EFFECTS OF COMBINED INOCULATION OF PEA PLANTS WITH ARBUSCULAR MYCORRHIZAL FUNGI AND *RHIZOBIUM* ON NODULE FORMATION AND NITROGEN FIXING ACTIVITY

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Summary. The response of pea (*Pisum sativum* cv. Avola) to arbuscular micorrizal fungi (AM) species *Glomus mosseae* and *Glomus intraradices* and *Rhizobium leguminosarum* bv. *Viciae*, strain D293 regarding growth, nodulation and nitrogen fixing activity was studied. Pea plants (*Pisum sativum* cv. Avola) were grown in a glasshouse until flowering stage (35 days) in 4 kg plastic pots using leached cinnamonic forest soil at low phosphorus level (60mg P_2O_5 kg soil^{-1}). The obtained results demonstrated that dual inoculation of pea plants increased plant biomass, nodulation parameters, N_2 fixation activity at varying levels compared to plants submitted to single inoculation with *Rhizobium leguminosarum*, strain D293, and depended on AM fungi species. Coinoculation increased significantly total P content in plant tissues and percentage of root colonization. Coinoculation efficiency of *Rhizobium* bacteria and *Glomus mosseae* was higher compared with *Glomus intraradices* regarding biological N_2 fixation and AM colonization at the tested P concentration.

Keywords: *Glomus intraradices, Glomus mosseae, Pisum sativum, Rhizobium leguminusarum*

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INTRODUCTION

The arbuscular mycorrhizal (AM) association between fungi and higher plants and N_2 fixing interaction between *Rhizobium* and legumes are the two most commonly studied symbioses. Microbes use photosynthetic products of the host plant and they supply it with P or N representing limiting factors in the soil. Phosphorus plays an important role in the plant energy metabolism and particularly in N_2 fixation as an energy requiring process (Dilworth, 1974). The processes of ammonium assimilation and the subsequent transformation into amino acids and ureides which occur in plant cell fraction of nodules are energy-consuming. Therefore, the way in which P deficiency or excess affect nodule ammonium metabolism and energy status is relevant to its function.

The AM fungi associated with legumes are responsible for adequate P nutrition. Increased P assimilation influence positively nitrogenase activity that in turn promotes root and mycorrhizal growth. The conducive effect of dual inoculation of roots with AM fungi and *Rhizobium* on growth, nutrient uptake and N_2 fixation in soybean (Bethlenfalvay et al., 1990), cowpea (Islam et al., 1990), and pea (Xavier and Germida, 2003) has been established. Olivera et al. (2004) reported that P application increased leaf area, plant dry biomass, nodule biomass, and shoot and root P content in common bean plants.

The present study aimed to assess the effect of tripartite symbiosis (pea plants, two cultures of AM fungi and *Rh. leguminosarum* bv. *Viciae*,) on nodule formation, N_2 fixation, percentage of colonization, tissue P and N content and growth under low P supply.

MATERIALS AND METHODS

AM fungi, *Glomus mosseae* and *Glomus intraradices*, originated from Spain, were received from the N. Poushkarov Institute of Soil Science, Sofia. Cultures were in the form of sand and soil (3:1) mixtures containing spores, hyphae and infected mycorrhizal root segments of *Avena sativa*. *Rhizobium leguminosarum* bv. *Viceae* strain D293 contained approximately 10^8 colony forming units per ml.

Pea plants (*Pisum sativum L.*) cv. Avola were grown in a glasshouse until flowering stage (35 days) in 4 kg plastic pots (three plants per pot) filled with leached cinnamonic forest soil (Chromic Luvisols – FAO) with the following agrochemical characteristics: pH (H_2O) – 6.2, total mobile N - 0.008 g kg^{-1}, P_2O_5 - 0.03 g kg^{-1}, K_2O - 0.120 g kg^{-1}. Mineral salts were applied prior to seeding: $Ca(NO_3)_2$ - 0.07g kg soil^{-1}; KH_2PO_4 - 0.13g kg soil^{-1}; $MgSO_4$ - 0.2 g kg soil^{-1}. Inoculation with AM fungi was done during seeding by the layering method (Jackson et al., 1972). Broth culture of *Rhizobium* inoculum was pipetted over the seeds (2 ml/seed). Water was added to make up about 60 % of water-holding capacity (WHC of soil – 30%).

