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ORIGINAL PAPER

BIOTIZATION WITH *GLOMUS FASCICULATUM* TO ENHANCE THE ACCLIMATIZATION AND ABSORPTION OF NUTRIENTS BY MICROPROPAGATED SAVORY (*SATUREJA KHUZISTANICA* JAMZAD) PLANTLETS

Leila Mirjani[1], Azam Salimi[2], Mohammad Matinizadeh[3], Khadijeh Razavi[4], Maryam Shahbazi[5]

[1,2] Faculty of Biological Sciences
Kharazmi University, Teheran, Iran
[3] Forest Research Department
Research Institute of Forests and Rangelands
[4] Department of Plant Biotechnology
National Institute of Genetic Engineering and Biotechnology (NIGEB)
[5] Molecular Physiology Department
Agricultural Biotechnology Research Institute of Iran (ABRII)

Abstract

Increasing the rate of acclimatization in micropropagated plantlets has always been followed by major limitations in the tissue culture technique. In this research, the biohardening using arbuscular mycorrhizal (AM) fungi, *Glomus fasciculatum*, was considered to improve the survival and growth of micropropagated plantlets. Savory, (*Satureja khuzistanica* Jamzad), as an endangered and valuable medicinal species, was used for this purpose. Thus, micropropagated plantlets were inoculated with AM in the soil and some physiological and biochemical parameters were evaluated at 5 stages of their life cycles (0, 15, 30, 60, and 90 days). The root colonization of the plantlets, at the time of transferring to *ex vitro* conditions, affected the physiological equilibrium, enabled the absorption of more nutritional elements and then contributed to the rapid restoration of plantlets during the acclimatization. It also improved the amount of biomass and nutrient absorption. According to the results, inoculation with AM had significant effects on survival, leaf water potential, total chlorophyll, *a*, *b* and carotenoid content, leaf area, biomass, the phosphorus, zinc, copper and potassium content, but there were no significant differences detected among the treatments with respect to the leaf relative water content, soluble sugars and protein. Our results emphasized the need to use AM inoculation in a tissue culture protocol

Leila Mirjani, PhD student, Department of Plant Sciences, Faculty of Biological Sciences, Kharazmi University, Postal Code: 15719-14911, Tehran, Iran. e-mail: sl.mirjani@gmail.com
Azam Salimi, Associate Professor, Faculty of Biological Sciences, Kharazmi University, Postal Code: 15719-14911, Tehran, Iran. E-mail: salimi@khu.ac.ir

in order to increase the survival and absorption more nutritional elements, growth of this valuable medicinal plant.

Keywords: Arbuscular mycorrhizal, ex vitro establishment, *Glomus fasciculatum, Satureja khuzistanica* and Survival rate.

INTRODUCTION

Micropropagation of plants is considered as one of the best techniques exploited to produce uniform and healthy plants regardless of the weather or season. However, the high mortality of micropropagated plants at the time of transferring to a field is counted as one of the major limitations in using this technology on a large scale. Saturated atmosphere, a constant temperature of 20-25°C, low rate of gas exchange, high concentration of carbohydrates and the external growth regulators have successfully prepared the best growing conditions for the plantlets. Thus, due to growing in the above conditions, some physiological, anatomical and morphological changes occur in plantlets which lead to a reduction in their survival in *ex vitro* conditions (Puthur et al. 1998).

Micropropagated plantlets are exposed to abiotic (changes in temperature, light intensity, humidity) and biotic (soil flora) stresses at the time of transferring to *ex vitro* conditions. Therefore, it is necessary to control the environmental conditions for their successful establishment (Deb, Imchen 2010).

'Biotization' or co-culturing plantlets with bacteria or arbuscular mycorrhizal (AM) fungi may induce metabolic changes in the plantlets and cause an increase in their tolerance to biotic and abiotic stresses (Chandra et al. 2010). The colonization of AM fungi has great potential in nutrient absorption, water absorption, biological control of pathogens, growth hormone synthesis, and the establishment of tissue culture plantlets under adverse weather and soil conditions (Rai 2001, Kapoor, Bhatnagar 2007, Chaudhary et al. 2008, Kaushih et al. 2012, Parkash et al. 2011*a*,*b*, Singh et al. 2012). Mycorrhization increases nutrient absorption in plants under water deficit stress (Busse, Ellis 1985). It also raises the effective water use and hydraulic conductivity (Graham, Syversen 1984). Mycorrhizal plants had high water potential in leaves (Nelson, Safir 1982, Kapoor et al. 2008). AM fungi can improve the growth of the host plant by increasing the absorption of minerals (Turk et al. 2006).

It has been reported that there is improved acclimatization of some micropropagated plantlets inoculated with AM, e.g.*Cynara cardunculus* L. var. *scolymus* (Fortunato et al. 2005), *Podophyllum peltatum* (Moraes et al. 2004), *Punica granatum* L. (Singh et al. 2012), and increased biomass and absorption of some elements in plantlets at the time of their transferring to *ex vitro* conditions, e.g. *Capsicum anulum* (Andrés et al. 2003), *Tapeinochilos*

ananassae (De Oliveira et al. 2011), *Vitis vinifera* L. (Krishna et al. 2005), *Glycyrrhiza glabra* L. (Yadav et al. 2013) and *Annona cherimola* Mill. (Padilla, Encina 2005).

