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Influence of arbuscular mycorrhiza in phosphorus acquisition efficiency and drought-tolerance mechanisms in barley (*Hordeum vulgare* L.)

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Abstract

Mycorrhiza can alleviate drought damage effects through osmoregulation improvement in plant cells. This research was conducted to investigate mycorrhiza on phosphorus content, phosphatase activity and physiological mechanisms on hulless barley against drought stress. Therefore, the seedling was saw in randomized complete block with two factors, *Glomus intraradices* (with and without inoculation) and three levels of water stress '90% FC (Control), 60%FC (mild water stress) and 30%FC (Sever water stress). Results showed that mild stress, mycorrhizal root volume and colonization percent were highest. Phosphorus leaves of mycorrhizal and phosphatase enzymes were highest against severe drought stress. Mycorrhiza improved phosphatase activity, carbohydrate and starch, the proline content of leaves and roots. Although mycorrhizal affects not only on plant water relations, but also improves P acquisition and host growth.

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Introduction

Drought stress is considered as major abiotic agents that limit plant growth and physiological mechanisms in semi-arid and arid regions of the world (Porcel and Ruiz-Lozano, 2004). Water potential gradient between the rhizosphere and root, affect on absorption process (Kramer and Boyer, 1995). Therefore root growth is less sensitive to water deficits than leaf growth and higher proportion of *assimilated carbon allocated* to root (Hsiao and Xu, 2000) and root dry mass is usually reduced under drought (Raziuddin *et al*., 2010).

Arbuscular mycorrhizal (AM) symbiosis can protect host plants against abiotic stress (Ruiz-Lozano, 2003). (Mycorrhizal association promotes plant absorption of scarce or immobile minerals, especially phosphorus, resulting in enhanced plant growth. Mycorrhiza fungi able to change plant structure, productivity and stabilization in soil aggregates (Miller and Jastrow, 2000). Phosphorus is lowest mobility in soil and thus prevents plant growth, when soil water potential and P is declined in dry or saline soils and mycorrhization increase plant growth and biomass under abiotic stress (Aggarwal *et al*., 2012). Moradi and Salimi (2013) believed that AM plants had lower resistances to water transport than non-mycorrhizal plants. AM symbiosis can diminish stress effects by using various defense mechanisms (Aggarwal *et al*., 2012). Aim of this research was evaluation barley responses with mycorrhiza symbiosis against salinity stress. To this order, phosphatase activity changes on phosphorus concentration and effects of mycorrhiza colonization of root, accumulation of osmotic adjustment such as proline and products of plant photosynthesis, carbohydrate and starch were important to study against drought stress.

Materials and methods

This research was conducted in randomized complete block with two factors in Plant Physiology Lab of Agriculture and Natural Resources Research Center, Golestan province, Iran. Factors of experiments were three levels of water stress'90% FC (Control), 60% FC (mild water stress) and 30% FC (Sever water stress) and other factor two levels (with the mycorrhizal fungus *Glomus intraradices* and without). Hulless barley seed were sterilized and saw in Syringes, 50 ml in incubator 20°C ± 2°C in sandy loam soil for 40 days (Table1). Soils were disinfected Field capacity and wilting point of soil was measured by pressure chambers. Two grams of inoculums were added to autoclaved soil in Syringes at sowing time just around seeds. After 40 days of growth under different water regimes, each syringes were emptied. Roots were separated from the soil manually and washed free of soil. Then each plant was separated into roots and shoots. Roots areas were obtained via scanning detailed in Bouma *et al*. (2000), Therefore roots were stained with Trypan blue and spended in water in a 20 cm 30 cm plexiglass tray, scanned at 400 dpi with an Epson 1680 flat bed scanner, and analyzed using GSA Image Analyzer (Regent Instruments, Quebec, Canada) with automatic threshold based on gray levels. Root and shoot samples were oven-dried (65°C, 24 h) and shoot, root weight, and volume were measured.

