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Research Paper

Studying the Morphology and some Mineral Nutrients Content of Artemisia of Karaj Habitat in Iran

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Abstract: Plants have various mechanisms helping them to prevent the collapse of thermodynamic or chemical balance between inside and outside of cells. Protective and resistance mechanisms which are used to cope with the stresses and the ability of protoplasm to alleviate the stresses are examples of these mechanisms. In this experiment, artemisia (*Artemisia sieberi*) was collected by plotting at the full flowering stage from a natural habitat at 30 km south west of Karaj, Iran, in 2011. The following traits were measured were measured on the collected samples: plant height, the number of tillers, minimum and maximum canopy diameter, root length and weight, soluble sugars content, proline Na, K, Mg, Ca, Cl, Fe and the essential oil content of each plot. Analysis of variance showed that the effect of sampling area (plot) was significant on plant height, root length, the number of tillers, minimum canopy diameter, flowering shoot yield, non-flowering stems weight, essential oil content, soluble sugars, proline, chlorophyll a, chlorophyll b, Na, K, Mg, Ca and Cl at $P \leq 0.01$, and on maximum canopy diameter and the number of lateral branches at $P \leq 0.05$. Mean comparison of the plots indicated that the highest value of plant height and root length was 57.52 and 54.21 cm, respectively. The highest number of tillers and minimum canopy diameter were 50.2 tillers/plant and 41 cm, respectively. The highest flowering shoot weight was 82.6 g/plant. The highest and the lowest essential oil content was 0.85% and 0.42%, respectively. The highest soluble sugars and proline content was 0.45 and 1.61 mg/l, respectively. The highest content of Na, K, Mg, Ca and Cl was 7.63, 19.82, 5.82, 27.64 and 28787.1 ppm, respectively. Determining the correlation of traits showed that plant height had significantly positive correlation with the number of tillers, minimum and maximum canopy diameter, number of lateral branches, flowering shoots yield and non-flowering stems weight, and significantly negative correlation with essential oil content. Root length had also a significantly positive correlation with essential oil content and significantly positive correlation with the number of tillers, flowering shoot yield and non-flowering shoot weight.
Keywords: artemisia, ICP, mineral nutrients, proline, soluble sugars.

Introduction

Different definitions are offered for salinity. According to the definition represented by Shannon and Grieve (1999), salinity is the condition of high concentration of salts and soluble minerals in water or soil around plant roots which disturbs plant water absorption. High concentration of neutral salts such as NaCl and Na_2SO_4 makes the osmotic pressure of soil solution higher than the osmotic pressure of plant root cells; so plants cannot absorb water from the soil. On the other hand, soil wilting point also increased as the result of high salt concentration and soil can provide lower water to plant roots. As the result, soil salts may disturb plant nutrition and plants cannot receive their required nutrients (Heidari Sharifabad, 2001).

Plants have various mechanisms enabling them to prevent the collapse of thermodynamic or chemical balance between inter/intracellular environment. This ability is very different among plant species and different species have different tolerance to salinity stress (Volkmar and Steppuhn, 1998). Sensitive plants such as the beans face 50% reduction in yield in 60 mM salinity level and rice faces yield reduction in 50 mM (Glenn *et al*., 1997). Sugar beet tolerates up to 260 mM salinity and the halophytes tolerate up to 500 mM salinity and can complete their life cycle in that condition (Mass, 1986). Neumann *et al*. (1997) reported that shoot fresh and dry weight and root dry weight are the factors severely affected by the salinity stress. Suriyan *et al*. (2003) in their experiment on sorghum indicated that salinity stress reduced LAR, germination percentage, leaf area, dry weight and fresh weight. Ryuichi *et al*. (2006) studied the effect of salinity on photosynthesis and morphology of *Nicotiana tabacum* by micro morphometric method and found that salinity stress affected plant stem diameter within few hours after the stress was applied; however, it took three to four days until the effect of salinity was observed on photosynthesis. They reported that carbon transfer in plant reduces quickly after salinity stress is applied; however, sugar accumulation in leaf and sodium accumulation in all plant organs increase.

