Global Advanced Research Journal of Agricultural Science (ISSN: 2315-5094) Vol. 5(9) pp. 354-360, September, 2016 Issue.
Available online http://garj.org/garjas/home


*Full Length Research Paper*

# Phenotypic studies on Wheat and Barley after colonization with mycorrhiza symbiosis Fungi

**Amal Ahmed Mohammed Al-Ghamdi**

Department of Botany, Environment Program, Faculty of Biological Sciences, King Abdul Aziz University, P.O. Box 35009, Jeddah 21488, Saudi Arabia.
Email: amalalgamdi@gmail.com

Accepted 12 September, 2016

**Symbiotic associations between endomycorrhiza and crops can provide several benefits to the host crops. An investigation was conducted to assess the impact of arbuscular mycorrhizal fungi (AMF) inoculation on seed germination, overall growth (growth rate and percentage) and the general morphology of barley and wheat plants. The experiment demonstrated that the height was significantly higher in barley plants grown in soil inoculated with AMF (45.0±1.6 cm versus 30.8±0.9 cm for those grown in non-inoculated soil; p=0.014). Similarly, stem and root length were significantly higher in barley plants grown in AMF-inoculated soil (p=0.009 and 0.04, respectively). Further, dry shoots of plants grown in AMF-inoculated soil weighed significantly more than those grown in non-inoculated soil (1.020±0.015 g versus 0.353±0.015 g for plants grown in non-inoculated soil; p=0.002). The dry shoots and dry roots of barley plants grown in AMF-inoculated soil weighed significantly more than those of plants grown in non-inoculated soil (p=0.0006 and 0.009, respectively). Both the growth rate and percentage were increased in AMF-inoculated wheat plants. A similar increase in growth rate was observed in barley plants inoculated with AMF (P<0.005). Overall, inoculation with AMF was beneficial in increasing plant growth parameters and plant biomass.**

**Keywords:** wheat, Barley arbuscular mycorrhizal fungi, root, shoot

## INTRODUCTION

The concept of communal living is a frequent phenomenon in the biological world, particularly among plants and sub terrain micro-organisms. Symbiotic associations have been described in the underground world, which has been reported to sustain one of the most common symbiotic associations between plant roots and mycorrhizal fungi (Allen *et al*., 2003). The mutualistic or parasitic interactions between plants and other micro-organisms have a strong influence on agriculture, forestry and the ecosystems (Strack *et al*., 2003), as well as to some extent cause disturbances by affecting the physical, chemical and biological processes in soil.

Arbuscular mycorrhizal fungi (AMF) affect the physical properties of soil in that they help bind soil particles and improve soil aggregation as well as play a part in soil conservation (Dodd, 2000). Owing to their widespread association with land plants, endomycorrhiza play a crucial role in the ecosystem by improving plant productivity (Fedderman et al*,* 2010). Studies should investigate how endomycorrhiza affect the growth rate of the host plant in an attempt to use these fungi in sustainable agriculture (Linderman, 1992).

Arbuscular mycorrhizal fungi can alter plant physiological and morphological characteristics such that plants can tolerate environmental stress (Miransari *et al.*, 2008). These mycorrhizae are highly concentrated in the uppermost soil layer and help extend the roots of the host plant; the external mycelia of the fungi directly connect plant roots and soil nutrients (Finlay and Soderstrom, 1992). Arbuscular mycorrhizal fungi improve growth of many plant species by increasing nutrient uptake, producing substances that promote plant growth, increasing plant tolerance to drought, and other stressful conditions such as salinity (Sreenivasa and Bagyaraj, 1989).

This research aimed to ascertain the form and structure of barley and wheat cultivars before and after inoculation with AMF. Several factors such as seed germination, overall plant development as well as the general morphology of the plants (plant height, root and root hair length, stem height, leaf number and number of grains per plant) were determined after treatment with AMF. Soils containing these cultivars were also analyzed to determine the pH level, organic matter content, soil texture and soil moisture level and whether soil elements such as phosphorus, nitrogen, potassium, calcium and magnesium had certain roles in plant growth.

