RESPONSE OF INOCULATED PEA PLANTS (*PISUM SATIVUM L.*) TO ROOT AND FOLIAR FERTILIZER APPLICATION WITH REDUCED MOLYBDENUM CONCENTRATION

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Summary. The effects of foliar absorbed nutrients on root processes related to assimilation of nitrogen under presence or absence of molybdenum (Mo) were studied. Pea plants (*Pisum sativum* L.), var. Avola were grown until the 15th day in a glasshouse on liquid nutrient solution both without and with Mo added to the media. Plants were inoculated with bacterial suspension of *Rhizobuium leguminosarum* bv. *Viciae* strain D 293 at approximately 10^8 cells per cm^3. Application of a liquid foliar fertilizer at a concentration of 0.3% was performed twice a week by spraying under high pressure. The effects of foliar supplied nutrients in addition to root nutrition on dry biomass accumulation, protein content, root nodulation and activities of the enzymes of primary nitrogen assimilation (nitrate reductase (NR – NADH, EC 1.6.6.1), and glutamine synthetase (GS: EC 6.3.1.2) during the early stage of nodulation were studied. Foliar application of nutrients had a positive effect on the activities of NR and GS enzymes in shoots of Mo-supplied plants. It was found that foliar application of nutrients reduced the inhibitory effect of Mo shortage on root nodulation, plant dry biomass and protein content. The negative influence of Mo exclusion from the nutrient media on biomass accumulation and nodule formation was diminished through the foliar absorbed nutrients.

Keywords: pea *(Pisum sativum L),* foliar fertilizer, nitrate reductase, glutamine synthetase, molybdenum.

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INTRODUCTION

The formation and functioning of nitrogen fixing symbiotic nodules of legume roots is known to be very sensitive to environmental changes. The early steps of nodule formation and N_2 fixation are especially sensitive to changes in mineral nutrition (Streeter, 1988). The negative interaction between NO_3^- supply and nodule N_2 fixation in higher plants is well established (Becana and Sprent, 1987). A perspective way to reduce the inhibitory effect of exogenously supplied inorganic nitrogen on nodulation and symbiotic nitrogen fixation is to change the place of direct N uptake and assimilation in plant. Foliar application of inorganic nutrients, including nitrogen, could be additional to normal root supply of nutrients and allows decreasing of the harmful direct action of inorganic nitrogen on nodule development and functioning. Foliar uptake of N is not only supplementary, but can influence N status of the whole plant. Changing the site of primary N assimilation can affect the activities of some key enzymes of nitrogen metabolism in roots and shoots. The most important highly regulated enzymes of N metabolism in higher plants are nitrate reductase (NR) and glutamine synthetase (GS) (Oaks, 1994). Their activities are closely coupled with the nutrition and metabolism of molybdenum (Mendel and Haensch, 2002). Molybdenum ions are important co-factors components of key enzymes of assimilatory nitrogen metabolism – nitrogen fixation, nitrate uptake and reduction (Gupta and Lipsett, 1981; Campbell, 1999). Moreover, Mo is an element that is translocated with low mobility inside plants, which is the main reason for its low utilization by plant organs during the period of starvation (Gupta and Lipsett, 1981).

There are insufficient data about the regulation of nitrogen assimilatory pathway under conditions of combined foliar and root nutrient application especially when plants are inoculated with the respective *Rhizobium* strain and starved for Mo during growth. Therefore, the aim of this study was to investigate the effects of root and foliar applied mineral nutrition on the background of different Mo supply, with respect to growth and nitrogen assimilation enzymes at early steps of nodule formation in *Rh. leguminosarum* bv. *Viciae* inoculated pea seedlings.

MATERIALS AND METHODS

Seeds of garden pea *Pisum sativum* L. var. Avola were surface sterilized with 4% sodium hypochlorite (NaOCl) and germinated in Petri dishes at 25 °C. Three-day-old seedlings were inoculated with bacterial suspension of *Rhizobium leguminosarum* bv. *Viciae* strain D 293 at approximately 10^8 cells per cm^3. On the 5th day, seedlings were transferred to 1.2 dm^3 pots (2 plants per pot) containing liquid nutrient solutions of Helriegel and were grown in a greenhouse until the 15th day. Solution was aerated continuously and refreshed twice a week. Plants were supplied with half strength Helriegel nutrient solution containing 0.5 mM NO_3^-. Complementation of nutrients with micronutrients was performed according to Hoagland-Arnon's medium (1950). Plants were designed as (+Mo) and Mo-deficient plants were designed as (-Mo). The following variants were tested: Mo-supplied plants with root nutrition (RN + Mo); Mo-supplied plants with root and foliar nutrition (RNF + Mo); Mo-deficient plants with root nutrition (RN-Mo); Mo-deficient plants with root and foliar nutrition (RNF-Mo).

