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Growth response and drought tolerance of *Lens Culinaris* inoculated with Arbuscular Mycorrhizal Fungi

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ABSTRACT

The use of Arbuscular Mycorrhizal Fungi (AMF) as bio-fertilization technology in the agriculture, which would contribute effectively to improving plant productivity is being discovered in the recent research. The influence of inoculation with AMF on growth processes is difficult to evaluate, in part because it varies with the inoculated plant and used AMF species as well as with pedoclimatic conditions. Our work is conducted in a greenhouse at well-defined water stress regime of 25%, 50% and 75% of field capacity (CC), to evaluate the effects of inoculation with AMF on growth and tolerance of Lens culinaris to lack water. Native mycorrhizal strains from rhizospheric soils and exogenous strains "Glomus spp" were used in this experiment. The effects of AMF were evaluated after two months of culture. The highest colonization rates on roots of plants were registered at severe stress water. AMF inoculation increased clearly all growths parameters of mycorrhizal plant compared with those uninoculated. AMF was showed a significant effect on the tolerance of the young legume to the water stress. It is concluded that the indigenous showed positive effects on growth stimulation and resistant to water stress.

Key words: *Arbuscular Mycorrhizal Fungi, Lens Culinaris, Water stress, Inoculation*

INTRODUCTION

Lens Culinaris (*L. Culinaris*) is among the important legume in the agricultural systems. This legume is source of human nutrition and animal consumption[1]. In Tunisia, the main production of *L. Culinaris* was around 1687 tonnes, covering an area of 3750 acres[2]. However, the cultivation and productivity of this legume in arid and semi-arid region confronted to certain edaphic stress, such as salinity and drought[2,3].

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Drought was one of the most severe and widespread problems as a result of the agricultural production in semi-arid regions[4].

As an adaptation strategy to water stress, symbiotic relationships between plants and benefic micro-organism especially, Arbuscular Mycorrhizal Fungi (AMF) are ubiquitous and play a very important ecological role in arid and semi-arid ecosystems. Mycorrhizal associations played a significant role in plant hydro-mineral nutrition[5,6]. AMF were significant drivers of nutrient cycling[7]. It was reported the significant effect of AMF in soil structures and their stability[8]. AMF played a crucial role in ecosystem dynamics and the adaptation of the major vascular plants to their environments[9].

Because beneficial effect of AMF would improve the plant development, the use of AMF as inoculum may be a key factor in agriculture system. Until now, several studies reported that AMF may be benefic in agricultural ecosystems[10]. The use of AMF as inoculum can be increase plant biomass and minimized the different effects of environmental stresses especially drought[11,12]. In the same context, previous works reported that AMF improved significantly the aerial[13] and root[14] biomass of inoculated plants. All these finding may be confirmed the evidence using AMF as inoculum in sustainable agriculture. Some AMF species could improve the tolerance of inoculated plants to drought stress. However, the growth and tolerance response to inoculation depend at the first the mycorrhizal strain used[15], second, the capacity of mycorrhizal strains to increase plant growth and health[16], and finally the pedoclimatic conditions[17].

However in Mediterranean ecosystem especially in Tunisia, the application of AMF as bio-fertilization technology in agriculture to enhance plant growth and stress tolerance is still under-exploited and few studies has been interested to this bio-fertilization. Indeed, the eff ectiveness of AMF as inoculum to improve the growth and productivity of some cultured plants in semi-arid ecosystem as Tunisia has not been well documented. Therefore, to determinate the impact of AMF in the agriculture at Tunisia ecosystem, our work aimed to evaluate the response of *L. culinaris* to different types of AMF inoculation under three water stress regime.

MATERIALS AND METHODS

Plant Material and Fungal inoculums

L. Culinaris was used in this study in order to evaluate the efficacy of AMF inoculum. It was among most cultivated legumes at arid region of Tunisia. For the fungal inoculum, composite native indigenous strains was selected at four rhizospheric soils at semi-arid sites "Bou-Hedma National Park, Tunisia", with distinct proprieties (type of soil, management practice...) and a plant species presenting high AMF colonization "*Medicago truncatula*". Three sites were taken inside the Park where the first site (Soil 1) was an open area near an *Acacia* population; the second (Soil 2) was located near a seasonal river; and the third (Soil 3) on the mountain summit (600 m altitude). While, the fourth site (Soil 4) was outside the Park and it was subject to different managements. Table 1 shows the proprieties of the four studied soils. Commercial inoculum of AMF (exogenous strains) containing some species of *Glomus* genus which is considered widespread AMF genera in arid and semi-arid regions[18,19], was obtained by French society "Inoculum plus". Mycorrhizal strains were propagated in association with *Zea mays* as host under standard pot culture conditions in greenhouses preparing each inoculum[20]. With three replicates per treatment, plant trap was grown in pots containing 250 g of soil (Rhizospheric of *M. truncatula* in different sites) and 1750 g a sterilized substrate. During three months of cultivation, solution of Long Ashton[21] was used regularly to water the plants. The obtained inoculum was consisted a mixture of spores and fragments of mycorrhizal roots.

