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EFFECT OF DIFFERENT FOLIAR FERTILIZER CONCENTRATIONS ON PEA PLANTS NODULATION AT REDUCED MO SUPPLY

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ABSTRACT. Garden pea *Pisum sativum* L. var. *Avola* inoculated with bacterial suspension of *Rhizobium leguminosarum bv. Vicae* strain D 293 were grown in the greenhouse until the 15th day on liquid half strength nutrient solutions of Hellriegel, contained 0,5 mM NO_3^- in the all experimental variants. The complementation of nutrients with micronutrients was done according to Hoagland and Arnon excluding Mo from the nutrient medium. The following variants were tested: (1) control plants with root nutrition only and presense of Mo; (2) control plants with root nutrition only without Mo (3) plants with combined root and foliar nutrition (0,1%Agroleaf); (4) plants with combined root and foliar nutrition (0,3%Agroleaf); (5) plants with combined root and foliar nutrition (0,5%Agroleaf). Application of foliar fertilizer in elevated concentrations had a positive effect on the Mo deficient pea plants. An increase of nodule number, plant dry weight, total shoot nitrogen and leaf soluble sugars have been found. The highest nodule number and leaf soluble sugar content was observed at spraying with 0.3% concentration of Agroleaf.

KEY WORDS: *Pisum sativum* L, *Rhizobium leguminosarum bv. Vicae*, foliar fertilization, plants with reduced Mo supply.

INTRODUCTION

The most importance of molybdenum (Mo) for plants, animals and microorganisms has been known for a long time (Coughlan, 1980). Molybdenum is a constituent of nitrate reductase and nitrogenase and is required for both synthesis and activity of the enzymes. In nitrate reductase it is bound to a unique pterin compound named molybdenum cofactor – Moco, which binds to diverse apoproteins. One of the two nitrogenase cofactors, MoFe –cofactor is related to the binding site and reduction of N_2 from the athmosphere (Orme-Jonson, 1985).

The early steps of nodule formation after inoculation with Rhizobium strains are especially sensitive to the changes of mineral nutrition both under conditions of nutrient starvation (Thorne and Williams, 1997) and nutrient excess (Streeter 1988; Marschner 1995). The negative effect of high soil NO_3^--N content on nodule formation, nitrogenase activity and synthesis of transport products is described (Streeter 1988, Oghoghorie and Pate, 1971). The application of nutrients by spraying a solution onto the leaves (foliar feeding) seems to be a perspective way to reduce the inhibitory effect of soil supplied inorganic nitrogen on the nodulation and symbiotic nitrogen fixation**.** Because the molybdenum in higher plants is a component of nitrate reductase, it might be expected that when plants are supplied with nitrogen in a form other than nitrate the plants would show no requirements for molybdenum and Mo shortage would not influence plant growth.

Fertilization at early stages could influence by different mechanisms compared with fertilization at reproductive stages. One of the established benefits of foliar fertilization is the increased uptake of nutrients from the soil because of the enhanced exretion of the exudates from the roots, which in turn stimulated bacterial population into the rhizosphere (Kuepper, 2003). High rates of foliar applied N would cause serious leaf damage while the small rates could stimulate growth without inhibiting nodulation.

The present study comprising glasshouse experiment was undertaken to determine the effect of Mo shortage on the nodule formation and other nitrogen fixation related parameters as plant dry weight, the content of tissue nitrogen, leave soluble sugars and free amino acids in foliar fed plants. In this reason we attempt to establish the most appropriate foliar fertilizer concentration that ensure normal plant growth and nodule development at early steps of nodule formation of the inoculated with a *Rh. Leguminosarum bv. Vicae* pea plant seedlings.

