Phosphorus Efficiency of Tagetes Plant Inoculated with Two Arbuscular Mycorrhizal Fungi Strains

Abou El Seoud, I.I.A.

Department of Soil and Agricultural Chemistry, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt.

Abstract: This research aimed to study P efficiency of tagetes plants (Tagetes patula nana, Yellow jacket) without and with two mycorrhizal strains (Glomus intraradices, M49 and M301) and to quantify the contribution of root growth and hyphae length in P uptake by tagetes plant. Plants were grown in potted soil with two levels of available P in soil (40 (low P) and 270 (high P) mg P / kg soil) in glasshouse with five replicates in randomized complete block design. Tagetes plants inoculated with mycorrhizal strains attained more than 80% of its maximum yield at the low level of P supply, (tagetes inoculated with mycorrhizal strains M49 and M301 attained 97% and 86% of its maximum yield respectively). In contrast, at low level of P supply, tagetes without mycorrhizae (NM) attained only 67% of their highest yield. This signified that tagetes inoculated with both mycorrhizal strains had high P efficiency, whilst tagetes without mycorrhizal fungi had low P efficiency. The shoot P concentration at which tagetes inoculated with mycorrhizal strains 49 and 301 attained its maximum yield were low, being only 1/2 of that observed for tagetes without mycorrhizae. Therefore, tagetes inoculated with mycorrhizal strains had high P use efficiency, whereas tagetes without mycorrhizal fungi had low P use efficiency. A significant correlation (R²= 0.95, p< 0.05) between hyphae length and shoot dry matter under limited P supply indicated that mycorrhizal hyphae length contribute to the acquisition of P from the soil. The mycorrhizal hyphae length of strain 49 was significantly longer than the other strain M301 at low P level. Then P uptake efficiency increased in the order of tagetes inoculated with M49 > M301 > NM fungi. A highly significant and positive correlation was found between P uptake and hyphae length at limited P supply (R²= 0.92, p< 0.05). This relation could explain the contribution of mycorrhizal hyphae to P uptake. Also, based on the results of the present study, we can concluded that the plants inoculated with mycorrhizal fungi had a highly P efficiency comparing to plants without mycorrhizal fungi.

Key words: Tagetes, mycorrhizal strains, phosphorus efficiency, root length, hyphae length.

INTRODUCTION

Phosphorus is a limiting element for plant growth particularly in low P soil of high Fe/Al or Ca contents, where P is strongly bound and largely unavailable for plant uptake (Marschner, 1995). Phosphorus efficiency can be generally defined as the ability of a crop plant to produce high yield in a soil (or other media) that is limiting in phosphorus supply (Gourley et al., 1994). Also, may be defined as the ability of a plant to produce a certain percentage of its maximum yield (80% of maximum yield) at low level of soil P (Föhse et al., 1988). Phosphorus efficiency can arise in two ways: I) the efficiency with which P is utilized to produce yield, i.e. the amount of P needed in the plant to produce one unit of dry matter. This is often called P utilization efficiency or internal P requirement and it is the P concentration in the plant to produce a given percentage of its maximum yield (for example 80 – 90% of maximum yield) (Föhse et al., 1988; and Bhadoria et al., 2002). II) The P uptake efficiency of the plant is the ability of the root system to acquire P from the soil and accumulate it in the shoots (Bhadoria et al., 2002). The most realistic solution according to Marschner (1995), is the use of mycorrhizal fungi that have the ability to acquire P and give high yield under limited P supply. Mycorrhizae are the most widespread association between microorganisms and roots of higher plants. Several studies confirmed the contribution of mycorrhizae to P uptake efficiency. The hyphae of the fungus have the radius approximately 1.5 μm (Tinker et al., 1992) and large surface area, leading to an increase in P absorbing
surface area, the production of organic acids and phosphatase which catalyze the release of P from organic complexes (Koide and Kabir, 2000; and Wang et al., 2004). Enhancement of P uptake by mycorrhizal hyphae can also be indirectly attributed to the faster uptake rate of P by the hyphae and the disturbance of the solution solid P equilibrium, which will increase the sorption of absorbed phosphate into soil solution (Nye and Tinker, 1977). Plants with coarsely branched roots and with few or no root hairs are expected to be more dependent on mycorrhizal fungi than are plants with finely branched root systems (Smith and Read, 1997). Therefore, the selected plant which used in present study (tagetes) is dependent on mycorrhizal fungi to acquire P from P-limited soil, whereas tagetes produce extremely short and stubby root hairs. That is help to study the contribution of mycorrhizal hyphae in P acquisition from the soil without any overlapping for root hairs in the total P uptake by plant. The mycorrhizal strains differ in the P efficiency under the same condition and the same plant.

This research was, thus, aimed to study phosphorus efficiency of tagetes plant grown in soil which inoculated with two mycorrhizal strains (M49 and M301) under two levels of available P in soil.