Arbuscular mycorrhizal fungi (AMF) root colonization and spores density

AMF colonization was determined according to Phillips and Hayman (1970). Root system of each plant were kept in 10% KOH solution at room temperature for 5days and stained with 0.05% (v/v) Trypan blue in Lactophenol. The calculation of AMF colonization was estimated using gridline intersect method (Giovannetti and Mosse 1980). Stained roots placed on the glass slides for studying by microscopic observations under 40×magnifications. Mycorrhizal Dependency (MD) was quantified by calculating formula (1).

(1) $$MD\ (\%) = \frac{(\text{Total dry weight M1} - \text{Total dry weight M0})}{\text{Total dry weight M1}} \times 100$$

Proline content

Leaf and root Samples (0.1 g) were homogenized with 1.5ml sulphosalicylic acid (3% w/v), and then the residue was removed by centrifugation at 18,000 g for 15 min. Supernatant (2 ml) were added to 2 ml of

glacial acetic acid and 2 ml ninhydrin solution (1.25 g ninhydrin dissolved in 30 ml glacial acetic acid and 20 ml 6 M orthophosphoric acid). Tubes were incubated for 1h at 100°C, and then allowed to cool in an ice bath. Finally toluene (4ml) were added and mixed on a vortex for 20 seconds. The test tubes were stand for at least 10 min and separated the toluene and aqueous phases. The absorbance of toluene phase was measured at 520 nm by spectrophotometer (WPA Biowave II). The concentration of proline was calculated from a standard curve in the range of 20–100 µg (Bates *et al*., 1973).

Total water soluble carbohydrate (WSC) and starch

Water-soluble carbohydrates and starch were analyzed by the phenol-sulfuric acid method. The dried plant matters (2 mg) were ground three times in ethanol (80% v/v) and centrifuged for 10 min at 10,000 g to pellet insoluble fraction. The centrifugal residues were used for starch. Then 10 ml distilled water was added to pellet dried and then, $Ba(OH)_2$ (0.3 N) and $ZnSO_4$ (5%) mixed with them. After centrifuge samples (3000 rpm for 10 min), 1ml phenol (%5) and 5 ml of sulfuric acid (98%) in 2ml Supernatant were added. Finally the absorbance of extraction for determining carbohydrate and starch were detected at 485 nm.

Phosphorus determination and alkaline phosphates activity

Phosphorus content was studied following Watanabe and Olsen (1965). Dried material (1g) was digested with HCl. Phosphate was measured with a spectrophotometer in absorbance at 470 mm 15 min after adding chromophoric solution (vanadate and ammonium molybdate) to samples. Alkaline Phosphatase activity assay was obtained using Lowry *et al*. (1954) method. Roots (1g) were ground in 50 mM Glycine NaOH buffer (pH 10.5) for alkaline phosphatase. Extraction buffer were centrifuged at 10000×g for 10 min. Supernatant was used for alkaline phosphatase activity and expressed in moles of PNP released g^{-1} fresh weight of sample.

Results and discussion

Root traits

Drought stress significantly decreased in root volume. Mycorrhizal root volume under 60%FC was highest than the other mycorrhizal root (Fig1a). Also Root area of inoculated in 60%FC was increased than mycorrhizal in other water treatments (Fig1b). Maximum root area was founded in non mycorrhizal under 90%FC (241.35Cm^2) that in comparison lowest root area inoculated in severe stress (30%FC) was 61.7%. Expansion in root area is an adaptive mechanism that accumulates more solutes in plant cell (Dichio *et al*., 2006). According to results, under mild water stress, root growth is restricted in comparison with mycorrhizal root. Reported that water deficiency decrease root surface and volume of poa, but mycorrhizal symbiosis, alleviates water shortage through increasing in root morphological properties (Moradi and Salimi, 2013) Extra radical AMF, outstretch the root surface area and improve the acquisition of Mineral nutrients and water by the roots (Bethlenfalvay *et al*., 1988). AM fungi induce drought tolerant by expanding root hairs area and plant growth, therefore promote plant growth against drought stress through enhance in shoot and root P concentration (Li *et al*., 2014). Effects of mycorrhiza symbiosism on some crops such as Sugarcane, Mungbean, Wheat and Tomato confirmed that mycorrhiza affect on uptake capacity and vegetative growth (Wu and Xia, 2004). Moradi and Salimi (2013) reported that Poa with mycorrhiza had greatest volume root than without AMF.

