The endomycorrhizal fungi produce a highly branched hyphal structure within the plant cell. This infection creates an absorptive structure with a very high surface area of transfer for nutrients between the plant and the fungus. Mycorrhizal fungi hyphae secrete acid and alkaline phosphatases (APA and ALP) into the rhizosphere. It was established that APA activity increases in roots growing under P stress (Woolhouse 1975). Therefore, the regulation of these enzymes is critical to a plant’s survival in soils with limited P resources (Duff et al. 1991). There is extensive evidence for a decrease in the number of arbuscules under high external P (Bethlenfalvay et al. 1990, Smith and Smith 1996).

Phosphorus has a key role in the energy metabolism of all plant cells, and particularly in nitrogen fixation (Dilworth 1974). It was established that nodulating legumes require more P than legumes growing on mineral nitrogen (Al-Niemi et al. 1997). The AM fungi associated with legumes are an essential link for effective phosphorus nutrition, leading to enhanced nitrogen fixation that in turn promotes root and mycorrhizal growth. Synergistic effect of dual colonization of roots with AM fungi and Rhizobium on growth, nutrient uptake and nitrogen fixation in soybean (Bethlenfalvay et al. 1990) and pea (Xavier and Germida 2003) were reported. The effectiveness of the tripartite symbiosis – AM fungi, Rhizobium and plant, depends on the competition of the three symbionts for carbon (Jakobsen and Rosendahl 1990). Roots with AM fungi receive about 4–20% more photosynthates than comparable non-mycorrhizal roots (Smith and Reed 1997). Jakobsen and Rosendahl (1990) estimated that AM fungi could use up...
to 20% of the total fixed $^{14}$CO$_2$ in young plants. Concomitant development of mature functional nodules and effective AM infection depended on microsymbiont species and strains (Saxena et al. 1997).

The synchronization between the two symbiotic systems needs an optimal P level in the nutrient medium to stimulate the nodulation and nitrogen fixation and not to slow down the formation of effective mycorrhizal associates.

As the information presented in the studies in this area is insufficient, the objective of our study was to evaluate the effectiveness of triple symbioses – pea plants, AM fungi (Glomus mosseae and Glomus intraradices) and Rhizobium leguminosarum bv. viceae on the nitrogen and phosphorus assimilation at low and higher soil phosphorus levels.

**MATERIAL AND METHODS**

Pea plants (Pisum sativum L.) cv. Avola were grown in a glasshouse until the flowering stage (35 days), in 4 kg plastic pots (3 plants per pot) using leached cinnamonic forest soil (Chromic Luvisols – FAO) with the following agrochemical characteristics: pH(H$_2$O) = 6.2, 8 mg/kg soil total mobile nitrogen (N-NO$_3^-$ + N-NH$_4^+$), 13.2 mg P/kg soil, 100 mg K/kg soil. Mineral nitrogen was determined spectrophotometrically after Kjeldahl digestion; assimilated P and K by the acetate – lactate method (Ivanov 1984).

Fertilizers were applied prior to the seeding as follows: 50 and 133 mg Ca(NO$_3^-$)$_2$/kg soil for the inoculated and non-inoculated variants, respectively; and 200 mg MgSO$_4$/kg soil. The low P1 level (13.2 mg P/kg soil) is a natural soil P reserve. The P2 level (33.6 mg P/kg soil) is achieved by adding 260 mg KH$_2$PO$_4$/kg soil. Water was added to make up about 60% of water-holding capacity (WHC).

Two cultures of arbuscular mycorrhizal (AM) fungi, Glomus mosseae and Glomus intraradices were used. Inoculation with AM fungi was done at seeding by the layering method (Jackson et al. 1972). The seeds were inoculated with the bacterial suspension of Rhizobium leguminosarum bv. viceae strain D 293 at approximately 10$^8$ cells per cm$^3$.

The following scheme was used: I. At low P1 level: 1. Control plants; 2. Plants inoculated with Rh. leguminosarum; 3. Plants inoculated with G. mosseae; 4. Plants inoculated with Rh. leguminosarum + G. mosseae; 5. Plants inoculated with G. intraradices; 6. Plants inoculated with Rh. leguminosarum + G. intraradices; II. At higher P2 level the variants 7–12 are the same as the variants at P1 level.

Nitrogen fixation activity of root nodules was assayed by the acetylene reduction assay (ARA, EC 1.7.99.2) immediately after harvesting according to Hardy et al. (1973). Acid phosphatase activity (APA, EC 3.1.3.2) was measured with a modification of the method of Tabatabai and Bremner (1969). Root tissue was homogenized with 0.1M sodium acetate buffer (pH 5.0). After centrifugation the supernatant was assayed for the enzyme activity by incubation in 5mM p-nitrophenyl phosphate and 0.1M sodium acetate buffer (pH 5.0). The reaction was stopped by the addition of 0.2M NaOH, and absorption was measured at 405 nm.