Navarro *et al*. (2006) reported that salinity stress reduced dry matter, leaf area, plant height, photosynthesis and intercellular space in plants endemic to Spain. Dashtakian (2000) found that salinity stress reduced root length, shoot length, leaf area, water consumption, root weight, stem weight, leaf weight and relative growth rate of madder. Afzali (2001) tested the effect of salinity stress on *Melilotus officinalis* and *Trifolium fragiferum* and concluded that the stress decreased germination rate, chlorophyll content, the number of leaves, leaf area and root and shoot length and dry weight. Amirian (2002) studied forage sorghum and reported that salinity stress reduced whole plant growth.

Artemisia genus has 34 species; *Artemisia sieberi* Bess. is reported to grow in desert area of Iran. Two species of this genus (*A. kermanensis* and *A. melaolepis*) are endemic to Iran (Mozafarian, 1988 and 1996). *Artemisia sieberi* Bess grows Iran, Palestine, Syria, Iraq, Turkey, Afghanistan and Central Asia (Farzaneh *et al*., 2006).

Artemisia sieberi Bess. is reported to have anti-parasitic features beneficial in curing intestinal worm infections, Malaria and fungal infections (Giao *et al*., 2001; Negahban, 2006). In the early studies on this plant some new compounds were detected such as derivatives of Bisabolene and Salsolene. The main compounds in the essential oil of *Artemisia sieberi* L. of Semnan province of Iran were comphor, 1-8,cineole and bornyl acetate (Sefidkon *et al*., 2002). Researches indicated that the essential oil of *Artemisia sieberi* L. is effective on the regulation of heart beat, reduction of blood sugar, enhancement of Insulin production in type II diabetes (Mansi and Lahham, 2008). There are also reports about the anti-bacterial and anti-acne effects of the essential oil (Konuklugil *et al*., 1997).

Regarding the importance of the medicinal plant *Artemisia sieberi* Bess., and the effects of environmental conditions plants growth, this experiment was conducted to evaluate the variation of morphology and nutrients absorption of *Artemisia sieberi* Bess. from different habitats.

Materials and Methods

In this experiment, artemisia (*Artemisia sieberi* Bess.) was collected by plotting at the full flowering stage from Rood Shoor (Saline River) area at 30 km south west of Karaj, Iran, in 2011. In the habitat, nine plots with the size of 10 m^2 were selected with the distance of 100-500 m from each other and 20-40plants were sampled from each plot at each stage. To select the plots place, the area with high salt concentration and very low vegetative cover was selected as the first plot and each 100 m from the river side was selected as the second and the third plots. The repetition of each plot was considered in a area with same distance from the river side.

At the reproductive stage (full flowering stage), morphologic traits such as plant height, the number of tillers and the minimum and maximum canopy diameters were measured first, and then, the flowering shoot and other parts of shoot were separately harvested and transported to the laboratory. To measure root weight and root length in each plot, eight samples were harvested with roots, dried in laboratory and the required traits were measured. In the habitat, some samples were separated from the harvested plants and were kept in ice in order to measure chlorophyll content. Other parts of the harvested plants were kept in paper bags and transported to the laboratory, dried and their dry weight was measured. The content of soluble sugars, proline, Na, K, Mg, Ca, Cl, and Fe in each plot was measured by the specific method. In addition to sampling from the plants, samples were taken from the soil of the rhizosphere and were analyzed in the laboratory (Table 1).

Table 1 (part 1). Physico-chemical properties of the test site soil.

pH	EC (ds/m)	P (ppm)	Cl (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)
8.33	7.4	11.63	14681.2	377.13	37147	5965.7c	23072.7

Table 1 (part 2). Physico-chemical properties of the test site soil.

