



## Vesicular-arbuscular mycorrhizal symbioses in some plants and their relationship with soil factors and seasons

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### Abstract

Mycorrhizal association in seven plants grown in Arasbaran forest located in the north west of Iran was investigated. The soil samples were collected from the plants rhizosphere. Physico-chemical properties of rhizosphere soils like soil texture, pH, EC and some extractable nutrients such as N, P and K were recorded. The number of spores in rhizosphere and percentage of root mycorrhizal colonization were found. Soil physico-chemical analyses were done on August while spore number and root colonization were studied in May and August. All the data collected in this research were analyzed by the statistical software, SPSS. Results showed that different plant species colonized differently by VAM fungi, and spore number and root mycorrhizal colonization increased through the time from May to August. Among the physico-chemical factors, soil N content had high correlation with root colonization and soil extractable P had high correlation with soil spore number. pH had a high negative correlation with spore number. Among the plant species, *Stachys pubescens* showed the most VAM fungi root colonization and *Cruciata laevipes* rhizosphere soil showed the highest spore number.

**Keywords:** vesicular-arbuscular mycorrhizae; seasonality; soil properties; forest

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### Introduction

The management and conservation of forest biomes is a recognized priority on a global scale. Integral to the understanding of forest ecology is the rhizosphere and in particular the mycorrhizal symbiotic associations between plant hosts and fungi. Mycorrhizal relationships are an example of mutual symbioses, involving plants and fungi whereby both organisms benefit through an exchange of nutrients at the root-soil interface (Pongrac et al., 2009). Arbuscular mycorrhizas (AM), sometimes referred to as

endomycorrhizas, are formed predominantly by the fungal group Glomeromycota (Schüßler et al., 2001). The association is identified by intracellular dichotomously branching haustorial structures called arbuscules, found in the cortical plant root cells and hyphal coils, as well as intercellular hyphal networks and external hyphae that extend into the soil. AM are found on a wide range of host species, predominantly colonizing herbaceous shrubs and tree species. AM colonization has no visible effect on root morphology (Sonjak et al., 2007). The arbuscular mycorrhizal (AM) symbiosis is the most commonly occurring underground symbiosis in plants. It can be found in a large majority of

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terrestrial plants and in almost a quarter of a million plant species (Gadkar et al., 2001). The primary abiotic factors known to influence the abundance and distribution of AM fungi are water, nutrient, and oxygen availability (Wang and Qiu, 2006). There are some evidences that plants can regulate the amount of carbon invested in the mycorrhizal symbiosis (Lingfei et al., 2005), suggesting that the controls on the abundance and distribution of AM fungi may depend on how much a plant benefits from the interaction. Arbuscular mycorrhizal (AM) fungi (Glomeromycota) are one of the most prominent soil microorganisms. They expand the interface between plants and the soil environment and contribute to plant uptake of macronutrients P and N as well as micronutrients Cu and Zn (Fuchs and Haselwandter, 2004). AM fungi are also involved in plant interactions with soil toxic metals, either by alleviating metal toxicity to the host or by accentuating it (Füzy et al., 2008). The specific role of arbuscular mycorrhizae in the host exposure to metal stress and in the progression of the host stress response depends on a variety of factors, including the plant species and ecotype (Kaligarič et al., 2008). In this study we were interested in finding the influences of soil extractable nutrients and seasonality on root colonization quantity, the correlation between them and how these kinds of symbiosis affect spore population in the rhizosphere.

## Materials and Methods

### Sampling site

Arasbaran or Qaradağ is a UNESCO registered biosphere in East Azerbaijan Province, Iran, with a varying altitude from 256m in the vicinity of Aras River to 2896 m and covers an area of 78560 hectares and geographically located at 38°40' to 39°08'N; 46°39' to 47°02'E. The biosphere is also home for 23,500 nomads who are living in transition buffer in 2000 m altitude. This biosphere reserve is situated in the north of Iran at the border to Armenia and Azerbaijan belonging to the Caucasus Iranian Highlands. In-between the Caspian, Caucasus and Mediterranean region, the area covers mountains up to 2,200 meters, high alpine meadows, semi-

arid steppes, rangelands and forests, rivers and springs. Economic activities in the biosphere reserve are mainly agriculture, animal husbandry, horticulture, apiculture, handicrafts and tourism, but business activities can also be found in urbanized areas. Sampling site (38°50'N, 47°00'E) was approximately 1620 m above sea level which receives an annual rainfall of 400-600mm (Shams Azgan and Alizade, 2012)

### Soil analyses and isolation of AM fungi spores

Three soil samples (1000 g of soil) were collected for each of the seven plants. Soil samples were collected from the rhizosphere around the roots. Soil samples were bagged in polythene bags, sealed, brought to the laboratory and stored at 5 °C until analysis for mycorrhizal spores was performed. Soil samples were also analyzed to determine pH, electrical conductivity (EC) and available N, P, K using AOAC and Olsen P protocols (AOAC, 1960). Mycorrhizal spore count of each soil sample was assessed by a modified wet-sieving and decanting technique of Gerdemann and Nicolson (1963). Spore number was expressed as the total number of spores per 1 g of soil. Identification was done with the help of synoptic keys adopted by Raman and Mohankumar (1988).

