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Phosphorus Inflow into Two Species of Clover Root with Different Morphology Colonized by AM Fungi

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ABSTRACT-The effects of arbuscular mycorrhizal (AM) fungi on growth and phosphorus (P) inflow into two species of clover plant with different root morphology were studied. The experiment was arranged as a randomized complete block design consisting of a 2×3×3 factorial combination of two clover species (*Trifolium alexandrinum* L. and *Trifolium pratense* L.), three mycorrhiza states (without mycorrhiza, *Glomus intraradices* and *Glomus mosseae*) and three harvests (20, 40 and 60 days after transplanting) with 4 replications. In this experiment, plant dry matter, root colonization and P uptake in terms of P inflow and mycorrhizal P response (MPR) were determined. Results showed that mycorrhizal growth response (MGR) of *T. alexandrinum* was greater than that of *T. pratense*. This was mainly attributed to the higher root length of *T. alexandrinum* which provides a greater surface area for colonization compared to *T. pratense*. The highest P inflow was observed during the first harvest period (0-20 d). In this harvest period and during the treatment with *T. alexandrinum*, P inflow into non-colonized roots, roots colonized by *G. mosseae* and *G. intraradices* were 1.9, 6.8 and 8.01 p*M* m^{-1} s^{-1}, respectively being 3.6 and 4.2 times greater than the control plants. The greater effect of *G. intraradices* compared to *G. mosseae* on increasing P inflow might be due to the superior ability of *G. intraradices* to spread into the soil and absorb more P beyond the P depletion zones around the roots and/or might be due to the higher intensity of arbuscules and intra-radical hyphae per unit length of colonized root. In conclusion, *T. alexandrinum* was more responsive to mycorrhizal colonization than *T. pratense* which, in turn, resulted in better P nutrition of *T. alexandrinum* colonized by *G. intraradices*.

Keywords: Clover, *G. intraradices*, *G. mosseae*, Mycorrhizal growth response, Phosphorus inflow

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INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are the most widely distributed type of mycorrhiza. They establish intimate associations with more than 80% of all plant species (35). It has been shown that mycorrhizal colonization can help plants to thrive in arid conditions (1, 2, 5), deter root pathogens (26), alleviate the effects of soil compaction on plant growth (21), increase soil aggregation (3,27, 39), and alleviate the effect of soil salinity on growth and nutrient uptake (4). The greatest beneficial effect of AM colonization on the growth of a host plant has been related to improved mineral nutrition, particularly P nutrition (35).

It is now well documented that mycorrhizal (M) plants absorb P and some other nutrients from soil more efficiently than non-mycorrhizal (NM) plants and that the external hyphae play key roles in effectively increasing the volume of soil available for the acquisition of these nutrients (17, 18, 19, 28, 29). Increased uptake of the nutrients is a direct effect of colonization and often leads to increasing plant growth when nutrients are limited. Differences in P content between M and NM plants have frequently been used in the assessment of mycorrhizal effects on the P supply of plants. Previous works have pointed out that this is a poor method of determining mycorrhizal contribution (14, 15), because the architecture of plant root systems is usually altered by mycorrhizal colonization (13) and this, in turn, influences the uptake characteristics of the roots. Comparison of P inflow (P uptake per unit length of root per unit time) into M and NM roots is more suitable for assessing the efficiency of P uptake by M plants (15, 29, 35). P inflow has previously measured into M and NM roots of *Allium cepa* and found to be larger 4 times on average than to NM roots (28). Such increases in P inflow into mycorrhizal roots have also been reported for subterranean clover (15, 38), and onion (28, 33). P inflow to mycorrhizal roots is influenced by many factors such as environmental conditions, type of AM fungi and host plant. For example, it was reported (38) that the increased P inflow into M clover (*Trifolium subterraneum* L.) roots was 5 times higher at high photon irradiance (450.0 μmol m^{-2} s^{-1}) than low photon irradiance (100 μmol m^{-2} s^{-1}). Similarly, it was found that low irradiance (250 μmol m^{-2} s^{-1}) decreased the flow of P into M onion (*Allium cepa* L.) roots, even in the presence of additional P (36). This reduction of P inflow might be due to the lower level of colonization, however, an effect of low irradiance on P uptake by hyphae or on the transport between the symbionts is also likely.

