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Mycorrhizal symbiosis increases reproduction and seed quality of summer squash in field conditions

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Abstract

Phosphorus is one of the most important elements for plant nutrition and mycorrhiza has the capability to improve plant yield by increasing nutrient supply to plants. We studied the effect of presence and absence of mycorrhiza on growth and productivity of summer squash under different phosphorus levels (0, 25, 50, 75 and 100 kg ha^{-1}) laid out in factorial randomized complete block design. Results showed that with increasing phosphorus fertilizers, fruit number per plant increased while seed number per fruit decreased. In addition, mycorrhiza inoculation with application of 50 kg ha^{-1} phosphorus fertilizer had maximum values for root colonization (54.33%), oil (45%) and linoleic acid (68.25%) percentage. Mycorrhiza symbiosis had a direct relationship with summer squash yield. Mycorrhiza acted as good substitute of phosphorus fertilizers. Therefore, it seems that the low rate of phosphorus fertilizer application leads to improved interaction with mycorrhiza.

Key words: Mycorrhiza, phosphorus, *Cucurbita pepo*, root colonization, yield components, seed quality

Introduction

Pumpkin (*Cucurbita pepo* L.) is an important annual plant belonging to the *Cucurbitaceae* family (Bavec *et al.,* 2002). Pumpkin seeds have medicinal properties and many other health benefits, since they are a good source of protein, zinc, and other vitamins, and they are even said to lower cholesterol. The value of vitamin E in pumpkin seeds is very high (Murkovic *et al.,* 1996). Pumpkin seeds are a good source of phosphorus, magnesium, manganese and phytosterols. Seed oil of pumpkin contains essential fatty acids that help maintain healthy blood vessels, tissues and nerves (Applequist *et al.,* 2006). The main characteristic in oil Pumpkin is its dark green color of the thin-coated seeds. The oil content of pumpkin seed varies from 30-50% (Stevenson *et al.,* 2007) and the composition of fatty acids is dependent on several factors. Since, production of pumpkin seeds is increasing; production techniques require further research (Bavec *et al.,* 2002).

Among the essential elements, phosphorus besides nitrogen is the most important element for cell division, root development and seed formation (El-Gizawy & Mehasen, 2009). Phosphorus plays a significant role in several physiological and biochemical activities like photosynthesis, transformation of sugar to starch, and developmental phase. One of the advantages of feeding the plants with phosphorus is to create deeper and more abundant roots (Sharma, 2002). Phosphorus causes early ripening in plants, decreasing seed moisture, improving crop quality and is the most sensitive nutrient to soil pH. However, the availability of phosphorus for plants is limited by different chemical reactions especially in arid and semi-arid soils (Mehrvarz *et al.,* 2008). A considerable proportion of phosphorus in chemical fertilizer becomes unavailable to the plants after its application in the soil. This takes place due to strong bonds between phosphorous with calcium and magnesium in alkaline pH and the same bonds with iron and aluminum in acidic soils. The mobility of phosphorus is very slow in the soil and cannot respond to its rapid uptake by plants. This causes the creation and development of phosphorus depleted zones near the contact area of roots and soil in rhizosphere. Therefore, the plants need an assisting system which could extend beyond the depletion zones and help to absorb the phosphorus from a wider area by developing an extended network around root system (Arpana *et al.,* 2002).