Preparation of Pot Experiment

About 20 g mycorrhizal inoculum was used by cultivating pot filled with autoclaved (120 min two consecutive days at 120°C) substrates[20]. Pots were treated either with: native AMF

strains, commercial AMF (*Glomus* spp.) or uninoculated control. The uninoculated received only the sterile substrate. The different treatments were subjected to three water stress levels of 25%, 50% and 75% compared to the capacity of the floor field. The experiment lasted two months of culture. As shown in Fig. 1 which explain the experimental procedure used in this study, three factors were elaborated: inoculation factor at three levels, water stress at three levels also and sampling data at three dates.

Plant harvest and analysis parameters

After 40, 50 and 60 days of growth, plants were harvested and some parameters were analyzed. The growth of *L. culinaris* in height was followed for each type of inoculation. Aerial and root parts of three plants were separated to determine their fresh biomass and after drying at 30°C for 48 hours, to evaluate their dry biomass. Mycorrhizal colonization (mycorrhizal frequency and intensity) were evaluated by microscope observation of 30 root fragments per plant, according to the technique of Trouvelot et al.[22]. Fines root segments were submerged in 10% potassium hydroxide (KOH) at 90°C for 45 min to clear them, bleached in Hydrogen peroxide (H_2O_2) for 3 min and acidified in 1% hydrogen chloride (HCL). Finally, root segments were stained for 90 min with 0.05% Trypan Blue at 60°C. Stained 30 root fragments were checked for AMF infection (hyphae, vesicles, and arbuscules) by examination under a compound microscope and the mycorrhizal colonization percentage were evaluated by the root slide technique[23].

Statistical analyses

Statistical analyses were performed by using the SAS statistical package. The statistical effects of AMF inoculums were subjected to ANOVA for repeated measures. The least significant difference values at 5% level of significance ($P \leq 0.05$) were calculated to assess differences between parameters. Three ways-ANOVA was used to determine relationships between variables.

RESULTS

Influences of different factors on the AMF Colonization of *L. culinaris*

As a response to different inoculum types, the roots of the studied plant were colonized with different AMF morphological structures (intraradical hypae, arbuscules, vesicules and extra-radical spores) as shown in Fig. 3. All of these symbiotic structures could confirm the natural mycorrhization of *L. culinaris*. Fig. 2 shows that the type of inoculums can be affected the infection rate of the root system by AMF. Statistical analysis showed that the mycorrhizal frequency (F %) and intensity (M %) differed among species the six types of inoculum. The highest colonization was recorded in the case of inoculation with *Glomus* spp (21%). and the rhizospheric soil on Site 1 (20%). The roots of uninoculated plant did not present any structures of the endomycorrhizal fungi.

Results illustrated also in Fig. 2 revealed that the impact of AMF inoculum is affected by various factors especially imposed drought stress and sampling dates. AMF morphological structures were apparent from the collection to 50 days of growth. Mycorrhizal infection rate appears clearly to be affected by the imposed regime water (Fig. 2). The highest Mycorrhizal intensity and frequency was recorded in the stress water by 25% CC than 50 and 75% CC. Regarding a moderate drought level, the application of 75% CC decrease AMF colonization in root cortex of this legume in the different inoculation types (Fig. 2). Whatever the level of applied water stress, plants inoculated with *Glomus* spp. and Soil 1 registered the higher percentage of roots mycorrhizal infection.