The primary advantage of P uptake by mycorrhizal hyphae as compared with roots is their ability to extend beyond the P-depletion zone surrounding the root. It was found that, by using a compartmented mesh system, the depletion of $NaHCO_3$-extractable P extended up to 11.7 cm (the length of the hyphal compartment) from the root surface of M white clover (*Trifolium repens* L.)\, but in the NM plants, the P depletion zone extended only about 1 cm from the root surface (18).

The results reported from different species of AM fungi under same experimental conditions indicated that mycorrhizal fungi differ significantly in their ability to spread into the soil and absorb P beyond the P depletion zones around the roots. The results clearly indicate that the ability of AM fungal hyphae to spread in the soil is an important

factor in improving the mineral nutrition of the host plant (14, 15). Researchers have found that hyphal density of *Scutellospora calospora* declined with increasing distance from the roots, whereas *Acaulospora laevis* had a constant hyphal density up to a distance of 11 cm from the roots at the final harvest. The superior ability of *A. laevis* to spread into soil beyond the P depletion zones resulted in a higher P inflow into mycorrhizal roots compared with the two other fungi. In another experiment, it was found that the ability of *A. laevis* to transport ^{32}P over soil-root distances longer than 1 cm (up to 7 cm) was greater than *Glomus sp*. and *Scutellospora calospora* (16). Despite the low hyphal length density of *S. calospora* in soil, this fungus accumulated more ^{32}P in its hyphae and failed to transport it to the host plant in comparison to the two other fungi.

A number morphological characteristics of roots such as root branching, total root length and root diameter may affect the uptake of less mobile nutrients like P and this, in turn, can affect mycorrhizal P and growth responses of the host plant as suggested (7, 11, 17). In our study, in a preliminary experiment, we found that root branching and total root length of berseem clover or Egyptian clover (*Trifolium alexandrinum* L.) are higher than those of red clover (*Trifolium pratense* L.). There is little information on the response of berseem clover to mycorrhizal colonization in comparison to subterranean clover (*Trifolium subterraneum* L.). To our knowledge there is no published report on how different root morphology of *Trifolium alexandrinum* and *Trifolium pratense* may affect P uptake in terms of P inflow and how this effect may interact with mycorrhizal colonization. Thus, the present study aimed at comparing P inflow into two species of clover roots (berseem and red clover) that differ in root morphology colonized by two species of AM fungi.

MATERIALS AND METHODS

A surface layer of a Typic Torrifluvent (USDA), Calcaric Fluvisols (FAO) soil with low P content was collected from a research field at Mollasani, Khuzestan province (south-western Iran). Some properties of the soil used in this study are shown in Table 1. A glasshouse experiment was arranged in a randomized complete block design consisting of a 2×3×3 factorial combination of two clover species: berseem or Egyptian clover (*Trifolium. alexandrinum* L.) and red clover (*Trifolium pratense* L.); three mycorrhiza states: *Glomus intraradices*, *Glomus mosseae* and without mycorrhiza and three harvests: 20,40 and 60 days after transplanting. The experiment had 4 replications. The soil was sieved and autoclaved at 121°C for 1 hour on two consecutive days. Seeds of two clover species were sterilized with NaOCl solution (5 g dm^{-3}) and germinated on moist filter papers at 23°C. Inoculum of *Glomus intraradices* and *Glomus mosseae* was obtained from pot cultures of *T. alexandrinum* grown for two months in a soil:sand mix (1:9) containing 10% dry inoculum from pot cultures of *Glomus intraradices* and *Glomus mosseae* Schenck & Smith (originally provided by NPI Utah). Six clover seedlings were transplanted into each pot. Each seedling was inoculated by placing enough dry inoculum in each planting hole. Plants were grown in a glasshouse under relatively controlled conditions. All pots received 10 ml nutrient solution weekly (22).

