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EFFECT OF ENDOMYCORRHIZAL COLONIZATION WITH *GLOMUS INTRARADICES* ON GROWTH AND ANTIOXIDANT CAPACITY OF *SIDERITIS SCARDICA* GRISEB

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Summary. The effect of *Glomus intraradices* inoculation on the growth and antioxidant activity of mountain tea (*Sideritis scardica* Griseb.) was determined. Two-months-old mountain tea seedlings were grown during 10 weeks on the soil/sand (w/w=3:1) substrate in a glass house. Mycorrhizal colonization improved shoot and root dry biomass accumulation and increased total phenols and flavonoid content. During the period of vegetative growth the level of antioxidant metabolites (ascorbate acid and reduced glutathione) and the antioxidant enzymes guaiacol peroxidase and catalase decreased as a result of the mycorrhizal colonization. A favorable effect of root colonization with G*lomus intraradices* was observed regarding the levels of ascorbate peroxidase and super oxide dismutase. We conclude that inoculation of *Sideritis scardica* Griseb. with *Glomus intraradices* resulted in enhanced plant dry biomass accumulation, but the antioxidant defense was not efficient enough during the period of vegetative growth.

Key words: Mountain tea (*Sideritis scardica* Griseb); *Glomus intraradices;* antioxidant capacity.

INTRODUCTION

Sideritis scardica belonging to the *Lamiaceae*, also known as mountain tea, is a perennial herb that grows in the mountainous regions between Southern Europe and Eastern Mediterranean. These plants are known to have antispasmodic, anti-feedant, carminative, analgesic, nervous system stimulant, sedative, antitussive, stomachic, anticonvulsant, antibacterial, antiinflammatory, antimicrobial and antioxidant activities (Ozturk et al., 1996; Navarro et al., 2001; Ozkan at al., 2005). Studies on the biological and ecological properties of four populations *S. scardica*, factors determining the optimal plant development and the growth of the species under cultivation

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conditions have been reported (Evstatieva and Koleva, 2006). The herb and leave exudates of *S. scardica* have been shown to possess anti-inflammatory, antimicrobial and anti-rheumatic activities. The influence of plant cultivation on the chemical composition and antibacterial activity against *Staphylococcus aureus* of the extracts of some cultivated *Sideritis* species was shown by Kostadinova et al. (2008).

Many species belonging to the family *Lamiaceae*, including mountain tea, form arbuscular mycorrhizas (Wang and Qiu, 2006). In addition to increasing uptake of poorly available nutrients such as phosphorus and nitrogen (Smith at al., 2003) or conferring protection against pathogens (Cordier et al., 1996), they can also induce changes in the accumulation of secondary metabolites, including phenolics, in host plants (Devi and Reddy, 2002). Although the ameliorative effect of arbuscular mycorrhizal fungi (AMF) on the host response to detrimental circumstances has been attributed to a wide variety of mechanisms, the antioxidant defense role of arbuscular mycorrhizas is generally considered (Wu et al., 2006). The objective of this work was to evaluate if colonization with AMF *Glomus intraradices* can provide an efficient and natural way to improve the growth of *S. scardica*, and at the same time, to increase the production of phenolic compounds and enhance the antioxidant activity.

MATERIAL AND METHODS

Mountain tea seeds were grown in a climatic chamber at a 12 h photoperiod, day/night temperature 25/18°C and photon flux density of 95 μmol m^{-1} s^{-1}. Two-months-old seedlings were transferred to 5 kg plastic pots in a glass house (1 plant per pot) and were grown 10 weeks on a soil/sand substrate (3:1). Leached cinnamonic forest soil (Chromic Luvisols – FAO) with the following agrochemical characteristics: pH(H_2O) = 6.2; 8 mg kg^{-1} soil total mobile N ($N\text{-}NO_3^-$ + $N\text{-}NH_4^+$); 30 mg kg^{-1} soil P_2O_5; 120 mg kg^- soil K_2O was used. Culture of *Glomus intraradices* was kindly provided by N. Pouskarov Institute of Soil Science microbial collection in Bulgaria. Inoculation with AM fungi was done at the seeding by the layering method (Jackson et al. 1972). The following treatments were applied: control plants (C); plants, inoculated with *Glomus intraradices* (Gi). The rate of mycorrhiza infection of the roots was determined microscopically by the gridline intersect method (Giovanetti and Mosse, 1980). The root samples were treated with 10% KOH and colored with 0.05% tripan blue (Philips and Hayman, 1983). The colored root samples were spread out evenly in a square plastic petry dish. A grid of lines was marked on the bottom of the dish to form squares. Vertical and horizontal gridlines were scanned and the presence or absence of infection was recorded at each point where the root intersected the line by stereomicroscope.