ESP (%)	HCO_3^{-2} (ppm)	OC (%)	N (%)	SO_4^{-2} (ppm)	Clay (%)	Silt (%)	Sand (%)
33.83	545.57	0.44	0.44	2117.6	23.33	25.33	51.33

Some elements in plants tissue (Na, K, Mg, Ca and Fe) were measured by Inductively Coupled Plasma (ICP). Plant Cl content was measured by the titration with silver nitrate solution method. Proline and the soluble sugars were measured by the method of Irrigoyen *et al*. (1992). The chlorophylls content were measured by the following equations:

$$\text{Chl a (mg/l)} = (12.25 \times a663) - (2.79 \times a647)$$
$$\text{Chl b (mg/l)} = (21.5 \times a647) - (5.1 \times a663)$$
$$\text{Chl a + b (mg/l)} = (7.15 \times a663) + (18.71 \times a647)$$

In this equation:
Chl a: the content of chlorophyll a based on milligram in liter of fresh weight,
Chl b: the content of chlorophyll b based on milligram in liter of fresh weight,
Chl a + b: the total content of chlorophyll a plus b based on milligram in liter of fresh weight,
a: the formula of absorption by the solutions in different wavelengths.

Titrometry method was used for soil Cl and Mg measurement, Kjeldahl method for total N, Turbidity method for sulfate, Walkley-Black method for organic carbon (Ghazanshahi, 1997), titration with silver nitrate solution method for Cl, bulk method for total lime, hydrometry method for soil texture, flame photometry method for Na and K, and titrometry method by methyl orange reagent for bicarbonate (Ghazanshahi, 1997).

The essential oil from dried flowering shoots was conducted with the method of hydrodistillation within 4 h. At the end, data were analyzed using SAS and means were compared according to the Duncan's multiple rang test.

Results

Analysis of variance (Table 2) indicated that the effect of repetition inside an area was significant on root length, the number of tillers, minimum canopy diameter, the number of lateral branches and flowering shoot yield at $P \leq 0.01$ and on plant height and root weight at P≤0.05. The effect of plot was also significant on plant height, root length, the number of tillers, minimum canopy diameter, flowering shoot yield, non-flowering stems weight and essential oil percentage at P≤0.01 and on maximum canopy diameter and the number of lateral branches at P≤0.05.

Table 2. Analysis of variance of the effect of sampling site on the measured traits.

SOV	df	Mean Squares										
		Plant height	Root length	The number of tillers	Minimum canopy diameter	Maximum canopy diameter	Lateral branches	Flowering shoot	Non flowering stem	Root weight	Total weight	Essential oil percentage
Repetition inside area	2	*	**	**	**	ns	**	**	ns	*	ns	ns
Plot	2	**	**	**	**	*	*	**	**	ns	ns	**
Error	4	4.89	2.74	0.93	0.11	11.11	19.93	30.24	30.23	519.23	639.55	0.01
CV (%)	-	13.32	14.26	7.59	5.94	6.86	3.46	9.56	6.12	14.78	8.40	7.10

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Analysis of variance (Table 3) indicated that repetition inside an area had significant effect on Na, Mg and Ca at P≤ 0.01 and on K at P≤0.05. Plot had also a significant effect on soluble sugars, proline, total chlorophyll 1, total chlorophyll 2, chlorophyll a, chlorophyll b, Na, K, Mg, Ca and Cl at P≤0.01.

Table 3. Analysis of variance of the effect of sampling site on the measured traits.

SOV	df	Mean Squares									
		Soluble sugars	proline	Total chlorophyll	Chlorophyll a	Chlorophyll b	Na	K	Mg	Ca	Cl
Repetition inside area	2	ns	ns	ns	ns	ns	**	*	**	**	Ns
Plot	2	**	**	**	**	**	**	**	*	**	**
Error	4	0.01	0.01	0.01	0.01	0.01	0.05	0.43	0.07	0.13	754689.7
CV (%)	-	26.86	9.32	7.65	10.88	9.08	3.27	3.67	5.18	4.45	4.23