Despite many reports on the effects of AM fungi on plantlets of certain crops and forest species (Rai 2001), there is little information about the effects of AM on the survival and growth of micro-propagated medicinal plants. Thus, in this research, *Satureja khuzistanica* Jamzad was selected as a valuable medicinal herb and natural food preservative containing high amounts of carvacrol (80.9-93.9%), but being low in toxicity and having an exquisite taste. *S. khuzistanica* plantlets were produced in media culture (Jamzad 2011). We aimed to assess the effectiveness of mycorrhization on the survival of micropropagated *S. khuzistanica* during acclimatization as well as their physiological and biochemical characterization during colonization.

MATERIAL AND METHODS

Plant material and *in vitro* culture of *S. khuzistanica* explants

The shoot apex and axiliary buds of *S. khuzistanica* wild type were collected from Pol Dokhtar region, N 33°00′19.7″, E 47°40′57″, located in Lorestan province in Iran, in the summer 2014. The collected specimens were kept in a cool box (4°C) in a laboratory.

The explants (shoot apex and auxiliary buds) were cultured on different basic culture media and hormones. Branching and rooting were carried out in a basic MS medium containing (0.5 mg l^{-1} BAP+ 0.3 mg l^{-1} 2iP+ 0.1 mg l^{-1} IBA) and an MS medium containing (1 mg l^{-1} IBA+ 0.1 mg l^{-1} NAA), respectively (Figure 1). All cultures were kept in a growth chamber at 22 ± 3°C with 16:8 photoperiods; with an average photosynthetic photon flux density (PPFD) of 200 µmol m^{-2} s^{-1}.

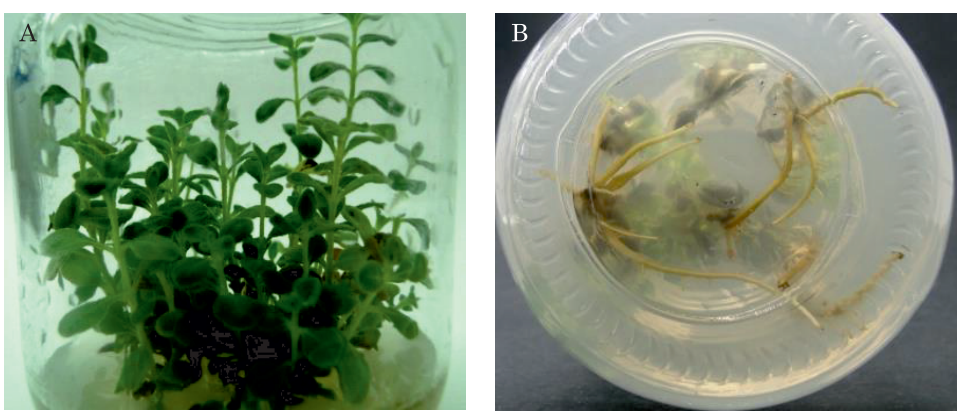

Fig. 1. Different stages of S. khuzistanica micropropagation, branching (A), rooting (B)

Substrate preparation

The substrate was prepared as a mixture of sand, peat, perlite and soil (1:1:1:1). Physicochemical properties of the soil were as follows: pH (at start), 6.3; EC, 6.0 dS m^{-1}; 0.11%, total N; P, 30.0 mg kg^{-1}; Ca, 5631 mg kg^{-1}; K, 548.7 mg kg^{-1} and organic carbon, 1.8%. The physical texture of the soil was 71%, Sand; 18%, Silt and 11%, Clay. The soil was sterilized in an autoclave at 125°C for 75 min.

***Ex vitro* establishment and mycorrhizal inoculation**

The plantlets of *S. khuzistanica* were washed with distilled water to remove the adhering agar from the root systems. Then they were transferred to plastic pots filled with a sterile potting mixture and inocula of *Glomus fasciculatum*. *G. fasciculatum* propagules, which were provided by Touran Ltd (P.O. Box, Iran). Each micropropagated plantlet received 300 spores as an inoculum, placed adjacent to and below the roots into the acclimatization substrates at the transplanting time. 300 pots with no fungal inocula were considered as the control.

Staining root and mycorrhizal colonization percentage

To assess AM colonization after 15, 30, 60 and 90 days of transferring micropropagated plantlet to *ex vitro*, roots were washed in tap water, and assayed according to Phillips and Hayman's (1970) method. The percentage of mycorrhizal colonization was also measured according to the gridline intersect method (Giovannetti, Mosse 1980).

Physiological parameters

The percentage of the leaf relative water content was measured according to Boyer (1968), while the leaf water potential was measured using the liquid immersion method (Michel 1972) after 0, 4, 7, 11, 15, 30, 60 and 90 days of transferring micropropagated plantlet to *ex vitro*.

The biomass, i.e. the fresh and dry weight of leaf, stem and root, was measured. The dry weight was determined after drying in an oven at 70°C for 48 hours. The chlorophyll content and carotenoids were assayed according to Lichtenthaler and Welburn's (1983) method.

Soluble sugars were analyzed according to the method by Irigoyen et al. (1992). The absorbance was measured after cooling on a spectrophotometer at 625 nm.

The leaf soluble protein content was evaluated by Bradford's (1976) method using bovine serum albumin (BSA) as the standard.