The content of phenolic compounds was determined spectrofotometrically using Folin-Ciocalteu reagent and calculated as caffeic acid equivalents (Pfeffer et al., 1998). Flavonoids in plant tissues were measured by Zhishen et al. (1999) spectrofotometrically using a standard curve of catechin. Spectrophotometric quantification of ascorbate (ASC), reduced glutathione (GSH) and vitamin E was

performed through the formation of phosphomolybdenum complex. The assay was based on the reduction of Mo (VI) to Mo (V) by the sample analysis and the subsequent formation of a green phosphate-Mo (V) at acidic pH. The extraction for ASC and GSH was done with a water solvent and for vitamin E it was done with hexan. An aliquot of 0.1 ml of water extract was combined in an Eppendorf tube with 1 ml reagent solution (0.6 M H_2SO_4, 28mM NaH_2PO_4, 4 mM $(NH_4)_6Mo_7O_{24}.4H_2O$). The tubes were incubated at 95°C for 90 min. The hexan extract was mixed with 1 ml of reagent solution and incubated at 37°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous phase was measured at 695 nm against blank. The method has been optimized and characterized with respect to linearity interval, repetitively and reproducibility, and molar absorption coefficients for the quantitation of ASC ((3.4±0.1) x 10^3 M^{-1} cm^{-1}), GSH ((2.7±0.2) x 10^3 M^{-1} cm^{-1}) and vitamin E ((4.0±0.1) x 10^3 M^{-1} cm^{-1}) (Prieto at al., 1999). Enzyme activities were determined spectrophotometrically according to the following protocols: superoxide dismutase SOD [EC 1.15.1.1] (Beauchamp and Fridovich, 1971) catalase CAT [EC 1.11.1.6] (Beers and Sizer, 1952), ascorbate peroxidase APX [EC 1.11.1.11] (Nakano and Asada, 1987) and guaiacol peroxidase GPO [EC 1.11.1.7] (Dias and Costa, 1982). One unit of SOD activity was defined as the amount of enzyme which causes 50% inhibition of riboflavin-mediated NBT (nitroblue tetrazolium) reduction. Soluble protein content was determined by the method of Bradford (1976). Data were expressed as means ±SE, where n=3. Comparison of means was performed by the Fisher LSD test ($P \leq 0.05$) after performing ANOVA analysis.

RESULTS AND DISCUSSION

The changes in roots, stems and leaves fresh and dry plant biomass and level of AMF colonization of plants are shown in Table 1. The higher percentage of root colonization of inoculated plants (63.60) resulted in increased FW and DW of leaves and roots in comparison with the non-mycorrhizal plants (control). For example, compared with the control, the

Table 1. Effect of *Glomus intraradices* root colonization on the fresh and dry root, steam and leaf biomass accumulation, and the extent of arbuscular mycorrhizal fungi (AMF) colonization (percentage of root length infected).

Treatments	FW root	DW root	FW stem	DW steam	FW leaves	DW leaves	Root colonization
	[g plant^{-1}]	[g plant^{-1}]	[g plant^{-1}]	[g plant^{-1}]	[g plant^{-1}]	[g plant^{-1}]	%
Control	67.39^a	14.19^a	12.87^b	3.72^a	35.40^a	10.94^a	26.37^a
G. intraradices	89.22^b	16.65^b	11.42^a	3.57^a	43.25^b	13.05^b	63.60^b
LSD($P \leq 0.05$)	8.96	1.75	1.38	0.41	4.47	1.36	1.2839

Values are means from three replications. The different letters in the same column indicate a significant difference at $P \leq 0.05$ by the Fisher LSD test after performing ANOVA analysis.

