Research Journal of Biological Sciences 10 (4-5): 56-61, 2015
ISSN: 1815-8846

Effect of Mycorrhiza in Adaptation of *Artemisia aucheri* to Altitude Changes

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Abstract: Despite wide distribution of *Artemisia* species in Iran, few investigations are available on ecophysiological mechanism(s) of its adaptation to various altitudes and soil properties. This study reports on the mycorrhizal fungal populations in the rhizosphere and degree of Arbascular Mycorrhizal (AM) symbiosis with *Artemisia aucheri* in Parvar Protected Area (PPA), located in Eastern Alborz mountain ranges, Iran. Samples of soil and plants were collected randomly in three altitudes at/and about 36° latitude. Soils samples from top 30 cm of rhizosphere and *A. aucheri* roots were collected and analyzed. Mycorrhizal fungal spores were isolated, identified and statistically analyzed. Soil type was sandy-loam significantly becoming loamy with increase in altitude. Spores of *Glomus* species formed the dominant mycorrhizal populations in the rhizosphere. *A. aucheri* roots developed arbuscular-vesicular mycorrhizae. Content of Ca in the roots and shoots of *A. aucheri* significantly was lower while contents of N and P significantly improved with hike in altitude. There were strong to moderate correlations between degree of AM mycorrhizal development, spore density and soil nutrients. Mycorrhizal symbiosis affects *A. aucheri* nutritional uptake and growth. Adaptive distribution potentials of *A. aucheri* makes it a reliable candidate for "designated indicator species" in steppe ecosystems and provides a means for well-planned sustainable green management in disturbed areas and conserving of protected areas. All ecological and conservation programs for Artemisia communities in PPA, need to take into account the role of mycorrhizal symbionts and the need to conserve mycorhizal fungi in the soil as well.

Key words: Adaptation, altitude, *Artemisia aucheri*, ecophysiological, mycorrhizal, Iran

INTRODUCTION

Many environmental stresses produce severe limitations on plant growth; for example, low and unpredictable precipitation, low relative humidity with desiccating winds, extremes of temperatures and low nutrient availability and accessibility. Despite such stresses, arid and desert plant communities often contain surprisingly large amounts of plant biomass, possess remarkable diversity of plant growth forms, produce variety of valuable chemicals (Noori *et al*., 2012) and accommodate microhabitats for many organisms, among other functions and services. Frequency of occurrence of plant species is usually thought to follow soil profile description generalities (Zare-maivan *et al*., 2014). Although, soil analysis helps to relate plant community changes to abiotic variations, it is the microtopography and genetic evolutionary past of species that define the scale and pattern of species distribution which may or may not be similar to the assumed distribution pattern. Overloking such criteria can lead to ecosystem management implications and misappropriate species conservation efforts.

Thirty four species of *Artemisia* (with English names worm wood and sage brush) are widely distributed in mid to upper latitudes as well as in different altitudes suitable for plant growth, exhibit medicinal and strong antioxidant properties (Zare-maivan *et al*., 2014) and are the most abundant perennial plant species in steppic and semi-arid prairie ecosystems of Iran. Artemisia plants are highly resistant against extreme environmental conditions and very effective in stabilizing the habitat (Torbatinejad *et al*., 2003). They also have great forage, conservation and aesthetic values. Despite wide distribution of *Artemisisa* species, ecophysiology of its adaptation to changes in altitude and soil properties has not been investigated under natural circumstances in Iran. Zare-maivan *et al*. (2014) studied succession of plant species in relation to altitude and indicated that distribution and behavior of *A. aucheri* were affected by altitude and soil abiotic properties. However, aspects on the effects of biotic factors such as mycorhizal spores were not reported on. Mycorrhizal fungi are distributed worldwide and develop symbiotic relationship with many plant species roots (Hedlund *et al*., 2003; Karimi *et al*., 2004). Mycorrhizal symbiosis could affect nutritional uptake and growth of *A. aucheri*, the dominant plant of PPA and as such deserves detailed study.