The presence of arbuscular mycorrhiza fungus (AMF) in rhizosphere provides an advantageous and interactive symbiosis relationship between a higher plant root and a nonpathogenic fungus. More than 80% of crops establish symbiotic associations with AMF, during which fungal hyphae expand the functional root-soil interface and enhance access to inorganic phosphate and other mineral nutrients (Brundrett, 2002). Through receiving energetic carbon resources from plant, fungus facilitates the uptake of many inorganic nutrients such as phosphorus, zinc, molybdenum, copper and iron for it. Inoculation of sorghum plants with AMF helped in absorbing enough micronutrients through chelate formation with sidrophores (Aliasgharzad *et al.,* 2009). The symbiotic relationship between AMF and plants is one of the most abundant symbiotic activities in plant kingdom which exists in most of the ecosystems. Unfortunately, the neglectful interference of human activities such as over application of fungicides and frequent chemical phosphorous fertilizers application, mainly in intensive agricultural systems, has seriously threatened this advantageous symbiosis. Efforts to produce inoculants from AMF and to use it in proper environmental conditions, is a significant environmental friendly way to help plant growth and development through the enhancement of this natural phenomenon (Mehrvarz *et al.,* 2008). The significance of this practice, especially under low fertility conditions, has been very obvious. Photosynthesis improvement in plants through mycorrhizal symbiosis is mainly due to the increase in transport of inorganic elements from soil to plants. One of the most important means to achieve the goals of sustainable agriculture is to extend the application of biological fertilizers. To this end, it is necessary to moderate the use of chemical fertilizers and pesticides, and in the mean time to increase the soil organic matter content. This study aimed at the evaluation of performance of AMF inoculation in the presence of P chemical fertilizer on pumpkin yield.

Materials and methods

The experiment was carried out at the Faculty of Agriculture, University of Bu-Ali Sina (48°31’ E, 35°1’ N and 1690 m height from sea level), Hamedan, Iran. To determine the soil characteristics, several samples from 0-30 cm depth were collected from the field and analyzed for their basic physical and chemical properties (Table 1).

The statistical analysis was performed in factorial design where the treatment (2×5) arranged in a randomized completely block design (RCBD), with three replications. The first factor was arbuscular mycorrhiza fungi at two levels (+M: inoculation and –M: non inoculation) and the second factor, phosphorus chemical fertilizer at five levels (P_0: 0, P_1: 25, P_2: 50, P_3: 75 and P_4: 100 kg ha^{-1}). Prior to planting, seeds were inoculated with AMF (*Glomus intraradices*) and P chemical fertilizer was utilized as strip takes under seed according to experimental treatments. All the seeds were sown soon after inoculation in experimental plots. Phosphorus fertilizer used was triple super phosphate and all P fertilizers were added at planting time. Also, urea was added half at planting and half at flowering stage. Each plot consisted of 5 rows, 6 m long with 140 cm spaced between rows and 30 cm distance between plants on the rows. All operations were done regularly during the different stages of plant growth, practices such as weeding, and irrigation were performed for all plots.

At the flowering stage, percentage of root colonization by mycorrhiza was measured according to the method of Phillips and Hayman (1970). A fraction of roots was carefully washed, cut into 1 cm pieces and fixed by formalin-acetic acid-alcohol solution (FAA). Root samples were cleared with 10% KOH solution and stained with 0.05% trypan blue in lactophenol, and the Gridline intersection technique was used to determine the percentage of root colonization. Oil content of seeds was measured according to Soxhlet method (AOAC, 1980). Leaf chlorophyll index was measured with SPAD meter (SPAD 502). Seed phosphorus content was measured according to Olsen and Sommers (1990). At the end of the harvest stage, 2 m^2 was harvested and traits of yield and yield components such as seed number per fruit, fruit number per plant, 100-seed weight, fruit yield and seed yield were measured after physiological maturity. At full maturity, fruits were harvested when become yellow-orange in color and their fresh weights were recorded.

Data analysis was done using SAS statistical software. Means of treatments were compared with the least significant difference (LSD) test (P <0.05).

Results

Fruit number per plant: The results showed that P chemical fertilizer had a significant effect on fruit number per plant, but the effect of AMF inoculation and interaction effect of AMF and P fertilizer on fruit number per plant was not significant (Table 2). With increasing P fertilizers from P_0 to P_4 treatment, fruit number per plant increased. The highest fruit number per plant (1.66) compared to the lowest in control (1.13) was obtained by application of 100 kg P; that was 32% more than control treatment (Table 2).

