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Symbiosis relationship between some arbuscular mycorrhizal fungi (AMF) and *Salsola laricina* and its effect on improving plant growth parameters

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Abstract

The aim of this study was to examine the symbiosis relationship between some arbuscular mycorrhizal fungi (AMF) and *Salsola laricina* (*Chenopodiaceae*), a non-mycotrophic plant species and its effect on improving plant growth parameters. Initially, the development of AMF density was monitored through two parameters including evaluation of mycorrhizal colonization of plant roots and density measurement in the soil under field conditions. Then, the spores were counted and the highest four morphotypes were isolated for morphological identification and preparation of inoculum to culture. The seeds of *S. laricana* were planted inside pots under greenhouse conditions and were inoculated by four isolates of AMF including *Archaeospora schenckii*, *Glomus deserticola*, *Scutellospora erythropa*, and *Septoglomus constrictum* and one treatment remained non-inoculated as control. High root colonization of the plants was found at six months after inoculation (46%) where the highest morphotypes density belonged to *S. constrictum* (No./20 g soil-1), suggesting the importance of AMF for plant growth efficiency. The AMF symbiosis generally improved the growth related to the height and weight of shoot and root of *S. laricana* that were significantly increased. The results led to the conclusion that, identification of interactions between plants, soil properties, and AMF colonization can contribute to improve management of ecosystems.

Keywords: AMF, colonization, *Chenopodiaceae*, morphological recognition, taxonomy

همزیستی برخی قارچ‌های میکوریزی آربوسکولار با گونه *Salsola laricina* و تاثیر آن بر بهبود پارامترهای رشد گیاهان*

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خلاصه

هدف از این مطالعه، بررسی همزیستی برخی قارچ میکوریزی آربوسکولار (AMF) با گونه *Salsola laricina* (اسفناجیان) از یک تیره گیاهی غیرمیکوریزی و تاثیر آن بر بهبود پارامترهای رشد گیاهان بوده است. در ابتدا، میزان کلونیزاسیون میکوریزی ریشه‌های گیاهی و تراکم AMF در خاک منطقه تحت مطالعه مورد ارزیابی و اندازه‌گیری قرار گرفتند. پس از شمارش هاگ‌ها در خاک اطراف ریزوسفر گیاه سالسولا، چهار مورفوتایپ که دارای بیشترین فراوانی بودند، به منظور تشخیص مورفولوژیکی جداسازی و برای تهیه مایه تلقیح کشت شدند. شناسایی هاگ‌ها براساس تحلیل ساختار میکوریزا با استفاده از میکروسکوپ ترکیبی در ×۱۰۰–۱۰۰۰ انجام شد. بذور گیاه *S. laricina* در گلدان‌هایی در گلخانه با چهار تیمار مایه تلقیح میکوریزی شامل: *Archaeospora schenckii*, *Glomus deserticola*, *Scutellospora erythropa*, *Septoglomus constrictum* و یک تیمار بدون تلقیح میکوریزی کاشته شده و مورد آزمایش قرار گرفتند. بیشترین کلونیزاسیون ریشه گیاه توسط قارچ‌ها در اوایل پاییز (۴۶٪) و بیشترین تعداد مورفوتایپ‌ها مربوط به تیمار قارچی *S. constrictum* (تعداد بر ۲۰ گرم خاک) بود. پارامترهای رشدی *S. laricina* به طور معنی‌داری در تیمارهای میکوریزی نسبت به غیرمیکوریزی افزایش یافت. با توجه به نتایج این تحقیق، می‌توان اظهار داشت که شناسایی تعاملات بین گیاهان، خصوصیات خاک و کلونیزاسیون AMF منجر به بهبود مدیریت در اکوسیستم‌ها می‌شود.

واژه‌های کلیدی: اسفناجیان، تاکسونومی، شناسایی مورفولوژیکی، کلونیزاسیون، AMF

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Introduction

The presence and identity of living plants are especially important for the communities of fungal groups that form a part of the root energy channel (Moore, Walter, Hunt 1988), e.g. mycorrhizal fungi, plant pathogens, and endophytes (Bahram *et al.* 2016). The arbuscular mycorrhizal (AM) symbiosis so far has been the most widely recognized association between the majority of terrestrial vascular plants and AMF in the phylum Glomeromycota (Smith & Read 2008). The pivotal role of AM symbiosis is evident in enhancing plants' uptake of poorly mobile nutrients such as Phosphorus and/or Zinc (Kiers *et al.* 2011).