MATERIALS AND METHODS

Description of the study site: Parvar Protected Area (PPA) is a unique conserved ecosystem in N. Semnan highlands and located between lush Hyrcanian forests to

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the North and arid and desert plant communities to the South. Annual precipitation varies between 140-450 mm and mean temperature of 10-15°C, making its climate regime categorized as cold steppic as it is affected by the North-westerly flow of polar air masses (Khalili, 1973). Much of annual rainfall is in winter and early spring lessening towards warmer seasons and extending towards lower altitudes and desert lowlands. The summer is arid, warm and sunny with intensive radiation most of the time. During the arid season water is supplied mainly by the melting snow and springs. Middle Jurassic to upper Cretaceous limestone formations are dominant and form some very high rock cliffs along the East-West directed thrust fault zones (Stocklin, 1974). The study area has temperate and Mediterranean continental climate in the lowland and high altitudes, respectively. Annual temperature amplitudes can be high. At 1500 m Above Sea Level (ASL), the mean annual temperature is 13°C ranging from 0.6°C in January to 26°C in August (Kamrani *et al*., 2011).

Sampling: Samples of soil and *A. aucheri* plants were collected in triplicates from 3 altitudes at/and about 36°C latitude line on the Southern slopes of Alborz mountains in Semnan Province, in a completely randomized design in late May 2012 (Table 1). Samples were transported in plastic containers to the laboratory and kept cool and dry until further analysis.

Ecological characteristics data collection: Method of Bonham was used to collect quantitative ecological characteristics data such as abundance, frequency, density, percentage of coverage and height of all plant species in each quadrate in all stations. Simpson's index of dominance was also calculated (Simpson, 1949).

Mycorrhizal roots and spores: Fine roots of *A. aucheri* were isolated and washed with distilled water before fixing in 60% Glycerin-Alcohol solution. Longitudinal sections of root tips, 10-15 μ thick were hand prepared and stained in of Lacto-Phenol Cotton Blue solution (Phillips and Hayman, 1970). Mycorhizal spores were stained with a 1:1 mixture of Polyvinyl Lactoglycerol (PVLG) solution of Melzer's reagent. Prepared slides were examined for mycorrhizal colonization under an Olympus BH2 light microscope equipped with 35 mm digital camera. Percentage of mycorhizal colonization, the average mycorrhizal density and intercellular arbuscular intensity percentage were determined.

Table 1: Geographic coordinates and distance between stations around 36° latitude

Stations	Altitude, above sea level (m)	Geographical coordinates	Distance between stations (m)	Slope (%)
Upper (U)	U	N: 35, 59, 55.2	U-L	U-L
		E: 53, 35, 46.5	16,619	2.55
Middle (M)	M	N: 35, 58, 41.3	M-U	M-U
		E: 53, 29, 0.97	10,053	3.27
Lower (L)	L	N: 36, 02, 16.6	M-L	M-L
		E: 53, 24, 48.9	6,566	3.33

Mycorrhizal fungal spores were isolated using Wet-Sieving Method and sucrose centrifugation (Gerdemann and Nicolson, 1963; Tommerup, 1992; Pacioni, 1992) in 10 g of soil from each station, filtered through 0.45 μm Milipore grid filter paper and counted under Olympus BH2 stereomicroscope. Mycorrhizal species were identified based on appearance and wall characteristics of spores INVAM.

Soil analysis: Soil samples were air dried for 72 h. Soil subsamples (3 g of 2 mm mesh) were power pressed and analyzed via WD-XRF Method using XRF Phillips 2404 in Geology Laboratory of Tarbiat Modares University. Soil texture was determined using a hydrometer after soaking 200 g of soil for 24 h. Soil pH was determined using potentiometry method and EC was measured using an EC meter.

Determining elements in plant samples: Parts of *A. auchery* plant roots, stems and leaves were dried to constant weight in a 70°C oven within 48 h. The 1 g of specimen was digested using wet ash method with perchloric acid-nitric acid solution (Adler and Wilcox, 1985; Zasoski and Buran, 1977; Ali *et al*., 1988). Mg, Ca, Na and K were determined using the method by Isaac and Kerber (1971) via atomic absorption spectrometry. P was determined using Vanadate Ammonium-Molybdate Ammonium Spectrophotometry and N was determined using blue Indophenol method (Nelson and Sommers, 1973).

Statitical analysis: All data were statistically analyzed using SPSS V. 16 and Excel programs.

RESULTS

Results showed that the abundance, frequency, density, coverage area, importance value and Simpson's dominance index of *A. aucheri* population hiked as altitude increased, proportionally with greater difference at 2300 m (Station U) ASL (Table 2).