Seed weight and seed number per fruit: The effect of phosphorus fertilizer on seed weight was significant (Table 2). Maximum seed weight (152.6 mg) was observed using 50 kg P fertilizer (Table 2). Also, results indicated that with reduction in the number of fruit per plant, seed weight increased. Analysis of variance showed that seed number per fruit of pumpkin was affected by P chemical fertilizer and mycorrhiza treatment (Table 2). However, interaction of P fertilizer and mycorrhiza was not significant. Moreover, the highest and the lowest seed number

Table 1. Physical and chemical characteristics of the soil

Soil texture	EC (dS m^{-1})	pH	OC (%)	P	K	Zn	Fe	Mn	Cu
				mg kg^{-1}					
Clay loam	1.45	7.7	0.72	8.2	220	1.06	4	5	0.94

Table 2. Analysis of variance for the effect of mycorrhiza and P fertilizer on yield and yield components of pumpkin

S.O.V§	df	Fruit $plant^{-1}$	Fruit yield	Seed $fruit^{-1}$	Seed weight	Seed yield
Replication	2	ns	ns	ns	ns	ns
Mycorrhiza (M)	1	ns	**	**	ns	**
Phosphorus (P)	4	**	**	*	**	**
M×P	4	ns	ns	ns	ns	ns
CV (%)	-	17.16	11.61	13.39	11.54	16.03
Means comparison¥		-	kg m^{-2}	-	mg	g m^{-2}
Mycorrhiza	–M	-	3.68b	195b	-	71b
	+M	-	4.94a (25.51)†	255a (23.53)	-	108a (34.26)
Phosphate fertilizer	0	1.13c	2.82c	215ab	127.30bc	68c
(kg ha^{-1})	25	1.22bc	4.08b	237a	144.80ab	84bc
	50	1.25bc	4.36b	248a	152.60a	95ab
	75	1.45ab	5.27a	234a	128.60bc	103a
	100	1.66a	5.02a	193b	119.60c	96ab

* Significant at P = 0.05.. ** Significant at P = 0.01.. ns Non significant.. § Source of variations.. † Increasing percentage in +M plants in comparison with –M plants. ¥ Different letters in a column denote a significant difference at the 5% probability level by LSD test.

per fruit were obtained by 50 and 100 kg P fertilizer treatments, respectively. AMF inoculated plants had 23.5% more seeds than control (Table 2).

Fruit and seed yields: The data (Table 2) indicated that there was a significant difference in inoculation and P fertilizer treatments on fruit and seed yield. The highest value (5.27 kg m^{-2}) of fruit yield was obtained with 75 kg P fertilizer and the lowest value (2.82 kg m^{-2}) belonged to control (0 kg P) treatment. Also, mycorrhizal plants had about 25.5% more fruit yield compared to non-inoculated plants (Table 2). Seed yield was significantly affected by AMF inoculation and P chemical fertilizer but, interaction of them had no significant effect on this trait (Table 2). Maximum seed yield (103 g m^{-2}) was revealed at 75 kg P fertilizer, although it was not significantly different with treatments of 25 and 50 kg P fertilizers (Table 2). Minimum seed yield was recorded in control treatment. Inoculation treatment increased seed yield to 108 g m^{-2} compared to 71 g m^{-2} in control (Fig 1a).

Root colonization: Mycorrhizal symbiosis had a direct relationship with seed yield. The interaction effect of P fertilizer and mycorrhiza on root colonization was significant (Table 3). In this study, mycorrhizal colonization fluctuated from 4.04% in control treatment (0 kg P and –M) to 54.33% in treatment of mycorrhizal plants with consumption of 50 kg P fertilizer ha^{-1} (Table 3).

Oil percentage: Analysis of variance showed that oil percentage was affected by treatments of mycorrhiza and P chemical fertilizer (Table 3). Mycorrhizal plants had the highest value of oil percentage (Fig 1b). The highest value of oil percentage (45%) was revealed at mycorrhizal plants under consumption of 50 kg P fertilizer ha^{-1}, while the minimum amount of oil percentage (30.33%) was achieved at control treatment (0 kg P×–M) (Table 3).