Nutrient analysis

After 90 days following the trasfer of the tissue culture plantlets to the *ex vitro* conditions, the concentrations of Zn, Ni, Fe, Cu, Ca, K, Mg and

Na in the leaf were measured by the wet oxidation method (Westerma 1990). Then, the nutrient extraction obtained was injected to a coupled plasma--optical emission spectrometer (model GBC, integral XL, Australia).

The concentration of nitrogen was determined by the Kjeldahl method (Bremner, Mulvaney 1982). The phosphorus concentration in leaves was measured by the colorimetric method (the yellow color of molybdate-vanadate) – Chapman, Pratt (1961).

Statistical analysis

The data analysis was carried for a factorial experiment based on a completely randomized design with three replications, with each replication for each treatment consisting of one hundred plantlets. The first factor (F1) contained two treatments: a) inoculated plantlet with AM, b) non-inoculated plantlet (control). The second factor (F2) consisted of the time after the establishment of plantlets in *ex vitro* condition (15, 30, 60 and 90 days).

The results of the evaluations underwent an analysis of variance. Significant differences among the means were ascertained by the Duncan's multiple range tests ($p < 0.05$).

RESULTS AND DISCUSSION

Mycorrhizal colonization

The mycorrhizal colonization percentage was determined after 15, 30, 60, and 90 days following the inoculation. The statistical analysis showed significant differences among colonization percentage ($p < 0.01$). The percentage of colonization increased 20% per month (Figures 2 and 3).

We were able to inoculate *S. khuzistanica* with *Glomus fasciculatum*. The progress and completion of infection is dependent on a fungi species (Monticelli et al. 2000).

In our research, the results indicated that the percentage of root colonization of the plantlets inoculated with *Glomus fasciculatum* increased over the time of treatment.

Physiological parameters

The results related to physiological traits are presented in Tables 1 and 2.

The transfer of AM plantlets to the field was enhanced by about 17.4%, which means that the survival rate was higher than that of non-inoculated plantlets ($P < 0.01$). Different results were observed in different times of the establishment in *ex vitro* conditions ($P < 0.01$), and the interaction effect of the time after the establishment and treatments ($P < 0.01$) was varied as well (Tables 1 and 2, Figure 4A). The enhancement of the rate of plantlets' survival was observed from the beginning of colonization (Figure 4).

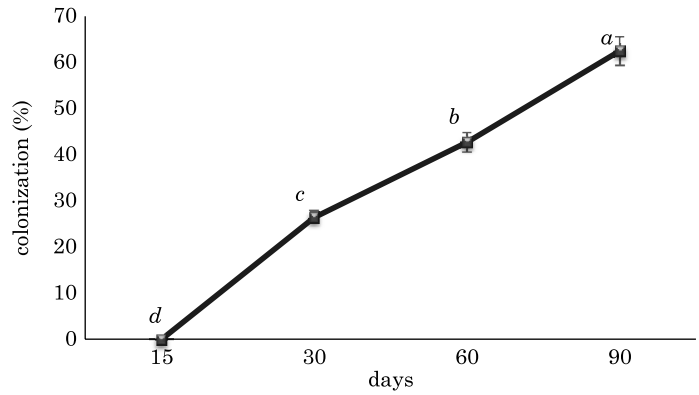

Fig. 2. The colonization percentage from 15 days to 90 days in AM plantlet of *S. khuzistanica*

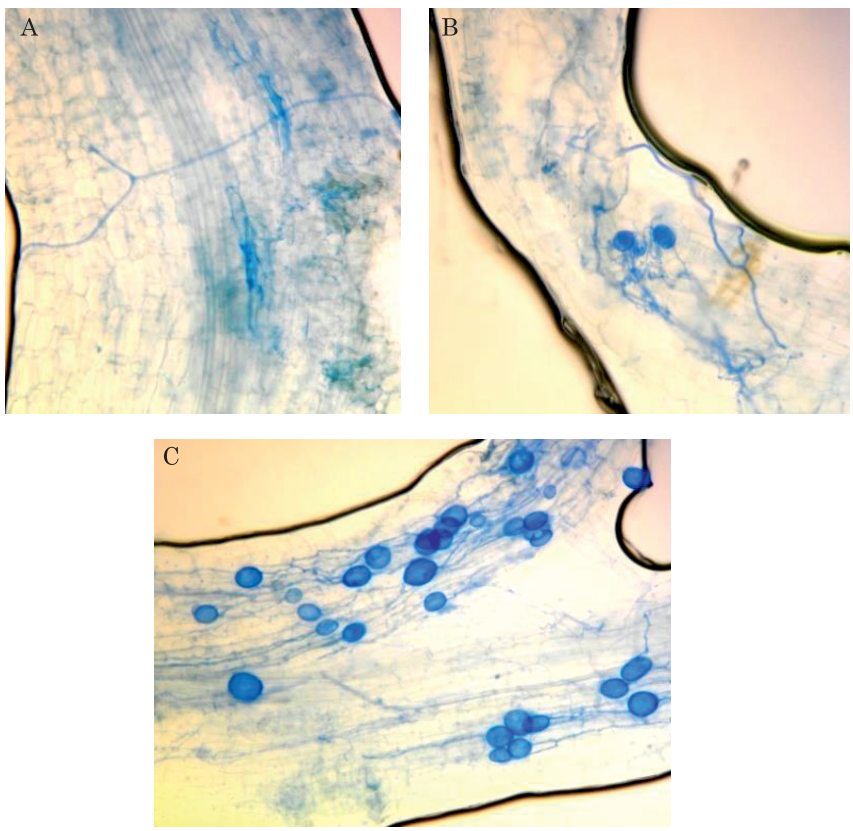

Fig. 3. The occurrence of AM fungi (vesicles, and intra hyphae) in the roots of *S. khuzistanica* plantlet by *G. fasciculatum*: A – 30 days, B – 60 days, C – 90 days, after inoculation under greenhouse conditions

