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EFFECT OF EXOGENOUS SUPPLY OF BORON ON NODULE DEVELOPMENT IN PEA (*PISUM SATIVUM* L.)

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Abstract

Exogenous supply of boron was evaluated on the seeds of *Pisum sativum* L. inoculated with *Rhizobium leguminosarum* and grown in aqua-culture. The liquid nutrition medium contained six boron concentrations (control, 1, 1.86, 2.86, 3.86, and 4.86 mg L^{-1}). The results obtained from the present work showed that number of nodules, size of nodules and weight of nodules exhibit great improvement in 2.86 mg L^{-1} boron treatment. Toxic effect of high concentration of boron (4.86 mg L^{-1}) was also recorded. There was significant reduction as compare to control in nodule weight, size and numbers. This fact becomes clear while observing the nodules growing in the boron free culture, which did not develop extensively. The comparison of the transverse sections of root-nodule area, from nutrient solutions with various boron concentrations showed that in the absence of boron, there was a considerable hypertrophy in cambial cells and a frequent disintegration of phloem and ground tissue along with xylem. The disintegration of the tissue seems to be linked with nodule bacteria. The nodular bacteria are considered to assume a parasitic habit in the absence of boron.

Introduction

Growth medium exaggerate the growth and developmental processes in the plant species in presence or absence of certain elements. Boron is one of these elements generally considered essential for various plant development processes especially in vascular plants (Reguera *et al.,* 2010). The boron effect on translocation was due to its complex with cell membrane, James *et al*., (2010). Boron controls the activity of enzymes that lead to the production of toxic phenolic substances (Ismail *et al.,* 1997)**.** It has also been suggested that boron may be involved in many facts of cell differentiation and development (Bonilla, *et al*., 1997). The role of boron in organic translocation in plants, plant growth regulator response, nucleic acid metabolism, and cell wall metabolism is important for normal growth of plant species (Dugger, 1973). Boron is essential not only for the development of the roots but also for the formation of root nodules of the leguminous plants (Bonilla, *et al*., 1997). Absence of boron reduced the growth of bacterial nodules which do not develop extensively and the bacteria tend to assume a parasitic habit (Yamagishi & Yamamoto, 1994). The nodules develop on the roots of pea and *Vicia faba*, responsible for nitrogen assimilation, are much reduced in the absence of boron.

Generally more than 80 percent species of fabaceae family have the ability of nodulation (Mahmood & Qadri, 2004). Symbiotic relationship between legume and *Rhizobium* is one of the main process of the biological nitrogen fixation and boron play a key role in this association (Lodwig *et al.,* 2003). It has been established that boron deficient nodules fix less N, and contain abnormal cells and aberrant cell walls. Under boron deficient conditions, nodule weight and N_2 fixation capacity of legumes is usually decreased (Serrano, 1989; Bolanos *et al.*, 1996; Brown & Shelp, 1997). However it was reported that higher N application to soil has depressive effect on the number of nodules in pea (Gill *et al.,* 2006). Nitrogenase activity is significantly inhibited by boron deficiency because the number, development and function of nodules in pea *(Pisum satívum* L.) is adversely affected (Bolanos *et al.,* 1994; 2001). Role of boron in the stability of cell wall under boron deficient soil is clear which caused dramatic changes in nodule cell wall and alterations in both peribacteroid and infection thread membranes (Bolanos *et al.*, 1994).

The present work was carried out to study the effect of various B concentrations on the nodulation in *Pisum sativum* L., and to find the B concentration more beneficial for nodule formation.