Oleic and linoleic fatty acids: Effect of P chemical fertilizer and mycorrhiza treatments on oleic acid was significant (Table 3). According to our findings (Fig. 2), oleic acid had a range from 22.57 in 100% recommended P fertilizer (100 kg P ha^{-1}) to 21.21% in control treatment (0 kg P ha^{-1}). With increasing P fertilizer consumption from 0 to 100 kg ha^{-1}, oleic acid increased. Also, mycorrhizal plants had more oleic acid in comparison to non-inoculated plants (Fig 1c). Also, our results indicated that application P fertilizer levels and mycorrhizal fungi had significant effect on trait of linoleic acid percentage in summer squash. Furthermore, the interaction effect of P fertilizer × mycorrhiza for linoleic acid was significant at 1% probability level (Table 3). Maximum value for linoleic acid percentage was revealed at mycorrhizal plants (Fig 1d). Also, means comparison for the combined treatments indicated that the highest value of

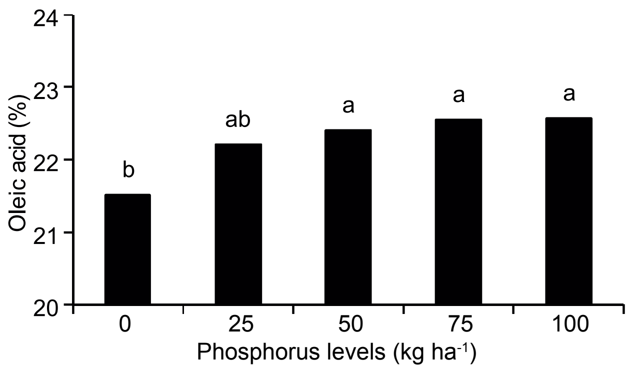

Fig. 2. Mean comparison for oleic acid at different phosphorus levels

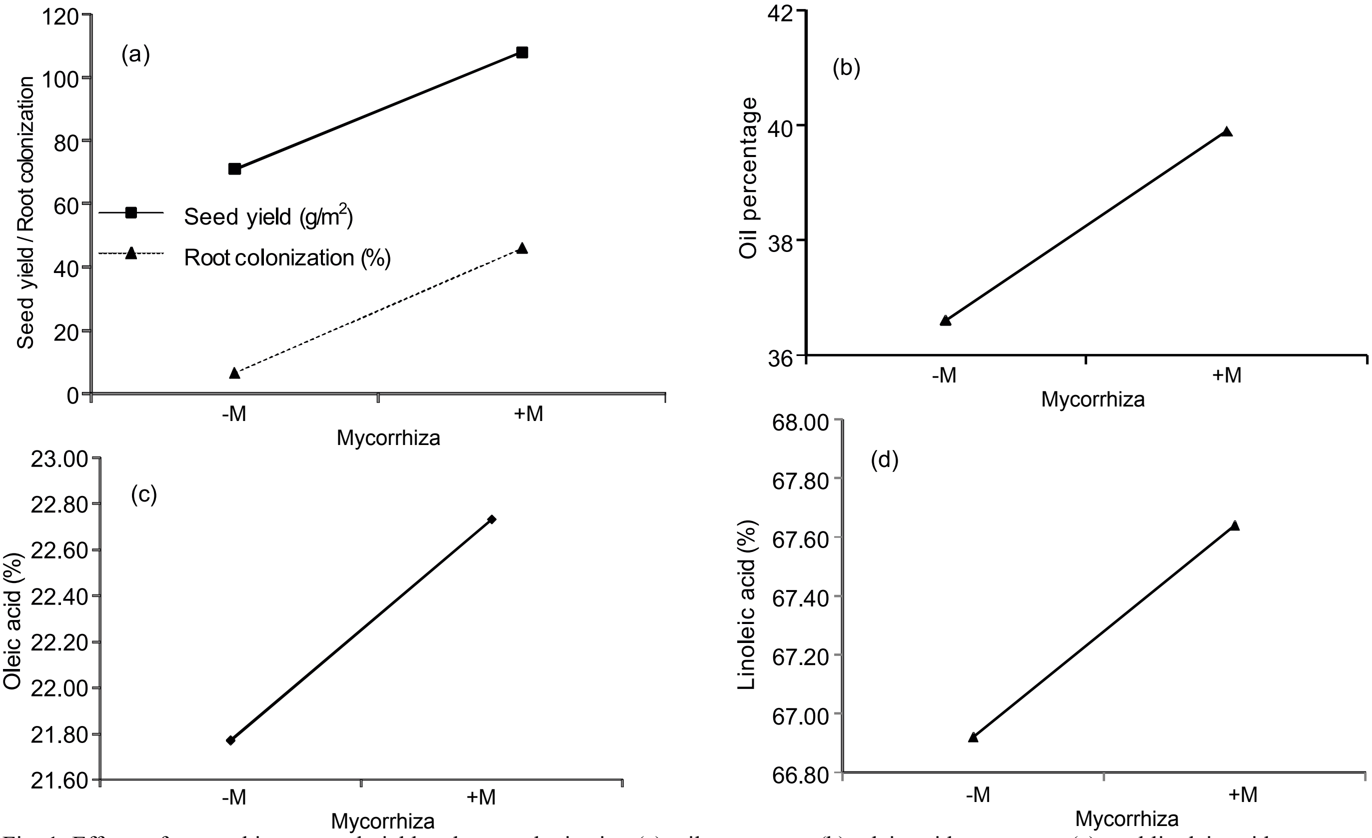

Fig. 1. Effects of mycorrhiza on seed yield and root colonization (a), oil percentage (b), oleic acid percentage (c), and linoleic acid percentage (d) in *Cucurbita pepo*

Table 3. Analysis of variance for the effect of mycorrhiza and P fertilizer on root colonization, and percent of oil, oleic acid and linoleic acid in pumpkin

S.O.V§	df	Root colonization	Oil	Oleic acid	Linoleic acid
Replication	2	ns	ns	ns	**
Mycorrhiza (M)	1	**	**	**	**
Phosphorus (P)	4	**	**	*	**
M×P	4	**	**	ns	**
CV (%)	-	16.64	5.10	2.85	0.79
Interaction means¥		%	%	ns	%
$-M \times P_0$		4.04d	30.33d	-	65.50e
P_1		6.00d	34.87bc	-	66.29de
P_2		7.00d	37.89b	-	67.06bcd
P_3		7.66d	37.89b	-	67.73abc
P_4		7.33d	42.12a	-	68.05a