### Analysis of mycorrhizal colonization

Seven plant species representative of 7 genera from 7 families were examined in this study. The plant species were identified taxonomically in the field, and root samples were collected. Approximately 15–20 g of root material was collected in each case. Sub-site of roots were immediately removed from each field soil core and carefully washed. Roots were cut into 1 cm segments, wrapped in mesh, placed into tissue capsules and stored in formalin acetic acid (FAA). Specimens were stained with acid fuchsin as in Kormanik and McGraw (1982), slightly modified by reducing the concentration of KOH to 5%. Root fragments were examined with light microscopy (Zeiss Axioplan), and AM fungal colonization assessed using the magnified intersections method (Mc. Gonigle et al., 1990). Each slide was scored twice using different

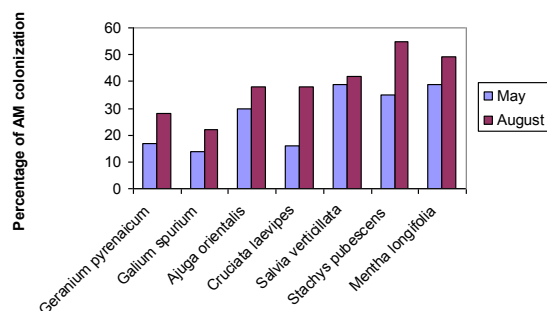


Fig. I. Comparison of root colonization in May and August

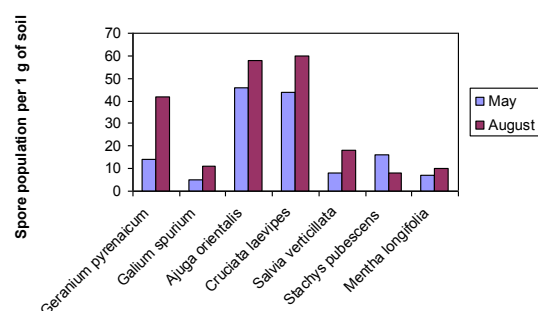


Fig. II. Comparison of spore number in May and August

starting points, for a total of 200 intersections per sample.

### Statistical analysis

Statistical tests were performed with SPSS version 18 (PASW statistic 18). The data were analyzed by T-test to test the effect of the factors. In this study  $P \leq 0.05$  was used to compare means. Also Pearson correlation was calculated to find correlation coefficient between factors.

### Results

Figure 1 compares root colonization at two times, May and August, and it is clear that it has increased in all seven plants from May to August. In May, percentage of arbuscular mycorrhizal colonization ranged from 14% in

*Galium spurium* to 39% in *Salvia verticillata* and *Mentha longifolia* besides in August it ranged from 22% in *Galium spurium* to 55% in *Stachys pubescens*. (Table 1). Table 2 compares spore population per 1 gram of rhizosphere soil through the time from May to August. It shows that in all plants except one of them (*Stachys pubescens*) spore population has increased. In May spore population in *Galium spurium* rhizosphere was least (5%) and in *Ajuga orientalis* (46%) it was the most besides on August in *Stachys pubescens* (8%) and in *Cruciata laevipes* (60%) it was the least and the most, respectively (Table 1). The rhizosphere soils of plants were analyzed only in August and found to be Loam, Clay Loam, Sandy, Sandy Loam and Loamy Sand which exhibited variation in pH, EC and variable N, P, K (Table 1). However, percentage of arbuscular mycorrhizal

Table 1  
Characteristics of the soil sampled from the study site

Plant species	Spore population per 1 g of soil		Percentage of AM colonization		EC*10 <sup>3</sup> (ds/m)	pH	Available N, P, K (mg/kg)			Soil texture			
	May	August	May	August			N	P	K	%Clay	%Silt	%Sand	Text
<i>Ajuga orientalis</i>	46	58	30	38	783	6.79	49	18.6	163	13	3	84	LSa
<i>Cruciata laevipes</i>	44	60	16	38	390	7.28	42	16.9	458	36	32	32	CL
<i>Stachys pubescens</i>	16	8	35	55	360	7.44	49	19.1	233	37	40	23	CL
<i>Geranium pyrenaicum</i>	14	42	17	28	1970	7	56	30.6	364	19	26	55	SaL
<i>Salvia verticillata</i>	8	18	39	42	470	7.62	42	11.4	238	34	36	30	CL
<i>Mentha longifolia</i>	7	10	39	49	530	7.67	45.5	15.2	332	26	33	41	L
<i>Galium spurium</i>	5	11	14	22	1200	7.44	12	3.4	140	2	3	95	Sa