Materials and Methods

The experiment was conducted at the Department of Botany, GC University, Lahore Pakistan during 2009-2010. There were six boron (0, 1, 1.86, 2.86, 3.86 and 4.86 mg L^{-1}) treatments. The seeds of *Pisum sativum* L., were obtained from the Federal Seed Certification Department, Lahore. The seeds were first surface sterilized with 0.2% solution of mercuric chloride and sown in the cotton pad in a dissecting tray. The seeds were irrigated with deionized water. After germination, the young seedlings were infected with the proper bacterial strain *Rhizobium leguminosarum* by bathing their roots in the broth medium containing the *R. leguminosarum* culture (obtained from CAMB, Center for Advanced molecular Biology, Lahore). The contents were kept at 28°C in oven and left overnight then this starter culture was added to the main sterilized medium. Latter was again kept at 28°C and left for overnight and so the culture medium obtained was used for root infection of the plants. Each plant was then separately transplanted to the Hoagland nutrient, (Hoagland, 1940) medium with different B concentrations viz., 0, 1, 1.86, 2.86, 3.86 and 4.86 mg L^{-1} .The solutions were replaced daily with fresh nutrient solutions of similar boron concentration. Photographs of the nodulated roots were taken and the transverse sections of nodules of each specific boron concentration were made with the help of microtome. The data was collected after 10 and 20 days after boron treatment. The data was analysed by analysis of variance technique (Steel & Torrie, 1980) at 5% level of significance.

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Table 1. The effect of B (conc.) on various parameters of nodules after 20 days.

Boron concentration (mg L^{-1})	Number of nodules after 20 days	Size (mm) of nodules after 20 days	Weight (g) of nodules after 20 days
Control (0)	4.00 ± 0.33	0.93 ± 0.03	1.01 ± 0.01
1	5.33 ± 0.33	1.07 ± 0.07	1.11 ± 0.01
1.86	6.00 ± 0.00	1.48 ± 0.02	1.55 ± 0.03
2.86	10.33 ± 0.33	1.87 ± 0.03	1.93 ± 0.03
3.86	6.67 ± 0.33	1.00 ± 0.00	1.28 ± 0.03
4.86	3.67 ± 0.33	0.57 ± 0.03	0.93 ± 0.02

Results

Difference in the number of nodules, size of nodules and weight of nodules at different boron concentrations showed clear demarcation. Variations in nodule number were great under various boron concentrations viz., control, 1.0, 1.86, 2.86, 3.86 and 4.86 mg L^{-1}. Nutrient solution with boron 2.86 mg L^{-1} favored maximum nodulation which showed 83% greater value over control (Fig. 1). Control and 4.86 mg L^{-1} gave minimum value leading to a toxic effect of boron in higher concentration.

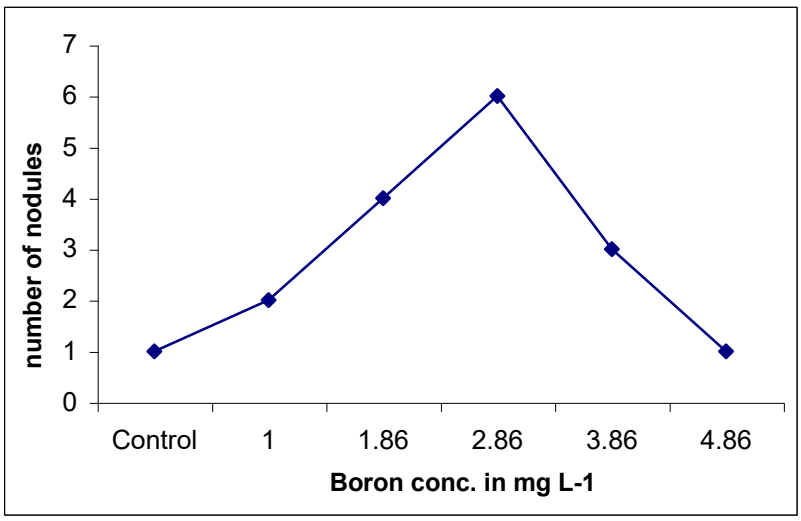

Fig. 1. Effect of B on the number of nodules of *Pisum sativum* after 10 days of treatment.

Comparison of the results from various boron concentrations viz., 1.0, 1.86, 2.86, 3.86 and 4.86 mg L^{-1} and control revealed that nutrient solution with Boron 2.86 mg L^{-1} favored maximum nodule size (Fig. 2). Increase in nodule size was 46% over control under such exogenous supply of boron showed significant influence. Higher concentration of 4.86 mg L^{-1} proved toxic, reducing the size of the nodule up to 28% as compare to control. Similar trend was also depicted in nodule weight. Boron concentration of 2.86 mg L^{-1} gave higher percentage of 50% over control. All other treatment also showed improvement as compare to control except 4.86 mg L^{-1}.