Table 1. Physical and Chemical Properties of Soil.

Soil texture	wilting point	Field Capacity	silt(%)	sandy(%)	clay(%)
Sandy soil	10.97	20.17	10	86	3.2

Barley root with AMF had lesser phosphorus content (Fig2a). Storage of phosphorus in root without AMF and 60%FC with 2.20 mgg^{-1} was higher than control. In a while, the highest values of phosphate were

founded in the leaves of mycorrhizal plants. Phosphorus Leaves of mycorrhizal against severe drought stress were highest significantly ($p<0.01$). (Fig2b) was shown, phosphorus accumulation in leaves was varied from 0.276 to 1.537. Phosphatase enzyme in mycorrhizal root was most active in 30%FC (Fig3). Roots with AMF in Medium water stress had 17.9 OD Min Fw^{-1} phosphatase enzyme activation that was lowest among all of treatments. Mycorrhizal symbiosism improve P availability in rhizosphere (Ingrid *et al.*, 2002). Generally, inadequate soil phosphorus (P) decreased the mycorrhizal colonization in the root. Lu *et al.* (2007) found that mycorrhiza hyphae enable to absorb fixed phosphorous from rhizosphere where plant roots couldn't. Also effects of mycorrhiza symbiosis on leaves phosphorus were approved (Al-Karaki *et al.*, 2004; Wu *et al.*, 2004).

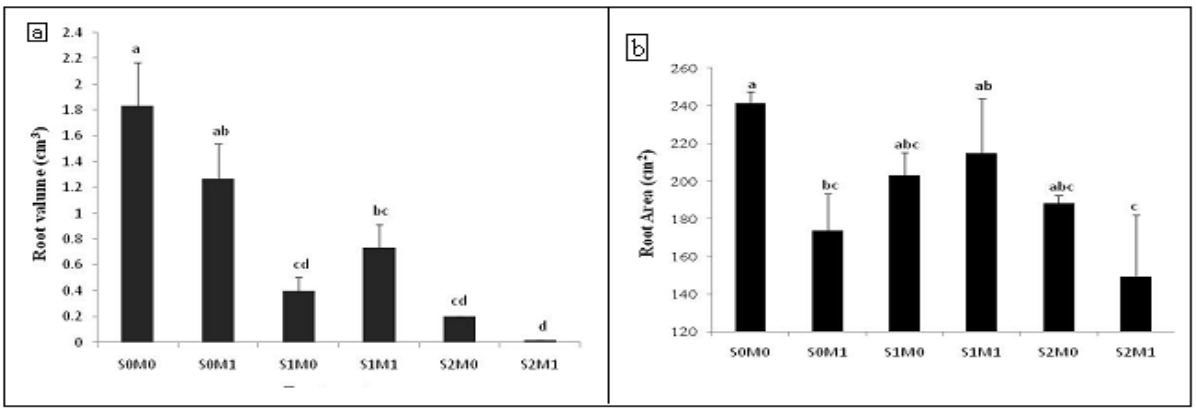

Fig. 1. a) Root volume (cm^2) and b) Root area(cm^2) in influence of inoculation with *Glomus mosseae* at three levels water stress (So(Control) 60% FC (mild stress) and 30% FC (sever stress).