Influences of different factors on the growth of *L. culinaris*

The conclusive information that has been obtained to Figs. 4, 5 and 6 showed that inoculated *L. culinaris* had a significantly higher growth for all analysis variables compared with non-inoculated plants. All growth parameters were more pronounced for inoculated plants (with native or commercial strains) than for non-inoculated plants. For the

sampling factor, the morphological studied parameters were characterized by rapidly and highly value after 50 days of growth. At the first date of harvesting, the inoculated plants have slightly increased than non-inoculated plants. The impact of AMF in the growth of *L. culinaris* has not yet been very significant at this date. Regarding inoculation factor, the types of used inoculation (native or commercial strains) have a significant effect on biomass and length of aerial and root part ($p<0.001$). Therefore, as indicates in Figs. 4, 5 and 6, the responses of the present plant to AMF was dependent highly the types of used inoculation. Forever, the result of this study shows that inoculation with Soil 1 and *Glomus* spp was registered the highest value. The most noticeable, statistical analysis confirmed the similar results between those two type inoculations for the major studied variables. Considering water stress, the drought had a negative effect on the most studied morphological parameters.

As first negative result of water deficient, the biomass and the elongation of aerial part of this plant is reduced according the accentuated of water stress level (Figs. 4 and 6). Contrary, root biomass and length were significantly higher with the applied stress at level of 50% and 25% CC (Figs. 5 and 6). Statistical analysis indicated that aerial and root biomass data for different inoculation types (Figs. 4 and 5) showed the different impact of water stress. Indeed, the aerial and root biomass of all inoculated plants was significantly greater than non inoculated plants.

Table 3 present the rapport of aerial biomass to root biomass. The result of this table demonstrated that the imposed water stress stimulated the increase of the underground biomass compared to the aerial biomass. The development of root system is associated with the water efficient.

Correlation and Interaction between the three studied factors

Table 2 registered the correlation and the interaction between inoculation types, water stress and sampling factors. From the obtained results, it is appearing clearly that mycorrhizal colonization (mycorrhizal frequency and intensity) correlated significantly with inoculation factors. The high value was registered at native AMF strains from Soil 1 and exogenous strains. More the positive correlation with inoculation types, the percentage of AMF colonization correlated the sampling date. At the first harvesting (40 days), mycorrhizal structures are not detected for any inoculations types or water stress even though at 25% CC. Also, Mycorrhizal parameters appear correlated with water stress factors (Table 2). The highest mycorrhizal colonization was registered in the inoculation with Soil 1 and *Glomus* spp. under limit deficiency water (25% CC) and after 60 days of growth. At the same context regarding growth parameters, growth response of *L. culinaris* was varied according to the correlation between the three studied factors: AMF inoculation, date of sampling and water stress.

DISCUSSION

In our present study, the growth and drought resistance of *L. culinaris* was studied by inoculation of AMF (native mycorrhizal and exogenous strains), under three drought stress.

As showing in the results of Figs. 2 and 3, the microscopic observation of root samples revealed that AMF successfully colonized the roots of our studied plant as evidenced from extensively ramified hyphae and presence of arbuscules and vesicles. All this structures confirmed the natural colonisation of plant[14]. The highest rate colonization of root samples could be explained by the high mycotrophy of this Fabaceae, which is formed naturally a dense mycelium filament at the soil[14]. The present legume was identified as highly mycotrophic plant.

L. culinaris responded in variance to AMF inoculation used in this study. This response to AMF inoculation is correlated generally with the performance of the treated host plant. However, the response degree to AMF inoculation depended on the types of fungal strain (Fig. 2). Similar finding were

reported with other plant species under semi-controlled conditions[13,14]. The highly percentage of root infection was registered in the inoculation with *Glomus* spp. and Soil 1. For *Glomus* it may be explained by the widespread of these genera in Mediterranean ecosystem[18,19]. For Soil 1 which was contained heterogeneous AMF species may be explained by the positive interaction of AMF species increasing plants growth and adaptation to their ecosystem [24,25]. The present result as indicated in Fig. 2 and Table 2 was showed that mycorrhizal colonisation of *L. culinaris* correlated significantly with water stress factor and sampling date. The impacts of drought are varied according AMF species and the available water level. The studies of Lozano et al.[26] and Guissou et al.[27] reported the same finding. Mycorrhizal colonization increases significantly with the level of drought stress. The accentuated water stress is accompanied with highly rate AMF colonization (Fig. 2). Radi et al.[28] registered the same correlation between water stress and AMF colonization in their study. On the contrary, the works of Kong et al.[29] and Mirshad and Puthur[30] concluded negative correlation between this two factors which indicates that water stress has inhibited the infection of AMF.

