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Effects of phosphorus levels on dry matter production and root traits of chickpea plants in presence or absence of Arbuscular mycorrhizal fungi

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ABSTRACT

To determine how chickpea plant's respond to colonization by mycorrhizal fungi and phosphorus application relates to its ability to acquire and utilize phosphorus for growth, a factorial experiment was carried out based on a randomized completely design in pot culture. Four treatments (rates) of phosphorus fertilizer, the first factor, were considered including 2, 5, 10, 15 mg P kg^{-1} soil. For the second factor that is mycorrhizal fungus the three components were the two species *Glomus mosseae*, *G. intraradices* of mycorrhiza species, and the control (no inoculation with mycorrhizal fungus) arranged in three replications. Results showed that above ground dry matter of chickpea inoculated with the two *Glomus* species produced higher dry matter (mean 382.92 mg/plant), more root fresh, and dry weights (mean 2452.50 mg/plant and 192.50 mg/plant, respectively), longer roots (mean 25.96 cm), and root volume (mean 3.11 cm^3) than the control. Highest mycorrhizal colonization by *G. mosseae* and *G. intraradices* was obtained at 2mg P kg^{-1} soil; 49.88% and 41.81%, respectively. *G. mosseae* and *G. intraradices* had led to maximum leaf phosphorus contents that are 343.98 and 338.47 mg/100g of leaf dry weight, respectively, at 15 mg P kg^{-1} soil. All parameters measured were positively correlated (r = 0.60 to 0.72) with above ground dry matter. Although phosphorus applications showed to be increasing above-ground dry matter and root characteristics, our study clearly demonstrated that mycorrhizal fungi play an important role in the enhancement of growth of chickpea plants under very low phosphorus conditions.

Key words: Chickpea, dry matter, mycorrhizal fungi, phosphorus, plant roots.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a cool season grain legume with high nutritive value and serves as an important cheap source of protein in developing countries diet in addition to improving land fertility. It is the third leading grain legume in the world and first in the South Asia. Its range of cultivation extends from the Mediterranean basin to the Indian sub-continent and southward of Ethiopia and the East African highlands (Muehlbauer and Singh, 1987). Chickpea is the readily available source of protein (19.5%), fats (1.4%), carbohydrates (57 to 60%), ash (4.8%) and moisture (4.9 to15.59%) (Huisman and Van der Poel, 1994). It also helps in replenishment of soil fertility by fixing of atmospheric nitrogen through symbiosis coupled with deep root system.

Phosphorus is one of the essential mineral macronutrients, which is required for maximum yield of cultivated crops. Most of the essential plant nutrients, including phosphorus, remain in insoluble form in the soil (Abd-Alla, 1994; Yadav and Dadarwal, 1997).

Table 1. The characteristics of the soil used to growth the chickpea plants.

Saturation (%)	Electrical Conductivity (ds m^{-1})	pH	Organic carbon (%)	Phosphorus (mg kg^{-1})	potassium (mg kg^{-1})	Soil texture
29	1.3	7.4	0.20	2.0	85	Sandy loamy

Phosphorus is vital for plant growth, and is a component of the nucleic acid structure of plants and bio-membranes. Therefore, it is important in cell division and tissue development. Phosphorus is also involved in the energy metabolism of cells and is required for the biosynthesis of primary and secondary metabolites in plants. Consequently, plants have evolved a range of strategies to increase phosphorus uptake and mobility (Marschner, 1996).

The ubiquitous arbuscular mycorrhizal (AM) fungi are an integral component of any soil system where they form obligate symbiosis with the roots of over 80% terrestrial plant species (Vander and Sanders, 2002). AM fungi occur in nearly all natural and agricultural soils, and commonly colonize roots of many plant species (Smith and Read, 1997).This symbiosis is based on the beneficial exchange of reduced carbon from the plant and mineral nutrients, especially phosphate and nitrogen as well as water from the fungus (Smith and Read, 1997; Dreyer et al., 2010). Mycorrhizal symbiosis plays a significant role in the nutrition and development of host plants. AM fungi have been apparent to recover soil composition (Miller and Jastrow, 2000).