Table 1

The analysis of variance for the effect of AM symbiosis on the plantlets of *S. khuzistanica* at the time of acclimatization to *ex vitro* conditions

Source	DF	*Ex vitro* survival (%)	RWC (%)	Leaf water potential (MPa)	Chlorophyll total (mg g^{-1} F.W)	Chlorophyll *a* (mg g^{-1} F.W)	Chlorophyll *b* (mg g^{-1} F.W)	Caratenoid (mg g^{-1} F.W)	Leaf area (cm^2)	Biomass (g)
Treatments (F1)	1	2066.7**	17.05ns	21.47**	252.12**	86.66**	8.3**	6.74**	0.007**	0.0009**
F2	4	2454.30**	213.02**	18.77**	522.34**	250.46**	40.07**	8.93**	0.036**	0.01**
F1*F2	4	336.54**	24.76ns	21.11**	21.97**	7.6*	0.76ns	0.63*	0.003**	0.002**
Error	20	1.79	10.5	0.79	4.53	2.49	0.45	0.15	0.0001	0.00003
C.V.		1.93	4.23	4.46	9.97	11.07	13.82	11.64	6.12	6.17

Explanations: F1 – inoculated plantlet with AM and non-inoculated plantlet, F2 – the establishment time in *ex vitro* conditions, ns, *, ** indicate non-significant or significant differences at $\alpha = 0.05$, and $\alpha = 0.01$, respectively

Table 2

The comparison of the means of effects of AM symbiosis on the plantlets of *S. khuzistanica* at the time of acclimatization to *ex vitro* conditions by the Duncan's Multiple Range Test

Item	*Ex vitro* survival (%)	RWC (%)	Leaf water potential (MPa)	Chlorophyll total (mg g^{-1} F.W)	Chlorophyll *a* (mg g^{-1} F.W)	Chlorophyll *b* (mg g^{-1} F.W)	Caratenoid (mg g^{-1} F.W)	Leaf area (cm^2)	Biomass (g)
AM plantlets	77.4*a*	77.48*a*	-19.05*a*	15.97*a*	5.39*a*	3.96*a*	24.25*a*	0.20*a*	0.096*a*
Control	60*b*	75.97*a*	-20.7*b*	12.57*b*	4.34*b*	2.92*b*	18.45*b*	0.17*b*	0.085*b*

Mean values followed by different letters within a column do not differ significantly from one another at $P \leq 0.05$ led by the Duncan's Multiple Range test.

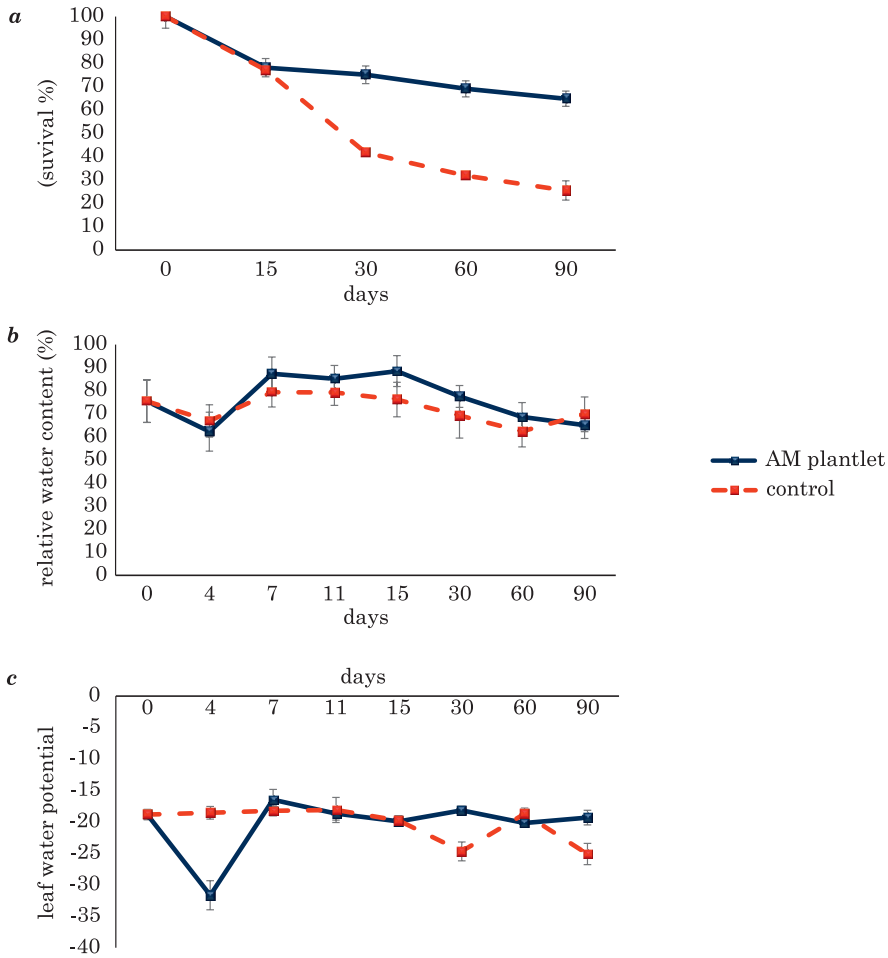

Fig. 4. Effects of AM fungal infection on survival percentage (*a*), relative water contents (*b*) and leaf water potential (*c*) in the plantlets at different times of the establishment in *ex vitro* conditions