L: Loam; CL: Clay Loam; SaL: Sandy Loam; Sa: Sandy; LSa: Loamy Sand

colonization in August did not tend to be higher at the soil with the greatest soil N but it significantly correlated ( $r=0.513$ ,  $p\leq 0.05$ ) with soil N. In comparison with root colonization, spore number had lower correlation with soil available N ( $r=0.361$ ,  $p\leq 0.05$ ). Root colonization did not significantly correlated with soil available P ( $r=0.139$ ,  $p\leq 0.05$ ) but spore number had higher correlation ( $r=0.452$ ,  $p\leq 0.05$ ) with soil available P. Both root colonization ( $r=0.132$ ,  $p\leq 0.05$ ) and spore number ( $r=0.391$ ,  $p\leq 0.05$ ) did not tend to have higher correlation with soil available K. The role of K in root colonization and spore number of AM fungi is little known as compared to N and P. Soil pH had positive correlation with the root colonization ( $r=0.379$ ,  $p\leq 0.05$ ) but negative correlation with spore population ( $r=-0.789$ ,  $p\leq 0.05$ ). Soil EC had negative correlation ( $r=-0.747$ ,  $p\leq 0.05$ ) with root colonization but positive correlation ( $r=0.156$ ,  $p\leq 0.05$ ) with spore number.

## Discussion

The extent of root length colonized by VAM fungi depends on root growth and spore number. In general, plants tend to allocate more resources for root production irrespective of soil nutrient levels (Konings et al. 1992). These confirm our results that root colonization and spore population increase through time. This was also the case with root growth which increased from May to August. Number and kind of VAM

fungi spores in the soil are related to some factors such as soil microbial activities, temperature and soil fertility (Ross, 1980). Some other factors like pH, available soil P, and spore germination affect spore production but do not affect its color, size and structure (Vestbery, 1995). Soil pH is one of the most important factors in growth of plants and mycorrhizal fungi but root colonization and spore production show different results under difference pH. The differential response of AM fungi to soil pH can be attributed to the species and strains constituting the indigenous AM flora (Robson and Abbott, 1989). The variation in response can also be attributed to the host-mediated changes of the rhizospheric pH. Johnson et al. (1991) showed that root colonization, more than spore production, is related to the soil pH but Porter et al. (1987), showed that soil pH has positive correlation with VAM fungi spore population too. Hayman and Stovold (1979) proved that VAM fungi especially *Glomus fasciculatum* are able to live in a broad range of pH and can reduce acidic stress in plants growth regions. In this research pH had a negative correlation with root colonization but a high positive correlation with spore population. Correlation between root colonization and plant P nutrition is so important that many researchers have focused on that. One of the most important characteristics of the VAM fungi is being able to absorb P from the soil with

Table 2  
Correlation coefficient (r) and significant at  $p\leq 0.05$  (p) between factors

		Percentage of AM colonization (May)	Spore population (August)	Soil EC	Soil pH	Available N	Available P	Available K	Soil texture		
									%Clay	%Silt	%Sand
Percentage of AM Colonization (August)	r	---	---	-0.747	0.379	0.513	0.139	0.132	0.761	0.694	-0.739
	p	0.013	---	---	---	---	---	---	---	---	---
Spore Population (August)	r	---	1.000	0.156	-0.789	0.361	0.452	0.391	-0.020	-0.280	0.163
	p	---	---	---	---	---	---	---	---	---	---
Spore Population (May)	r	---	---	---	---	---	---	---	---	---	---
	p	---	0.004	---	---	---	---	---	---	---	---

P deficiencies. This helps plants to be stable at soils with P deficiencies. Xiao-lin et al. (1997) showed that in P deficiencies conditions, more than 80% of plant P can be absorbed by external hypha of the VAM fungi. According to Marschner and Bell (1996) reports, concentration of soil available P affects root colonization by VAM fungi; therefore, high concentration of soil available P reduces mycorrhizal status and as a results, root colonization. It can be a supportive factor for our results in which soil available P had a low correlation with root colonization. Previous studies indicated that soil extractable N can either stimulate or suppress root colonization and spore production through modification of the soil pH and Muthukumar and Udaiyan (2000) believe that soil extractable N affects root colonization and spore number. In our research there was a high correlation with spore population and specially root colonization with soil available N. Furlan et al (1989), reported that soil K has stimulatory effect on mycorrhization. Ouimet et al (1996) showed that a minimum soil K is needed for mycorrhization in some plant species. It seems that the effect of soil K on mycorrhizal fungi depends on its own availability and also on concentration of other exchangeable ions like Ca and Mg in the soil (Mengel and Kirkby, 1980). In this study we found that soil available K had low correlation with spore number and specially root colonization.

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