AMF inoculation has influence on the growth of plants and their resistance to environmental stress[26,27]. The growth of plants is generally regulated by water and nutrients. AMF inoculation can promote plant absorption of mineral elements and water nutrition[31]. However, mycorrhizal plants were able to absorb the water beyond several centimeters to roots absorption zone[32,33]. AMF can be influence the water holding capacity of the soil[34] and facilitates water uptake even at low water level by extra radical hyphal growth[35]. AMF affect positively plant nutrition which is generally improved in the aerial part of mycorrhizal plants compared those non-mycorrhizal[29,6]. Biomass and length of aerial parts (Figs. 4 and 6) of mycorrhizal *L. culinaris* are very important compared to those non mycorrhizal (uninoculated). Following the works of Khan et al.[36] and Fan et al.[37] which confirmed that AMF stimulates the biomass of root system, our results (Fig. 5) showed clearly that AMF inoculation improved significantly the length and biomass root of mycorrhizal plant.

In the present study, drought had a negative effect on the growth of *L. culinaris* (Figs. 4, 5 and 6). The morphological studied parameters of this plant reduced following the applied water stress. Confirming to our results, the study of Radi el al.[28] concluded that lack water decreased the growth parameters of plants. AMF inoculations improved the drought resistance of our studied legume. The mycorrhizal plant will acquire a better tolerance to imposed drought[28]compared those uninoculated. As a first response, the increase of root biomass is generally associated with the drought condition. Moreover, AMF inoculation provided extensive extra-radical mycelium, which would increase surface areas of mycorrhizal roots in order to acquire more nutrients and water improving resistance to drought stress[38]. According our result (Fig. 5), it is evident that an increase in the root biomass allows obtaining high yields under conditions of water stress[39].

Further, the positive impact of AMF to improve plant drought resistance (Figs. 4, 5 and 6) is closely associated with AMF species (inoculation types). Previous study of Guissou et al.[27] concluded that root growth (density and length) were positively correlated with AMF strains. What appear clearly from Figs. 4, 5 and 6 the mixed AMF species of Soil 1 affected positively the major growth proprieties of *L. culinaris*. The native AMF strains were effective as *Glomus* spp. for plant growth and biomass production.

Under stressful water regime, the inoculation of AMF can enhance root growth than that aerial part so as to accelerate plant tolerance to lack water (Table 3). The development of root part may be a resistance strategy to water stress. The highly root

growth can be explained by the extra-radical mycelium formed by AMF under stress water to explode soil and increase the absorption surface of root system[28]. Furthermore, the inoculation of AMF can increase of osmotic pressure, reduces water loss, and slows down lack of water caused by the imposed drought[27,29]. Drought stress accelerates the accumulation of some composed (sugar and protein) play a significant role to maintaining osmotic pressure and reducing water loss as possible[28,29].

Tables and Figures

Table 1: Description of the four studied Soils as indigenous inoculum. Mean of three replicates and ± standard error

Sites	*Soil texture	*Spore density (spores/ 100 g of soil)
Soil 1	Sandy loam	517± 28
Soil 2	Sandy	437± 12
Soil 3	Sandy loam	464± 07
Soil 4	Sandy loam	369± 14

Soil texture determined according the method of Naanaa and Susini[40]. AMF spores occurring in soil samples were extracted following the wet sieving method described by Gerdemann and Nicolson[41].

Table 2: three way- ANOVA for the three studied factors (Inoculation types, water stress and sampling date) and their interactions

	Inoculation types	Water Stress	Sampling date	Inoculation types * Water Stress	Inoculation types* Sampling date	Inoculation types * Water Stress* Sampling date
Mycorrhizal frequency F%	1766,07 ***	2968,27 ***	2097.32 ***	130.93 ***	93.43 ***	8.2 ***
Mycorrhizal Intensity M%	10123,36 ***	45788,37 ***	12176.4 ***	2095.76 ***	677.76 ***	97.72 ***
Weight of Aerial part	787,27 ***	1040,69 ***	7167.43 ***	8.62 ***	124.25 ***	1.89 *
Dry Weight of Aerial part	13,7 ***	51,5 ***	1238 ***	8.3 ***	6.8 ***	9.5 ***
Weight of root part	1983,18 ***	505,8 ***	5129 ***	7.32 ***	63.7 ***	4.35 ***
Dry Weight of root part	57,6 ***	28 ***	175 ***	4 ***	28.6 ***	8 ***
Weight of Aerial part / root part	290,05 ***	1518,07 ***	194.73 ***	29.07 ***	33.94 ***	4.62 ***
Length of Aerial part	1642,01 ***	417,51 ***	1672.57 ***	80.35 ***	10.94 ***	4.39 ***
Length of root part	684,03 ***	695,77 ***	6022.73 ***	6.51 ***	7.72 ***	2.2 **

