Influence of Arbuscular Mycorrhizal Fungi (AMF) Inoculation on Growth and Mycorrhizal Dependency of (*Lens culinaris* L.) Varieties

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To cite this article:

Shah Wali Khan, Tabassum Yaseen, Falak Naz, Syed Abidullah, Mohammad Kamil. Influence of Arbuscular Mycorrhizal Fungi (AMF) Inoculation on Growth and Mycorrhizal Dependency of (*Lens culinaris* L.) Varieties. *International Journal of Bioorganic Chemistry*. Vol. 4, No. 1, 2019, pp. 47-52. doi: 10.11648/j.ijbc.20190401.17

Received: February 9, 2019; Accepted: March 25, 2019; Published: April 22, 2019

Abstract: Arbuscular mycorrhizal fungi (AMF) are soil fungi, obligate biotrophic fungi and form the most common mutualistic symbiosis in nature: the arbuscular mycorrhiza (AM). This association occurs on the roots of most plants, promoting improvements in plant growth and development. The present study was aimed to investigate the effect of arbuscular mycorrhizal fungi (*Glomus aggergatum*s, *Glomus fasciculatum*, *Glomus mosseae* and *Sclerocystis niger*) inoculation on growth and mycorrhizal dependency of *Lens culinaris* L. varieties. The experiment was carried out under natural condition with two treatments (inoculated and control) in triplicates. Inoculation of lentil plants with arbuscular mycorrhizal fungi resulted a significant increase in various plant growth parameters compared to plants without inoculation. In addition, fungal inoculation significantly increased mycorrhizal dependency as compared to un-inoculated plants. It is concluded that fungal inoculation improve plant growth parameters under phosphorous deficient soil.

Keywords: AM Fungi, Growth, Mycorrhizal Dependency, *Lens culinaris*, Inoculation

1. Introduction

The word mycorrhiza is originated from two Greek words myco meaning fungi and Rhiza meaning root and its' meaning in reality means symbiosis between a fungus and root of the other plants. Mycorrhiza is described as a mutual sharing of life; whereby the fungal is the major partner of the plant has the duty to supply food, growth hormones and protection of plants' root from pathogens and a fine plant will offer high energetic material to the fungus [1]. Mycorrhizal fungi are one of the soil organisms that form a direct link between the soil and plant root systems [2].

It is well known that AM symbiosis can increase plant growth and nutrient uptake, improve fruit quality and enhance several abiotic stresses such as low temperature stress, drought, salt stress, etc. [3]. Arbuscular mycorrhizal fungi (AMF) colonization increased growth of chickpea up to +43% of total dry matter [4]. Colonization of roots by AM

fungi has been shown to improve growth and productivity of several field crops [5-7] by increasing nutrient element uptake [8]. Mycorrhizal fungi are beneficial association of microorganisms with their extra metrical hyphae, which increase plant growth and yield through increase absorption of relatively immobile elements in soil such as P, Cu and Zn by substantially extending the area of absorption beyond that of root hairs [3]. Many workers studied that arbuscular mycorrhizal (AM) fungi inoculation had a significant effect on the growth and productivity of legumes when compared with non-mycorrhizal plants [9-13].

2. Materials and Methods

This experiment was carried out at the Department of Botany, Bacha Khan University Charsadda Khyber Pakhtunkhwa-Pakistan during winter season 2014-2015. The site lies between 34°N to 38°N latitude and between 71°E to

53°E longitude. In the experimental work, rhizospheric soil of maize field having 123/100 gm spores number of different AMF species *Glomus fasciculatum*, *Glomus mosseae* and *Glomus aggregatum* and roots of infected with AMF were used as inoculum. These roots along with soil used as an AMF inoculum and spread uniformly in layer at a depth of 3-6 cm before sowing. Each pot was filled with 4 kg of soil.

Physio-chemical properties of soil was done by the methods of [14]. Soil had (70% sand, 25% silt, 10% clay, texture sandy loamy soil, 24.4% CaCO_3 , pH 7.41, E.C. 1.2 dS m^{-1} and ionic concentrations were, Ca^{++} 11.9; Mg^{++} 8.1; Na^+ 2.35; K^+ 1.28; CO_3^{--} traces; HCO_3^- 8.52; Cl^- 1.6; NO_3^- traces and SO_4^{--} 6.1 mg Kg^{-1} soil. Total nitrogen and phosphorus were 2.21 and 1.43 mg Kg^{-1} soil, respectively.

The experiment was laid out in a randomized block design (RCBD) following two treatments (control and inoculated), each treatment was replicated three times (Two treatments $\times$ 3 varieties of lentils $\times$ 3 replicates). The following parameters were recorded: Agronomic parameters including plant height, fresh and dry weight and number of branches/plant was known at two different stages (vegetative and harvesting). Mycorrhizal dependency (MD) value of selected legumes was calculated by the formula given by Plechentte & Furlan [14].