Our results confirm earlier findings by KRISHNA et al. 2005 (grape), SINGH et al. 2012 (*Punica granatum* L.), YADAV et al. 2013 (*Gloriosa superba* L.), KASHYAP, SHARMA 2006 (*Morus alba*), MORAES et al. 2004 (*Podophyllumpeltatum* L.) and CAMPANELLI et al. 2014 (*Cynara cardunculus* L. var. *scolymus*). The higher survival rates determined for AM plantlets might have been due to the rhizosphere expansion through mycorrhiza mycelium, which can improve the water and nutrient uptake, and this contributed to a better acclimatization owing to higher tolerance against plant pathogens and stress due to transplant stress (PARKASH et al. 2011*a*).

RWC in the plantlets was increased, although this increase (Tables 1 and 2) and interaction effects between the time of establishment and treat-

ment were not significant ($P < 0.05$) – Figure 4*b*. The leaf water potential showed significant differences between the plantlets inoculated with AM and the control group (non-inoculated plantlets) ($P < 0.01$) – Table 1. The leaf water potential in the plantlets which were inoculated with AM was higher than in the non-inoculated plantlets (Table 2 and Figure 4*c*). Moreover, significant differences ($P < 0.01$) were found in different times of establishment in *ex vitro* conditions and also in the interaction effects of establishment times and treatment (Table 1). The RWC and leaf water potential have been considered in studies on water relationships of plants. As seen in Figure 4, a sharp decline was observed in the leaf relative water content and leaf water potential in both treatments until the fourth day after transferring the plantlets to the *ex vitro* conditions, which indicates a stress condition at the time of transfer, and the parameters became normal after the seventh day. Although no significant differences were found in RWC between inoculated and non-inoculated plantlets, RWC showed an obvious increase in AM plantlets from the fourth day onwards. AM fungi significantly increased RWC in the peak of six-day dehydration. ESTRADA-LUNA and DAVIES (2003) also reported a decline in RWC of *Capsicum annuum* L. cv. San plantlets during the early days of transfer to an *ex vitro* environment. A higher RWC was recorded in the leaf of the colonized plantlet during the peak of dehydration (6 days after the transferring). It may also be a plant's response to a decrease in RWC, which may be related to a reduction in stomatal conductance and transpiration through the stomata control. These mechanisms prevent rapid dehydration (ESTRADA-LUNA, DAVIES 2003).

The inoculation of the plantlets with AM affected the leaf water potential, which was higher in the mycorrhizal plantlets (Table 3 and Figure 4). Higher water potential indicates an increased amount of available water in AM plantlets. It may also be counted as a reason for water retention in the inoculated plantlets and consequently higher acclimatization and survival. According to incomplete stomatal activities in tissue culture plantlets, the increased water potential and the stability in mycorrhizal plants led to a decrease in the need for stomatal functionality. Therefore, AM plantlets were under less stress and for this reason they had reached more stability. Mycorrhization enabled the plants to absorb water by increasing the hydraulic conductivity (GRAHAM, SYVERSEN 1984, KAPOOR et al. 2008).

Table 3

Effects of AM fungi infection on the content of soluble sugars and proteins in inoculated and non-inoculated plantlets

Treatment	N	Soluble sugar ($\mu g\ g^{-1}$ F.W.)	Protein ($\mu g\ ml^{-1}$)
AM plantlet	15	527.2*a*	600.88*a*
Control	15	526.88*a*	594.55

Mean values followed by different letters within a column do not differ significantly from one another at $P \leq 0.05$ lead by the Duncan's Multiple Range Test.

The leaf area in AM plantlets was significantly ($P < 0.01$) increased in comparison to the non-inoculated plantlets (Tables 1 and 2). Significant differences ($P < 0.01$) were also found at different times of establishment in *ex vitro* conditions and for the interaction effects of establishment times and treatment (Table 1, Figure 5*a*). In our results, the leaf area per plant