$+M \times P_0$		28.33c	34.08c	-	66.87cd
P_1		52.66ab	42.10a	-	67.86ab
P_2		54.33a	45.00a	-	68.25a
P_3		49.33ab	41.71a	-	67.65abc
P_4		45.33b	36.66bc	-	67.55abc

*Significant at P = 0.05. ** Significant at P = 0.01. ns: Non significant. § Source of variations. P_0 to P_4: phosphorus levels; 0, 25, 50, 75 and 100 kg ha^{-1}, respectively. ¥ Different letters in a column denote a significant difference at the 5% level by LSD test.

linoleic acid (68.25%) was achieved at mycorrhizal plants with application of 50 kg P ha^{-1}. However, the lowest value (65.5%) for linoleic acid was in control (non-mycorrhizal plants without P fertilizer) (Table 3).

Discussion

Pumpkin is an important vegetable species, rich in oil and can also be used medicinally. One of the possible options to reduce the use of chemical fertilizers or a substituent of P fertilizer in sustainable agriculture could be the use of mycorrhiza.

Our results showed that P chemical fertilizer had a significant effect on fruit number per plant (Table 2). Although former research couldn't find any significant effect of chemical fertilizer on fruit number of pumpkin (Habibi *et al.,* 2011), here, it is clear that with increasing P fertilizers from 0 to 100 kg ha^{-1}, fruits number per plant increased. Effect of P chemical fertilizer and mycorrhiza treatments on pumpkin fruit weight was significant (data not shown). The highest fruit weight was obtained by 75 kg P ha^{-1}. With increasing P fertilizer >75 kg ha^{-1} although fruit number increased but fruit weight decreased. Moreover, AMF inoculation increased fruit weight. It seems that AMF inoculation and 75% of chemical fertilizer had the same effect on fruit weight. So, AMF could be a good substitute for phosphorus fertilizers (Duponnois *et al.,* 2006).