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Mean comparison of plots (Table 4) showed that area three had the highest plant height (57.5 cm) and area one had the lowest plant height (32.4 cm). Area one and area three had the highest and the lowest root length (54.2 and 28.8 cm, respectively). The number of tillers was the highest in area three (50.2) and the lowest in area one (25.9). Area three and area one had the highest and the lowest minimum canopy diameter (41 and 27 cm, respectively). Area two had the highest (55 cm) and area one had the lowest maximum canopy diameter (45 cm). The number of lateral branches was the highest in area three (139.9) and the lowest in area one (115.22). On the other hand, area three had the highest flowering shoot weight (82.6 g) and area one had the lowest (24.2 g). Stem weight was the highest in area three (121.9 g) and the lowest in area one (41.54 g). Root weight was the highest in area one (249.1 g) and the lowest in area three (188.8 g). Moreover, area three had the highest total weight (310.7 g) and area one had the lowest (290.6 g).

Table 4. The effect of plotting area on the measured traits.

Treatments	Plant height (cm)	Root length (cm)	The number of tillers	Minimum canopy diameter (cm)	Maximum canopy diameter (cm)	The number of lateral branches	Flowering shoot weight (g/plant)	Stem weight (g/plant)	Root weight (g/plant)	Total weight (g/plant)
Plot 1	32.4b	54.2a	25.9c	27.0c	45.0b	115.2b	24.2c	41.54c	249.08a	290.63a
Plot 2	34.8b	33.3b	35.5b	37.6b	45.6b	131.4a	65.6b	106.07b	195.70b	301.77a
Plot 3	57.5a	28.8c	50.2a	41.0a	55.0a	139.9a	82.6a	121.89a	188.83b	310.72a

Means in a column followed by the same letter are not significantly different at P≤0.01.

Mean comparison of the plots (Table 5) indicated that area one had the highest (0.85%) and area three had the lowest (0.42%) essential oil percentage. Area one had the highest soluble sugars (0.6 mg/l) and area three had the lowest (0.45 mg/l). Proline content was the highest in area one (1.61 mg/l) and the lowest in area three (0.36 mg/l). Total chlorophyll was the highest in area three (0.9 mg/l) and the lowest (0.49 mg/l) in area one. Chlorophyll a was the highest in area three (0.4 mg/l) and the lowest in area one (0.21 mg/l). Chlorophyll b was also the highest in area three (0.49 mg/l) and the lowest in area one (0.27 mg/l). Area one had the highest and area three had the lowest Na content (7.63 and 5.81 ppm, respectively). Area one had the highest and area three had the lowest K content (19.82 and 15.89 ppm, respectively). Mg content was the highest in area two (5.82 ppm) and the lowest in area three (4.6 ppm). Ca content was also the highest in area one (27.64 ppm) and the lowest in area three (24.24 ppm). Area one had the highest and area three had the lowest content of Cl (28787.1 and 15096.8 ppm).

Table 5. The effect of plotting area on the measured traits.

Treatments	Essential oil percentage (%)	Soluble sugars (mg/l)	Proline (mg/l)	Total chlorophyll (mg/l)	Chlorophyll a (mg/l)	Chlorophyll b (mg/l)	Na (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	Cl (ppm)
Plot 1	0.85a	0.39a	1.61a	0.49c	0.21c	0.27b	7.63a	19.82a	5.38a	27.64a	28787.1a
Plot 2	0.68b	0.09b	0.91b	0.73b	0.31b	0.42a	7.46a	18.20b	5.82a	24.70b	17598.3b
Plot 3	0.42c	0.03b	0.36c	0.90a	0.40a	0.49a	5.81b	15.89c	4.60b	24.24b	15096.8c

Means in a column followed by the same letter are not significantly different at $P \leq 0.01$.