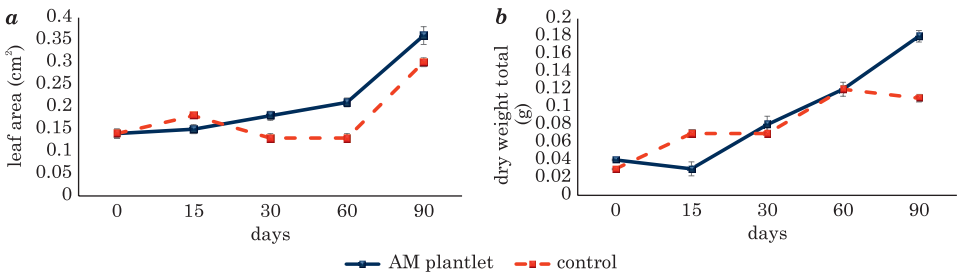

Fig. 5. Effects of AM fungal infection on the leaf area (*a*) and biomass (*b*) at different times of establishment of the plantlet in *ex vitro* conditions

was significantly larger in AM plantlets, which means that the leaves might be protected against osmotic stress by mycorrhiza (Figure 5). This effect is consistent in terms of the RWC and higher leaf potential in AM plantlets, which is in agreement with previous research (Yano-Melo et al. 1999, Estrada-Luna et al. 2000, Yadav et al. 2013). The raised leaf area in AM plantlets might have arisen from the increased anabolic processes (especially photosynthesis) owing to improved transfer of various essential nutrients and water (Sbrana et al. 1994). Others claim that the increased leaf area and chlorophyll content in plants that had been colonized by AM might be correlated to enhanced photosynthesis and higher absorption of phosphorus (Abbaspour et al. 2012).

Colonization by AM fungi did not lead to significant differences ($P < 0.05$) in biomass (Table 1, Figure 5*b*). The biomass of non-inoculated plantlets was higher until the 30th day after transfer to the *ex vitro* conditions. Higher biomass in AM plants was also reported in pomegranate (Rupnawar, Navale 2000), banana (Decklerk et al. 2002) and *Psidium guajava* L. (Estrada-Luna et al. 2000).

As a result, changes in the dry weight are associated with a high percentage of colonization, which could lead to the enhancement of nutrient uptake.

Higher biomass production was attributed to the improved intake of nutrients and possibly an increased rate of photosynthesis (Mathur, Vyas 1999).

Biochemical parameters

The content of total chlorophyll, *a* and *b*-chlorophyll and carotenoids in the AM plantlets was significantly higher than in the non-inoculated plantlets. Such an increase was observed after 60 days (Table 2 and Figure 6).

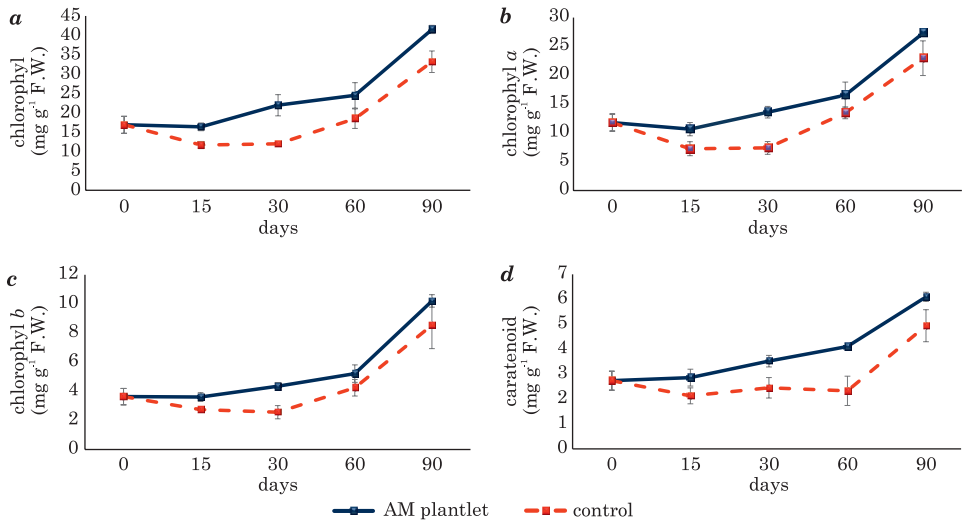

Fig. 6. Effects of AM fungal infection on the contents of total chlorophyll (*a*), chlorophyll *a* (*b*), and *b* (*c*) and carotenoids (*d*) at different times of plantlet establishment in *ex vitro* conditions

The inoculation with AM induced significant impact on biochemical attributes of *in vitro* grown plantlets compared with the control group. In this study, all AM plantlets showed a higher chlorophyll content than in the control group, which is in line with previous findings (Giri et al. 2003, Kapoor, Bhatnagar 2007, Krishna et al. 2005). It seemed that mycorrhiza formation can lead to an increased transpiration rate of leaves, reduced leaf temperature and inhibited chlorophyll degradation (Abbaspour et al. 2012). Higher levels of chlorophylls may cause increased photosynthesis in inoculated tissue culture plantlets (Krishna et al. 2005).

Carotenoids, as a secondary auxiliary antenna, serve a principal role in photosynthesis. AM plantlets showed higher carotenoid contents, therefore, carotenoid like chlorophyll caused increasing photosynthesis.

The results of the mean comparison of the effect of AM symbiosis on the soluble sugar and protein contents in the plantlets at the time of acclimatization in *ex vitro* conditions are presented in Table 3. Since no significant differences ($P < 0.01$) were found in both traits, then they were placed in the same statistical group. The soluble sugar contents increased in both treatments after 30 days of transferring to *ex vitro* conditions (Figure 7*a*).

AM fungal symbiosis in *S. khuzistanica* plantlets at the time of acclimatization to *ex vitro* conditions had no effect on soluble sugars and proteins, whose content changed similarly in both inoculated and non-inoculated plantlets. The total soluble sugar content during acclimatization slightly decreased in the mycorrhizal treatments of micropropagated plantlets of *Gloriosa superba* (Yadav et al. 2013). According to Subramanian (2000), the fungus uses 10-15% of the host's photosynthetic carbon for proliferation.