The mutualistic nature of AM symbioses relies on the ability of the fungal mycelium to take up mineral nutrients from the soil solution and to transfer them to the symbiotic roots in exchange for carbohydrates. Plant benefits from AM symbiosis are traditionally recognized as improved access to limiting soil resources, mainly immobile nutrients such as P, Cu, Zn and ammonium. On the other hand, the carbon supplied from the host plant to the fungus is essential to the formation and functioning of arbuscular mycorrhiza and for the completion of the fungal life cycle. Although the mutualism of the AM association is based on this bidirectional nutrient exchange, this does not necessarily mean that nutrient transfer from the fungus to the plant is directly linked to C transfer to the fungus. In fact, different AM fungi in symbiosis with the same host plant, can considerably differ in their C–P exchange ratio and, consequently, in their degree of functional compatibility with the plant (Pearson and Jakobsen, 1993). In mycorrhizal mungbean plants grain yield, biological yield, leaf phosphorus, leaf nitrogen, protein percentage, protein yield, harvest index of protein, and ecosystem water use efficiency were improved compared with the non-mycorrhizal plants. Two species of mycorrhiza, *G. mosseae* and *G. intraradices* significantly improved the yield (grain, protein) and reduced the water-deficit stress in the field (Habibzadeh et al. 2013). In legume crops, mycorrhizal fungi were found to increase the vegetative growth and seed yield (Lambert and Weidensaul, 1991; Mathur and Vyas, 2000). Mycorrhizal fungi such as *G. intraradices* and *G. mosseae* were reported to improve the performance of both blume *Nothofagus dombeyi* (Mirb.) and soybean [*Glycine max* (L.)Merr.] plants under drought conditions (Porcel and Ruiz-Lozano, 2004; Alvarez et al., 2009).

This experiment was conducted in a greenhouse condition to compare the effects of different phosphorus levels in association with AM fungi on dry matter production and root traits of chickpea plants with an aim to reduce the application of chemical fertilizer for sustainable system.

MATERIALS AND METHODS

A trial was conducted in a greenhouse at the agricultural research center of west Azarbaijan province, Urmia in Iran.The greenhouse located in longitude 37° 35' 32'' north, latitude 45° 3′39'' east and 1330 m altitude. Soil was collected from a low P (2 ppm Olsen extractable P) field in the region of Shaharchay River around Urmia. Some physicochemical properties of the soil used in the experiment to grow the chickpea plants were determined (Table 1). The greenhouse's day/night cycle was 16 h at 22°C and 8 h at 19°C. The relative humidity was maintained at 50 – 70%.

A factorial experiment arranged in a randomized completely design was carried out with three replications. Four phosphorus fertilization treatments in this study, that is 2, 5, 10, 15 mg P kg^{-1} soil (KH_2PO_4) composed the first factor and two mycorrhizal fungi inoculums (*G. mosseae* and *G. intraradices*) plus a control (zero inoculation of AM) the second factors. The fertilizers were incorporated into the soil by hand.

The two species of AM fungi used in this study were *G. mosseae* and *G. intraradices*, which were produced on maize (*Zea mays* L.) host plants by Dr. E.M. Goltapeh at Tarbiat Modarres University, Tehran, Iran. The mycorrhizal inoculum was a mixture of sterile sand, mycorrhizal hyphae, spores (20 spores g^{-1} inoculums), and colonized root fragments. Seeds of the chickpea cultivar Gazvin were provided by the Agricultural

Table 2. Mean squares traits of chickpea affected by mycorrhizal infection under different levels of phosphorus.