MATERIAL AND METHODS

Seeds of garden pea *Pisum sativum* L. var. *Avola* were surface sterilized with 4% NaOCl and germinated in the Petri dishes at 25 ^oC. Three days old seedlings were inoculated with the bacterial suspension of *Rhizobium leguminosarum bv. Vicae* strain D 293 at approximately 10^8 cells per cm^3. On the 5^{th} day the seedlings were transferred to 1.2 dm^3 pots (2 plants per pot) containing liquid nutrient solutions of Hellriegel and were grown in the greenhouse until the 15^{th} day. The solution was aerated continuously and replaced twice weekly. Nutrient solution of Hellriegel supplied to plants was with half strength of nutrients. The NO_3^- concentration was 0,5 mM in all-experimental variants. The complementation of nutrients with micronutrients was done according to Hoagland and Arnon excluding Mo from the nutrient medium. The following variants were tested: (1) control plants with root nutrition only and presence of Mo; (2) control plants with root nutrition without Mo (3) plants with combined root and foliar nutrition (0,1%Agroleaf); (4) plants with combined root and foliar nutrition (0,3%Agroleaf); (5) plants with combined root and foliar nutrition (0,5%Agroleaf). The liquid foliar fertilizer Agroleaf®, Scotts Co, Ohio, USA contains the main elements in the proportion of N:P:K equal to 20:20:20 and all important micronutrients in the chelated form presented by the unique Scotts M77 formula: 0.1% Fe, 0.06% Mn, 0.06% Cu, 0.06% Zn, 0.02%. Agroleaf was applied with spraying under high pressure at above-mentioned rates - 5 cm^3 per plant. Supplied nitrogen consisted of 2,46 mM NO_3^- and 6,11 mM NH_4^+. The Agroleaf application was done at changing of nutrient solutions. Spray-solution pH has been adjusted in the neutral range (7,0-7,5).

In order to prepare total crude extract for determination of sugars and amino acids 1,0 g fresh plant material was ground with 3,0 ml heated 96% alcohol. The homogenate was centrifuged at 4000 g for 10 minutes and the supernatant removed. Ethanol soluble extract was dried and soluble compounds were redissolved with 1,0 ml distilled water and 1,0 ml chloroform. The obtained solution was used for the soluble sugars assay according to Dubois et al. (1956).

The total N content was measured after Kjeldahl and determined with automatic analyzer “Contiflo” (Hungary). Our results are expressed as means ± standard error where n=8.

RESULTS AND DISCUSSION

The favorable effect of applied foliar fertilizer on the nodule formation in the plants grown without Mo has been shown on figure 1. The nodule number and nodule fresh weight of the foliar fed plants increased in comparison with the control plants with root nutrition only. The highest nodule number was observed at 0,3% concentration of Agroleaf as it was established in our previous study with normal supply with Mo (Hristozkova et al, 2005). The high nodule number in these variants could due to the supplied NH_4NO_3 with foliar fertilizer in an appropriate concentration, because the lower (0,1%) and the higher (0,5%) applied concentrations resulted in a reduced nodule number. According to Gulden and Vessey, (1997) low levels of NH_4^+ in nutrient solution can stimulate nodulation in pea. On the other hand the number of the nodules in the control variants with lack of Mo is higher compared to the Mo supplied control plants. Inoculated with Rh. Leguminosarum pea plants grown without Mo had the higher nodule number (figure 1) but less nodule fresh weight (table 1) compared to the Mo supplied plants (Hristozkova et al, 2005). Gupta and Lipsett (1981) pointed out that on soils containing little Mo, plants developed many nodules on roots but N_2 is not fixed.

Shoot and root dry weight decreased in Mo free variants in comparison with the control plants supplied with Mo (Table 1). As Jongruaysup et al., (1994) have shown low Mo supply rates symptoms typical of Mo deficiency appeared and dry weight was depressed. Foliar fertilizer application resulted in higher than the Mo free control shoot and root biomass accumulation and shoot biomass gradually increased within the range of Agroleaf concentrations 0,1-0,5%. Root dry biomass did not significantly differ among the variants with different concentration of Agroleaf. In the plants grown without Mo a decrease of shoot biomass only was observed while root biomass did not considerably change (table 1) in contrary to the Mo-adequate plants (Hristozkova et al, 2005).

The similar changes have been observed in the tissue nitrogen content (figure 2 A). Nitrogen in shoots and roots in Mo deficient plants had lower values than the Mo supplied plants. According to Jongruaysup et al., (1994) under conditions of Mo deficiency N deficiency symptoms appeared and shoot nitrogen content was depressed. The treatment with foliar fertilizer have as a result increased total nitrogen content in the shoots while in the roots N content slightly increased regarding the Mo deficient control. The great variance in shoot and root nitrogen content among the plants treated with elevated foliar fertilizer concentrations is not found which is in correspondence with the little differences in shoot and root biomass.