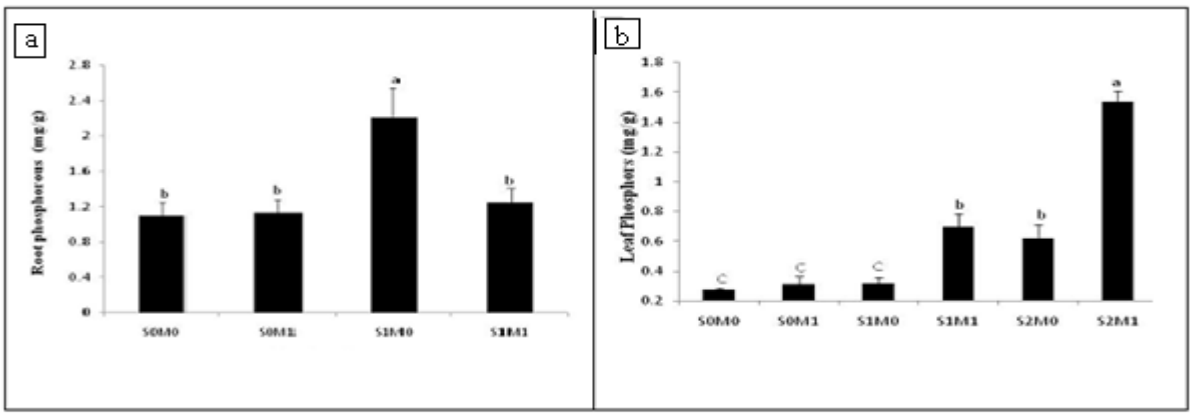

Fig. 2. a) Root phosphorus (mg/g) and b)leaf phosphorus(mg/g) in influence of inoculation with *Glomus mosseae* at three levels water stress(So(Control) 60% FC (mild stress) and 30% FC (sever stress).

Also, AMF improve phosphatase secretion and efficiency of P uptake (Ezawa *et al.*, 2005). Deep rooted mycorrhizal plants, allows absorbing sufficient water and minerals supplements from dry soil (Mastrangelo *et al.*, 2011). Wu *et al.* (2011) in studies of *Glomus diaphanum*, *Glomus mosseae* or *Glomus versiforme* inoculated seedlings concluded that total phosphatase activities were increased significantly in mycorrhizal seedlings. Phosphatase activity (ALP) have correlated positive and strong on H^+ and improve drought resistance (Tang and Chen, 1999). Wu *et al.* (2011) on evaluation effect of mycorrhizal symbiosism on ALP *trifoliate orange* approved that total phosphatase activities increased with AMF and leaf and root P content were higher significantly than Non-AMF, but soil available P contents was decreased.

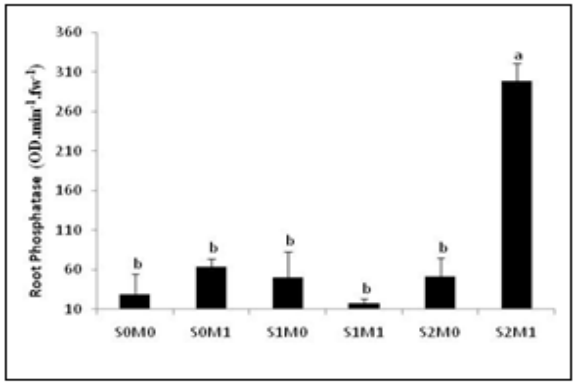

Fig. 3. Root phosphatase (OD.min^{-1}fw^{-1}) in influence of inoculation with *Glomus mosseae* at three levels water stress (So (Control) 60% FC (mild stress) and 30% FC (sever stress).

Mycorrhizal symbiosis and mycorrhiza dependency

As results showed the symbiosis of plant roots with AM fungi was induced in 60% FC (mild stress) and 30% FC (sever stress) (Fig4). However, percent root colonization in moderate stress with 34.5% was greatest than others (Fig5) Percentage of colonization without AMF in 30%FC was 9%. Dependency effects of AM symbiosis on mineral absorption increase with reducing root area (Porcel and Ruiz-Lozano, 2004). Plant dependency on AMF was varied extremely among crops (Al-Karaki, 2013).