Determining the correlation of the measured traits (Table 6) indicated that plant height had significantly positive correlation with the number of tillers (r = 0.94**), minimum canopy diameter (r = 0.72*), maximum canopy diameter (r = 0.84**), lateral branches (r = 0.76*), flowering shoot (r = 0.76*) and stem weight (r = 0.70*) and negative correlation with essential oil percentage (r = -0.72*). Root length had significantly positive correlation with essential oil percentage (r = 0.85**) and negative correlation with the number of tillers (r = -0.68*), flowering shoot (r = -0.73*) and stem weight (r = -0.82**). The number of tillers had significantly positive correlation with minimum canopy diameter (r = 0.68**), maximum canopy diameter (r = 0.82**), lateral branches (r = 0.84**), flowering shoot (r = 0.91**) and stem weight (r = 0.88**) and negative correlation with root weight (r = -0.69*) and essential oil percentage (r = -0.90**). Minimum canopy diameter had significantly positive correlation with maximum canopy diameter (r = 0.74*), lateral branches (r = 0.95**), flowering shoot (r = 0.94**) and stem weight (r = 0.90**) and negative correlation with root weight (r = -0.89**). Maximum canopy diameter had significantly positive correlation with lateral branches (r = 0.67*) and flowering shoot (r = 0.68*) and negative correlation with root weight (r = -0.66*) and essential oil percentage (r = -0.67*). Lateral branches had significantly positive correlation with flowering shoot (r = 0.91**) and stem weight (r = 0.85**) and negative correlation with root weight (r = -0.81**). Flowering shoot had significantly positive correlation with stem weight (r = 0.98**) and negative correlation with root weight (r = -0.76*) and essential oil percentage (r = -0.72*). Stem weight had significantly positive correlation with root weight (r = 0.70*) and negative correlation with essential oil percentage (r = -0.84**).

Table 6. The correlation coefficient of the measured traits.

	Plant height	Root length	The number of tillers	Minimum canopy diameter	Maximum canopy diameter	Lateral branches	Flowering shoot	Stem weight	Root weight	Total weight	Essential oil percentage
Plant height	1										
Root length	-0.48ns	1									
The number of tillers	0.94**	-0.68*	1								
Minimum canopy diameter	0.72*	-0.54ns	0.86**	1							
Maximum canopy diameter	0.84**	-0.36ns	0.82**	0.74*	1						
Lateral branches	0.76*	-0.44ns	0.84**	0.95**	0.67*	1					
Flowering shoot	0.76*	-0.73*	0.91**	0.94**	0.68*	0.91**	1				
Stem weight	0.70*	-0.82**	0.88**	0.90**	0.61ns	0.85**	0.98**	1			
Root weight	-0.53ns	0.28ns	-0.69*	-0.89**	-0.66*	-0.81**	-0.76*	0.70*	1		
Total weight	0.05ns	-0.54ns	0.91ns	-0.21ns	-0.23ns	-0.16ns	-0.05ns	0.15ns	0.59ns	1	
Essential oil percentage	-0.84**	0.85**	-0.90**	-0.65ns	-0.67*	-0.63ns	-0.82**	-0.84**	0.38ns	-0.43ns	1

ns, non significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

The correlation of the measured traits (Table 7) indicated that soluble sugars had significantly positive correlation with proline (r = 0.93**), Ca (r = 0.79**) and Cl (r = 0.96**) and negative correlation with total chlorophyll (r = -0.92**), chlorophyll a (r = -0.88**) and chlorophyll b (r = -0.92**). Proline had significantly positive correlation with Cl (r = 0.92**) and Ca (r = 0.70*) and negative correlation with total chlorophyll (r = -0.94**), chlorophyll a (r = -0.89**) and chlorophyll b (r = -0.94**). Total chlorophyll had significantly positive correlation with chlorophyll a (r = 0.97**) and chlorophyll b (r = 0.98**) and negative correlation with K (r = -0.81**), Ca (r = -0.80**) and Cl (r = -0.93**). Chlorophyll a had significantly positive correlation with chlorophyll b (r = 0.91**) and negative correlation with Na (r = -0.69*), K (r = -0.82**) and Cl (r = -0.88**). Na had significantly positive correlation with K (r = 0.84**), Mg (r = 0.90**) and Ca (r = 0.76*). K had significantly positive correlation with Mg (r = 0.67*), Ca (r = 0.86**) and Cl (r = 0.76*). Ca had also significant correlation with Cl (r = 0.83*).