S.O.V.	Df	Mycorrhizae Colonization	Above-ground dry matter	Leaf phosphorus	Root fresh weight	Root dry weight	Root length	Root volume
Irrigation (I)	3	351.24**	52787.96**	3814.84**	1611774.07**	10434.03**	102.07	2.67**
Mycorrhizae (M)	2	4889.27**	45758.33**	2691.50**	20988802.78**	12702.08**	230.53**	5.25**
M × I	6	91.09**	2665.74	371.17**	56399.07	1190.97	5.38	0.17
Error	24	2.11	3808.33	39.23	21445.00	1079.86	13.56	0.19
CV (%)	-	6.29	17.76	2.02	20.93	18.97	16.32	15.91

* Significant at the 5% probability level, ** Significant at the 1% probability level.

Research Station of Urmia. They were surface sterilized with 0.05% sodium hypo-chloride for 45 min. before sowing them. Seeds were sown at 3 cm depth of plastic pots' soils (12 x 12 cm; 180 pots) in 25th June 2013. Thirty grams of the appropriate inoculums were placed into the hole below each seed, and then covered with sterile soil. For control plants were sown with no inoculation. The plants were grown in a greenhouse under natural photoperiods for 6 weeks during which only distilled water was applied. In addition, twice a week, each pot was supplied with 100 ml of a nutrient solution containing 720 mg of $MgSO_4.7H_2O$, 295 mg of $Ca(NO_3)_2.4H_2O$, 240 mg of KNO_3, 0.75 mg of $MnCl_2.4H_20$, 0.75 mg of KI, 0.75 mg of $ZnSO_4.7H_2O$,1.5 mg of $H3BO_3$, 0.001 mg of $CuSO_4.5H_20$, 4.3 mg of FeNaEDTA and 0.00017 mg of $Na_2MoO_4.2H_2O$ supplemented without phosphorus (Vosatka and Gryndler, 1999).

The root fresh weights (five samples per treatment) were measured before drying at 72 °C for 24 hours for the determination of root dry matter weights. In fact, all the parameters (five samples per treatment) including length and volume of roots, root dry weight, sand above-ground dry matter of seedlings were determined after harvesting. At 6 weeks after planting, the percentage of colonization of chickpea roots by AM fungi was determined per experimental unit. Root colonization was measured in fresh roots cleared in 10% KOH for 10 min. at 90°C and stained in 0.05% lactic acid–glycerol–Trypan Blue (Phillips and Hayman, 1970). The percentage of root colonization by arbuscular mycorrhizal fungi was microscopically determined using the gridline intersection method (Giovannetti and Mosse, 1980). To measure leaf Phosphorus, dried leaves were milled, digested, and analyzed as described by Watanabe and Olsen, (1965) and Ohnishi et al., (1975). The method described for Phosphorus involves drying, homogenization, and combustion (4 h at 500°C) of the leaf sample. The plant ashes (5 mg) are digested in 1mL of concentrated HCl. The samples are then filtered, and total P is quantified as PO_4^- using the ascorbic acid method (Watanabe and Olsen, 1965). The amount of PO_4^- in solution was determined colorimetrically at 882 nm (Graca et al., 2005).

Analysis of variance of data was performed using MSTATC software. The effects of phosphorus, inoculation with mycorrhizae, and that of their interaction were analyzed by ANOVA and the means compared by Student Neuman-Keul test. Correlations between the different vegetative growth parameters and mycorrhizal colonization were assessed.