The following scheme was used: 1) control plants; 2) plants inoculated with *Rh. leguminosarum* bv. *Viciae*; 3) plants inoculated with *G. mosseae*; 4) dual inoculation with *Rh. leguminosarum* bv. *Viciae* and *G. mosseae*; 5) plants inoculated with *G. intraradices*; 6) dual inoculation with *Rh. leguminosarum* bv. *Viciae* + *G. intraradices*. Each variant consisted of three replications.

Mineral soil nitrogen was determined spectrophotometrically after Kjeldal digestion, and assimilated P and K were measured according to the acetate – lactate method (Ivanov, 1984). Nitrogen fixation activity of root nodules was assayed by the acetylene reduction technique (ARA) according to Hardy et al. (1973). Total N content was measured after Kjeldal digestion. Total P content was determined according to Lowry and Lopez (1946).

Results are 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.

RESULTS AND DISCUSSION

The supply of pea plants with 60 mg P_2O_5 kg soil^{-1} specifically changed nodule number, nodule fresh weight, ARA and plant DW under conditions of tripartite symbiosis between pea, *Rhizobium* bacteria and *Glomus* sp. AM fungi (Fig.1, Fig. 2). Priority of dual inoculation with *G. mosseae* and *Rhizobium* over the other treatments regarding nodule number and biomass, ARA (Fig.1) and plant dry biomass (Fig.2) was observed. Better compatibility between *Rhizobium* and *G. mosseae* was obtained at P content of 60 mg P_2O_5 kg soil^{-1} while *Rhizobium* and *G. intraradices* appeared to be a less compatible pair under the described conditions.

Specific compatibilities between AM fungi and *Rhizobium* have been reported in cowpea (Thiagarajan et al., 1992) and pea plants (Xavier and Germida, 2003). Jia and Gray (2004) reported that inoculation with AM fungi promoted biomass production and influenced positively photosynthetic rate in *Vicia faba* due to the enhanced P supply resulting from AM inoculation. Studies with several legumes have consistently shown a positive response to P application – plant DW and N content were found to increase (Pereira and Bliss, 1987).

The rate of mycorrhizal colonization in control plants and in plants inoculated with *Rh. leguminosarum* only, was low and could be due to natural diversity of AM fungi presented in soil (Fig.2). A higher number of AM structures (compared to plants inoculated only with N_2 fixing bacteria and the controls) developed as a result of colonization with *G. mosseae* and *G. intraradices* separately or in combination with *Rh. leguminosarum*. Mycorrhizal status was best manifested in plants submit-

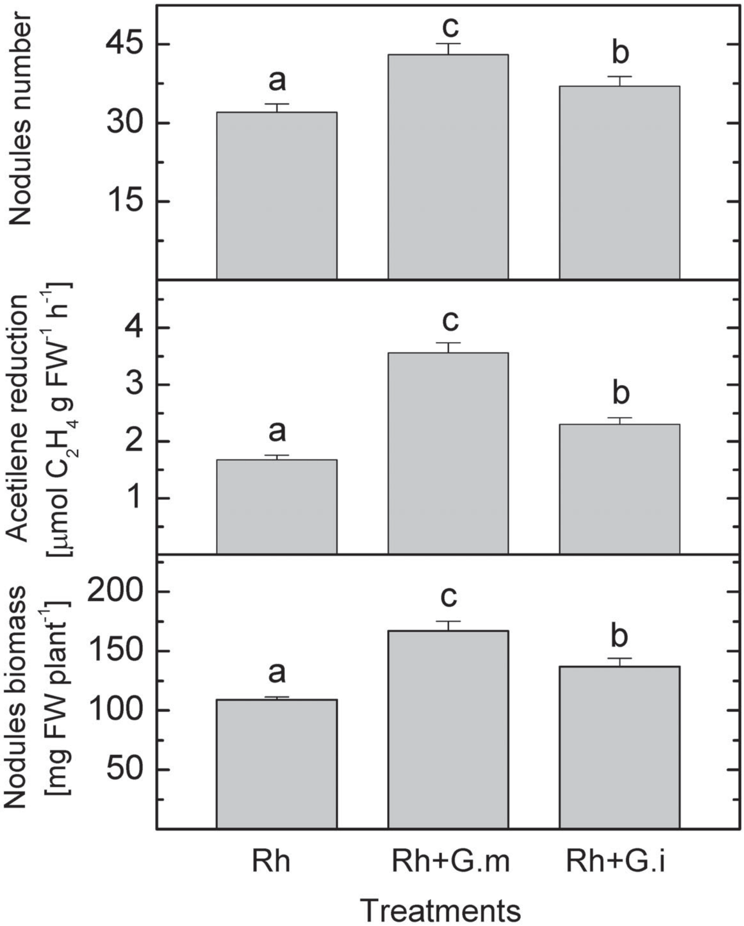

Figure 1. Nodule number, biomass and nitrogenase activity in inoculated pea plants at low phosphorus level (60mg P_2O_5 kg $soil^{-1}$). Values are means ± SE, *n* = 3; different letters indicate significant differences assessed by Fisher LSD test (*P* d ≤ 0.05) after performing ANOVA multifactor analysis.

ted to dual inoculation with *Rh. leguminosarum* and *G. mosseae*, as it is obvious from N_2 fixing parameters (Fig.1).