The effect of 50% of recommended P fertilizer on 100-seed weight was significant. Also, with reducing the number of fruit, 100-seed weight was increased. Such effect is also seen in sunflower (Soleimanzadeh, 2010). It is reported that some agricultural practices like over application of chemical fertilizers, have negative effects on life and development of arbuscular mycorrhiza fungus (Arpana *et al.,* 2002). Based on this study, it seems that combination of high level of P fertilizer with AMF, did not have much positive effects on seed number per fruit of pumpkin (Table 2). As it is seen from Table 2, AMF plays an important role in pumpkin generative growth and therefore makes a significant increase in the seed number per fruit, most likely because AMF greatly increase the plant uptake of phosphorus and nitrogen (Chen *et al.,* 2005). This is in accordance with earlier results (Wagner, 2000).

The highest value of fruit yield was obtained with 75 kg P ha^{-1} (Table 2). Also, mycorrhizal plants had about 25.5% more fruit yield than the control. Bio-fertilizer treatment significantly enhances the induction of female flowers on squash, which consequently reduced the sex ratio of squash plants (Abdel-Fattah & Sorial, 2000). Seed yield was significantly affected by AMF inoculation and P chemical fertilizer (Table 3). AMF inoculation increased seed yield to an acceptable level. So it can be considered as a suitable substitute for P chemical fertilizer in organic agricultural systems. There is the beneficial effect of AMF on phosphate nutrition of crop plants in soil with low phosphorous concentration (Chen *et al.,* 2005). This means that the adequate amount of phosphorus in the soil provided enough resources for phosphate solubilizing microorganisms. But, high levels of P, led to an antagonistic interaction with fungus.

Mycorrhizal symbiosis had a direct relationship with pumpkin yield (Fig 1a). It can be seen that the low rate of P chemical fertilizer application, leads to improved plant interaction with AMF. So, it seems that inoculation of pumpkin with AMF significantly improves its yield and nutrient uptake. Hence, combined application of AMF and low amount of P chemical fertilizer can be practical and helpful to increase P availability and production for medicinal pumpkin and also, to reduce the environmental pollution. Extensive research has shown that phosphorous uptake by plant roots can be enhanced when they are infected by AMF. The amount of mycorrhizal symbiosis depends strongly on the availability of phosphorus and the presence of high phosphorus decreases this symbiosis (Covacevich *et al.,* 2007).

P fertilizer significantly increased percentage of oil, and oleic and linoleic fatty acids. As seen in Table 3, application of mycorrhizal fungi had significant effect on quality traits of squash plants. Our results revealed that maximum values for oil percentage and linoleic acid were obtained in inoculated plants by mycorrhiza under application of 50% P recommended fertilizer (50 kg P ha^{-1}). Earlier researches reported could find significant effect of combined application of phosphate solubilizing microorganisms and P chemical fertilizer on quality traits of squash plants (Habibi *et al.,* 2011). Our results suggest that plants inoculated by mycorrhiza under application of 50% P recommended fertilizer has a great potential to increase oil content of squash plants. Based on our findings, with increasing seed oil content, linoleic acid increased, indicating a positive correlation between oil content and linoleic acid (Gholipouri & Nazarnejad, 2007).

The methodology described in this study is suitable for the production of summer squash under field conditions. Under field conditions, it is possible to establish a functional mycorrhizal symbiosis between AMF and summer squash plants and this condition is more important for shoot biomass production and seed quality of this plant. Inoculation of summer squash plants with mycorrhiza did not only strongly influence biomass accumulation, fruit, seed and oil production but also reduced the chemical fertilizer input in the growth stages.

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