The values of total soluble sugars (fig. 2B) differed significantly among the studied variants, but the positive effect of the foliar fertilization on the accumulation of soluble sugars was observed. Since Tejada and Gonzales (2003) have reported that the foliar fertilization increased leaf carbohydrate contents as well as leaf N concentration. The sugar content reached maximal values when plants were sprayed with 0,3% foliar fertilizer where the number of nodules was the highest. The number of nodules is usually adapted to the plant capacity to produce carbohydrates, because nitrogen fixation is energetically very expensive (Vance and Gant, 1992). However little is known about the relationship between nodule number and tissue sugar concentration under conditions of Mo deficiency, where it is supposed that the nodules are ineffective. Increased sugar content in Mo deficient control plants could due to the sugar accumulation because of the disturbance in N assimilation when Mo is omitted from the nutrient medium. Pea plants grown under conditions of root and combined root and 0.3% foliar nutrition are shown on figure 3.

The treatment of Mo deficient pea plants with elevated concentration of foliar fertilizer resulted in increase of nodule number, plant dry biomass, total shoot nitrogen and leaf soluble sugars. The highest nodule number and leaf soluble sugar content is observed at spraying with 0.3% concentration of Agroleaf.

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Table 1. *Effect of different concentrations of foliar fertilization on plant dry weight shoot to root ratio and nodule weight.*

Variants	Shoot	Root	S:R	Nodule
	g DW $plant^{-1}$	g DW $plant^{-1}$		g FW $plant^{-1}$
1. Root nutrition with Mo	0,525±0,02	0,260±0.01	2,02	0,0199
2. Root nutrition without Mo	0.170±0,05	0,240 ±0,02	0.71	0,0196
3. Combined nutrition with 0,1% Agroleaf (-Mo)	0,244±0,04	0,141 ±0,03	1.73	0,105
4. Combined nutrition with 0,3% Agroleaf (-Mo)	0,264±0,03	0,152±0,01	1.74	0,344
5. Combined nutrition with 0,5% Agroleaf (-Mo)	0,297±0,03	0,203±0,02	1.47	0,064

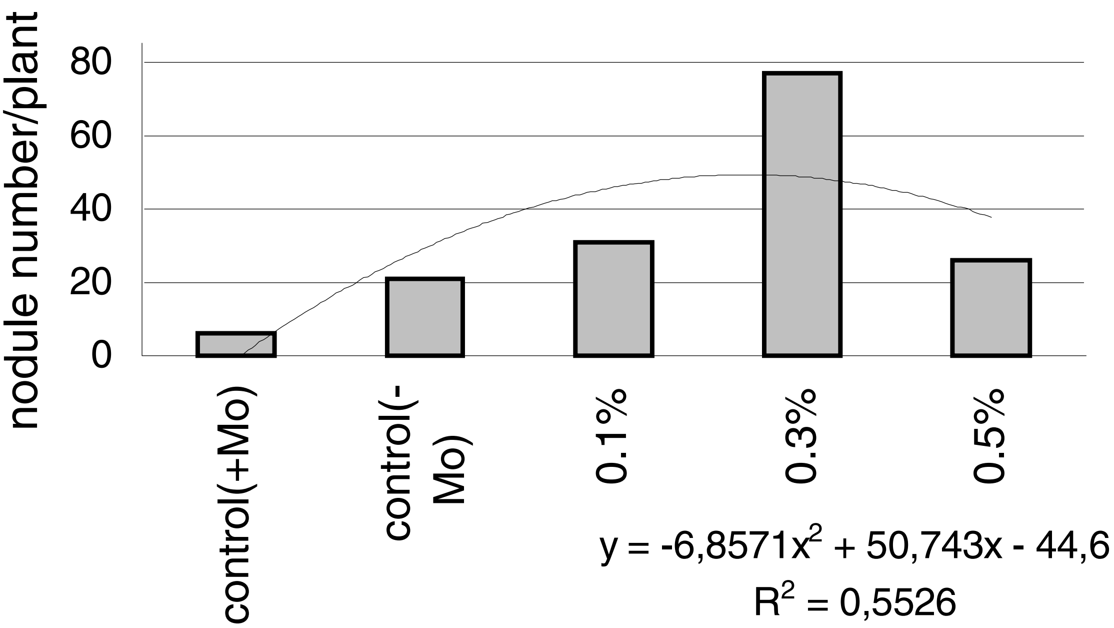

Figure 1. *Effect of different foliar fertilizer concentrations on pea plants nodulation.*