AMF colonization increased plant root and leaf FW by 32.3% and 22%, respectively. Only stem biomass decreased. The observed AMF colonization in the roots of the non-mycorrhizal control may be due to the natural diversity of AM fungi in the soil different from species applied in the experiment. Jia et al. (2004) reported that inoculation with AM fungi promoted biomass production and photosynthetic rates in *Vicia faba* because of the enhanced P supply due to AM fungi inoculation.

The favorable effect of coinoculation of pea plants with AMF and *Rhizobium* on N and P assimilation and plant biomass was reported by Geneva et al. (2006). Toussaint et al. (2007) reported that AMF potentially represented an alternative natural way of promoting growth of this important medicinal herb.

The phenolic content, expressed as μg caffeic acid g^{-1} DW and flavonoids content expressed as μg catechin g^{-1} DW were higher in the plants inoculated with *Glomus intraradices* compared with non-inoculated plants (Table 2).

The potential of three arbuscular mycorrhizal fungi, including *Glomus intraradices* to enhance the production of phenolic compounds with antioxidant activity (rosmarinic and caffeic acid) was established in sweet basil by Toussaint et al. (2007). The *Sideritis* species have been found to be rich in phenolic compounds, especially flavonoids, which have been proved to possess a valuable antioxidant activity (Gabrieli et al., 2005). This fact is especially important considering the pharmacological interest and the traditional use of mountain tea in folk medicine for its anti-inflammatory and anti-rheumatic properties. The genus *Sideritis* contains antimicrobial and antioxidant polyphenolics such as flavonoids (Ozkan et al., 2005). Mycorrhizal infection resulted in reduced levels of ASC and GSH in the leaves of mountain tea, but the level of vitamin E slightly increased (Figure 1A). The plants which have higher foliar ascorbate content possessed improved tolerance to oxidative stress (Foyer et al., 1995). Glutathione in plants has a great physiological significance in defense mechanisms. Glutathione is a precursor of phytohelatins, which are crucial in controlling cellular heavy metal concentration (Grill at al., 1985). Therefore, increased glutathione levels are connected with enhanced plant tolerance to stress. Thus, reduced ASC and GSH levels indicated that mycorrhizal infection did not cause oxidative stress. Although ASC and GSH are believed to participate in the free radical scavenging cycle, the

Table 2. Content of total phenols and flavonoids in the leaves of *Sideritis scardica.*

Treatments	Total phenols	Total flavonoids
	[μg gDW^{-1}]	[μg gDW^{-1}]
Control	28.12±0.76[a]	14.67±0.09[a]
Glomus intraradices	51.31±4.15[b]	23.70±1.42[b]
LSD(P≤0.05)	4.68	2.22

Values are means from three replications ±SE. The different letters in the same column indicate a significant difference at P≤0.05 by the Fisher LSD test after performing ANOVA analysis.

antioxidant defense of mycorrhizal *S. scardica* plants was based mainly on the increased content of total phenols and flavonoids. Some authors (Navarro et al., 2001) reported that the extent of the antioxidant and antibacterial effects of the genus *Sideritis* could be attributed generally to their phenolic composition such as flavonoids. Our results regarding antioxidant enzyme activities (Fig. 1B) showed that mycorrhizal infection resulted in a decrease of CAT and GPO by 22% and 27%, respectively. The activities of APX and SOD changed reciprocally after plant mycorrhization. APX and SOD increased by 67% and 33%, respectively. Therefore, experimental treatments with mycorrhizal fungi resulted in a reduction of CAT and GPO, while APX and SOD activities increased. The antioxidant defense system controls the level of highly toxic reactive oxygen species (ROS) in the cell. Both enzymatic and non-enzymatic systems protect tissue against ROS generated as a result of external influences. The enzymatic antioxidant defense system includes SOD, CAT, APX, phenolic peroxidases such as GPO, but only APX and SOD were activated. The superoxide radical (O_2^-) is transformed into H_2O_2 by the elevated SOD activity. The higher APX activity in the leaves of mycorrhized plants could result in a faster removal of H_2O_2 trough the ascorbate-gluthatione cycle, thus helping to alleviate oxidative damage. Therefore, it would be