## MATERIAL AND METHODS

*Germination*

Barley and wheat seeds were placed into a container of water to ascertain their viability. Viable seeds were selected and planted separately in pots, with five seeds planted in soil containing AMF and five in soil without devoid of AMF, respectively. The seeds were then watered every day for twelve weeks and observed for signs of germination. The time of germination was recorded for each seed. After 15 weeks, the growth rate of the seedlings was determined. The young plants were then harvested to measure the stem length. The shoots and roots were also weighed, both in the fresh and dry states.

*Characterization of arbuscular mycorrhizal fungi*

The roots of the host plants were cleared and stained using the technique described by Brundrett *et al.* (1996). Similarly the method used by Brundrett *et al.* (1996) was used to isolate and identify spores.

*Mycorrhizal inoculation*

The sample soil was collected from a field and sterilized in an autoclave at 121 °C for 1 hr (Toshihiro *et al.*, 2004). Each pot (measuring 15 cm for the upper rim, 10 cm for the bottom rim, and 15 cm in height) contained 100 g of soil, and a thin layer of AMF inoculums (50 g) were placed underneath the seed layer soil for soils treated with mycorrhiza; seeds in the non-AMF soils did not receive any AMF inoculums.

*Measurement of soil physiochemical properties*

Soil pH was measured using the technique described in a previous research (Conklin, 2005). The soil sample was treated as described by Wilde *et al.*, (1972) and its organic matter content was determined using the formula below:

$$\left(\frac{\text{Dry soil (gm)-incinerated soil (gm)}}{\text{Dry soil (gm)}}\right) \text{X100}$$

To determine the amount of moisture in soil, 100 g of the soil were treated using the method described by Yousef (1999) and Conklin (2005). The following formula was used to determine the quantity of water in the soil sample:

$$\left(\frac{\text{Wet sand (gm)-Dry sand (gm)}}{\text{Wet sand (gm)}}\right) \text{X 100}$$

Standard procedures were used to determine the composition of Ca, K, Mg, N (Ryan *et al.*, 2001), and P (van Lierop, 1988) in the soil samples.

*Measurement of root length*

A ruler was used to measure root lengths of the sample plants. All readings recorded in centimeters. The process was repeated thrice for both barley and wheat plants in each treatment group and the mean length was reported in cm.

*Measurement of the dry root and shoot weight*

The roots and shoots were individually set on metal sheets, then heated to a temperature of 80°C until no change in their weight was observed. The dry samples were then weighed and the result was reported in grams.

*Measurement of fresh root and shoot weight*

The fresh roots and shoots of barley and wheat plants were measured reported in grams. For each treatment group, the samples were weighed thrice and the mean weight was documented.

*Biochemical Analysis*

*Estimation of plant growth rate*

The plants were measured as they grew in order to determine the growth rate; the growth percentage was estimated by taking into consideration the time intervals and percentage of growth. For each treatment group, the procedure was replicated and the mean was recorded (Robinson *et al.*, 2014).

$$\text{Growth rate} = \frac{\text{Final length of the plant} - \text{Initial length of the plant}}{\text{Time interval}}$$

$$\text{Growth [\%]} = \frac{\text{Final length of the plant} - \text{Initial length of the plant} \cdot 100\%}{\text{Time interval}}$$

*Data analysis*

The differences in plant height, stem length, root length, and plant biomass in AMF and non-AMF inoculated soils were subjected to paired t-test at $\alpha = 0.05$. Similarly, differences in the rate of plant growth as well as the proportion of plant growth were compared using the paired t-test. The data were analyzed using Statistix® (Tallahassee, FL, USA).

## RESULTS AND DISCUSSION

In this study, the soil acidity or alkalinity and its composition in organic material, as well as its capacity to conduct electricity were assessed in both AMF- and non-AMF-inoculated soil. Prior to inoculation, the pH of the soil in AMF- and non-AMF-inoculated soil was close to slightly acidic. AS shown in Table 1, the soil was composed mainly of organic matter and had an electrical conductivity of 1.00 $MMOs/cm^3$.