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 (NH_4^+ - N: NO_3^- -N ratio = 2.5:1.0) and all important micronutrients in 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 as spraying under high pressure at a concentration of 0.120 g per 40 cm^3 H_2O (0.3% solution). Agroleaf® application was done after nutrient solution refreshment at days 11th and 14th.

In order to prepare crude extracts for NR and GS determination, 1.0g fresh harvested samples (leaves or roots) were ground in liquid nitrogen with chilled mortars and pestles using 5 cm^3 extraction medium containing: 50 mM Tris-HCl (pH 8.0), 1 µM Na_2MoO_4, 10 mM $MgSO_4.7H_2O$, 1 mM EDTA, 10 mM L-cysteine, 1% PVP-40, 1g Dowex (Freshilla et al., 2000). The extract was filtered through one layer of Miracloth, centrifuged at 10 000g for 20 min (4 °C), and the supernatant was used for the assays. Nitrate reductase (NR: NADH, EC 1.6.6.2) activity was measured according to Hageman and Reed (1980). Glutamine synthetase (GS: EC 6.3.1.2) activity was determined by a biosynthetic assay based on γ-glutamil hydroxamate synthesis (O'Neal and Joy, 1973). Protein content was determined according to Bradford (1976) with BSA as a standard. Results were expressed as means ± standard error where n=3. Comparison of means was performed by the Fisher LSD test ($P \leq 0.05$) after performing multifactor ANOVA analysis. The STATISTICA (version 6.0) package was used for statistical analysis.

RESULTS AND DISCUSSION

The present study is related to the early stage of nodule formation, which begins with exchange of molecular signals between symbiotic partners, proceeds with infection and nodule formation, and ends with the appearance of the first visible nodules. The most obvious changes in root tissue brought about by inoculation with *Rhizobium* included appearance of different number of nodules on pea roots of all experimental variants (Table 1). Combined foliar and root nutrient application to Mo-deficient plants (RNF-Mo) resulted in a highly increased number of nodules compared to roots of Mo-deficient plants (RN-Mo). Therefore, the negative influ-

Table 1. Number of visible nodules and shoot and root dry biomass of 15-day-old pea plants

Treatments	Nodules per plant	Shoot dry biomass g/plant	Root dry biomass g/plant
RN+Mo*	12±2[a]	0.18±0.009[b]	0.16±0.007[c]
RFN+Mo	18±3[ab]	0.23±0.011[d]	0.18±0.008[d]
RN-Mo	22±4[b]	0.12±0.007[a]	0.08±0.003[a]
RFN-Mo	40±6[c]	0.20±0.008[c]	0.13±0.005[b]

* RN+Mo – Mo supplied plants with root nutrition; RFN+Mo – Mo supplied plants with root and foliar nutrition; RN-Mo – Mo deficient plants with root nutrition; RFN-Mo – Mo deficient plants with root and foliar nutrition.

Values are means ± S.E., n=3. Different letters indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA multifactor analysis.

ence of direct application of high nitrate concentration on nodule formation was avoided when nutrients were supplied though the leaves. The nodule number in plants lacking Mo was higher compared to the Mo-supplied ones. Previously it was reported that inoculated pea plants grown without Mo had a higher nodule number but the fresh weight of nodules was lower compared to the Mo-supplied plants (Hristozkova et al., 2005). Gupta and Lipsett (1981) have reported that on soils containing low amounts of Mo, plants had developed many nodules on roots but N_2 fixation had not been observed.

A reduction of dry biomass accumulation in shoots and roots of pea plants with Mo shortage in the medium was registered (Table 1). However, biomass reduction in plants with added foliar fertilizer was less pronounced. Root-fed plants with reduced supply of Mo (RN-Mo) showed the most significant reduction in shoot and root dry biomass, 33% and 40% respectively, compared to root-fed Mo-supplied plants (RN+Mo). When Mo-deficient plants were grown at combined leaf and root nutrition (RNF-Mo) such a reduction reached only 13% in the shoots and 28% in the roots compared to (RNF +Mo) plants. Therefore, combined root and foliar nutrition affected favorably plant biomass under Mo shortage.

Foliar supplied nutrients in addition to root nutrition had also an advantage regarding protein content both in Mo-supplied and Mo-deficient plants - shoots and roots. Moreover, protein content in organs of Mo-deficient foliar-fed plants (RNF-Mo) reached the values of RFN+Mo-plants. The advantages of foliar application on protein content and dry biomass in plants grown with the restrictive Mo concentration in the medium could be explained by lower Mo requirements when nitrate metabolism is by-passed by use of other nitrogen sources (besides nitrates Agroleaf® contained 6,11 mM NH_4^+).