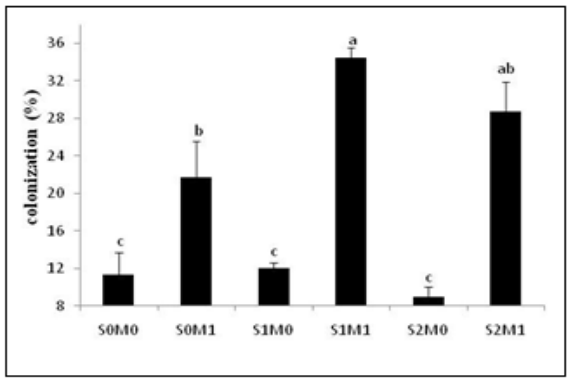

Fig. 4. Colonization (%) in influence of inoculation with *Glomus mosseae* at three levels water stress (So(Control) 60% FC (mild stress) and 30% FC (sever stress).

Therefore, plant response to AMF has affected on management in Cropping Systems (Bouamri *et al.*, 2006). Mycorrhizal efficiency is important for improving plants growth, but under high levels of P fertilization, mycorrhiza dependency has been slowed down (Ezawa *et al.*, 2000). Also, Amaya-Carpio *et al.* (2009) on effects of P manure on *Ipomoea carnea ssp*. founded that colonization of mycorrhiza with P in soil was inhibited and phosphorus absorption by root from rhizosphere of mycorrhizal plants will drop (Ghazi and Al-Karaki, 2013). pH changes of rhizosphere will restrict AM establishment and germination of *Glomus mosseae* will stimulate between pH 5-6 (Aggarwal *et al.*, 2013).

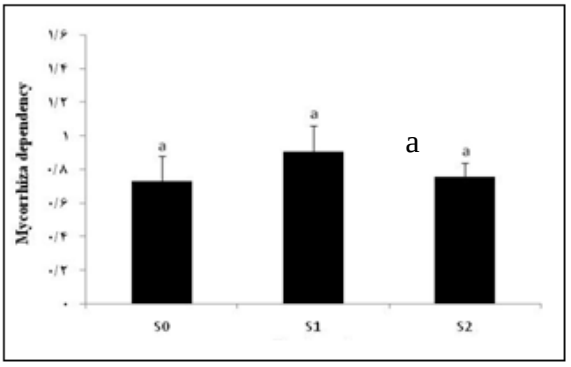

Fig. 5. Mycorrhiza dependency in influence of inoculation with *Glomus mosseae* at three levels water stress(So(Control) 60% FC (mild stress) and 30% FC (sever stress).

Carbohydrate and starch

Carbohydrate content in root enhanced in mycorrhizal roots in severe water stress significantly ($p<0.05$) (Fig6a). (Fig6b) showed the addition of mycorrhiza fungi at tillering stage induced a conspicuous increase in carbohydrates in leaves of hulless barley. The results indicated that arbuscular mycorrhizal fungi inoculation could increase carbohydrate of leaves in severe water stress in comparison with the other treatments. Porcel and Ruiz-Lozano (2004) founded that the effect of high sugar content in AM roots under well-watered conditions related to carbohydrate-demanding from shoot and transportation to roots. Therefore sugar content of shoot with AMF was lower than non-AM plants (Schellembaum *et al.*, 1998). Garmendia and Mangas (2012) were showed total soluble sugars of cut flower roses with mycorrhiza were not significant. Starch status of VA mycorrhizal root rose exposed to 30% of FC (Fig7), the roots of plants with mycorrhiza under moderate stress reduced starch content. Decrease in hexose accumulation in leaves of plant

with mycorrhiza in case of lower photosynthetic compounds and strong competitor of root for receiving a carbon supply (Schellembaum *et al.*, 1998). Wu *et al.* (2011) suggested that starch concentration of root and shoot of *Cynodon dactylon*L. With mycorrhiza reduced that related to hydrolyze starch to soluble carbohydrate in AMF plants. But they found total non-structural carbohydrate increased in mycorrhizal root and shoot.