Soil sample analysis showed that the most frequent elements were phosphorus, calcium and nitrogen (Table 2). Amongst the different types of elements found in the soil sample, molybdenum comprised the least proportion. Certain elements, including phosphorous, potassium and nitrogen are the main nutrients that are crucial for plant growth. These nutrients are primarily exchanged at the arbuscules, which support the plant with the necessary mineral nutrients (Guether *et al.*, 2009). In exchange, the fungi collect carbon compounds that are necessary to sustain its life cycle. This symbiotic relationship has a multifaceted and multifunctional character since the fungi also contributes substantially by making the host plant more tolerant to biotic and abiotic stress (Aroca *et al.*, 2008). It has been suggested that the increased growth rate observed in plants inoculated with AMF is mainly due to increased amassing and absorption of nutrients, including phosphorus, manganese, iron, zinc, etc., especially is soils that lack nutrients (Beltrano et al., 2013). AMF can regulate the uptake of water (Treseder and Allen, 2000) and nutrients (Goicoechea *et al.*, 2000; Treseder and Allen, 2000) by host plants and consequently facilitate its interaction with its surrounding. Further, AMF discover soil that is out of the range of the host plant's roots and make them accessible to the plant (Torrisi *et al.*, 1999).

The current study shows changes in the morphology of both wheat as well as barley plants inoculated with or without AMF (Table 3 and Table 4). Morphological parameters such as the length of the stem as well as plant height were not significantly different between AMF- and non-AMF-inoculated wheat crops. Conversely, the height was significantly higher in AMF-inoculated barley AMF versus those grown in non-inoculated soil ($P < 0.05$). Other researchers also reported increases in plant height following treatment with AMF (Safapour et al. 2011). In a recent investigation by Robinson *et al.* (2014), it was found that treatment with VAM significantly increased root and shoot lengths. A study by Otgonsuren *et al.* (2015) reported that plant height, biomass and concentrations of chlorophyll, minerals and proline were significantly higher in mycorrhizal (M+) than non-mycorrhizal (M-) crested wheatgrass. In addition, by forming associations with AMF, plants can be protected from soil pathogens and recover from herbivore attacks (Muchovej, 2001).

Similar to changes in plant height, stem and root length were significantly higher in barley plants grown in AMF-inoculated soil ($P < 0.05$). This finding is consistent with that reported by Zou *et al.* (2015), who found that inoculation with AMF significantly increased all plant growth characteristics, including plant height and stem diameter. Furthermore, similar results were obtained with crested wheatgrass grown with and without mycorrhizal fungi. Otgonsuren and his colleagues (2015) reported that plants inoculated with AMF performed better in terms of height compared with non-AMF inoculated plants. According to Beltrano *et al.* (2013), the increase in growth parameters after AMF inoculation is mainly due to improved mobilization and uptake of nutrients, including Fe, Mn, P and Zn. However, contradictory findings have been reported by other researchers who found that while inoculation with mycorrhizae favoured root growth in one plant species, it caused a reduction in root length (of up to 40%) in another species (Allen and Allen, 1984). This discrepancy was suggested to be due to the ability of AMF-inoculated plants to compete for resources that are unavailable to neighbouring non-mycorrhizal ones.

Little is known about the morphological changes brought about when plants are inoculated with AMF (Souza *et al.*, 2000). According to one report (Cooper, 1984), substantial morphological modification are not brought about in roots due to inoculation with AMF; however, findings from a study conducted by Norman *et al.*, (1996) showed that

**Table 1. Mean (± S. E.) physicochemical properties of soil sample.**

| Type of soil | Soil physicochemical properties | | |
|---|---|---|---|
| | pH | Organic matter (%) | Electrical conductivity |
| Soil | 5.93±0.07 | 90% | 1.00 MMHos/cm$^3$ |

