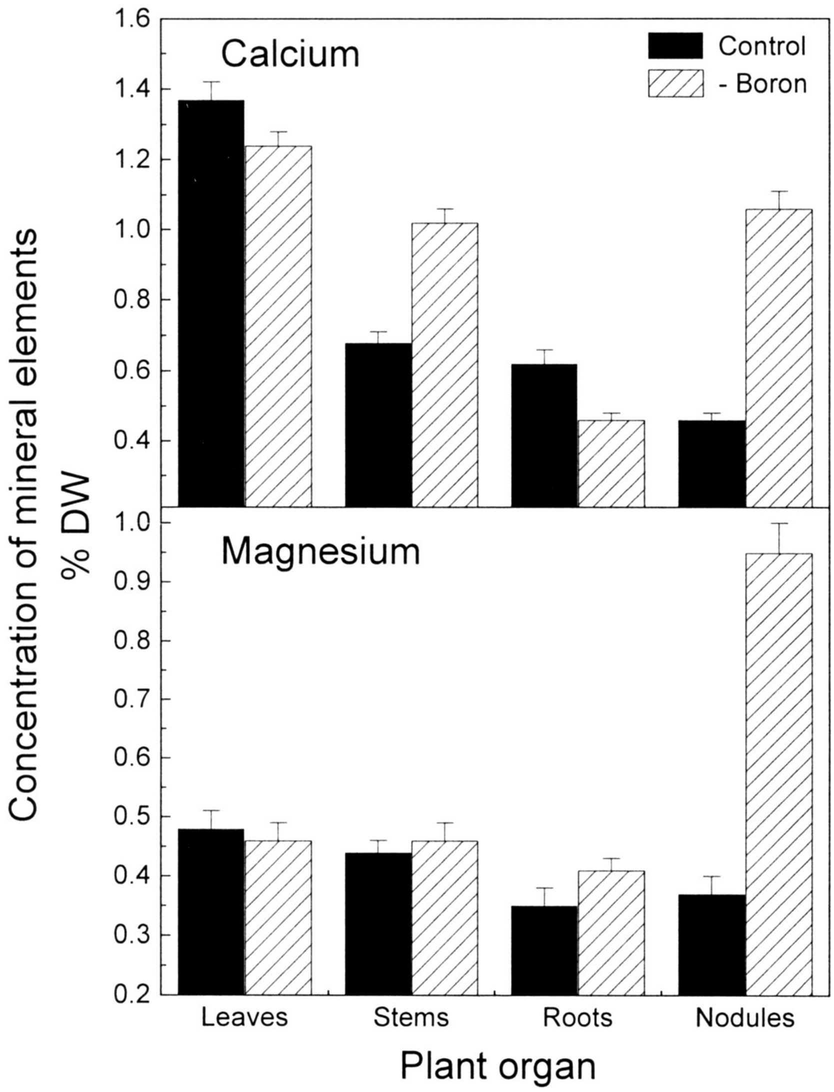

Figure 2. *Influence of boron starvation on Ca and Mg concentrations in plant organs of symbiotic soybean plants. Bar represent SE*

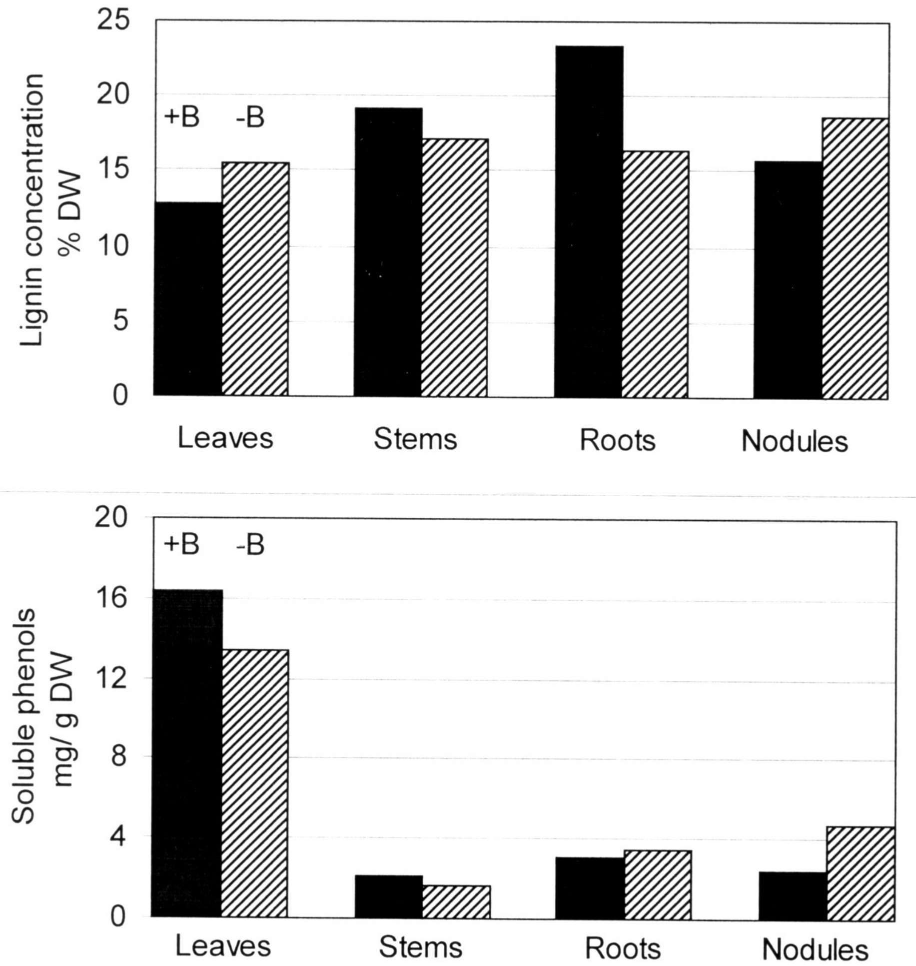

Figure 3. *Influence of boron starvation on lignin and soluble phenols in plant organ of symbiotic soybean plants. Significant at 5% level of probability.*