Previously, the identification of arbuscular mycorrhizal fungi (AMF) was based on spore morphology, spore formation, and spore wall structure (Gerdemann & Trappe 1974). In 1990, regardless of molecular aspects, the AMF were organized in three families (*Acaulosporaceae*, *Gigasporaceae*, and *Glomeraceae*) and six genera (*Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Scutellospora*) (Oehl *et al*. 2011) within one order, *Glomerales* of the fungal phylum Zygomycota (Morton & Redecker 2001). Today, the academicians accept three classes (*Archaeosporomycetes*, *Glomeromycetes*, and *Paraglomeromycetes*), five orders (*Archaeosporales*, *Diversisporales*, *Gigasporales*, *Glomerales*, and *Paraglomerales*), 14 families, 29 genera, and approximately 230 species (Walker & Schüßler 2004, Oehl *et al.* 2011). Understanding the biology and ecology of these fungi requires a robust classification that reflects their evolutionary history.

Prior researchers reported that, typical representatives of non-mycotrophic plants belong to the families *Brassicaceae*, *Chenopodiaceae*, and *Polygonaceae* (Allen *et al.* 1989). *Salsola* genus, as one of the largest genera in *Chenopodiaceae* family, contains 40 recognized species in Iran which often grow in saline and desert areas. *Salsola laricina* Pall. is a perennial plant in nature, with a height of 25–60 cm; its branches are covered with rough hair; leaves are alternate with one flower at the centre of the leaves and semi-dense branches which look like spikes in arid pastures (Assadi 2001). In addition, it is one of the most important plants for grazing the livestock in arid and semiarid regions (Assadi *l.c.*). *Salsola laricina* is predominantly considered as non-mycotrophic (Allen & Allen 1988), but interestingly field observations have evidenced significant root colonization (RC) by AMF in this species (Nouri *et al.* 2018). As such, we decided to recognize AMF using morphological keys in the current study. This would allow confirming the hypothesis that a response of the AMF-*S. laricina* symbiosis is associated with improvement of plant growth, which can be useful in the restoration of degraded ecosystems.

Materials and Methods

- Sampling

Soil and root samples were collected from field soil of Khoshkerood site in Markazi province in central Iran (50° 35′ to 50° 49′ E, 35° 23′ to 35° 30′ N, 1400 m a.s.l.). The climate is characterized by a mean annual temperature of 19.3 °C and average annual precipitation of 190 mm. This site is a protected habitat of rangeland plant species such as *S. laricina* without herbivore grazing over the entire year within a fence.

The samples were picked up and maintained in an ice-box and immediately transferred to the laboratory of forest research at the Research Institute of Forests and Rangelands (RIFR), Tehran, Iran. They were passed through a 2 mm sieve to remove roots and rock particles.

- Inoculum preparation and cultivation conditions

AMF spores were collected from six replications of soils around the rhizospheres of *S. laricina* and extracted from 100 g soil using the wet-sieving technique (Gerdemann & Nicolson 1963). They were then fixed with polyvinyl alcohollactic acidglycerol (PVLG) or in a mixture of PVLG + Melzer's reagent.

Analyses of the spore structures such as wall, surface, saccule etc. were performed using compound microscopes at 100–1000×. Photographs were taken by a digital camera (Olympus model DP73). The morphological characteristics have been presented in a series of recent publications dealing with different species groups of Glomeromycota.

The four most common spore morphotypes were collected and propagated by multispore culture technique on maize (*Zea mays* L.) for 16 weeks (according to INVAM) in the greenhouse. The cultivation substrate which was used to initiate the culturing contained a mixture of sand, soil, and perlite (1:2:1, v:v:v). After this cultivation period, the substrate of these pot cultures was homogenized and 200 g of the homogenized substrate, containing AMF-infected root segments and spores, was used as inoculum in the form of a layer within each pot of the experiment.

Seeds of *Salsola laricina* were collected at the same site as the spore inoculum. The plants were cultivated for 6 months (March to August) in greenhouse conditions. No fertilization was applied.