MATERIALS AND METHODS

Soil Preparation:
The soil used in this work was collected from El-Banger, Alexandria, Egypt from a depth of about 40 – 120 cm. The soil was air-dried, sieved through a 2 mm sieve to homogenize and separate roots from soil. Main soil physical and chemical properties are shown in Table (1).

<table>
<thead>
<tr>
<th>Sand %</th>
<th>Silt %</th>
<th>Clay %</th>
<th>Soil texture</th>
<th>pH</th>
<th>EC dSm⁻¹</th>
<th>OM %</th>
<th>CaCO₃ %</th>
<th>N⁺⁺ mg/kg</th>
<th>P⁺⁺ mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>19</td>
<td>24</td>
<td>Sandy clay loam</td>
<td>8.2</td>
<td>2.1</td>
<td>0.2</td>
<td>30.8</td>
<td>9.52</td>
<td>5.61</td>
</tr>
</tbody>
</table>

(1) Available N and P

Basal applications of N, K and Mg fertilizer were corporate with each kg soil at a rate of 150 mg N as NH₄NO₃, 150 mg K as K₂SO₄, and 40 mg of Mg as MgSO₄ per kg soil. Two rates of phosphorus fertilizer were applied to obtain low and high levels of available P (40 and 270 mg P/kg soil) as determined by using sodium bicarbonate method (Olsen et al., 1954).

Pot Experiment:
Each plastic pot (Ø = 9 cm) was uniformly filled with 280 g of the prepared soil and compacted to the bulk density of about 1.4 g cm⁻³. One week before planting, all pots were watered to the volumetric moisture content 0.25 cm cm⁻³, which correspond to the field capacity. Two strains of mycorrhizae (Clomus intraradices), 49 and 301 were used in this experiment. The mycorrhizal strains were obtained from Hanover University, Germany. The soil was mixed with 20 ml mycorrhizal inoculum one week before planting. Also, another inoculum (10 ml) was added in the hole before transplanting the seedlings.

The tagetes cultivar (Tagetes patula nana, Yellow jacket) was used as indicator crop. Uniform seedlings of tagetes each having two pairs of fully grown leaves, were selected and transplanted into the pots at the rate of one seedling per pot. The N fertilizer (in the form of ammonium nitrate NH₄NO₃ (35%)) was applied in splits as required in the time course for plant growth at the rate of 50 mg/20 ml water for each pot.

The tagetes plants were harvested 57 days after transplanting. At harvest, shoots were separated from roots. The shoots were then dried at 70°C/48 hours (Steyn, 1959) to constant weight in a forced-draft oven for 48 hours and then weighed and milled for analysis.

Phosphorus was extracted from soil samples at harvest by sodium bicarbonate (0.5N) method according to (Olsen et al., 1954) and determined by ascorbic acid – molybdenum blue method at wavelength of 406 nm as described by Murphy and Riley (1962). For determination of P in plants, plant material was digested with H₂SO₄ – H₂O₂ (Lownther, 1980). Total phosphorus was estimated by vanadomolybdophosphoric method (Jackson, 1967).

Quantifying Roots and Mycorrhizal Hyphae Length:
Root length was measured using the line intersect method according to Tennant (1975) method. Mean half distance between neighboring roots (r₁) was calculated according to the following formula (Schenk and Barber, 1979):
Where:
\[ V = \text{volume of the soil in the pot (cm}^3) \]
\[ RL = \text{root length per pot (cm)} \]

The length of mycorrhizal hyphae in the soil was measured using the grid interception method after collecting the hyphae on nitrate cellulose filters with grid (Brundrett et al., 1994).

Statistical Methods:
The treatments were arranged in a randomized complete block design and replicated five times. Data were analyzed by using analysis of variance in SAS (SAS institute INC. Cary, USA 1996). Tukey test was used to compare treatment means. Comparisons of different parameters were analyzed by regression analysis. A significance level of \( \alpha = 0.05 \) was used in all analysis.

RESULTS AND DISCUSSIONS

Shoot Yield and P Concentration:
At harvest, the shoot yield (g/plant) of the different treatments was compared. At low level of P supply, shoot yield of tagetes inoculated with mycorrhizae strain 49 was significantly increased in comparison with the uninoculated treatment (Figure 1). At high levels of available P, there was no significant difference between all treatments. On the other hand, the response of tagetes without mycorrhizae to increase of P supply was vigorous, whereas that of tagetes inoculated with mycorrhizal fungi was small. Similar results were observed in soybean (Sefik et al., 2006). Tagetes inoculated with mycorrhizal strains attained more than 80% of its maximum yield already at the lower level of available P, (tagetes inoculated with mycorrhizal strain 49 and 301 attained 97% and 86% of its maximum yield respectively). In contrast, at the same level of available P, tagetes without mycorrhizae attained 67% of their highest yield. This signified that tagetes inoculated with both mycorrhizal strains (49 and 301) had high P efficiency, whilst tagetes without mycorrhizal fungi had low P efficiency. Then tagetes inoculated with mycorrhizal fungi was tolerant to low available P in soil compared to tagetes without mycorrhizal fungi. This is in line with the suggestion of Cavagnaro et al. (2003) that, colonization by A-mycorrhizal fungi greatly enhanced host plant growth at low P concentration.