Table 1. Chemical and physical characteristics of the soil

pH	ECe ($dS\ m^{-1}$)	Available P ($mg\ kg^{-1}$ soil)	Organic C ($g\ kg^{-1}$ soil)	Mechanical composition (%)		
				Sand	Silt	Clay
7.6	2.8	3.2	3.1	25.2	33.4	41.4

Plants were harvested 20, 40 and 60 days after transplanting. At each harvest, roots from each pot were carefully washed free of soil and cut into 1 cm segments. Fresh weights of roots and shoots were recorded. Subsamples of roots were used to determine root dry weight and the percentage of root colonization using line intersection method (37) after staining with trypan blue (25). Shoot and root P concentrations were determined by the phosphovanado-molybdate method (23).

P inflow (P uptake per root length unit per time unit) into the roots during the three harvest periods (0-20, 20-40, 40-60 d) was calculated using equation 1 (9):

$$I = (P_2 - P_1) \times \ln (L_2 L_1^{-1})[(T_2 - T_1)(L_2 - L_1)]^{-1} \quad 1$$

where P refers to plant phosphorus content, T to plant age and L to total root length.

Mycorrhizal growth response (MGR) and Mycorrhizal P response (MPR) were calculated using equation 2 (6) and equation 3 (20), respectively.

$$\text{MGR} = \frac{\text{Shoot dry weight of M plants} - \text{Shoot dry weight of NM plants}}{\text{Shoot dry weight of NM plants}} \times 100 \quad 2$$

$$\text{MPR} = \frac{\text{P content of M plants} - \text{P content of NM plants}}{\text{P content of NM plants}} \times 100 \quad 3$$

P content equals plant P concentration× plant dry weight

Statistical analysis was performed using SAS statistical package (30). Significant statistical differences of all means were determined by using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The main effects of mycorrhizal colonization, harvest time and plant species on shoot dry weight of clover plants and the two- and three-way interactions of the main effect were found to be significant ($p \leq 0.05$). At the final harvest (60 days after transplanting), shoot dry weights of both species of M clover plants were significantly greater than those of the NM plants (Fig. 1A). No significant difference was observed between shoot dry weight of M plants and NM plants at the first harvest (result not shown). At the third harvest, no significant difference was found between the effect of *G. intraradices* and *G.*

mosseae on shoot dry weight of *T. alexandrinum*, while shoot dry weight of *T. pratense* colonized by *G. intraradices* was greater than that of *G. mosseae* (Fig. 1A). A trend similar to that of shoot dry weight was observed for total root length of clover plants with and without mycorrhizal fungi (Fig. 1B). Mycorrhizal growth response of *T. alexandrinum* was greater than that of *T. pratens* (Fig. 2).