**Table 2. Mean (± S.E.) amount of element in soil samples collected.**

| Elements | Weight (mg/kg) |
|---|---|
| Nitrogen | 103.00±2.89 |
| Phosphorus | 17.70±0.38 |
| Potassium | 189.60±1.16 |
| Calcium | 122.50±0.87 |
| Magnesium | 43.30±0.38 |
| Zinc | 0.13±0.04 |
| Copper | 0.13±0.03 |
| Manganese | 1.00±0.05 |
| Molybdenum | 0.0013±0.0003 |
| Boron | 0.07±0.01 |

**Table 3**. Effect of endomycorrhiza on crop growth parameters of wheat.

| Conditions | Parameters (cm) | | |
|---|---|---|---|
| | Plant height | Stem length | Root length |
| With AMF inoculations | 46.3±4.1a | 37.4±3.8a | 8.9±0.7a |
| Without AMF inoculations | 41.7±1.2a | 33.3±1.8a | 8.3±1.5a |

a Means in the same row followed by same letters were not significantly different at α=0.05 (Paired t-test).

**Table 4.** Effect of endomycorrhiza on crop growth parameters of barley.

| Conditions | Parameters (cm) | | |
|---|---|---|---|
| | Plant height | Stem length | Root length |
| With AMF inoculations | 45.0±1.6a | 37.9±1.0a | 7.1±0.7a |
| Without AMF inoculations | 30.8±0.9b | 27.7±0.8b | 3.2±0.2b |

a Means in the same row followed by different letters were significantly different at α=0.05 (Paired t-test).

AMF caused architectural changes in the roots of various plant species. In addition, Bressan and Vasconcelos (2002) found that AMF caused anatomical changes in the roots of the host plant.

Filion *et al.* (1999) demonstrated that plants inoculated with AMF suffered fewer damages than non-mycorrhizal plants. It has been proposed that mycorrhizal establishment is followed by several morphological changes within the roots that are typically brought about by the interaction of antagonistic hormones produced by both AMF and its host plant (Reboutier, 2002). The morphological changes that occur in the roots involve the decrease of tap root development, accompanied by the stimulation of sideways root growth, which is later followed by changes to formation of epidermal cells and hindrance of root hair growth (Barker *et al.*, 1998). The main benefit of the host plant from its association with AMF is the expansion of its root system by the fungus. Fungal hyphae act as an additional catchment and assimilating surface in soil (Sharma and Adholey, 2004; Bonfante and Genre, 2010).

The dry shoots of wheat plants inoculated with AMF was not significantly different from those devoid of AMF ($P > 0.05$) (Table 4). Similarly, no significant difference was

**Table 5.** Effect of endomycorrhiza on wheat biomass.

| Conditions | Parameters (g) | | | |
|---|---|---|---|---|
| | Fresh shoots weight | Dry shoots weight | Fresh roots weight | Dry Roots weight |
| With AMF inoculations | 2.003±0.003a | 1.020±0.015a | 1.007±0.007a | 0.603±0.009a |
| Without AMF inoculations | 2.006±0.007a | 0.353±0.015b | 1.003±0.003a | 0.417±0.009b |

a Means in the same row followed by different letters were significantly different at α=0.05 (Paired t-test).

**Table 7.** Influence of arbuscular mycorrhizal fungi on biochemical analysis of wheat.

| Conditions | Parameters | |
|---|---|---|
| | Growth Rate | Growth percentage |
| With AMF inoculations | 3.62±0.31a | 361.9±31.0a |
| Without inoculations | 3.26±0.13a | 326.0±12.7a |

a Means in the same row followed by different letters were significantly different at α=0.05 (Paired t-test).