Nitrate reductase activity in the young pea plants was lower under Mo-deficiency compared to Mo-supplied plants (Fig. 1C, D). It is known that Mo ions are involved in the formation of Mo-pterin, which is a cofactor of an enzyme, completing Mo-Co domain in the nitrate reductase electron transport chain (Mendel, 1997). Combined root and foliar nutrition of plants with normal Mo content in the medium (RN+Mo) resulted in higher shoot and root enzyme activities compared to root-fed plants (RN+Mo) (Fig. 1C, D). Additional foliar fertilization did not produce any advantage regarding nitrate reductase activity in the organs of Mo-deficient plants since this element plays a crucial role for the structure and function of NR enzyme. Similar changes in shoot-localized glutamine synthetase were observed (Fig. 1E).

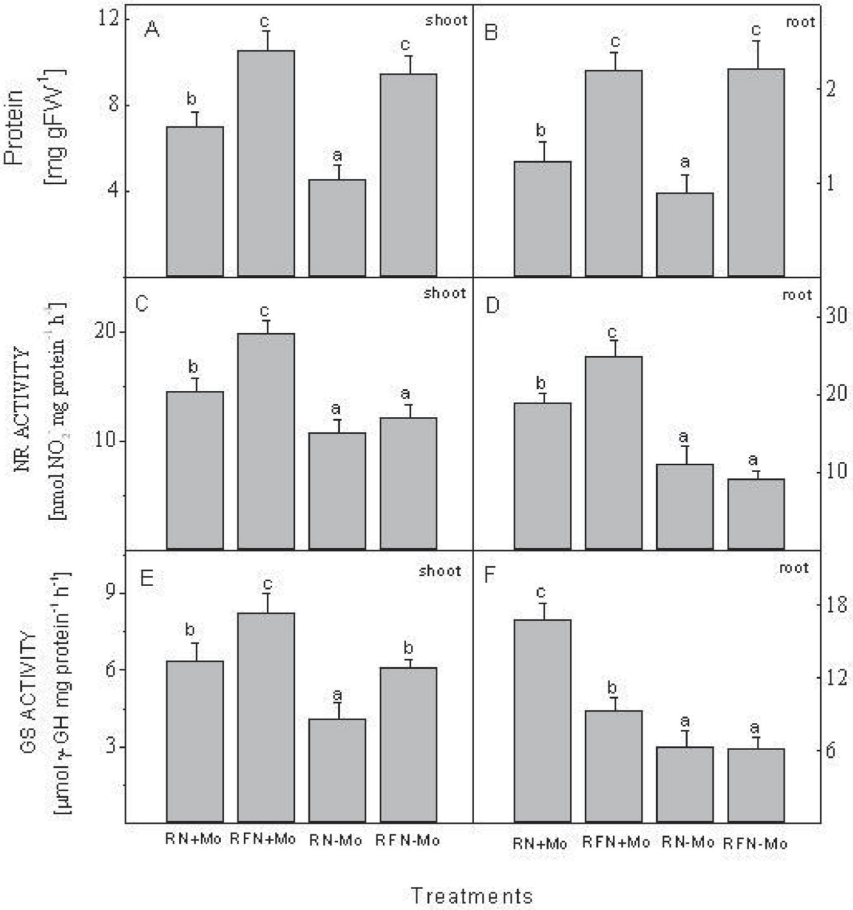

Figure 1. Protein content of shoots (A) and roots (B); nitrate reductase activity in shoots (C) and roots (D); and glutamine synthetase activity in shoots (E) and roots (F) of pea plants. Treatments: RN+Mo – Mo-supplied plants with root nutrition; RFN+Mo – Mo-supplied plants with root and foliar nutrition; RN-Mo – Mo-deficient plants with root nutrition; RFN-Mo – Mo-deficient plants with root and foliar nutrition. Values are means ± S.E., n=3. Different letters indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA multifactor analysis.

According to Peuke et al. (1998) ammonium has been taken up by the foliage more readily than nitrate. Thus, foliar feeding had a positive effect mainly on shoot GS activity both in the presence and absence of Mo (Fig. 1E). Root-localized GS activity did not increase in Mo-deficient plants (Fig. 1F). Foliar supplied nutrients in addition to ordinary root nutrition in inoculated young plants affected positively plant growth and nodulation. Reduced NR and GS activities were observed in plants grown on Mo restrictive medium compared to Mo-supplied plants. Combined foliar and root nutrient application to Mo-deficient plants resulted in an increased number of nodules, higher protein content and plant dry biomass compared to root-fed Mo-deficient plants. Therefore, the negative effect of Mo exclusion from the nutrient media on biomass accumulation and nodule formation could be diminished through foliar absorbed nutrients**.**

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