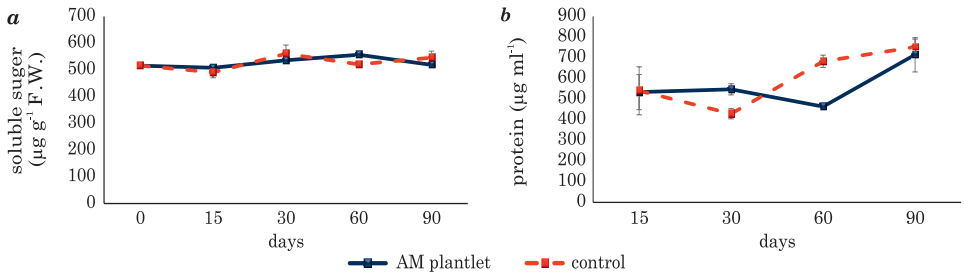

Fig. 7. Effects of AM fungal infection on soluble sugars (*a*) and protein (*b*) content at different times of establishment of the plantlet in *ex vitro* conditions

However, since the photosynthesis level had increased in AM plantlets, the carbon content did not decrease despite a higher uptake of carbohydrates. The protein content increased in non-inoculated and inoculated plantlets after 30 and 60 days, respectively, showing no significant differences. Therefore, both treatments caused a similar change in the content of proteins (Figure 7*b*).

Nutrient uptake

Significant differences were found between AM plantlets and the control group (non-inoculated plantlets) with respect to the concentration of zinc, copper ($P < 0.05$), phosphorus, calcium and potassium ($P < 0.01$) – Table 4.

The concentrations of zinc, phosphorus, copper, and potassium in the AM plantlets were actually higher than those in the non-inoculated plantlets (Table 5, Figure 8).

The enhanced uptake of low mobility elements P, Zn, Cu, and Fe from the soil has been reported in AM plants. The high density of extra radical hyphae in the soil and a greater uptake area lead to the stronger impact of mycorrhizal plants on the uptake of these low mobility metal nutrients (Azevedo-Neto et al. 2006).

In this study, 83% augmentation of the Zn content in the leaves was observed in mycorrhizal plantlets of *S. khuzistanica*. The Zn content was increased up to 22%, and AM increased the Zn transfer from hyphae to roots and then from roots to shoots (Saranya, Kumutha 2011). Many enzymes need zinc for their activation. This ion may also be required for the biological synthesis of chlorophyll in some plants (Taiz et al. 2015). There may be a relationship between the higher absorption of Zn and increased levels of chlorophylls, observed in AM plantlets.

The mycorrhizal structure effectively absorbs P from its lower concentrations in soil. Natural plant roots are incapable of this process (Jefferies et al. 2003). In this study, AM enhanced the uptake of P in inoculated plantlet considerably. Phosphorus, a major component of plant compounds, is a sugar-phosphate intermediate in photosynthesis, respiration and making the phospholipids membrane. This element is also an integral component

Table 4

Effects of AM fungal infection on the concentration of some nutrient elements (mg g^{-1}) in inoculated and non-inoculated plantlets after 90 days of transferring to ex vitro conditions

Item	DF	Zn^{++}	Ni	Fe^{++}	Cu^{+}	Ca^{++}	K^{+}	Mg^{++}	Na^{+}	P	N%
Source	1	0.012 **	0.0007 ns	0.0001 ns	0.00001 **	18 *	19.42 *	1.35 ns	3.44 ns	0.13 **	0.0008ns
Error	4	0.0004	0.0001	0.001	0.0000005	1.96	1.93	0.94	2.55	0.001	0.0006
C.V.		11.6	97.65	9.9	21.2	13.97	16.39	33.25	9.43	4.33	5.41

ns, *, ** indicate significant or non-significant difference at $P = 0.05$, and $P = 0.01$, respectively

Table 5

Effects of AM fungal infection on the concentration of some nutritient elements in inoculated and non-inoculated plantlets after 90 days of transferring to *ex vitro* conditions

Elements concentration (mg g^{-1})										
Item	N	Zn^{++}	Ni	Fe^{++}	Cu^{+}	Ca^{++}	K^{+}	Mg^{++}	Na^{+}	P
AM plantlet	3	0.22*a*	0.02*a*	0.33*a*	0.001*a*	8.30*b*	10.28*a*	3.39*a*	16.17*a*	0.88*a*
Control	3	0.12*b*	0*a*	0.32*a*	0*b*	11.77*a*	6.68*b*	2.44*a*	17.68*a*	0.58*b*

Mean values followed by different letters within a column do not significantly differ from one another at $P \leq 0.05$ led by the Duncan's Multiple Range Test.