**Table 8.** Influence of arbuscular mycorrhizal fungi on biochemical analysis of barley.

| Conditions | Parameters | |
|---|---|---|
| | Growth Rate | Growth percentage |
| With AMF inoculations | 3.58±0.19a | 350.0±12.8a |
| Without inoculations | 2.14±0.08b | 213.7±7.8a |

a Means in the same row followed by different letters were significantly different at α=0.05 (Paired t-test).

observed between the fresh roots of plants grown with and without AMF (P > 0.05). On the contrary, dry shoots of plants grown in AMF-inoculated soil weighed significantly more than those grown in non-inoculated soil (P < 0.05). While a significant difference in weight was observed for dry barley shoots and roots inoculated with AMF and those grown in non-AMF inoculated soils, no difference was observed for fresh shoots and roots (Table 5). The dry shoots and roots of AMF-inoculated barley crops weighed significantly more than those cultivated in soil devoid of AMF (P < 0.05). Kyriazopoulos *et al.* (2014) studied the effect of two Glomus species on plant roots. In their report, they found that AMF-inoculated plants showed a significantly higher shoot dry length compared to non-AMF inoculated plants. These findings are in accordance with those reported by Robinson *et al.* (2014), who observed an increase in shoot dry weight in AMF-inoculated plants. On the contrary, they found an increase in fresh shoot weight in plants treated with AMF. It is believed that the increase in fresh and dry shoot weight is probably due to the effects of AMF on the plants' ability to absorb nutrients such as nitrogen, potassium, zinc, calcium, and sulphur (Robinson *et al.*, 2014).

In the present study, the growth rate and growth percentage were increased in AMF-inoculated wheat (P < 0.05) (Table 6). While the growth rate was increased in barley crops treated with endomycorrhiza (P < 0.05), there was no significant difference in growth percentage between AMF and non-AMF-inoculated barley plants (P > 0.05; Table 7). Plant growth is increased under treatment with AMF (Chalk et al., 2006; Robinson et al., 2014). Recently, it was reported that the growth rate in plants treated with VAM was high since the fungi acted as growth regulators (Enteshari and Hajbagheri, 2011). In a more recent research, Rizvi et al. (2015) reported an increased in stem length and crop height as a result of inoculation with AMF. A similar increased was reported regarding other parameters, including root and shoot biomass.

Crops such as wheat, maize, onion, leek, sunflower, soybean and potato can benefit from AMF. This is particularly true in case of restricted plant growth due to nutrient deficiency. Besides affecting plant growth directly, AMF can also indirectly affect growth by stimulating soil quality and hinder the proliferation of organisms that decrease crop productivity (Carey *et al.*, 1992).

## CONCLUSION

Endomycorrhiza have been shown to positively impact root length, plant height, and stem length in both wheat and barley plants. Specifically, a significant difference in growth parameters was observed, with the roots, stem length, and plant height being greater in AMF-inoculated barley plants. Furthermore, the dry shoots and roots of AMF-inoculated barley crops differed significantly from non-AMF crops in terms of weight, with the dry shoots and dry roots of AMF-inoculated crops weighing more.. For wheat plants grown in AMF-inoculated soil, the dry shoots weighed significantly more than those grown in non-inoculated soil. This analysis also demonstrated that the growth rate and growth percentage were increased in AMF-inoculated wheat; however, AMF-inoculated barley plants only showed a significant elevation of the growth rate. Generally, AMF has a much more significant and positive impact on the growth of barley plants than compared to wheat plants.


## ACKNOWLEDGEMENT

The author would like to thank King Abdul-Aziz University-Biology department.


### REFERENCES


Allen EB, Allen MF (1984). Competition between plant of different successional stages: mycorrhizae as regulators. Canadian Journal of Botany. 62: 2625-2629.

Allen MF, Swenson W, Querejeta JL, Egerton-Warburton LM, Treseder KK (2003). Ecology of mycorrhizas: A conceptual framework for complex interactions among plants and fungi. Annual Review of Phytopathology. 41: 271-303.

Aroca R, Vernieri P, Ruiz-Lozano JM (2008). Mycorrhizal and non-mycorrhizal *Lactuca sativa* plants exhibit contrasting responses to exogenous ABA during drought stress and recovery. Journal of Experimental Botany. 59: 2029-2041.

Barker SJ, Tagu D, Delp G (1998). Regulation of root and fungal morphogenesis in mycorrhizal symbioses. Plant Physiology. 116: 1201-1207.