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**Figure 1.** Nodules number, biomass and nitrogenase activity in inoculated pea plants at low (P1) and higher (P2) phosphorus levels; FW – fresh weight; values are means ± SE, n = 3; different letters indicate significant differences assessed by Fisher LSD test (P ≤ 0.05) after performing ANOVA multifactor analysis.
The rate of photosynthesis was measured under conditions of natural irradiance with portable photosynthetic equipment Li-Cor 6000 (USA), equipped with a leaf chamber. The total N content was measured after Kjeldahl digestion. Total tissue P content was determined according to Lowry and Lopez (1946). The rate of mycorrhiza infection of the roots was determined microscopically (Giovanetti and Mosse 1980).

Data are expressed as means ± SE 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

Pea plants inoculated only with \textit{Rh. leguminosarum} and supplied with elevated P levels exhibited a significant increase in the nodule number and fresh biomass and its nitrogen fixing activity, respectively (Figure 1). Moreover, promotion in plant dry biomass and photosynthetic rate at elevated phosphorus level was observed (Table 1). At low P1 level without additional P application, coinoculation with \textit{Rhizobium} and \textit{Glomus mosseae} resulted in the statistically highest levels of nodule number, nodule fresh weight and ARA (Figure 1), plant dry biomass and photosynthesis (Table 1) as compared to the variants with single \textit{Rhizobium} inoculation and with dual inoculation with \textit{Rhizobium} and \textit{Glomus intraradices}. At the higher P2 level supplied a change of priority was observed: the statistically most significant and highest nodule number, maximal nodule fresh biomass and ARA values (Figure 1), plant dry biomass and photosynthesis (Table 1) were obtained for treatments inoculated with \textit{Rh. leguminosarum} and \textit{G. intraradices}. Therefore a better compatibility between \textit{Rh. leguminosarum} and \textit{G. mosseae} was obtained at variants with P1 level while \textit{Rh. leguminosarum} and \textit{G. intraradices} appeared to be more compatible pairings at variants with P2 level. An increase of parameters that quantified the \( \text{N}_2 \)-fixing organ – nodule number and dry weight, as well as specific ARA activity with an increase of P applied was observed also in common bean by Olivera et al. (2004). Jia et al. (2004) reported that inoculation with AM fungi promoted biomass production and photosynthetic rates in \textit{Vicia faba} because of the enhanced P supply due to AM fungi inoculation. Some authors (Saxena et al. 1997) reported that tripartite symbiosis be-