Note: *** Significant at P<0.001, ** Significant at P<0.01, *Significant at P<0.05, ns: non-significant

Table 3: Weight of Aerial part/ root part of *L. culinaris*. Mean of three replicates and ± standard error. Differences between groups were significant P<0.05

		Inoculation types					
Water stress	**Sampling date**	*Glomus* sp.	Soil 1	Soil 2	Soil 3	Soil 4	Control soil
75% CC	After 40 days	2.27±0.29	2.10±0.14	2.01±0.15	1.91±0.10	1.92±0.19	1.51±0.14
	After 50 days	1.81±0.02	2.16±0.08	1.94±0.03	1.82±0.09	1.81±0.14	1.61±0.09
	After 60 days	2.07±0.23	2.06±0.07	2.65±0.22	2.29±0.04	2.60±0.10	2.56±0.14
50% CC	After 40 days	1.61±0.13	1.41±0.14	1.47±0.09	1.61±0.08	1.55±0.03	1.61±0.04
	After 50 days	1.77±0.08	1.96±0.12	1.62±0.03	1.48±0.10	1.56±0.01	1.48±0.16
	After 60 days	1.72±0.03	1.66±0.04	1.99±0.11	1.79±0.10	1.97±0.05	1.76±0.16
25% CC	After 40 days	1.19±0.06	1.26±0.05	0.98±0.05	1.14±0.01	0.78±0.11	0.95±0.10
	After 50 days	1.18±0.05	1.74±0.06	1.15±0.02	1.41±0.08	1.17±0.04	1.19±0.12
	After 60 days	1.54±0.04	1.56±0.01	1.26±0.03	1.50±0.08	1.24±0.06	1.24±0.04

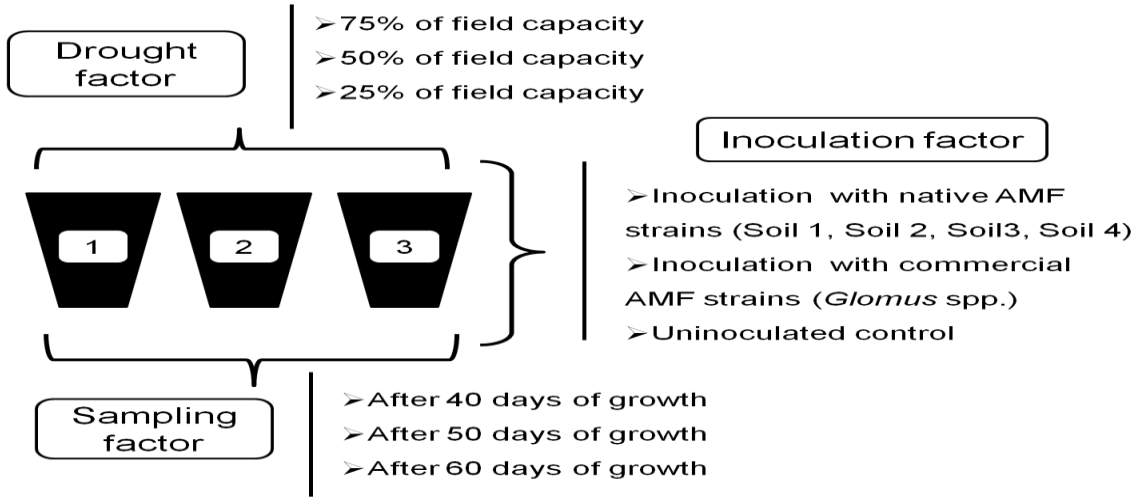

Fig. 1: Description of the experimental procedure (Inoculation, water stress and sampling factors) used in this study