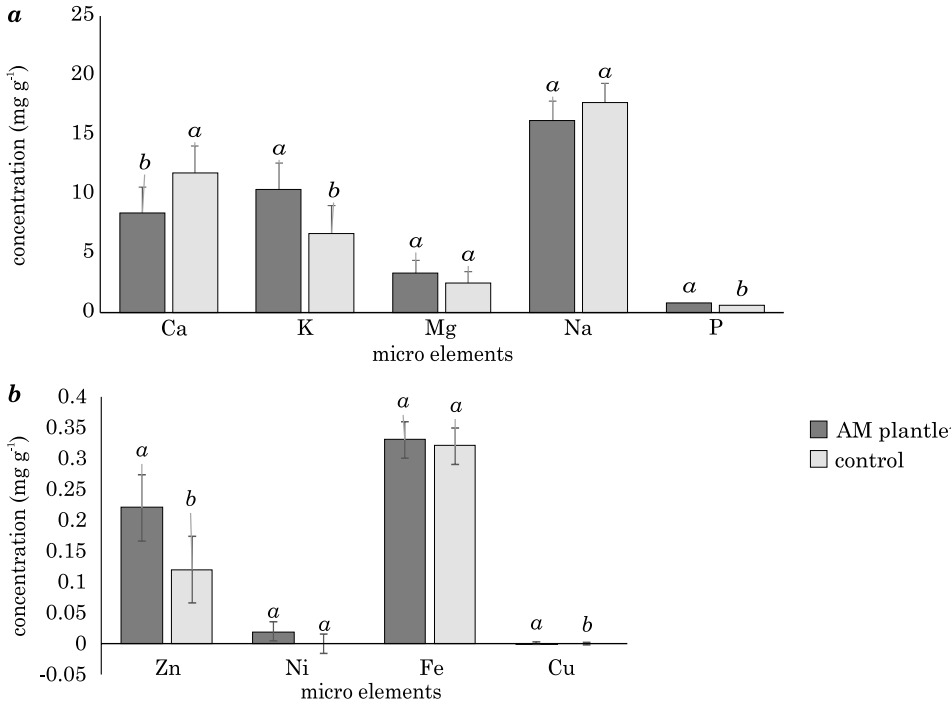

Fig. 8. Effects of AM fungal infection on some macro- (*a*) and micronutrients (*b*) in inoculated plantlets after 90 days of transferring to *ex vitro* conditions

of the nucleotides used in plant energy metabolism (ATP) as well as in DNA and RNA (Taiz et al. 2015).

Potassium in AM plantlets of *S. khuzistanica* appeared in elevated amounts compared to non-inoculated plantlets. Potassium has a pivotal role in drought tolerance, which corresponds to the stomata movement in response to leaf water changes (Ruiz-Lozano, Azcón 1995). On the other hand, it might play a significant role in osmotic adjustment of plant cells. Therefore, the protection of mycorrhizal plants against dehydration is somewhat related to the uptake of potassium, which also activates many enzymes involved in photosynthesis and respiration (Taiz et al. 2015). As a result, the increased content of potassium, phosphorus and zinc in mycorrhizal plantlets leads to augmented photosynthesis, respiration and osmoregulation as well as better acclimatization of plantlets.

As shown in Table 7, magnesium concentrations were not affected by the AM symbiosis. Magnesium is usually abundant in most soils and its deficiency is rare. This finding agrees with previous reports in other plants (Wu et al. 2007). Extra-radicular hyphae are less likely to contribute to the uptake of magnesium when this element is in good supply in the soil (Liu et al. 2002).

The concentration of calcium showed a decrease in inoculated plantlets. Calcium ions have many roles. They act as a second messenger in different plant responses to environmental and hormonal messages and for this reason the content of calcium ions increases in stress conditions. Its rise in non-inoculated plantlets may represent a stress response.

The N, P, and K content in AM plantlets of *Capsicum annuum* L. cv. San Luis was found to have increased, which was in accordance with the present findings, but no significant differences were observed in the Ca, Fe, B, and Cu levels. Moreover, Mg, Zn, and Mn reached higher levels in the control group than in the plants from the mycorrhizal treatment (Estrada-Luna, Davies 2003). The higher K and unchanged Fe levels reported by the cited authors were similar to the ones determined in the present study. Tissue culture plantlets of grapes showed higher concentrations of N, P, Mg and Fe in response to six AM species during acclimatization (Krishna et al. 2005). The symbiosis effect of AM fungi on plantlets of *Psidium guajava* L. led to the increased level of P, Mg, Cu, and Mo (Estrada--Luna et al. 2000). Sohn et al. (2003) reported the increased concentrations of P, K, Mg, Ca, Fe, Mn, and Cu in the leaves of seedlings in symbiosis with *Glomus* sp. Different accumulation was reported for N, P, and K in the tissue culture plantlets of banana inoculated with *G. clarum* (Leal et al. 2005). This information indicates that in addition to mycorrhizal fungi species, plant species play a role in the effective uptake of elements.

Differences in the uptake of some nutrients by plants may be related to the competition between the host and the environment (Cavagnaro et al. 2005), or to the preferential symbiosis of certain combinations of plant genotypes and AM species (Sanders 2004).

CONCLUSION

The rhizosphere area enlarged by the mycorrhizal mycelium leads to the increased absorption of water and low-mobility nutrient elements, which subsequently improved the survival rate and biomass as well as the levels of chlorophyll and carotenoids in the tissue culture plantlets. As a result, higher levels of photosynthesis and respiration led to better acclimatization to the *ex vitro* conditions, which is very important in the micropropagation of this valuable medicinal plant. On the other hand, our biochemical findings showed that AM fungi could be beneficial by limiting injuries related to stress during the transfer of plantlet to the *ex vitro* conditions. To sum up, our results clearly confirm the need to develop a tissue culture protocol with AM inoculation which would enable better survival and growth of this valuable medicinal plant.

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