- Root colonization

For determining the root colonization by AMF, the collected root subsamples were cut into 2 cm pieces and cleared in 10% KOH at 90 °C for 2 h, treated with 7.5% H_2O_2 for 5 min and acidified in 1% HCl for 5 min. The cleared roots were stained with 0.05% Trypan blue in lactoglycerol at 90 °C for 20 min (Phillips & Hayman 1970). Root colonization was quantified according to the gridline intersect method (McGonigle *et al.* 1990) on 100 intersections at 100x magnification using an Olympus CH2 dissecting microscope.

- Statistical analysis

Spore density and plant growth parameters data were analyzed using analysis of variance (ANOVA). One-way ANOVA was utilized to determine the difference between morphotypes treatment by Tukey's test, while the T-test was employed for RC% between two sampling times. Some of the data sets were logarithmically transformed to fulfil the requirement of ANOVA on normality and homogeneity of variance. All statistical analyses were performed in R (R_Core_Development_Team 2008).

Results

- Description of the species

All possible characteristics which appeared to discriminate the groups more inclusively were identified from prior studies. The name of fungi was coded with A, B, C, and D with observations illustrating the characteristics in figure 1.

Archaeospora schenckii (A) develops inside the neck of a soporiferous saccule, at some distance from the saccule; the saccule wall consists of one layer, hyaline, sometimes with an outer coating of granular material. The spore wall consists of two or three hyaline layers. *Glomus deserticola* (B) consists of one wall comprising two layers and the hyphae that do not break easily so that spores are often clustered together very loosely or are closely associated with root fragments. It is similar to *G. fasciculatum* but lacks the flexible innermost layer (Walker & Koske 1987). *Scutellospora erythropa* (C) walls are separated into two groups when spores are crushed, where the outer and inner wall groups and suspensor-like cell thin-walled are formed terminally on a wide, thin-walled septate hypha. Finally, *Septoglomus constrictum* (D) consists of one wall containing two layers. This species can be recognized easily because of the distinctive color of its spores which is one of the most frequently AMF found in cultivated and uncultivated soils.

Table 1. The name of isolated AMF and their characteristics

Groups	**A**	**B**	**C**	**D**
Name of taxon	***Archaeospora schenckii***	***Glomus deserticola***	***Scutellospora erythropa***	***Septoglomus constrictum***
Character				
Color	Completely hyaline (sparkling white)	Pale yellow to orange	Orange-brown to dark red-brown	From red-brown to almost black
Shape	Mostly globose, subglobose, but also ellipsoid to ovoid	Globose to subglobose	Globose to subglobose, obovoid, ellipsoid, irregular	Globose to subglobose, rarely irregular
Size (µm in diam.)	74–89	96–97	137–141	89–120
Thickness	Thick walled and all of which exhibit some flexibility in broken section	Frequently with side thickenings	Thick inner wall	Thick walled with the outer layer adherent until it degrades and sloughs
Spore formation	Single in the soil	Born in the soil singly or in loose aggregates lacking a peridium	Single in the soil or occasionally within roots	Single in the soil