Similarly, spores of *Glomus* species formed the dominant mycorrhizal populations in the rhizosphere. *A. aucheri* roots developed arbuscular-vesicular mycorrhizae. Glomus spore abundance increased with

Table 2: Ecological characteristics of *Artemisia aucheri* plant at three different stations in parvar protected area, Iran during 2012 sampling

Stations	Abundance	Density (n/100 m^2)	(Coverage, %)*	Importance value*	Simpson's dominance index**
U	44.25±2.0^a	177±14.6^a	16.5±2.5^a	138.40±11.9^a	0.26^a
M	36.25±2.5^b	145±24.5^a	9.41±1.7^{ab}	98.04±9.20^b	0.11^b
L	32.00±2.0^c	128±14.9^a	3.87±0.9^b	40.35±5.12^c	0.02^c

*Values represent measurement at the samling plots relative to other species present in the plots (data not included); **Different letters in each column indicate significant differences between treatments at the 5% level

Table 3: Comparison of means of mycorrhiza in *Artemisia aucheri* at three stations in parvar protected area, Iran, 2012

	Stations		
Treatments	L	M	U
Spore density (n/g soil)	44.90±3.34^b	54.80±3.780^{ab}	57.63±8.030^a
Frequency (F %)	25.00±3.23^b	33.43±2.410^a	33.85±3.590^a
Mycorrhizae density (%)	5.54±0.51^b	6.64±0.490^a	6.80±0.580^a
Arbusculer density in the total roots (%)	24.43±3.98^b	27.66±2.920^{ab}	35.10±5.590^a
Arbuscule mycorrhizal density (%)	396.06±3.98^b	422.58±55.25^b	539.77±41.69^a

Different letters in each row indicate significant difference by Duncan's test at 5% level

Table 4: Comparison of content of elements in vegetative organs of *Artemisa sucheri* at three stations in parvar protected area, Iran, 2012

Organ	Stations	P (µg/g DW)	N (mg/g DW)	Mg (mg/g DW)	Ca (mg/g DW)	K (mg/g DW)	Na (mg/g DW)
Leaf	U	130±14^a	2.36±0.07^a	2.32±0.11^a	4.03±0.23^{bcd}	52.31±3.65^a	3.69±0.21^{dc}
	M	130±2^a	2.18±0.05^b	2.28±0.17^a	4.31±0.20^b	45.99±3.68^b	4.13±0.24^{bc}
	L	110±44^b	1.54±0.02^c	2.04±0.16^{ab}	5.32±0.24^a	44.25±1.43^{bc}	4.07±0.21^{bcd}
Stem	U	110±7^b	1.55±0.08^c	2.08±0.10^{ab}	3.81±0.15^d	40.66±3.80^{cd}	3.22±0.24^f
	M	60±3^e	1.25±0.20^e	2.02±0.17^{ab}	3.96±0.13^{bcd}	39.92±1.62^{cde}	4.32±0.19^b
	L	40±3^f	0.76±0.01^h	1.86±0.14^b	5.11±0.07^a	31.71±3.71^f	3.83±0.27^{cd}
Root	U	80±5^c	1.41±0.10^d	2.24±0.06^a	3.94±0.16^{cd}	36.60±3.70^{def}	3.36±0.22^{ef*}
	M	70±5^{cd}	1.13±0.06^f	2.20±0.16^a	4.19±0.22^{bc}	35.02±1.66^{ef}	4.21±0.19^{bc}
	L	60±5de	0.90±0.03^g	2.10±0.15^{ab}	5.30±0.21^a	31.47±1.54^f	5.10±0.22^a

Different letters in columns indicate significant difference by Duncan's test at 5% level

increasing altitude and was significantly different between Lower (L) and Upper (U) stations at (Table 3). Density of arbuscules in roots of *A. aucheri* at U station was greater, though not signifianltly different than that of other stations (Table 3).

Contents of elements differed significantly among plant organs in all altitudes (Table 4). Content of Ca in the roots and shoots of *A. aucheri* was significantly lower and N and P significantly higher with increase in altitude. Mg content did not differ much between organs in all stations. Contents of K, Ca, N and P in leaves of *A. aucheri* were significantly lower at M stationl than either of the other stations (Table 4). There were strong to moderate correlations between degree of AM mycorrhizal development, spore density and soil nutrients. N and P contents were greater in upper station and in leaves more than stems and roots, respectively (Table 4).