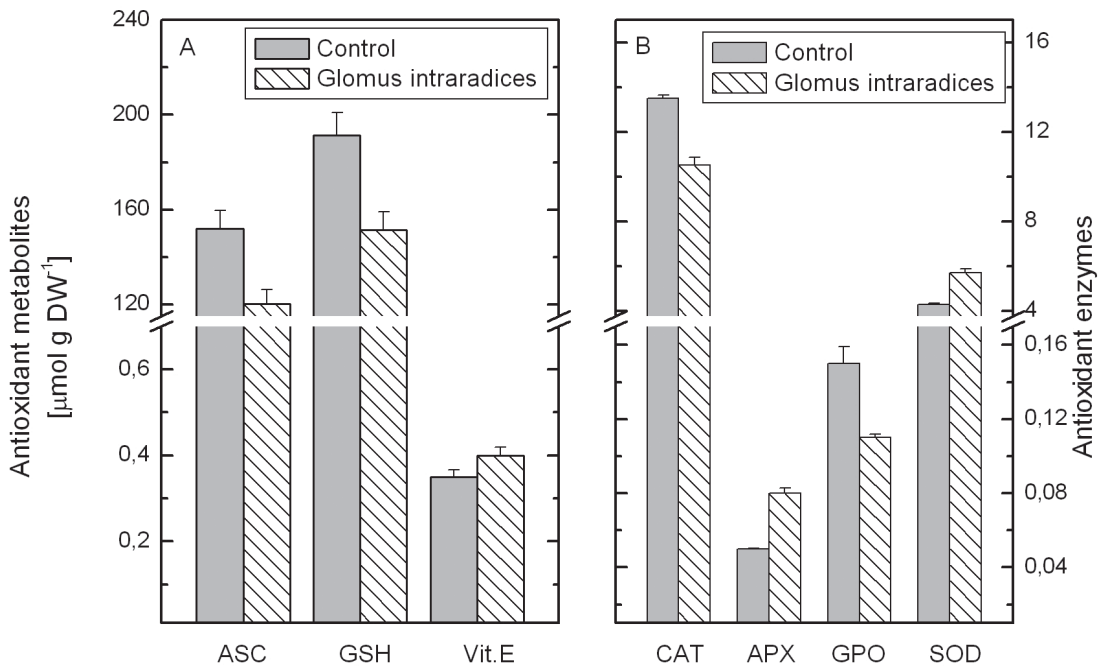

Figure 1. Content of antioxidant metabolites and changes in the activities of some antioxidant enzymes in *Sideritis scardica* leaves as a result of mycorrhizal colonization. CAT, APX and GPO activities are expressed as [µmol mg^{-1} protein] while SOD activity is expressed as [units mg^{-1} protein]. Values are means from three replications ±SE. The different letters in the same column indicate a significant difference at $P \leq 0.05$ by the Fisher LSD test after performing ANOVA analysis.

suggested that the ROS generated after mycorrhization were removed mainly by APX and SOD. The observations of some authors (Schützendübel and Polle, 2002) suggest that the stress reaction is diminished or perhaps the stress not perceived in mycorrhizal pine roots. This fact would imply that mycorrhization of plant roots plays a role of a link in the defense system.

CONCLUSIONS

Arbuscular mycorrhizal fungi colonization promoted root and leaf fresh and dry biomass of *Sideritis scardica* plants and increased total phenols and flavonoid content. The level of antioxidant metabolites (ascorbate acid and reduced glutathione) and some antioxidant enzymes (GPO and CAT) were suppressed as a result of mycorrhizal colonization during the period of vegetative growth. A favorable effect of root colonization with G*lomus intraradices* was observed regarding the levels of ascorbate peroxidase (APX) and super oxide dismutase (SOD). Therefore, the antioxidant defense of mycorrhized *Sideritis scardica* plants is based mainly on the increased content of total phenols and flavonoids and the enhanced APX and SOD activities.

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