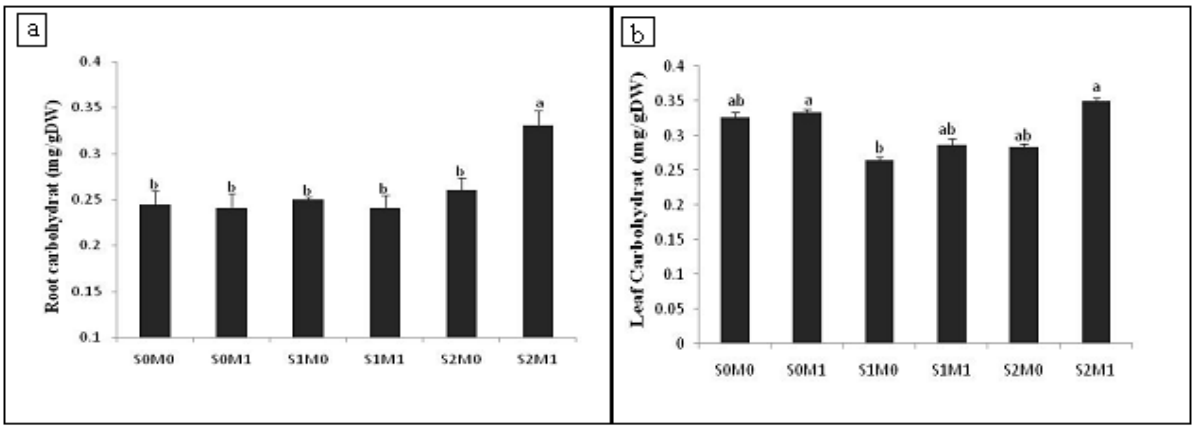

Fig. 6. a) Root Carbohydrate(mgg^{-1}Dw) and b) leaf Carbohydrate (mgg^{-1}Dw) in influence of inoculation with *Glomus mosseae* at three levels water stress(S0(Control) 60% FC (mild stress) and 30% FC (sever stress).

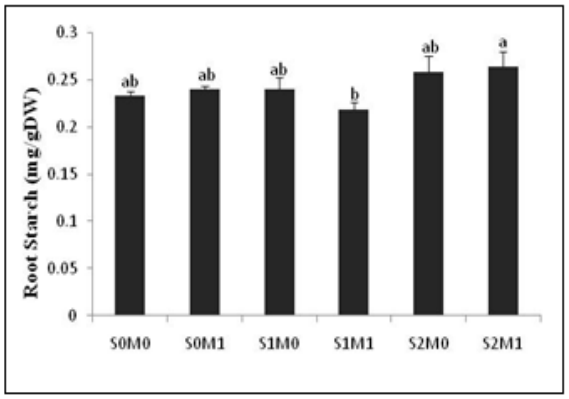

Fig. 7. Root Starch (mgg^{-1}Dw) in influence of inoculation with *Glomus mosseae* at three levels water stress (S0(Control) 60% FC (mild stress) and 30% FC (sever stress).

Proline content

Amount of proline in root with AMF significantly reduced in mild and severe drought stress in the comparison of 90% FC. Leaves proline accumulation of mycorrhizal plant in 90% and 60% FC enhanced (Fig8). Porcel and Ruiz-Lozano (2004) concluded proline as an osmoregulator can increased in shoots with AMF than in non-AM plants, then protect plants against stress damage. Amerian and Stewart (2001) showed leaf area of mycorrhizal plant improved in comparison non mycorrhiza. High proline content in shoot drove deeper soil moisture extraction (Chimenti*et al.*, 2006). Proline accumulation in shoot and root enhanced osmotic adjustment (Ruiz-lozano *et al.*, 1995). Fitter (1988) believed that protection of mycorrhizal plants against water stress related to osmotic balance in plant cell. Basically, according the results of Pavla Doubková *et al.* (2013) Increase in proline content in the roots of mycorrhizal plants during water shortage can be benefit in drought intensity. Proline prevents protein denaturalization and preserves pH levels in cell and then lead to maintain nodule metabolism (Aggarwal *et al.*, 2012).