Our data indicated that P content increased in plants inoculated solely with N_2 fixing bacteria or AM fungi and at dual inoculation with both microorganisms (Fig. 2). The highest total P content was observed in plants subjected to dual inoculation with *Rh. leguminosarum* and *G. mosseae*. Plant growth correlated positively with the total P concentration measured in tissues. Similarly, P absorption ability was reported to be strongly connected with dry matter production (Lynch et al., 1991). However, total N was not significantly influenced by the different treatments with

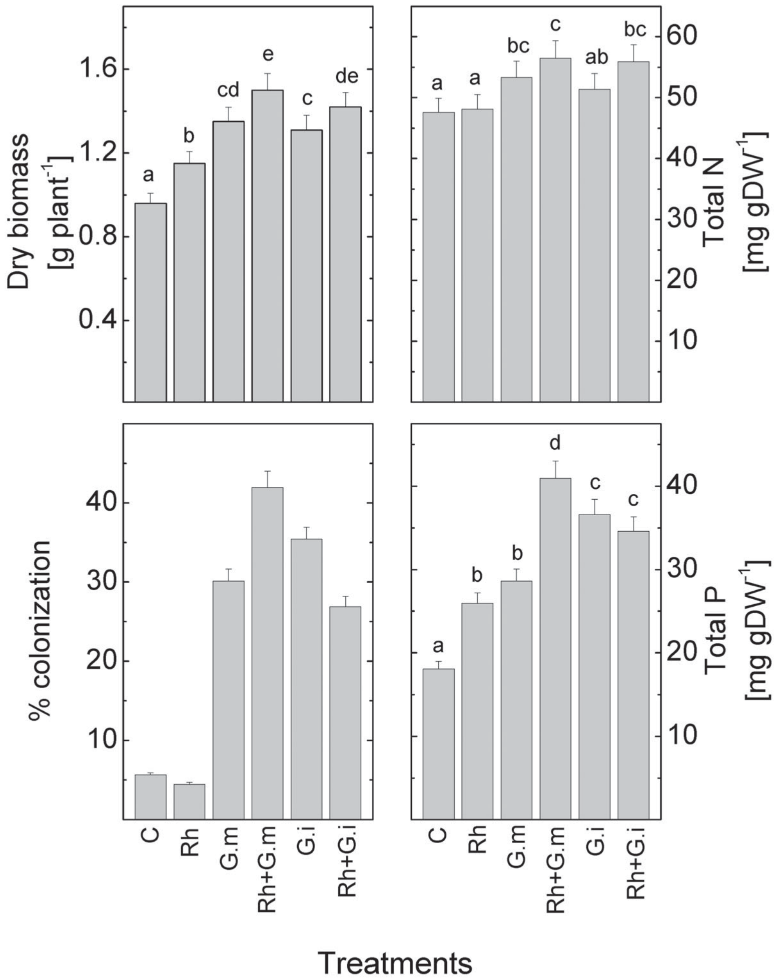

Figure 2. Arbuscular mycorrhizal colonization, plant dry biomass, total N and P content in inoculated pea plants at low phosphorus level (60mg P_2O_5 kg soil^{-1}). Values are means ± SE, $n = 3$; different letters indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA multifactor analysis

Rhizobium and AM fungi (Fig. 2). Total N content was slightly increased in plants submitted to dual inoculation with *Rh. leguminosarum* and *G. mosseae*. The absence of relationship between shoot N content and additional P levels in the nutrient medium was established by Olivera et al. (2004), which was confirmed by our results.

The present study demonstrated that dual inoculation of pea plants increased growth, mycorrhizal colonization rate, nodulation parameters, N_2 fixation activity and P content, compared to single inoculation of the host with *Rh. Leguminosarum* and depended on AM fungi species. The obtained results suggest that the dual inoculation of pea with *G. mosseae* in combination with *Rh. leguminosarum* is more competitive at low phosphorus supply than the coinoculation with *G. intraradices* and *Rh. leguminosarum*.

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