RESULTS AND DISCUSSION

Colonization percentage by *G. mosseae* was more than that by *G. intraradices* and less reduced with increasing phosphorus levels. Variations of this trait were for *G. mosseae* between 27.51 to 49.88 and *G. intraradices* 22.47 to 41.81. Colonization mycorrhiza reduced due to increasing phosphorus fertilization (Table 3). Both species of mycorrhiza had higher root fresh and dry weight, longer roots, and higher root volume with 2502.50 mg, 199.17 mg, 26.33 cm, 3.23 cm^3, respectively over the control (non-inoculated chickpea plants) (Table 4). Root fresh and dry weight, root length and root volume increased with improved rates of phosphorus fertilizer. Phosphorus levels of 2 and15mg kg^{-1} soil were 1755.56 mg, 132.78 mg, 18.22 cm, 2.37 cm^3 and 2677.79 mg, 197.78 mg, 26.00 cm, 3.51 cm^3 values of them, respectively (Table 5). Expanded roots of mychorrhizal plants enhanced root area (Allen et al., 1981). Therefore, nutrient uptake in mycorrhizal plants was due to more root expansion than control (Huang et al., 1985).The most common among which is AM symbiosis. In AM fungi symbiosis with plant roots, the enhanced uptake of phosphorus is attributed to the fungal partner, and the increase in phosphorus uptake by the colonized roots in turn leads to increased plant growth (Burleigh et al., 2002).

Analysis of the phosphorus accumulation in leaves of the chickpea plants showed that the highest phosphorus accumulation in leaves (343.67mg/100g dry leaf) was obtained from the plants inoculated with *G. mosseae* and

Table 3. Comparison of colonization percentage and leaf phosphorus accumulation of chickpea affected by different levels of phosphorus and mycorrhiza species.

Phosphorus mg/kg^{-1} soil	Arbuscular mycorrhizal fungus	Mycorrhizae colonization (%)	Leaf phosphorus (mg/100g dry leaf)
2	Non-mycorrhizal	0.00g	277.67e
	Glomusmosseae	49.88a	311.67c
	G. intraradices	41.81b	294.67d
5	Non-mycorrhizal	0.00g	283.33e
	G. mosseae	40.11b	307.70c
	G. intraradices	37.10c	302.14cd
10	Non-mycorrhizal	0.00g	276.53e
	G. mosseae	31.75d	326.74b
	G. intraradices	26.74e	324.74b
15	Non-mycorrhizal	0.00g	336.83ab
	G. mosseae	27.51e	343.98a
	G. intraradices	22.47f	338.47ab

Means followed by the same letter(s) in each column are not significantly different.

Table 4. Means comparison of chickpea traits by mycorrhizae species.

Mycorrhizal symbiosis	Above-ground dry matter(mg/plant)	Root fresh weight(mg/ plant)	Root dry weight (mg/plant)	Root length (cm)	Root volume (cm^3)
Non-mycorrhizal	276.67b	1733.33b	137.92b	17.75b	2.03b
Glomusmosseae	375.83a	2502.50a	185.83a	23.58a	3.33a
G. intraradices	390.00a	2402.50a	199.17a	26.33a	2.89a

Means followed by the same letter(s) in each column are not significantly different.

Table 5. Comparison of means of chickpea traits for different levels of phosphorus.

Mg Phosphorus kg^{-1} soil	Above-ground dry matter(mg/plant)	Root fresh weight(mg/plant)	Root dry weight (mg/plant)	Root length (cm)	Root volume (cm^3)
2	243.33b	1775.56c	132.78b	18.22b	2.37b
5	344.44a	1944.44c	137.78b	21.78ab	2.34b
10	378.89a	2475.33b	190.00ab	24.22ab	2.78b
15	423.33a	2677.79a	197.78a	26.00a	3.51a

Means followed by the same letter(s) in each column are not significant differences.

phosphorus treatment 15 mg P kg^{-1} soil (Table 3). The minimum phosphorus accumulation in leaves (277.67 mg/100g dry leaf) was obtained from the non mycorrhizal and 2 Mg P kg^{-1} soil, followed by the non mycorrhizal plants 5 and 15 mg P kg^{-1}soil. The phosphorus concentration in the leaves of chickpea plants in each treatment was significantly higher than that in the control (Table 3). Furthermore, phosphorus concentration in plants inoculated with AM fungi was significantly higher than that in plants treated with phosphorus fertilizer. The significance of sufficient phosphorus availability during early crop growth has been reported in different crop species (Grant et al., 2005). It has been reported that enhanced early-season phosphorus nutrition in maize increased dry matter at early stages partitioned to the grain at later development stages (Parewa et al. 2010). Plenets et al. (2000) reported a greater difference in dry matter accumulation of maize under phosphorus

Table 6. Correlation coefficients between chickpea traits.