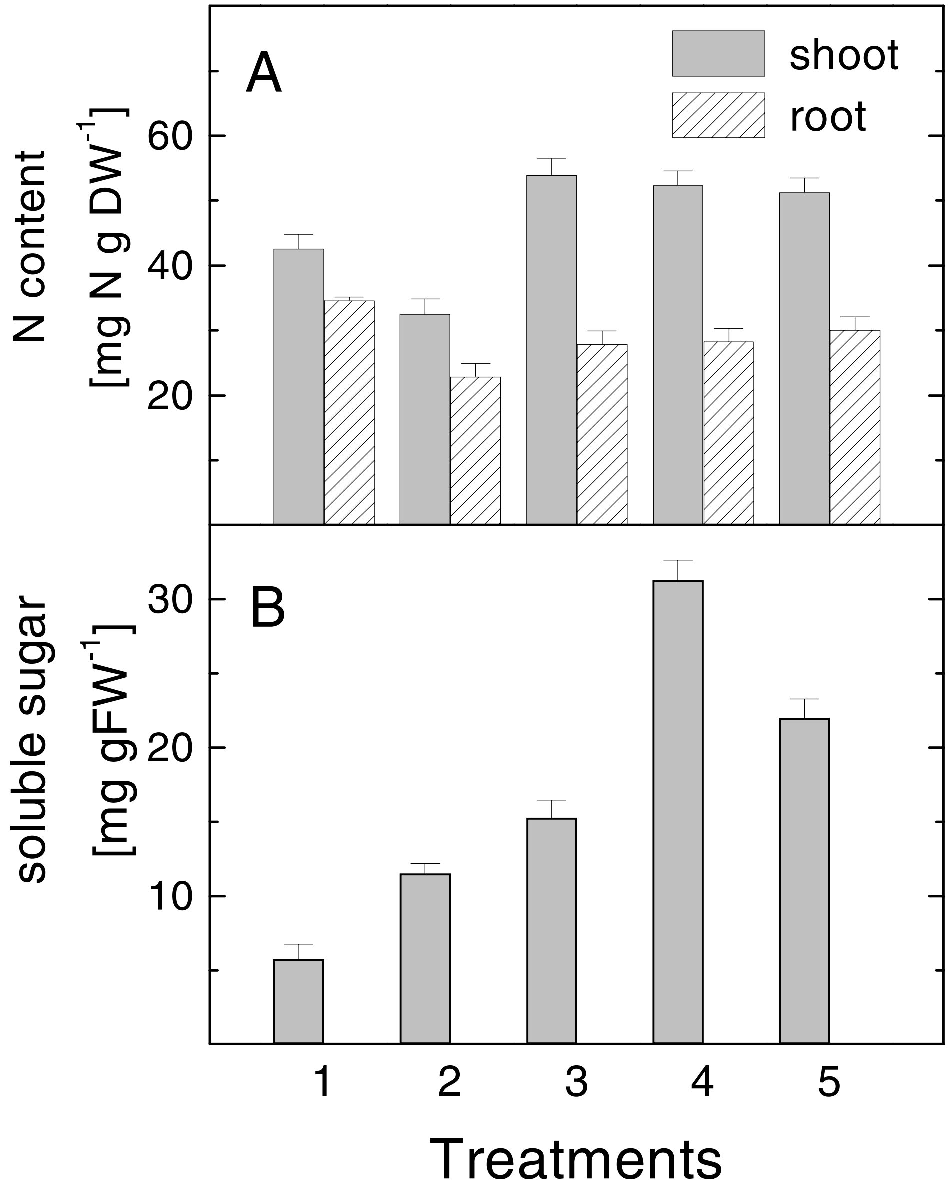

Figure 2. *Content of total nitrogen and soluble sugars in shoot and root pea plants. (1) control plants with root nutrition and presense of Mo; (2) control plants with root nutrition and without Mo; (3) plants with combined root and foliar nutrition (0,1%Agroleaf); (4) plants with combined root and foliar nutrition (0,3%Agroleaf); (5) plants with combined root and foliar nutrition (0,5%Agroleaf)*

Figure 3. *Pea plants grown without Mo with combined root and foliar nutrition (0.3%) – A and with root nutrition – B.*