Characters defined and formatted according to recommendations by INVAM

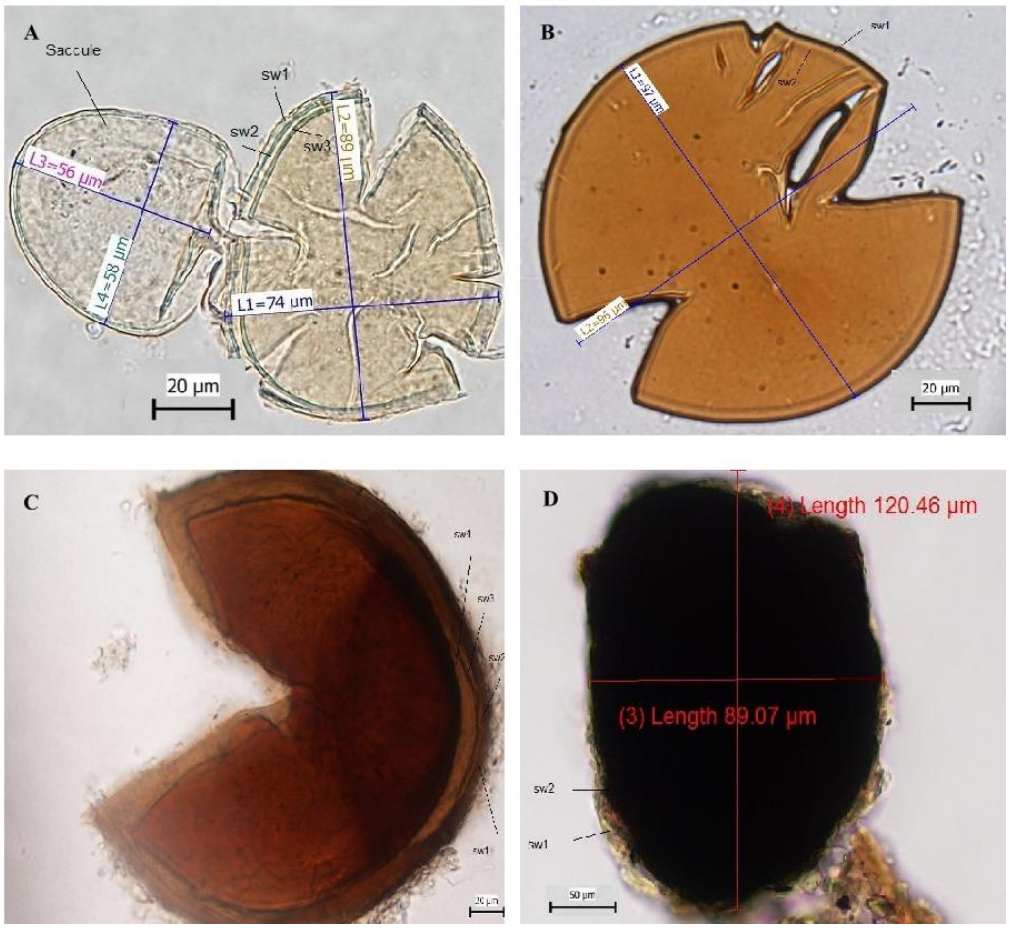

Fig. 1. A. Different morphological characteristics of *Archaeospora schenckii*, B. *Glomus deserticola*, C. *Scutellospora erythropa*, D. *Septoglomus constrictum* (SW = Spore wall layer, L = Diameter of spore in PVLG).

- Root colonization by AMF and spore density in the field study

AMF root colonization of *S. laricina* was detected in two sampling times (Fig. 2). In general, root colonization in spring decreased significantly by about half (average values/treatment) compared to fall.

The morphotypes spore density in the soil around *S. laricina* roots were isolated and then counted. Specifically, there were several morphotypes of spores, but the major counted spores were only four types. The D and A isolates maintained the same level along the whole spores. They formed significantly more spores than B and C isolates.