Fig. 1: Shoot dry matter (g/plant) of tagetes plant as affected by inoculation with different strains of mycorrhizae and P supply. (different letters indicate significant differences; small letters between P levels, capital letters between NM, M49 and M301, P < 0.05; d.m. = dry matter, NM = without mycorrhizal fungi, M49 = inoculated with mycorrhizal strain 49, M301 = inoculated with mycorrhizal strain 301).

Phosphorus concentration in plant is very important as an indicator of mycorrhizal activity. At low level of available P, shoot P concentration of tagetes inoculated with both mycorrhizal strains 49 and 301 were significantly increased than that of tagetes without mycorrhizal (Figure 2). That could be due to increase
acquisition of P by increasing the extension of depletion zone around the root. The mycorrhizal fungi extend a network of hyphae several centimeters out the surrounding soil, thereby expanding the effective volume of soil that plant can exploit (Frank, 2002). On the other hand, there was no significant difference between both mycorrhizal strains (Figure 2). In contrast, at high level of P supply, there was no significant difference between all treatments. That could be due to high available P in soil which inhibits both spore germination and early hyphae growth (Miranda and Harris, 1994). That could be attributed to increase phospholipids levels, which decrease membrane permeability and reduce exudation of organic acids, amino acids, and sugars, which are the source of food for growth and development of germinating mycorrhizal spores (Ratnayake et al., 1978). Therefore, the role of mycorrhizal fungi at high available P is limited. The shoot P concentration of all treatments increased significantly with increasing available P. The P concentration in shoot of tageotes without mycorrhizae at the higher P level was about 2.3-fold higher than at the lower P level. However, when tageotes was inoculated with mycorrhizal strains 49 and 301, the concentration in shoot at the higher P level was about 1.8 and 1.9-fold respectively higher only than at the lower P level. The same results were obtained for Spanish (Föhse et al., 1988).

The shoot P concentration at which tageotes inoculated with mycorrhizal strain 49 achieved more than 80% of its maximum yield amounted to 1.682 mg P/g d.m.. Tagentes inoculated with mycorrhizal strain 301 attained 80% of its maximum yield at about 1.552 mg P/g d.m. In contrast, tagentes without mycorrhizal fungi attained its highest yield at the relatively higher shoot P concentration of 2.824 mg P/g d.m.. The shoot P concentration at which tagentes inoculated with mycorrhizal strains 49 and 301 attained its maximum yield were low, being only 1/2\(^{th}\) of that observed for tagentes without mycorrhizal fungi (Figure 2). Therefore, based on the conventional P use efficiency definition, tagentes inoculated with mycorrhizal strains 49 and 301 had high P use efficiency, whereas tagentes without mycorrhizal fungi had low P use efficiency.

Tagentes plant inoculated with mycorrhizal strain 49 required the low level of P to obtain 97% of the maximum yield, where the tagentes plant which inoculated with mycorrhizal strain 301 obtained 86% only of the maximum yield at the same P level. That may be influenced by root growth and/or mycorrhizal hyphae length.

**Root Growth and Hyphae Length:**

**Root Growth:**

At low P level, only the root growth of tagentes inoculated with mycorrhizal strain 49 increased significantly than tagentes without mycorrhizae. Similarly, Neumann and George (2005) reported that, the root length of tomato was increased with inoculation with mycorrhizal fungi at low available P level (Figure 3). At high level of available P, there was no significant difference between all treatments.

Phosphorus supply significantly increased root length of tagentes without mycorrhizal fungi (Figure 3). This is in line with Bloom et al., (1993) who reported that if the rhizosphere is poor in nutrients or too dry, root growth is slow. With improving rhizosphere conditions, root growth becomes more extensive. In contrast,
there was no significant difference between tagetes inoculated with mycorrhizal fungi at the two levels of P supply. In the same line, Powers et al., (2005) showed that, root length of tropical forests plants inoculated with arbuscular mycorrhizal fungi were not related to amount of soil nutrient especially phosphorus.

Root:Shoot
Ratio is an important factor in P uptake efficiency of plants. The root : shoot ratio of tagetes with and without mycorrhizal strains at the low level of P supply were significantly higher than that at high P level (Figure 4). In the same line, Bhadoria et al., (2002) reported that, phosphate shortage increased root/shoot ratio of wheat, because shoot growth was more reduced than development. Similar results were observed in lettuce (Buso and Biss, 1988), and maize (Gaume et al., 2001).