Treatment	Mycorrhizae colonization	Above-ground dry matter	Leaf phosphorus	Root dry weight	Root Length	Root Volume
Above-ground dry matter	0.23					
Leaf phosphorus	0.29	0.60**				
Root dry weight	0.31	0.69**	0.62**			
Root Length	0.35*	0.72**	0.59**	0.69**		
Root Volume	0.46**	0.67**	0.74**	0.56**	0.62**	
Root fresh weight	0.30	0.72**	0.60**	0.63**	0.68**	0.85**

* Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$.

deficiency during early stages of growth. The above ground dry matter accumulation was observed to be severely reduced (up to 60%) during early stages of maize growth, while there were only slight differences on dry matter accumulation at harvest and grain yield. The effect of early phosphorus deficiency on decline in shoot growth occurs because of slight stimulation of root growth (Mollier and Pellerin, 1999). The initial reduction in growth related to phosphorus deficiency has an ultimate effect on the final crop yield, which is experienced by the crop throughout the remaining of the growing period. Phosphorus is critical for plant growth and makes up about 0.2% of dry mass, but it is one of the most difficult nutrients for plants to acquire. In soil, it may be present in relatively large amounts, but much of it is poorly available because of the very low solubility of phosphates iron, aluminium, and calcium, leading to soil solution concentrations of 10 mM or less and very low mobility (Ryan et al. 2005). The ability of AM fungi to enhance host-plant uptake of relatively immobile nutrients, in particular P and Zn (Balakrishnan and Subramanian 2012), and their requirement for up to 20% of host-plant for establishment and maintenance, is well accepted (Subramanian et al., 2009).

Between different levels of phosphorus application, 15, 10 and 5 mg had the most above-ground dry matter with 423.33, 378.89 and 344.44 mg/plant. Under lower phosphorus application (5 mg) reduced above-ground dry matter. Both Species with 390.00 and 375.83mg/plant above-ground dry matters had the most values than control (Tables 5 and 6). Subramanian et al. (2006) observed that root colonization by the arbuscular mycorrhizal (AM) fungus significantly increased dry matter yield and ultimate increased the production. Total dry weight differences in mycorrhizal treatments are related to water absorption and mineral nutrients (AL-Karaki et al., 2004; Demir, 2004; Kaya et al., 2003; Pelletier and Dione, 2004; Sanches-blanco et al., 2004).

Correlation coefficients of traits showed that mycorrhiza colonization with root length (r=0.35*) and root volume (r=0.46**) were significant different (Table 6). In addition, leaf phosphorus (r = 0.60**), root dry weight (r = 0.69**), root Length (r=0.72*), root volume (r = 0.67**) and root fresh weight (r =0.72*) had significant differences with above-ground dry matter. These observations indicate that plants having a higher leaf phosphorus and root dry weight produce higher total dry weight.

Conclusions

Inoculated plants with *G. mosseae* and *G. intraradices* showed more leaf phosphorus, root fresh and dry weight, root length and volume than control. Root related traits such as root fresh and dry weight,root length and root volume increased in more phosphorus application and consequently will lead to increase above-ground dry matter. Relationships between traits showed that with increasing leaf phosphorus, root dry weight and root volume in inoculated mycorrhizal chickpea plants enhanced above-ground dry matter. Furthermore, since the formation of mycorrhizae often leads to increases in root traits and above-ground dry matter, the effect of mycorrhizae on leaf phosphorus is also probably partly caused by the enhanced phosphorus nutrition. *G. mosseae* is recommended due to produce further colonization percentage in chickpea inoculated plants. Effective use of these symbiotic soil fungi is an essential element for sustainable agriculture.

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