3. Results and Discussion

In this research work *Lens culinaris* varieties mycorrhizal inoculated and control plants were grown in P-deficient soil. The objectives of the present study was to investigate the responsiveness of lentil, a legume to arbuscular mycorrhizal fungi inoculation on the growth and mycorrhizal dependency. The result showed that mycorrhizal inoculated plants increased plant growth parameters of inoculated plants as compared with control. Inoculated plants significantly ($P < 0.05$) enhanced the growth parameters including plant height, fresh and dry weight, number of branches/plant of lentils. Our results agree with Yaseen *et al.*, [15] studied the effects of mycorrhizal fungal inoculation on seed germination and plant growth of Bambara groundnut (*Vigna subterranea*). The fungal strains significantly increased plant growth parameters e.g. number of leaves, leaf length, leaf surface area, stem girth and number of shoots respectively. The present study also confirm the findings that the inoculation of *Rhizobium*, rock phosphate and Vesicular Arbuscular Mycorrhizae (VAM) significantly increased the growth parameters and growth yield of Burgundy (*Macroptilum bracteatum*) under “P” deficient soil [17]. The endophytic fungi significantly increased shoot growth, chlorophyll contents, and plant biomass and leaf area of pepper plant as compared to fungal-free plants [16]. AM fungi inoculation had a significant effect productivity of Chickpea attributed to growth, viz., and plant height, no. of nodules, mycorrhizal dependency and flowers per plant [32]. In the present study the following growth parameters mycorrhizal and non-mycorrhizal plants of lentil varieties were recorded and compared.

3.1. Plant Height

Results of AM fungi inoculation on plant height of three

different varieties of lentil (NARC 11-4, Masoor Markaz 09 and Masoor 2002) following two treatments inoculated and control are given in LSD Table 1 and Figures 1-2. The maximum plant height at vegetative stage was (9.3167cm) recorded in variety Masoor 2002 followed by NARC 11-4 (8.5250cm) and Masoor Markaz 09 with (7.8750cm). At harvesting maximum (16.667cm) plant height was recorded for NARC 11-4 followed by Masoor 2002 with (15.583cm). The results revealed that mycorrhizal application in nutrient deficient soil increased plant height as compared with control.

Figure 1. Influence of AMF on plant height at vegetative stage.

The interaction between varieties and the effect of treatments were significantly different regarding plant height. The results showed that mycorrhizal plants were taller as compared with non-inoculated plants. Our results correlate with the findings of Smith SE *et al.* [18] who compared uninoculated mycorrhizal plants with AM fungal inoculated plants, plant height was significantly increased by 30.3% in red tangerine (*Citrus tangerine*). Our results also correlate with the findings of Oluwatomiwa, [19] observed that inoculation of plant with (AM) fungi improved plant growth due to increase N_2 fixation and similarly by Thakur *et al.* [20] resulted that the symbiosis between plants and arbuscular mycorrhizal fungi efficiently promote plant growth especially in growth limiting environment.

Figure 2. Influence of AMF on plant height at harvesting stage.

3.2. Fresh and Dry Weight

Results of AM fungi inoculation on plant fresh and dry weight of three different varieties of lentil (NARC 11-4, Masoor Markaz 09 and Masoor 2002) following two treatments inoculated and control are given in LSD Table 1 and Figures 3-6. Analysis of the data showed that mycorrhizal inoculation increased fresh and dry weight of plants at both stages and had a significant effect compared to control plants. The interaction between varieties and the effect of treatments were significantly different regarding plant dry weight and the effect of treatments were significantly different in terms of plant fresh weight. The results were consistent to Udaiyan [21] that the inoculation of onion plants with AM fungi can significantly increase bulb diameter, bulb yield, shoot dry and fresh weights and shoot phosphorus content. The present results are also agree with the findings of Mudalagiriappan *et al.* [22] reported that the AMF inoculation increased the root, shoot and total dry matter production in Mungbean. Inoculation of *Glomus fasciculatum* with plants of *Casuarina equisetifolia*, results in the higher growth and biomass [23]. AMF inoculation significantly increased in dry matter production, improved growth rate and net assimilation rate [24].

Figure 3. Influence of AMF on fresh shoot weight at vegetative stage.

Figure 4. Influence of AMF on fresh shoot weight at harvesting stage.

Figure 5. Influence of AMF on dry shoot weight at vegetative stage.

Figure 6. Influence of AMF on dry shoot weight at harvesting stage.

3.3. Number of Branches/Plant

Figure 7. Influence of AMF on number of branches/plant.