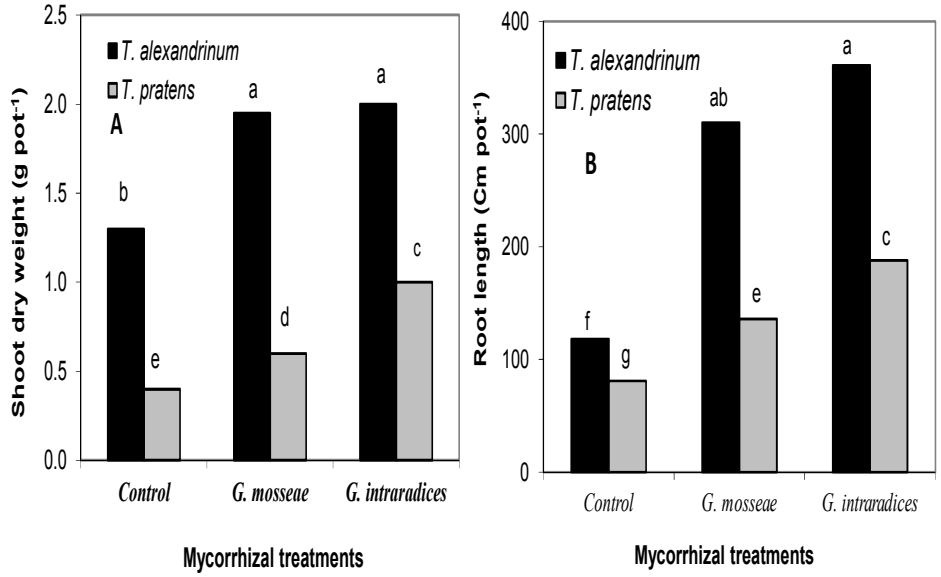

Fig. 1. Shoot dry weight (A) and total root length (B) of *T. alexandrinum* and *T. pratens*e colonized by *G. mosseae* and *G. intraradices* at the third harvest. Means with the same letters are not significantly different at 5% probability according to the Duncan's Multiple Range Test

Mycorrhizal dependency might be controlled by the physiological and morphological characteristics of the host plant roots and/or the AM fungus. It has been shown that plant species with coarse root systems and few root hairs are more responsive to mycorrhizal colonization than plant species with fine roots and dense root hairs (7, 10, 12, 17). Although the main difference between the two species of clover roots used in our study was total root length (not root diameter or root hair density), *T. pratense* (with lower total root length) was expected to have higher mycorrhizal dependency compared to *T. alexandrinum*. Attempt was made to detect relationships between root diameter of plant species in British flora and mycorrhizal colonization (11). The results indicated that plant species with coarse roots do emerge from this as being typically mycorrhizal, but plant species with fine roots had a wide range of colonization (8, 11), suggesting that other factors including physiological and ecological parameters may also influence mycorrhizal dependency.

The percentage of root length colonized by *G. intraradices* was significantly greater than that colonized by *G. mosseae* for both clover plants, although this increase was higher for *T. alexandrinum* in comparison to *T. pretense (*Fig. 3 A*)*.

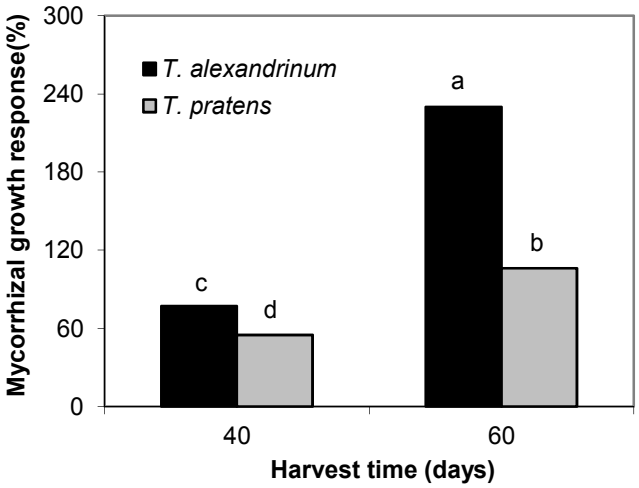

Fig. 2. Percentage of mycorrhizal growth response (MGR) of *T. alexandrinum* and *T. pratense* at 40 and 60 days after transplanting. Means with the same letters are not significantly different at 5% probability according to the Duncan's Multiple Range Test

The percentage of root colonization increased with increasing harvest time and this increase was greater for *T. alexandrinum* than for *T. pratense* at all harvest times (Fig. 3 B).This led to greater mycorrhizal growth response of *T. alexandrinum* in comparison to *T. pratense* (Fig. 2B). In most studies positive correlations between mycorrhizal growth response and root colonization have been reported (22, 31). A similar trend to that of the percentage of root colonization was observed for total root length colonized (result not shown).