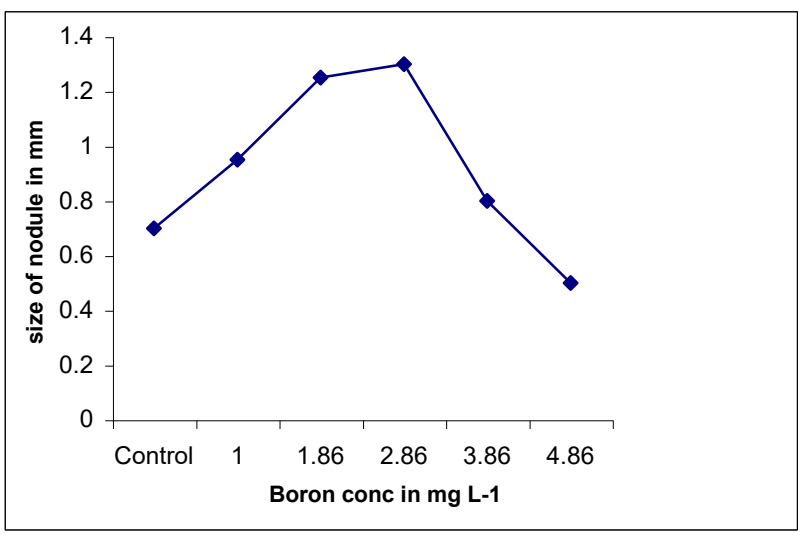

Fig. 2. Effect of B on the size of nodules of *Pisum sativum* after 10 days of treatment.

Transverse sections of the root area with nodule taken from water cultures with various boron concentrations revealed another fact, that root nodule section from boron free nutrient solution shows abnormal bacterial development and absence of vascular strands due to hypertrophy. It was also observed that boron concentration more than 2.86 mg L^{-1} did not favor the good development of nodule. It was also observed that overall growth of plant shoots is good in boron concentration 2.86 mg L^{-1}. , while concentrations higher than this and control one not much favorable.

Discussion

It is clear that data depicted in figure 1, 2 and 3 is in agreement with the work of Sommer and Lipmann (1926), and Grigor and Georgiev (2001), found that Boron is essential for the development of corn, pea and sunflowers. They also pointed out that dicotyledons respond more quickly to boron than monocotyledons. Warrington (1923) failed to find any stimulating effect of B on Barley (monocotyledon). Mulder (1948) working with peas, using water culture technique established that boron is not only essential for root development but also for nodulation in Leguminosae, Bolanos *et al*., (1994) investigations with beans showed that in the absence of boron, the nodules formed were not of normal size and were also unable to fix nitrogen.

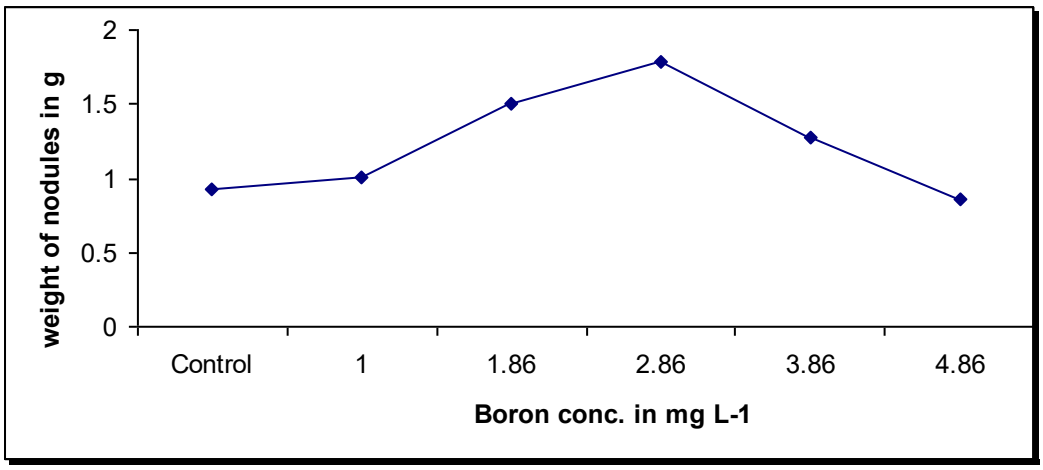

Fig. 3. Effect of B on the weight of nodules of *Pisum sativum* after 10 days of treatment.