Results of AM fungi inoculation on number of branches/plant of three different varieties of lentil (NARC

11-4, Masoor Markaz 09 and Masoor 2002) following two treatments inoculated and control are given in LSD Table 1 and Figure 7. It is observed that maximum number of branches (1.5833) was found in Masoor 2002 followed NARC 11-4 with (1.5000) as compared with control plants. The present study evaluate the significant effect of AMF on number of branches/plant. Our results is a good conformity that the AM fungi (*Glomus intraradices*) and (*Glomus mosseae*) have been studied for various plant growth

promoting activities and reported to positively influence the growth of various plant species [3, 25-26]. AM fungal inoculation had greater shoot number and diameter than non-AM seedlings, because mycorrhizal fungi are known to improve growth and nutrients particularly P [8-27]. Arbuscular mycorrhizal fungi inoculation increased plant growth in inoculated plants as compared with non-inoculated plants [28].

Table 1. Effect of arbuscular mycorrhizal fungi (AMF) inoculation on growth parameters of *Lens culinaris* M.

Varieties	Treatments	Stages	Height (cm)	Fresh	Dry	Fresh	No. of branches
				Weight of shoot mg/kg	Weight of shoot mg/kg	Root weight mg/kg	
NARC 11-4	AMF	Vegetative	8.5250	1.1093	0.5984	0.0854	1.5000
		Harvesting	16.667	0.5434	0.1220	0.2403	
	Control	Vegetative	7.7250	0.5690	0.2382	0.0315	0.3333
		Harvesting	13.510	0.0908	0.0376	0.1550	
Masoor markz 09	AMF	Vegetative	7.8750	0.3740	0.3311	0.1412	0.7500
		Harvesting	15.417	0.1656	0.2084	0.2000	
	Control	Vegetative	7.4000	0.3785	0.1284	0.0411	0.2500
		Harvesting	11.583	0.0835	0.0483	0.1547	
Masoor 2002	AMF	Vegetative	9.3167	0.6665	0.1645	0.2948	1.5833
		Harvesting	15.583	0.1095	0.2011	0.2667	
	Control	Vegetative	8.2417	0.1591	0.0433	0.0328	0.6667
		Harvesting	12.917	0.0923	0.0717	0.1733	

All values mean $\pm$ SEM of 3 determinations (replicates)

3.4. Mycorrhizal Dependency (M.D)

Mycorrhizal dependency is a value of measurement that, how much plant depends on Arbuscular mycorrhizal (AM) fungi for its growth and development [29]. Results of arbuscular mycorrhiza (AM) fungi inoculation on mycorrhizal dependency of lentil varieties following two treatments inoculated and control are given in Table 2 and Figure 8. Results revealed that the maximum dry weight was (0.2084gm/kg) recorded for variety Masoor markaz 09 as compared to control plants having less weighed at harvesting stage than the inoculated plants. Maximum mycorrhizal dependency value was recorded (76.82%) for variety Masoor markaz 09 followed by NARC 11-4 with (69.18%) and Masoor 2002 with (64.34%) respectively in mycorrhizal inoculated plants. Masoor markaz 09 and NARC 11-4 varieties are more dependent on mycorrhiza and variety Masoor 2002 are less dependent on mycorrhiza. Our result agree with Nazir *et al.*, [30] reported that, high mycorrhizal dependency value increased plant growth by arbuscular mycorrhiza activity in *M. lacerata*, *M. luisana*, *M. polyantha* and *M. texana* species. From the present study we found that legumes were more dependent on mycorrhizal association for better survival, in nutrient deficient soil. Our results also correlate with Wu *et al.*, [31] observed, that plant species and even cultivars of same species having different mycorrhizal dependency value due to soil type, root geometry, plant growth rates, mycorrhizal species and soil phosphorus.

Table 2. Means of dry weight of four replicates of mycorrhizal dependency.

Varieties	Treatments	Means of dry wt. of four replicates	M.D%
NARC 11-4	AMF	0.1220	69.18%
	Control	0.0376	
Masoor markaz 09	AMF	0.2084	76.82%
	Control	0.0483	
Masoor 2002	AMF	0.2011	64.34%
	Control	0.0717	

Mean dry weight of four replicates

Figure 8. Mycorrhizal dependency index (M.D).

4. Conclusion

In conclusion, our results have confirmed the important effects of AM fungal inoculation on lentil growth parameters and mycorrhizal dependency. These results show a significant application of AM fungal inoculation for agricultural development in areas having P-deficient soil. The symbiosis with mycorrhiza is a biological technology that may improve growth parameters and mycorrhizal dependency in leguminous plants for an agricultural development and to maintain friendly ecosystem.

Acknowledgements

The authors thank to all staff of Agriculture Chemistry Department, University of Agriculture, Peshawar, Pakistan.

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