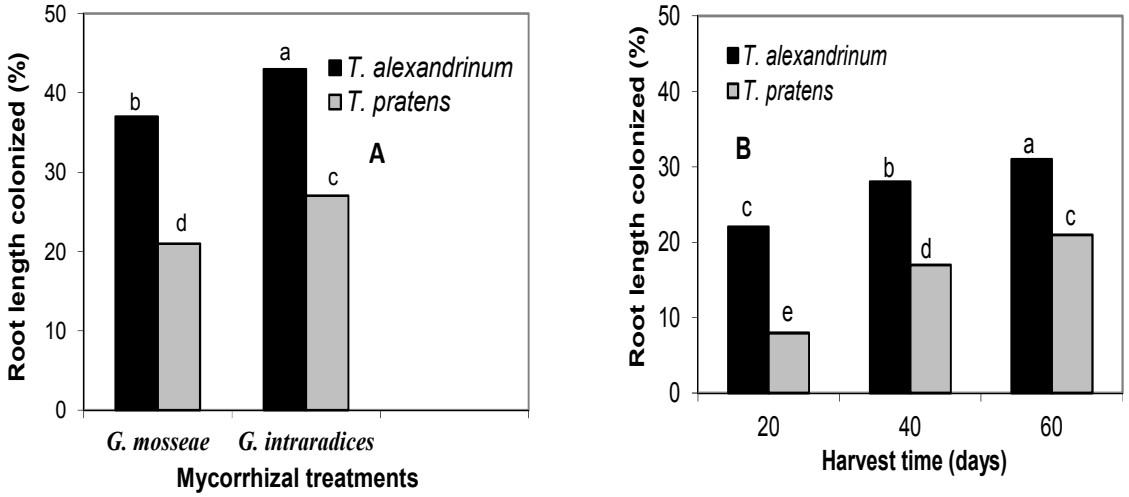

Fig. 3. Percentage of root colonization of clover plants colonized by *G. mosseae* and *G. intraradices* (A) at three harvest times (B). Means with the same letters are not significantly different at 5% probability according to the Duncan's Multiple Range Test

Shoot P concentrations of both species of M plants were greater than those of NM plants. A similar trend to that of shoot P concentration was observed for root P concentration of M and NM clover plants (result not shown).

The effect of mycorrhizal colonization on P uptake was determined in terms of (MPR). The result indicated that MPR (Fig. 4) followed a pattern similar to MGR (Fig. 2). Mycorrhizal P Response (MPR) of *T. alexandrinum* was greater than that of *T. pratens*. This was attributed, at least in part, to the higher root colonization of *T. alexandrinum* (Fig. 3). Mycorrhizal P Response (MPR) of both clover species increased as root colonization increased. This may explain why MGR increased as the plant aged.