These results are in accordance with the proposition that plants grown in P limited soils allocate a greater proportion of assimilates to the root growth and tend to have more root length than shoot growth to improve P acquisition (Marschner, 1995; and Paterson et al., 1999). On the other hand at low P level, the root/shoot ratio of tagetes inoculated with mycorrhizal strain 49 decreased significantly than tagetes without mycorrhizal fungi. At high level of P supply, there was no significant difference between different treatments (Figure 4). In the same trend, Rohyadil et al., (2004) reported that the growth improvement by mycorrhizas often includes a decrease in root/shoot ratio.
Mycorrhizal Hyphae Length:

The mycorrhizal hyphae length of plants decreased significantly with increasing P levels in soil (figure 5). Similarly, Liu et al. (2000) found that, the quantity of extraradical hyphae produced in soil were greatest at the lowest P level, and the highest level of P application reduced extraradical hyphae length compared to the other P levels. On the other hand, the mycorrhizal hyphae length of strain 49 was significantly longer than the other strain (strain 301). In the same line, Jakobsen et al. (1992) reported that, the total length of the external hyphae differs much between AM species as well as spread of the hyphae in the soil and their uptake rate of P per unit hyphae length. That might be related to poor development and activity of the external hyphae, low hyphal transport rates, and poor solute interchange at the arbuscule-host root cell interface (Bethlenfalvay and Franson, 1989). In general, the high P uptake efficiency of tagetes plant inoculated with mycorrhizal strains was due to long hyphae length under limited P supply.

The relationship between shoot dry matter and mycorrhizal hyphae length was analyzed to determine the effect of hyphae length on the production of dry matter yield. A significant correlation ($R^2 = 0.95$, $p < 0.05$) between hyphae length and shoot dry matter under limited P supply indicated that mycorrhizal hyphae length contribute to the acquisition of P from the soil (Figure 6). The increase in plant growth by mycorrhizal association is largely due to increase absorption of nutrients from soil solution (Son and Smith, 1988). Also, the hyphal mycelium increases the total absorption surfaces of infected plants and thus improves its access of immobile elements in areas beyond the root's depletion zone.
Phosphorus Uptake:

At low available P level, phosphorus uptake (mg P/plant) of tagetes which inoculated with mycorrhizae strain 49 was highly significant increased compared to that of tagetes without mycorrhizae. The P uptake of tagetes with strain 49 was increased about 2.2 times compared to tagetes plant without mycorrhizae (Figure 7). In the same trend, many workers reported faster rate of P uptake in mycorrhizal plants than that in non-mycorrhizal roots (Bolan, 1991; Marschner, 1995; Manske et al., 2000; Dechassa, 2001; and Neumann and George, 2005).

![Figure 7: Phosphorus uptake (mg P/plant) of tagetes plant as affected by inoculation with different strains of mycorrhizae and P supply. (different letters indicate significant differences; small letters between P levels, capital letters between NM, M49 and M301, P ≤ 0.05; NM = without mycorrhizal fungi, M49 = inoculated with mycorrhizal strain 49, M301 = inoculated with mycorrhizal strain 301).](image)

P uptake of tagetes inoculated with mycorrhizal strain 49 was significantly higher than that of the other strain by about 25%. On the other hand, P uptake of tagetes inoculated with mycorrhizal strain 301 significantly increased than that of tagetes plants without mycorrhizal fungi by about 68% (Figure 7). The P uptake in shoot of tagetes inoculated with mycorrhizal strains (49 and 301) and without mycorrhizae was increased with increasing available P in soil. However, the response of P supply was small for tagetes inoculated with mycorrhizal strains compared to the response of tagetes without mycorrhizae.

The relationship between P uptake and mean half distance between roots (r₁ cm) was analyzed to investigate the effect of r₁ on P taken up by plants. A highly significant correlation (R²= 0.997, p<0.05) at low P level observed that the P uptake by plants could be explained by root length density (Figure 8). By other words, when the root length density increased (= decrease mean half density between roots), P uptake of plant also increased. Increasing in root length density may be one of the possible mechanisms of P efficiency in maize (Alves et al., 2001).
A highly significant and positive correlation was found between P uptake and hyphae length at limited P supply (R² = 0.92, p<0.05). This relation could explain the contribution of mycorrhizal hyphae to P uptake (figure 9). Manske et al., (2000) reported that external hyphae of mycorrhizal fungi, which were about 100 times finer than wheat roots and 10 times finer than root hairs, access sites normally not permeable by roots or root hairs, thus reducing the P diffusion distances and increasing the surface area for nutrient absorption. Also, the length of external hyphae of mycorrhizal fungi can be a good predictor of its relative ability to take up P (Jones et al., 1990).

REFERENCES