Beltrano J, Ruscitti M, Arango MC, Ronco M (2013). Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and P levels. Journal of Soil Science and Plant Nutrition. 13: 123-141.

Bonfante P, Genre A (2010). Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nature Communications. 1: 2041-1723.

Bressan W, Vasconcellos CA (2002). Alterações morfológicas no sistema radicular do milho induzidas por fungos micorrízicos e fósforo. Pesquisa Agropecuária Brasileira. 37: 509 -517.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996). Chapter 4: Examining mycorrhizal associations. Brundrett, M.; Bougher, N.; Dell, B.; Grove, T.; Malajczuk, N. (ed). Working with Mycorrhiza in Forestry and Agriculture, Canberra: Australian Centre for International Agricultural Research. 173-215 pP.

Carey PD, Fitter AH, Watkinson AR, Url S, Carey D (1992). A field study using the fungicide benomyl to investigate the effect of mycorrhizal fungi on plant fitness. Oecologia. 90: 550-555.

Chalk P, Souza R, Urquiaga S, Alves B, Boddey R (2006). The role of arbuscular mycorrhiza in legume symbiotic performance. Soil Biology and Biochemistry. 38: 2944-2951.

Conklin AR (2005). Introduction to Soil Chemistry. Analysis and Instrumentation. Vitha M. F. (ed). Hoboken: John Wiley and Sons. 218 pP.

Cooper KM (1984). Physiology of VA Mycorrhizae associations. In: VA Mycorrhiza. CL Powel, C.L.; Bagyaraj, D.J. (ed), CRC, Boca Raton, FL, USA. 155-186 pP.

Dodd JC (2000). The role of arbuscular mycorrhizal fungi in natural ecosystems. Outlook On Agriculture. 29: 55-62.

Enteshari Sh, Hajbagheri S (2011). Effects of mycorrhizal fungi on some physiological characteristics of salt stressed *Ocimum basilicum* L. Iranian Journal of Plant Physiology. 1: 215-222.

Fedderman NR, Finally T, Boller F, Elfstr (2010.) Functional diversity in arbuscular mycorrhiza the role of gene expression, Phosphorurs nutrition and Symbiotic efficiency. Fungal Ecology. 3:1-8.

Filion M, Arnaud M St, Fortin JA (1999). Direct interaction between the arbuscular mycorrhizal fungus Glomus intraradices and different rhizosphere microorganisms. New Phytologist. 141: 525-533.

Finlay R, Soderstrom J (1992). Mycorrhiza and carbon flow in the soil. Allen, M.F. (ed), Mycorrhizal Functioning: An Integrated Plant Fungal Process. Chapman and Hall, New York, London. 134-160 pP.

Goicoechea N, Antolin MC, Sanchez-diaz M (2000). The role of plant size and nutrient concentrations in associations between *Medicago*, and *Rhizobium* and/or *Glomus*. Biologia Plantarum. 43: 221-226.

Guether M, Neuhäuser B, Balestrini R, Dynowski M, Ludewig U, Bonfante P (2009). A mycorrhizal-specific ammonium transporter from *Lotus japonicus* acquires nitrogen released by arbuscular mycorrhizal fungi. Plant Physiology. 150: 73-83.

Kyriazopoulos AP, Orfanoudakis M, Abraham EM, Parissi ZM, Serafidou N (2014). Effects of Arbuscular Mycorrhiza Fungi on Growth Characteristics of *Dactylis glomerata* L. under Drought Stress Conditions. Notulae Botanicae Horti Agrobotanici. 42:132-137.

Linderman RG (1992). Mycorrhizae in Sustainable Agriculture. Bethlenfalvay, G.J. (ed). Amer Society of Agronomy, Madison, WI 124 pP.

Miransari M, Bahrami, HA, Rejali F, Malakouti MJ (2008). Using arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth. Soil Biology and Biochemistry. 40: 1197-1206.

Muchovej RM (2001). Importance of Mycorrhiza for Agricultural Crops, Florida. *PhD Thesis* Institute of Food and Agricultural Science University of Florida. 170 pP.