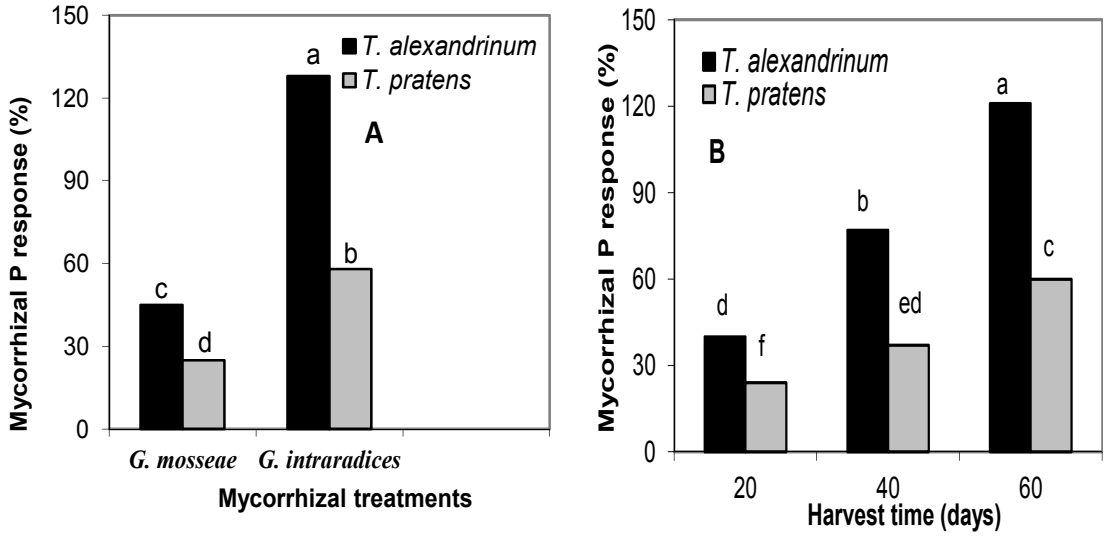

Fig. 4. Percentage of mycorrhizal P response (MPR) of *T. alexandrinum* and *T. pratense* colonized by *G. mosseae* and *G. intraradices* (A) at three harvest times (B). Means with the same letters are not significantly different at 5% probability according to the Duncan's Multiple Range Test.

The effect of mycorrhizal colonization on P uptake was also determined in terms of P inflow (P uptake per unit length of root per unit time). The comparison of P inflow into M and NM roots is more suitable for assessing the efficiency of P uptake by mycorrhizal plants (15, 28, 35). The result of our study indicated that P inflow was higher in both species of M plants than in NM plants (Fig. 5). During the treatment of *T. alexandrinum* and at the first harvest period (0-20 d), P inflow into non-colonized roots, roots colonized by *G. mosseae* and *G. intraradices* were 1.9, 6.8 and 8.01 pmole m^{-1} s^{-1}, respectively (Fig. 5). These were 3.6 and 4.2 times larger than the control plant, a finding which is similar to those reported previously (30). The higher P inflow into mycorrhizal root of *T. alexandrinum* led to the greater mycorrhizal growth response of *T. alexandrinum* compared to *T. pratens* (Fig. 2).