Fig. 4. T.S of root- nodule area showing disorganized vascular structures as well as cortical cells in the nodules of the plant growing in the boron free nutrient culture medium.

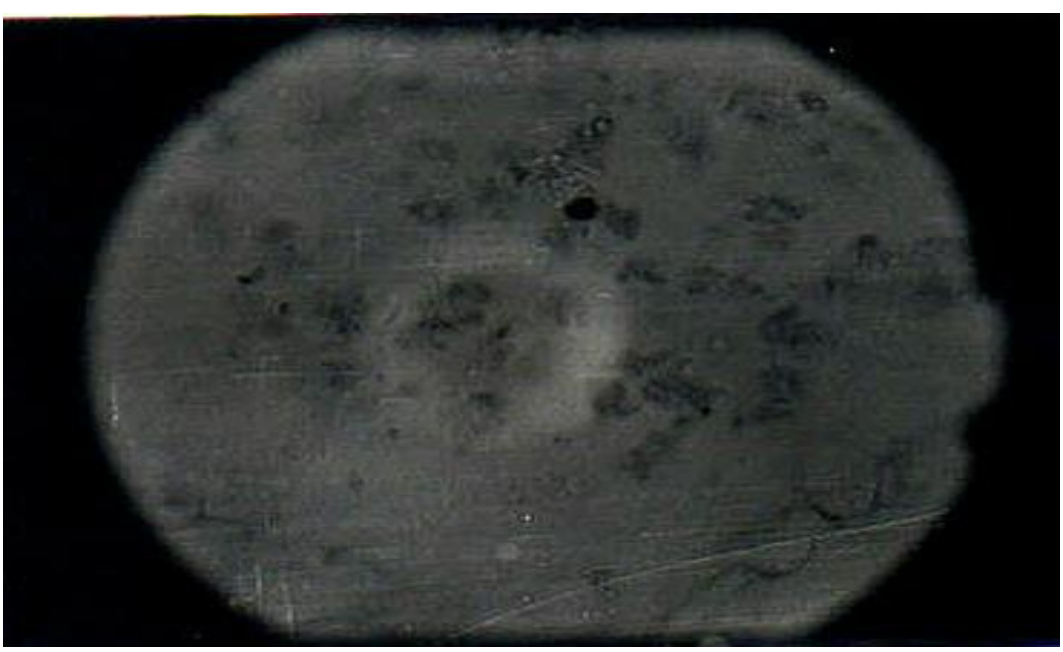

Fig. 5. Transverse section of root (*Pisum sativum*) growing in boron free medium, showing cells (I) infected with nodule bacteria and cells (d & e) not infected with nodule bacteria.

It has been observed also that although nodulation occurred in boron free solution, but is less as compared to the nodulation in other concentrations. Grigor & Georgiev (2001) observations concerning the development of nodules fully coincide with the results of these experimental proceedings. It is observed that in culture solution having boron concentration 2.86 mg L^{-1} the average size of nodule is 1.30 mm; average weight is 1.79g and the number of nodules is greater that in the solutions with other different B concentrations (Hasnain *et al*., 2011).

The results obtained from this experimental work indicated that boron concentration 2.86 mg L^{-1} is ideal for nodulation in *Pisum sativum* L. Observations of the slides of transverse section of root- nodule area revealed an important fact, that cells show a healthy growth in the nutrient medium containing Boron in different concentrations viz., 1.0, 1.86, 2.86, 3.86 and 4.86 mg L^{-1} but the vascular structures as well as cortical cells became defective in the nodules of the plant growing in the boron free nutrient culture medium. Bonilla *et al*., (1997) found hypertrophy of cambial cells in bean in B free medium. The strands ran short way into the nodule, possibly due to attack of bacteria. The masses of bacteria broke down the cells of the host. This observation is possibly linked with the change in the behavior of bacterium from symbiotic to parasitic, which may be due to absence of B.

Conclusion

Based on all the morphological attributes measured in this study, it can be concluded that the boron plays significant positive role in nodule development up to a certain threshold value, above and below that it has negative impact on number, size and weight of nodules with passage of time.

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