Analysis of soil samples showed soil type was loamy significantly becoming more sandy-loam with reducing altitude (Table 5). Silt content was greater in the lowest altitude. pH and EC did not differ ($p = 0.05$) amongst stations. Soil samples were slightly alkaline and haline. Soil elemental analysis showed presence of oxides of silica, aluminum, magnesium, sodium, potassium and phosphorus in upper altitudes except calcium which had greater content in the lowest altitude. Oxide of silicone (SiO_2) occurred in greater quantities in all stations. Such a trend was not observed for iron and sulfur (Table 5).

Table 5: Comparison of means and standard deviation of soil sampled at three stations in parvar protevcted area, Iran, 2012

	Stations		
Characteristics	L	M	U
Sand (%)	76.4±1.632^{a*}	70.4±1.41^c	72.4±1.63^{b*}
Clay (%)	5.6±0.00^a	7.35±0.5^a	5.6±0^a
Silt (%)	18±1.632^a	22.25±1.25^b	22±1.63^b
pH	8.0775±0.026^a	8.0475±0.05^a	8.13±0.073^a
EC (Ds/m)	1.1875±0.0629^a	1.175±0.05^a	1.1±0.081^a

Different letters in each row indicate significant difference by Duncan's test at the 5% level

DISCUSSION

Plants adapt themselves to dominant environmental conditions by changing their morphology for example leaf shape and size but mostly altering their physiology through multi-faceted biochemical adjustment of inner-cell environment via maintaining cellular homoeostasis, lowering competition (for example, via establishing symbiotic mycrrorhizal relationships or producing defensive chemicals), avoiding disrupting effects of light (excessive photo-excitation) by photo-inhibition and scavenging ROSs and temperature (Chill and heat) through non-photochemical quenching and ROSs quenchers (Zare-maivan *et al*., 2014).

A. aucheri plant genome responds to biotic symbiosis and abiotic stresses via biosynthesis of polyphenol compounds such as flavonoids, phenylpropanoids and lignin, all of which play strategic

role in enabling plants to confront environmental stresses. *A. aucheri* avoids harmful effects of UV radiation in open and higher altitudes via production of strong antioxidants (Rice-Evans, 2004; Soon *et al*., 1977) such as Phenylpropanes (p-coumaric acid) and increasing anthocyanine pigments that filter out Uv radiation, depending on light intensity and duration. Flavonoids apparently play a more protective role consequent to biotic interactions such as mycorrhizal development, competition with other plant species (such as *Astragalus* sp., *Acanthophyllum* sp., *Tanacetum* sp.) and herbivorey and is secondary in protection from radiation. Anthocyanines most likely play primary role in protection from radiation and are secondary in protection from biotic interactions as they are produced in lower quantities in roots.

Interaction of plant species with environmental parameters determines plant abundance and distribution (Hix and Pearcy, 1997) as well as diversity (Zare-maivan *et al*., 2014). Many studies have shown that ecological factors, particularly, topography (Badano *et al*., 2005; Bennie *et al*., 2006; Pourbabaei and Haghgooy, 2011; Fattahi and Ildromi, 2011; Vahdati *et al*., 2014) and soil can be determining sources of plant diversity and physiological response (Zare-maivan *et al*., 2014) in many ecosystems indifferent parts of the world. Interactions at the individual level usually add up to a community response which is manifested in ecosystem as succession of plant species to complete their life cycle over short time (phenology) and achieve niche specialization and trait development (for example producing a secondary compound) over long period. Ionic interactions take place within the cell (for example, ions of S and N) in different tissues of different organs at all times and are determining factors for the successful establishing of a plant in any given habitat.