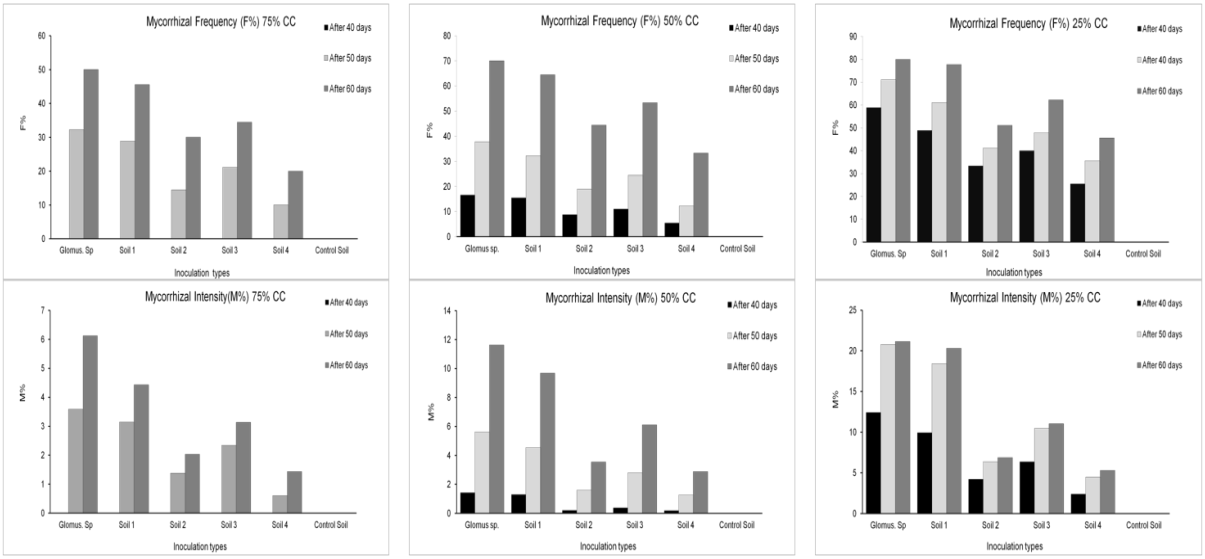

Fig. 2: AMF root colonization (mycorhization intensity (M) and mycorhization frequency (F)) of *L. culinaris*. Mean of three replicates and ± standard error. Differences between groups were significant P<0.05

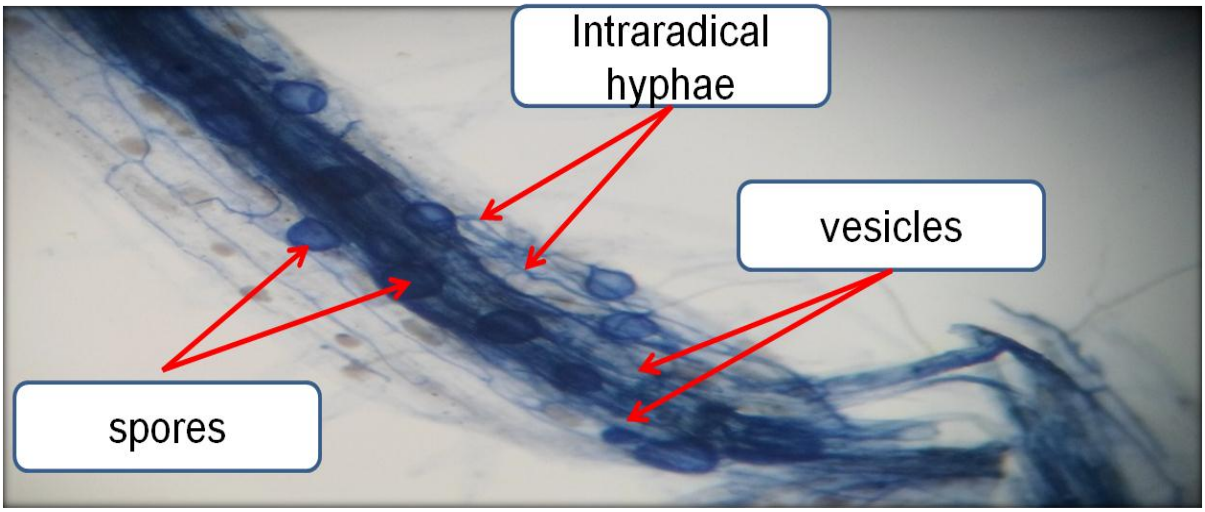

Fig. 3: *L. culinaris* root colonisation by AM fungi (40× magnification) inoculated with Soil 1 and collected after 60 days of growth at 25% CC