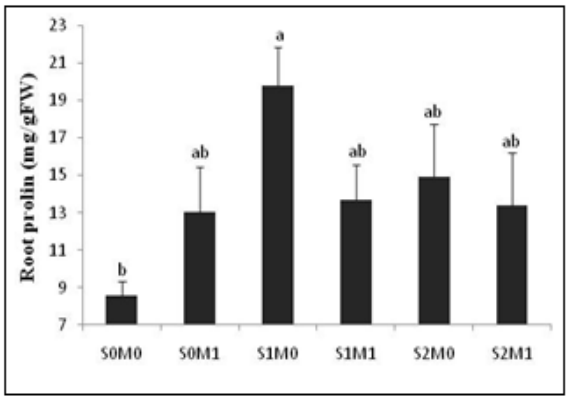

Fig. 8. Root Prolin (mg/gFW) influence of inoculation with *Glomus mosseae* at three levels water stress(S0(Control) 60% FC (mild stress) and 30% FC (sever stress).

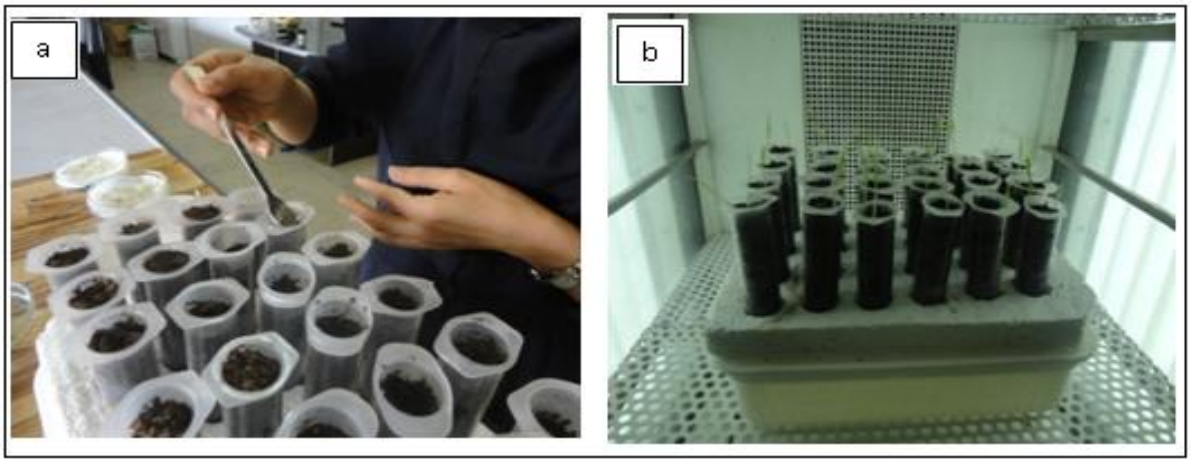

Fig. 9. a) Sowing hulless barley with *Glomus mosseae* at three levels water stress (S0 (Control) 60% FC (mild stress) and 30% FC (sever stress) b) growth period of plant in Incubator (20°C ± 2°C).

Conclusion

Our results suggest that plants benefit from the AM symbiosis under saline soil conditions mainly in terms of an improved phosphorus uptake in leaf with activation phosphatase enzyme. This may greatly facilitate the recovery of plants using decreasing proline accumulation root and enhancing proline content leaves under mild stress. Results further demonstrate that is strongly dependent on the association with AM fungi for growth on a saline soil. Plants were only able to recover from a period of salinity stress when AM colonized. It is recommended to monitor AM root colonization of such plants in the field, and to inoculate the soil with salinity tolerant AM fungal strains such as *Glomus intraradices* in case the naturally occurring AM infective potential should be too low to initiate appropriate symbiosis development. The overall data show that both root and shoot tissues are influenced by AM symbiosis by means of salinity-avoidance and salinity-tolerance mechanisms

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