In vegetative plants in which secondary compounds are not a significant sink for S, the molar N:S ratio is maintained at approximately, 30 reflecting the N and S composition of protein. Therefore, plants require N (acquired mainly as nitrate) and S (acquired mainly as sulphate) in a similar molar ratio (Anderson and MacMAhon, 2002). Regulation of sulphate and nitrate uptake must originate with a sensory mechanism in the root as this is the main site of uptake (Cram, 1990). Nitrate assimilation occurs predominantly in the root of some species and in the shoot of most plants (Brunold, 1993). Sulphate assimilation occurs mainly in the shoot in most plants (Brunold, 1993). Several processes are involved in coordinating nitrate and sulphate assimilation. The primary principle in all of these systems is that low levels of the reduced form of one element (say S) has the effect of enhancing the assimilatory capacity for that element (S) and shutting down the assimilatory capacity of the pathway for the other element (N). Results showed the lowest N in roots and the highest N in leaves in all altitudes. Similar pattern was observed for P. This finding shows that N and P are rapidly translocated to the sinks (leaves) to be assimilated into proteins or energy molecules. This argument is further supported because of basic soil pH and limestone rock formations of the study area (Table 5). In addition, colonization of *A. Aucheri* roots with mycorrhizae and leaching of acid phosphatases from fungal mycelia facilitates uptake of P in soil and regulates cationic balance in the root. Further, research will elucidate a better understanding of Sulfate (S):Nitrate (N) balance and physiological mechanisms of *A. aucheri* that regulate cellular ionic and nutrtient balance, particularly for allocating nutrients towards structural, regulatory, defensive or storage compounds under extra-stressful circumstances of aridity.

As such access to ecological knowledge such as flora and vegetation of an area, climate soil and topography is necessary for proper and sustainable management and conserving of protected and fragile habitats (Shumar and Anderson, 1986; Guisan and Zimmermann, 2000). Findings of this research, although corroborated findings by Shumar and Anderson (1986) in regards to the distribution of *A. aucheri* species and the effects of topography (the 1,738 m elevation above sea level), it concludes that it is the collective effects of slope and gradient, temperature fluctuations, humidity (all related to altitude differences) and soil nutrient balance and availability in microhabitat scale that inserts adaptation strategy and so, Artemisia plants, although might look physiognomically similar but show markedly different functional traits at different altitudes. Physical traits do not necessarily reflect upon functional traits.

Productivity has a role to play in species ability to distribute. For Artemisia, the crucial role of abiotic stresses in determining species richness (success) at low productivity and the increasing role of biotic interactions with increasing productivity is established. This is a finding similar to findings by Keleman *et al*. (2013) and Karimi *et al*. (2004). Furthermore, dominance of Artmisia plants provide microhabitats that accommodate higher competitive ability and organic matter recharge to the rhizosphere as depicted by elements content in different organs (Table 4).

Artemisia plants shift their morpho-physiological potentials across the season and along the soil water potential. In other words, in plants, water deficit contributes to shifts in selecting principal metabolic pathway and eventual species distribution with

establishing mycorrhizal symbiosis, particularly in well-aerated sandy loam soils (Table 5) or adapting and optimizing photosynthetic system to remove radical oxidants.

Mycorrhizal symbiosis, in exchange for carbon source, provide valuable P for host plant and therefore enhance competitive advantage of *A. aucheri* against other plants, especially under drought and arid circumstances and in light of climate getting warmer, semi-arid and arid ecosystems may face greater or longer evaporation episodes. Limited water supply leads to stomatal closure and CO_2 shortage in the assimilating mesophyll tissues of plants. Under these conditions, an important adaptation of the photosynthetic system is photo respiration. In the course of this reaction sequence, H_2O_2 is produced in peroxisomes where it is scavenged by high activities of catalase. Zare-maivan *et al.* (2014) reported on the antioxidant enzymes as well as other antioxidant compounds of *A. aucheri* in PPA. Adaptive behavior of plants to environmental stress is rather evident in two dominant *Artemisa* species in Iran. *Artemisia aucheri* responds more to rainfall and chilling factor and *A. siberie* more to heat and water deficit factor (Yaghmaei *et al*., 2008; Friedjung *et al*., 2013).

CONCLUSION

Researchers try to investigate plant communities for their habitat characteristics and quality, environmental tolerance, geographical distribution and niche determination. Usually, an indicator species is identified that characterize the biology of the ecosystem as a whole being. In light of climate change as well as increasing incidences of human-caused disturbance such as grazing, medicine and food production, adaptive distribution potentials of *A. aucheri* makes it a reliable candidate for "designated indicator species" steppe ecosystems and provides a means for well-planned sustainable green management in disturbed areas and conserving of protected areas such as PPA. Establishing comprehensive researched-based ecosystem management guidelines for each plant species in fragile ecosystems is a fundamental necessity.

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