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total N (mg/plant)</th>
<th>Total P (mg/plant)</th>
<th>Plant dry biomass (g/plant)</th>
<th>Photosynthesis (mg CO(_2)/m(^2)/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. control plants</td>
<td>( a^{17.40 \pm 0.90} )</td>
<td>( b^{5.87 \pm 0.29} )</td>
<td>( b^{0.83 \pm 0.04} )</td>
<td>( b^{13.08 \pm 0.65} )</td>
</tr>
<tr>
<td>2. \textit{Rh. leguminosarum}</td>
<td>( a^{22.15 \pm 1.11} )</td>
<td>( b^{12.39 \pm 0.62} )</td>
<td>( b^{0.97 \pm 0.05} )</td>
<td>( a^{11.57 \pm 0.58} )</td>
</tr>
<tr>
<td>3. \textit{G. mosseae}</td>
<td>( a^{27.81 \pm 1.39} )</td>
<td>( b^{14.88 \pm 0.74} )</td>
<td>( d^{1.27 \pm 0.06} )</td>
<td>( c^{16.52 \pm 0.83} )</td>
</tr>
<tr>
<td>4. \textit{G. mosseae} + \textit{Rh. leguminosarum}</td>
<td>( b^{35.90 \pm 1.80} )</td>
<td>( c^{20.46 \pm 1.03} )</td>
<td>( c^{1.34 \pm 0.06} )</td>
<td>( c^{15.88 \pm 0.79} )</td>
</tr>
<tr>
<td>5. \textit{G. intraradices}</td>
<td>( a^{26.26 \pm 1.31} )</td>
<td>( b^{11.05 \pm 0.55} )</td>
<td>( c^{1.15 \pm 0.06} )</td>
<td>( a^{10.92 \pm 0.55} )</td>
</tr>
<tr>
<td>6. \textit{G. intraradices} + \textit{Rh. leguminosarum}</td>
<td>( b^{32.00 \pm 1.60} )</td>
<td>( d^{16.96 \pm 0.85} )</td>
<td>( c^{1.20 \pm 0.06} )</td>
<td>( b^{13.04 \pm 0.65} )</td>
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<tr>
<td><strong>P2</strong></td>
<td></td>
<td></td>
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<tr>
<td>7. control plants</td>
<td>( a^{28.80 \pm 1.44} )</td>
<td>( b^{22.67 \pm 1.13} )</td>
<td>( a^{1.19 \pm 0.06} )</td>
<td>( a^{17.10 \pm 0.86} )</td>
</tr>
<tr>
<td>8. \textit{Rh. leguminosarum}</td>
<td>( a^{34.18 \pm 1.71} )</td>
<td>( b^{26.38 \pm 1.32} )</td>
<td>( b^{1.33 \pm 0.07} )</td>
<td>( a^{18.58 \pm 0.93} )</td>
</tr>
<tr>
<td>9. \textit{G. mosseae}</td>
<td>( a^{35.47 \pm 1.77} )</td>
<td>( b^{33.33 \pm 1.67} )</td>
<td>( b^{1.45 \pm 0.07} )</td>
<td>( b^{21.29 \pm 1.06} )</td>
</tr>
<tr>
<td>10. \textit{G. mosseae} + \textit{Rh. leguminosarum}</td>
<td>( b^{42.53 \pm 2.13} )</td>
<td>( d^{43.91 \pm 2.20} )</td>
<td>( c^{1.54 \pm 0.08} )</td>
<td>( c^{27.46 \pm 1.37} )</td>
</tr>
<tr>
<td>11. \textit{G. intraradices}</td>
<td>( b^{40.92 \pm 2.05} )</td>
<td>( c^{38.36 \pm 1.92} )</td>
<td>( c^{1.52 \pm 0.08} )</td>
<td>( c^{27.89 \pm 1.39} )</td>
</tr>
<tr>
<td>12. \textit{G. intraradices} + \textit{Rh. leguminosarum}</td>
<td>( c^{52.42 \pm 2.62} )</td>
<td>( d^{60.10 \pm 3.02} )</td>
<td>( d^{1.80 \pm 0.09} )</td>
<td>( d^{31.78 \pm 1.59} )</td>
</tr>
</tbody>
</table>

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.

Table 1. Total N and P content, plant dry biomass and photosynthetic rate in inoculated pea plants

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Figure 2. Arbuscular mycorrhizal (AM) colonization in inoculated pea plants at low (P1) and higher (P2) phosphorus levels; values are means ± SE, n = 3; different letters indicate significant differences assessed by Fisher LSD test (P ≤ 0.05) after performing ANOVA multifactor analysis

Figure 3. Root acid phosphatase activity in inoculated pea plants at P2 phosphorus level

Treatments: 1. control plants; 2. plants inoculated with Rh. leguminosarum; 3. plants inoculated with G. mosseae; 4. plants inoculated with Rh. leguminosarum + G. mosseae; 5. plants inoculated with G. intraradices; 6. plants inoculated with Rh. leguminosarum + G. intraradices. FW – fresh weight; values are means ± SE, n = 3; different letters indicate significant differences assessed by Fisher LSD test (P ≤ 0.05) after performing ANOVA multifactor analysis
absence of the relationship between shoot N content and elevated P levels in the nutrient medium was established by Olivera et al. (2004), which is in agreement with our results. The obtained results indicated that the highest total P content at 39.6 mg P/kg soil was observed in the variants with dual inoculation with \textit{Rh. leguminosarum} and \textit{G. mosseae}. The maximal P concentration in the variants with phosphates application is a result of the enhanced P uptake despite the reduction in mycorrhizal colonization and acid phosphatase activity.

The present study demonstrated that the dual inoculation of pea plants increased growth, photosynthetic rate, nodule parameters, nitrogen fixation activity and phosphorus content to different levels over single inoculation with \textit{Rh. leguminosarum} and depended on the level of phosphorus in the soil. The efficiency of coinoculation with \textit{Rhizobium} and \textit{G. mosseae} was higher at 13.2 mg P/kg soil phosphorus level. A higher efficiency of coinoculation with \textit{G. intraradices} at 39.6 mg P/kg soil appeared to reveal parameters connected with nitrogen fixation, photosynthesis and total dry biomass, despite the lower percent of mycorrhizal colonization and lower phosphatase activity. The obtained results allow us to suggest that the dual inoculation of pea with \textit{G. mosseae} in combination with \textit{Rh. leguminosarum} is more competitive at low phosphorus level while coinoculation with \textit{G. intraradices} and \textit{Rh. leguminosarum} is more effective at higher P content. Therefore the usage of \textit{G. intraradices} is more appropriate at higher phosphorus levels, because of its favourable effect on the biological nitrogen fixation and plant dry biomass.

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