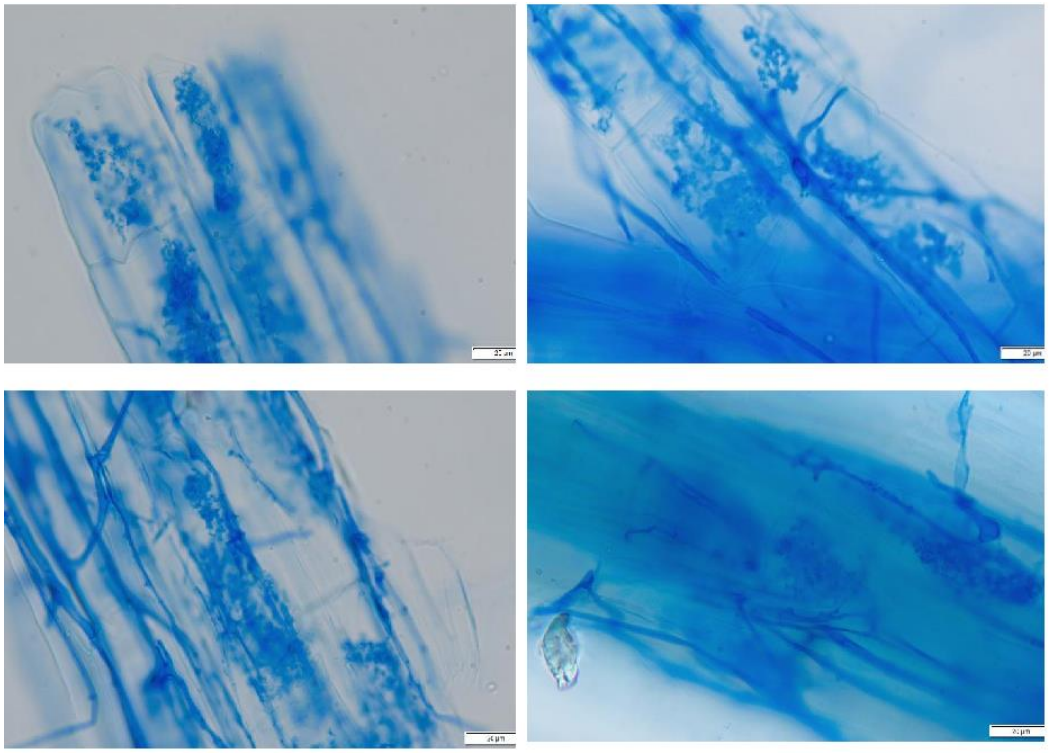

Fig. 2. Root colonization of Salsola laricina with hyphae and arbuscules of arbuscular mycorrhizal fungi.

Table 2. Root colonization of *S. laricina* (Fall, Spring) by four common mrphotypes spore density of arbuscular mycorrhizal fungi (A–D)

Morphotypes spore density (No./20 g soil-1)				**Root colonization**	
A	**B**	**C**	**D**	**Fall**	**Spring**
60.9 (0.5) a	9.3 (0.0) b	7 (0.0) c	70(0.8) a	46.0 (1.5) a	29.6 (1.1) b

Values are means of six replicates (S.E.). Values marked by the same letter are not significantly different

- Plant growth parameters in the greenhouse

All the determined parameters were significantly affected by inoculation treatment**.** In general, plant shoot as well as root height and weight were significantly reduced at NI treatment. The shoot to root ratio also significantly decreased. The different inoculation treatments, however, responded to better growth (Fig. 3), but there was no significant difference among them in the most parameters. Generally, B inoculant had the best result in height of shoot while D had a smaller height of root and shoot to root ratio than other treatments. Also, the number of branches of *S. laricina* was consistently higher up to four times in the inoculated than in non-inoculated plants.

Table 3. Some plant growth parameters of *S. laricina* as affected by inoculated treatment

Inoculation	Shoot height (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot/root ratio	Branch (No./plant)
A	31.10(0.17) b	27.45(0.3) a	2.28(0.03) a	0.32(0.00) a	7.53(0.08) a	8.00
B	42.03(0.26) a	31.47(0.23) a	2.51(0.03) a	0.28(0.00) a	9.04(0.12) a	7.00
C	34.80(0.21) b	28.25(0.16) a	2.59(0.03) a	0.35(0.00) a	7.29(0.05) a	12.00
D	32.93(0.61) b	19.21(0.36) b	2.70(0.06) a	0.29(0.01) a	8.18(0.17) b	11.00
NI	19.60(0.31) c	17.76(0.31) b	0.73(0.03) b	0.11(0.00) b	4.84(0.08) c	3.00
df			F-values and significances			
4	39.23 ***	12.42 ***	71.33 ***	36.49 ***	11.54 ***	-

Values are means of six replicates (S.E.). F-values and significances are given according to one-way ANOVA: *** P<0.001; ** P<0.01. Values marked by the same letter are not significantly different according to Tukey's HSD test