Table 7. The correlation coefficient of the measured traits.

	Soluble sugars	Proline	Total chlorophyll	Chlorophyll a	Chlorophyll b	Na	K	Mg	Ca	Cl
Soluble sugars	1									
Proline	0.93**	1								
Total chlorophyll	-0.92**	-0.94**	1							
Chlorophyll a	-0.88**	-0.89**	0.97**	1						
Chlorophyll b	-0.92**	-0.94**	0.98**	0.91**	1					
Na	0.43ns	0.21ns	-0.62ns	-0.69*	-0.54ns	1				
K	0.70*	0.75*	-0.81**	-0.82**	-0.75*	0.84**	1			
Mg	0.06ns	0.10ns	-0.29ns	-0.37ns	-0.21ns	0.90**	0.67*	1		
Ca	0.79**	0.70*	-0.80**	-0.78*	-0.79*	0.76*	0.86**	0.54ns	1	
Cl	0.96**	0.92**	-0.93**	-0.88**	-0.93**	0.48ns	0.76*	0.16ns	0.83**	1

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Discussion

As it is represented in Tables 3 and 4, salinity stress resulted in the reduction of morphologic traits and flowering shoot yield of the plant which is in agreement with the findings of Neumann *et al*. (1979) who reported that plant dry and fresh weight and root dry weight are affected by salinity stress. Suriyan *et al*. (2003) conducted experiments on sorghum and reported that salinity reduced LAR, germination percentage, LAI, dry weight and fresh weight. Ryuichi *et al*. (2006) studied the effect of salinity on the photosynthesis and morphology of *nicotiana tabacum* using micro morphometric method and found that the effect of salinity on plant stem diameter was observed within few hours after the stress was applied. Navarro *et al*. (2006) found that salinity stress reduced dry matter, leaf area, plant height, photosynthesis and intercellular spaces. Dashtakian (2000) observed that salinity stress reduced root length, shoot length, leaf area, water consumption, root weight, stem weight, leaf weight and relative growth rate of madder. Afzali (2001) tested the effect of salinity stress on *Melilotus officinalis* and *Trifolium fragiferum* and reported that the stress resulted in the reduction of germination rate, chlorophyll content, the number of leaves, leaf area and root and shoot length and dry weight. Amirian (2002) also studied forage sorghum under salinity stress and reported that the stress reduced plant growth; however, increased root length, root weight and the content of soluble sugars, proline, Na, K, Mg, Ca and Cl.

Comparing the content of the nutrients in soil and plant showed that some elements such as Na, K, Mg and Cl had higher concentrations in soil than the plant tissue; this may be attributed to the reduced absorption rate because of the mechanisms which disturb absorption. However, the content of Cl was higher in plant than the soil which may be because of Cl negative charge. Other elements are absorbed in positively charged forms.

In addition, essential oil percentage soluble sugars and proline contents increased under salinity stress (Table 4). Plants have different mechanisms to cope with the salinity stress (Volkmar and Steppuhn, 1998). Enhancement of essential oil, soluble sugars and proline content under salinity stress are plant mechanisms that help plants to tolerate saline condition better by the enhancement of regulating osmolytes. Chlorophyll content in plants reduced under salinity conditions (Table 4).

Significantly positive correlation between plant height and the number of tillers may be attributed to increased plant density when each plant has higher number of tillers. In this situation, plants will compete in vertical growth to reach higher sunlight. Moreover, evaporation reduces in that condition and auxin content increases in plant shoot. Negative correlation between flowering shoot weight and root may be because root is an organ which consumes photosynthetic products; so, when weight and length of root increase, higher photosynthetic products are needed to support its growth and maintenance.

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