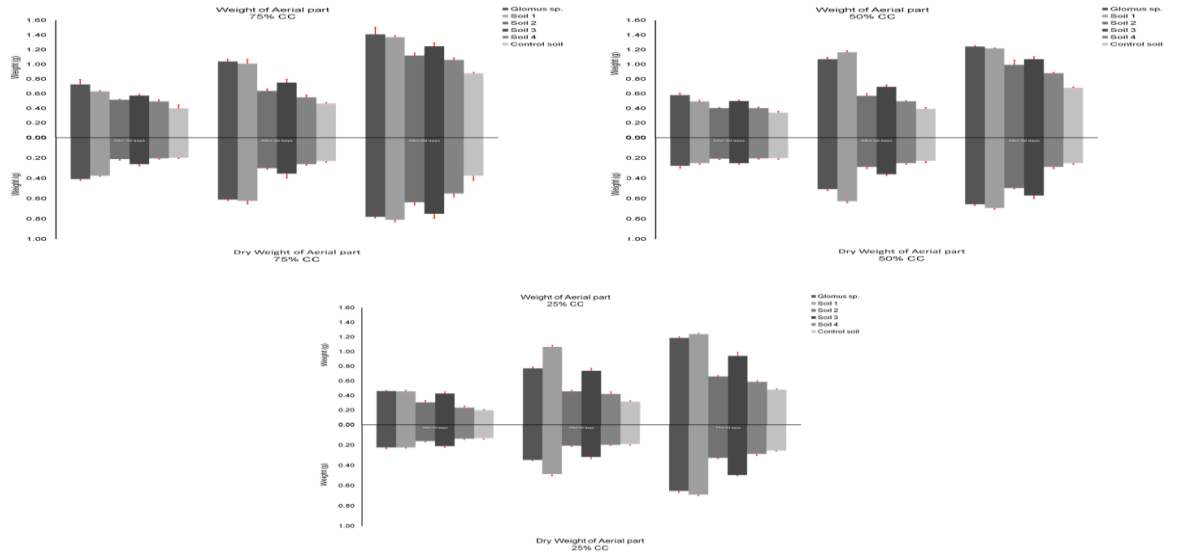

Fig. 4: Effect of AMF on weight and dry weight of Aerial part of *L. culinaris*. Mean of three replicates and ± standard error. Differences between groups were significant P<0.05

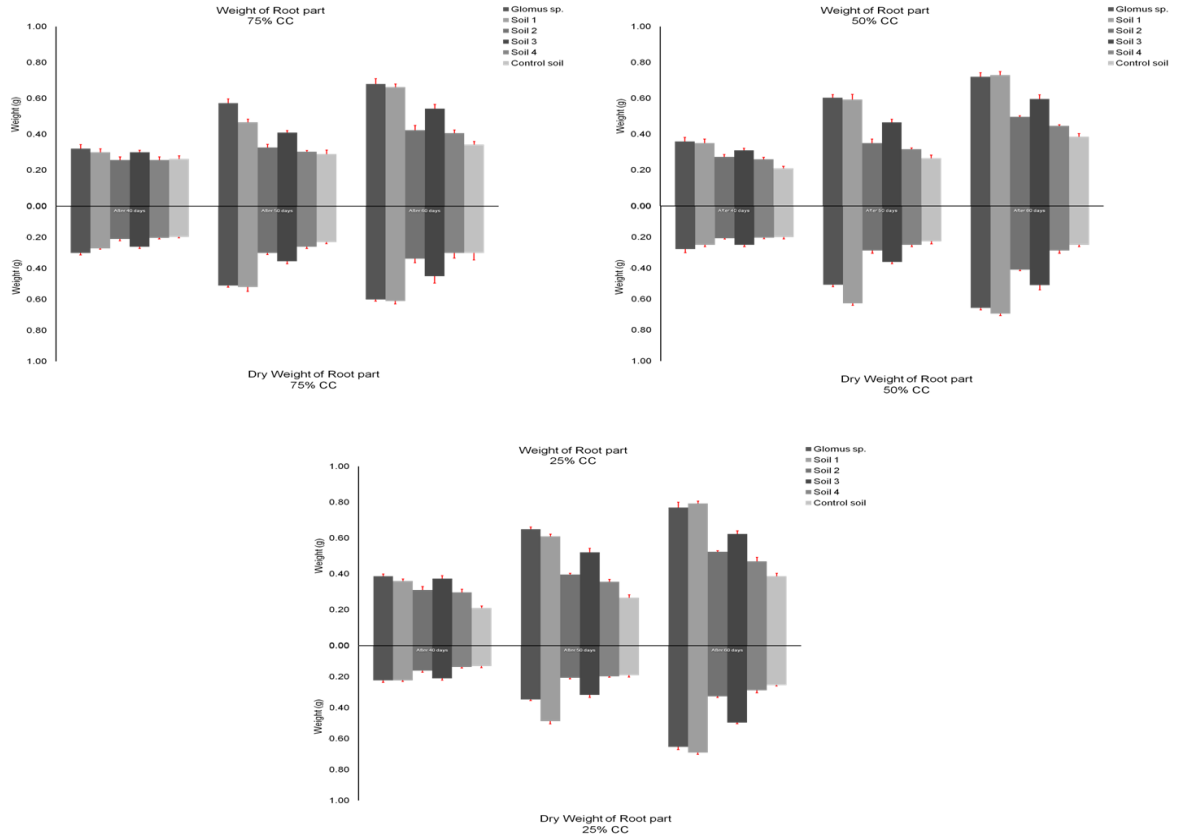

Fig. 5: Effect of AMF on weight and dry weight of root part of *L. culinaris*. Mean of three replicates and ± standard error. Differences between groups were significant P<0.05