Norman JR, Atkinson D, Hooker JE (1996). Arbuscular mycorrhizal-fungal-induced alteration to root architecture in strawberry and induced resistance to the pathogen *Phytophthora fragariae*. Plant and Soil.185: 191-198.

Otgonsuren B, Lee J-T, Lee M-J (2015). Arbuscular mycorrhizae enhanced the growth and freezing tolerance of Mongolian crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.). International Journal of Advanced Research in Biological Sciences. 2: 191-204

Reboutier D (2002). The indolic compound hypaphorine produced by ectomycorrhizal fungus interferes with auxin action and evolves early responses in nonhost Arabidopsis thaliana. Molecular Plant Microbe Interactions. 15: 932-938.

Rizvi R, Iqbal J, Mahmood I, Ansari RA (2015). Comparative efficacy of different arbuscular-mycorrhizal fungal spp. (AMF) on tomato (*Lycopersicon esculentum* Mill.). e-Journal of Science and Technology. 1: 1-14.

Robinson J, Nithya K, Ramya R, Karthikbalan B, Kripa K (2014). Effect of Vesicular Arbuscular Mycorrhiza *Glomus fasiculatum* on the growth and Physiological response in *Sesamum indicum* L. International Letters of Natural Sciences. 23: 47-62.

Ryan J, Estefan G, Rashid A (2001). Soil and plant analysis laboratory manual. Jointly published by the International Center for Agriculture Research in the Dry Areas (ICARDA). Syrian, Aleppo. 172 pP.

Safapour M, Ardakani M, Khaghani S, Rejali F, Zargary K, Changizi M, Teimuri M (2011). Response of yield and yield components of three red bean (Phaseolus vulgaris L.) genotypes to co-inocu-lation with Glomus intraradices and Rhizobium phaseoli. American-Eurasian Journal of Agricultural & Environmental Sciences. 11: 398-405.

Sharma MP, Adholey A (2004). Effect of AM Fungi and P fertilization on the micro propagated strawberry grown in a sandy loam soil. Canadian Journal of Botany. 82: 322-328.

Souza PVD, Agustí M, Abad M, Almela V (2000). Desenvolvimento vegetativo e morfologia radicular de Citrange Carrizo afetado por ácido indolbutírico e micorrizas arbusculares. Ciência Rural. 30: 249-255.

Sreenivasa MN, Bagyaraj DJ (1989). Use of pesticide for mass production of vesicular arbuscular mycorrhizal inoculum. Plant and Soil. 119: 127-132.

Strack D, Fester T, Hause B, Schliemann W, Walter MH (2003). Arbuscular mycorrhiza: Biological, chemical and molecular aspects. Journal of Chemical Ecology. 9: 1955-1979.

Torrisi V, Pattinson, GS, McGee PA (1999). Localized elongation of roots of cotton follows establishment of arbuscular mycorhizas. New Phytologist. 142: 103-112.

Toshihiro A, Maldonado-Mendoza IE, Dewbre GR, Harrison MJ, Saito M (2004). Expression of alkaline phosphatase genes in arbuscular mycorrrhizas. New Phytologist. 162: 525-534.

Treseder KK, Allen MF (2000). Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO2 and nitrogen deposition. New Phytologist.147: 189-200.

van Lierop W (1988). Determination of available phosphor using acid and calcareous soils with the Kewlona multi-element extractants. Soil Science. 146: 284-291.

Wilde SA, Voigt GK, Iyer JG (1972). Part 1: Analysis of physical properties of soils. In: Soil and Plant Analysis for Tree Culture. New Delhi: Oxford and IBH Publishing Co. 6-34 pP.

Yousef AF (1999). Analysis methods and devices for soil and water. In Arabic: أجهزه وطرق تحليل التربه والمياه. King Saud University, Riyadh.

Zou Y-N, Srivastava AK, Ni Q-D, Wu Q-S (2015). Disruption of mycorrhizal extraradical mycelium and changes in leaf water status and soil aggregate stability in rootbox-grown trifoliate orange. Frontiers in Microbiology. 16: 6-203.