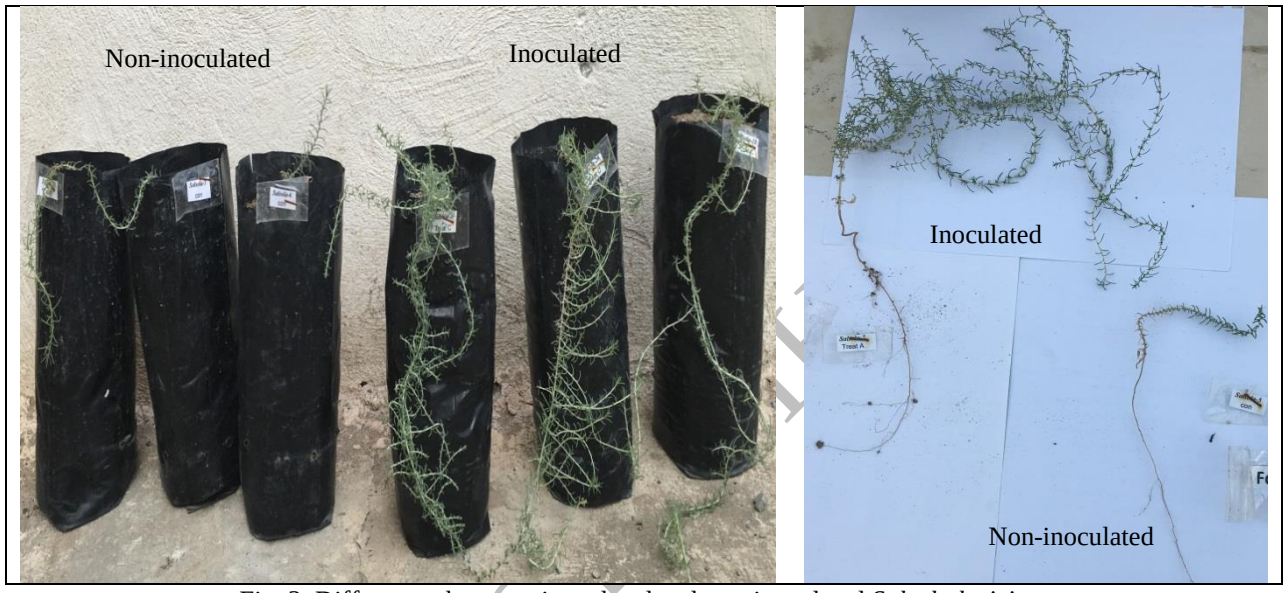

Fig. 3. Differences between inoculated and non-inoculated *Salsola laricina*.

Discussion

Our field experiment surprisingly revealed that, one species of the *Chenopodiaceae* family had a significant percentage of AMF symbiosis, while in last research, Allen *et al*. (1989a) showed that, a large percentage of this family is non-mycorrhizal. The present field experiment revealed that, *Salsola laricina* was colonized by AMF. From the review, it was clear that, taxonomic keys to discover more about AMF-plant preferences and types of AMF have difficulties to identify morphologically. Molecular approach is more authentic to identify the exact taxonomy of different species of AMF (Lone *et al*. 2014). However, this study provides a reference guide for morphological identification that could be a good start for understanding what we have in protected sites of Iran, after which we can compare it with the molecular approach in future studies. Our findings showed at least four morphotypes around the soil of *S. laricina* roots. *Septoglomus constrictum* is one of the most frequently found arbuscular mycorrhizal fungi in soils of our study field, confirming Błaszkowski & Czerniawska's (2011) study.

The high level of colonization in the fall may be due to the fact that, there are better conditions for *S. laricina* to grow, which seems to be less tense than spring, which is rapidly facing high-temperature heat. This season is likely to be a more appropriate time for AMF and *S. laricina* to make a symbiotic relationship.

The variations of AMF spores morphotypes in the field were associated with changes in soil

physiochemical properties and density of the dominant species as well as plant community (Ba *et al.* 2012).

In the study reported here, the effects of AMF inoculant on *S. laricina* performance were investigated. AMF enhanced the plant biomass, where this increase in plant growth can be attributed to the improvement of direct water uptake and transport via external hyphae (Augé 2001). Furthermore, the AM mycelium is able to transfer water both internally and on external hyphal surfaces that is not directly available to the roots (Marulanda *et al*. 2003). Moreover, the association between plants and fungi is strongly influenced by the photosynthetic capability of the plants (Hartnett & Wilson 2002), as AMF provides more benefits to plants supplying more carbohydrates (Kiers *et al*. 2011).

Root growth seems to be less affected by AMF inoculant between mycorrhizal and non-mycorrhizal plants compared with shoot growth. Under these conditions, the root/shoot ratio increased significantly in inoculated plants, which might be a way to improve water use efficiency (Tardieu *et al*. 1902).

Our results confirmed that, the beneficial effects of inoculated plants by AMF improved the growth indices. The inoculated plants with all AMF had up to four times more branches, which can be a good index for forage plants like *S. laricina*. These results strongly suggest that, the plants that form AMF symbiosis benefited more for enhanced restoration success against stresses such as climate changes, drought, salinity, and unmanaged grazing. Our suggestion for future is an investigation of the effects of these stresses on the mycorrhizal community in the field and plant-AMF interactions. It could be useful if, the recognition of AMF is done by molecular systematics including analyses based on deep sequencing in future in Iran.

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