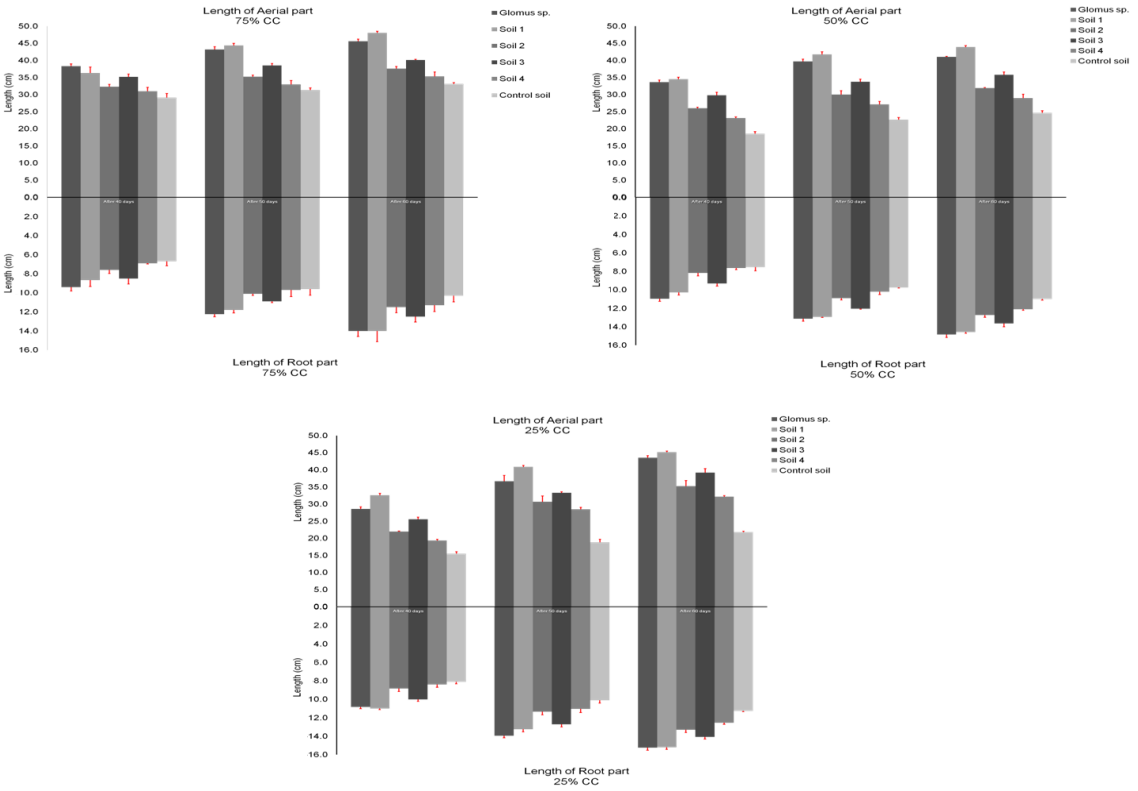

Fig. 6: Effect of AMF on length of Aerial part and root part of *L. culinaris*. Mean of three replicates and ± standard error. Differences between groups were significant P<0.05

CONCLUSIONS

The present study confirms the ability of mycorrhizal inoculum to improve growth of *L. Culinaris* by increasing their tolerance to stress drought. The response of this plant varied according to the different treatments. AMF effects were varied depending AMF species. The heterogeneity of indigenous strains "Soil 1" was remained more effective on the growth of the present legume as the exogenous inoculum (*Glomus* spp.). The highest mycorrhizal colonization was detected at water stress of 25% CC which is pointed that AMF effect was depended the water disponibility. Drought stress can involve a depressive effect on the growth of the uninoculated plants. Indigenous AMF as bio-inoculants can be used in agriculture for sustainable growth improvement of *L. Culinaris* at arid and semi-arid ecosystem.

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