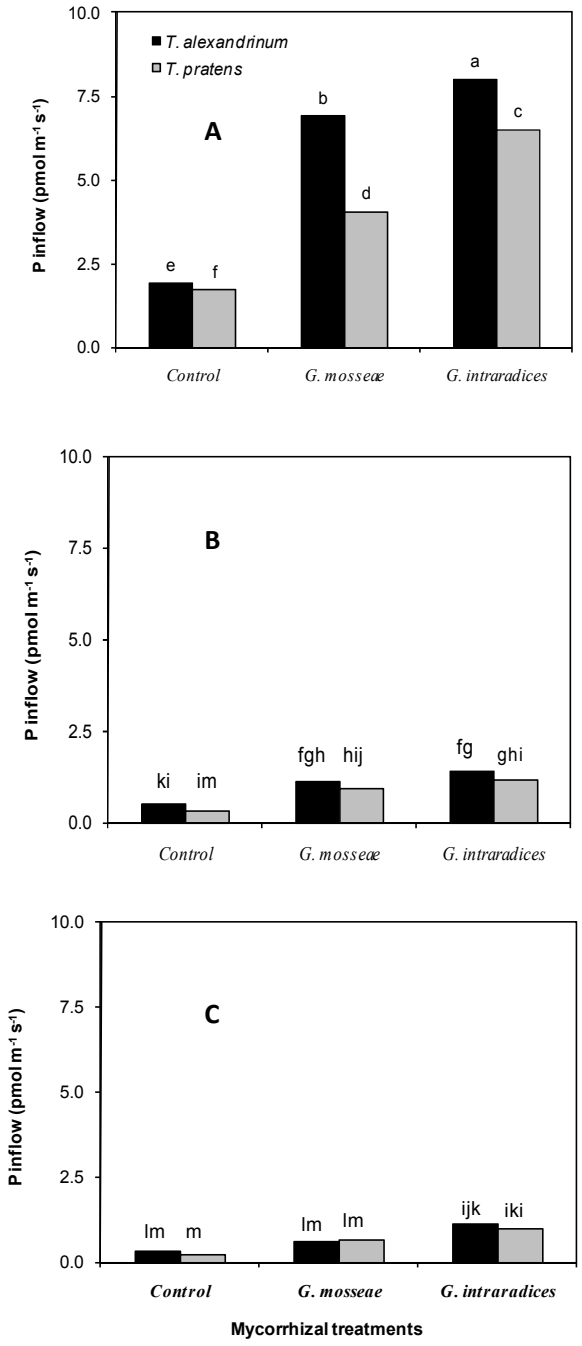

Fig. 5. P inflow into *T. alexandrinum* and *T. pratense* colonized by *G. mosseae* and *G. intraradices* at the first harvest (A), second harvest (B) and third harvest (C). Means with the same letters are not significantly different at 5% probability according to the Duncan's Multiple Range Test

Despite the low percentage of root length colonized, the inflow of P was higher at the first harvest than the second and third harvests for both species of clover. Similar declines in P inflow with plant age have also been reported elsewhere (15, 24, 33). Several factors may have contributed to these observations, although their relative

importance is not clear. One explanation for the higher P inflow at the first harvest might be the higher hyphal biomass per unit length of colonized root as shown earlier (21, 22). Another possible explanation is that the depletion of P from the soil is relatively small during early growth when the density of young roots is low and external hyphae may confer a greater advantage in terms of P inflow into colonized roots than later when rooting density and inter-root competition is greater. Moreover, it has been shown that in both *Trifolium subterrneum* and *Allium cepa*, the proportion of the root colonized by arbuscules declined as mycorrhizal roots aged (32) and this may influence the transfer of P from fungus to plant.

At the first harvest, P inflow into *T. alexandrinum* root was significantly higher than *T. pratense* colonized by both AM fungi (Fig. 5 A). This was attributed to the higher total root length colonization of *T. alexandrinum* in comparison to *T. pratense*. In our study, the frequency of arbuscules and intra-radical hyphae was not measured. However, another reason for the higher P inflow into mycorrhizal *T. alexandrinum* roots might be the higher frequency of arbuscules and intra-radical hyphae per unit root length colonized compared to *T. pratense.* The higher P inflow into *T. alexandrinum* root than *T. pratense* root (irrespective of mycorrhizal colonization) might be due, at least in part, to the high root length and high root proliferation of *T. alexandrinum*. Root length is one of the most important root characteristics which can influence the amount of P uptake from soil (22). Moreover, the type of root exudates, particularly carboxylates and phosphatases (17), expression of high-affinity and plasmamembrane-bound Pi transporters in roots (34) can also be involved in the higher P inflow into *T. alexandrinum* compared to *T. pratense* (Fig. 5 A).

The effect of *G. intraradices* on increasing P inflow was greater than *G. mosseae* (Fig. 5). With respect to previous findings regarding AM fungi have different abilities to spread into soil (15, 16), one possible reason for this observation might be the superior ability of *G. intraradices* (compared to *G. mosseae*) to spread into the soil and absorb more P beyond the P depletion zones around the roots. The production of external hyphae per unit length of colonized root might also be another reason for the higher P inflow in the treatment of *G. intraradices* as reported earlier for *Glomus intraradices, Glomus sp.* City Beach, *Glomus etunicatum* and *Glomus mosseae* (22).

CONCLUSION

The results of our study indicated that *T. alexandrinum* is more responsive to mycorrhizal colonization than *T. pratense*. This can be mainly attributed to the high root length of *T. alexandrinum* which provides a greater surface area for colonization compared with *T. pratense*. However, other physiological and ecological parameters might also be involved in the greater mycorrhizal dependency of *T. alexandrinum*. This resulted in better P nutrition of *T. alexandrinum* colonized by *G. intraradices* in terms of MPR and P inflow. Nevertheless, further research is needed to compare the development of internal colonization (intensity of arbuscules and intra-radical hyphae) and external colonization (length of external hyphae and hyphal biomass particularly per unit root length colonized) in relation to the ability of *G. intraradices* and *G. mosseae* to spread into soil.

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سرعت ورود فسفر به داخل ریشه دو گونه شبدر متفاوت در ریخت شناسی ریشه همزیست با قارچ های میکوریزا آربسکولار

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چکیده- اثر قارچهای میکوریزا آربسکولار بر رشد و سرعت ورود فسفر به داخل ریشه دو گونه شبدر متفاوت در ریخت شناسی ریشه مورد مطالعه قرار گرفت. این آزمایش گلدانی به صورت فاکتوریل در قالب طرح بلوکهای کامل تصادفی شامل دو گونه شبدر (*تریفولیوم الکساندرینوم* و *تریفولیوم پراتنس*)، سه وضعیت میکوریزا (بدون حضور میکوریزا، *گلوموس اینترارادیسز* و *گلوموس موسیه*) و سه زمان برداشت گیاه (۲۰، ۴۰ و ۶۰ روز بعد از کشت گیاهچه ها) در چهار تکرار به مرحله اجرا در آمد. در این مطالعه وزن ماده خشک گیاه، کلونیزاسیون ریشه و جذب فسفر بر حسب سرعت ورود آن به ریشه و پاسخ میکوریزایی آن اندازه گیری شد. نتایج نشان داد که پاسخ رشد میکوریزایی شبدر برسیم (*تریفولیوم الکساندرینوم*) به طور معنی داری از پاسخ رشد میکوریزایی شبدر قرمز (*تریفولیوم پراتنس*) بیشتر بود. این نتیجه عمدتاً به مجموع طول بیشتر شبدر برسیم که توانست سطح بیشتری را برای کلونیزاسیون فراهم کند نسبت داده شد. بیشترین سرعت ورود فسفر به گیاه در دوره اول برداشت گیاه (۲۰-۰ روز) مشاهده گردید. در این دوره برداشت و در تیمار شبدر برسیم (*تریفولیوم الکساندرینوم*)، سرعت ورود فسفر به داخل شبدر شاهد (بدون حضور میکوریزا) و شبدر کلنی شده با *گلوموس موسیه* و *گلوموس اینترارادیسز* به ترتیب برابر با ۱/۹، ۶/۸ و ۸/۰۱ پیکومول بر متر بر ثانیه بود که به ترتیب ۳/۶ و ۴/۲ مرتبه بیشتر از سرعت ورود فسفر به داخل گیاه شاهد بود. تاثیر بیشتر قارچ *گلوموس اینترارادیسز* در افزایش سرعت ورود فسفر ممکن است به توانایی بیشتر قارچ *گلوموس اینترارادیسز* در انتشار به داخل خاک و جذب بیشتر فسفر از منطقه دور تر از اطراف ریشه گیاه باشد و یا ممکن است به تراکم بیشتر آربسکول و ریسه های داخلی در واحد طول ریشه کلنی شده در مقایسه با *گلوموس موسیه* باشد. نتیجه گیری کلی اینکه، شبدر برسیم در مقایسه با شبدر قرمز پاسخ بیشتری به کلونیزاسیون میکوریزایی داشت و این به نوبه خود به بهبود بیشتر تغذیه فسفری شبدر برسیم کلنی شده با قارچ *گلوموس اینترارادیسز* منجر شد.

واژه های کلیدی: پاسخ رشد میکوریزایی، سرعت ورود فسفر ، شبدر، گلوموس اینترارادیسز، گلوموس موسیه

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