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Research Paper

Path and stepwise analysis of morphological and physiological traits of Artemisia sieberi

Sara Baghery[1], Bohloul Abbaszadeh[2*]
1 Department of Horticulture Science, Karaj Branch, Islamic Azad University, Karaj, Iran
2*Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran
E-mail: babaszadeh@rifr-ac.ir

Abstract: In order to investigate causal relationship between morphological traits, physiological traits and some elements of medicinal – pasture herb of *Artemisia sieberi* in the natural habitat, this project was conducted. *Artemisia sieberi* in the natural habitat and full bloom stage was sampled with the nine plots. Some morphological traits such as plant height, tiller number, small and big canopy diameter, total shoot and flowering shoot yield of 20 to 40 plants were measured. Plants chlorophyll, soluble sugars, proline, sodium, potassium, magnesium, calcium, Clare and iron were measured in each plot. Traits affecting shoot yield and oil percent were evaluated by simple correlation, factor analysis, path and stepwise analysis. Simple correlation results showed that flowering shoot yield had positive correlation with shoot yield (r=0.90**), total dry matter weight (r=0.89**), total chlorophyll (r=0.67*), chlorophyll a (r=0.69*) and potassium (r=0.89**), also negative correlation with soluble sugars (r=-0.68*) and proline (r=-0.81*). There was positive correlation between oil percent and sodium (r=0.74*). Principal component results indicated that four of first indicators were justified 52.24, 29.93, 8.57 and 4.08%, respectively and totally showed more than 94% of total variance changes. The first indicator was justified in total more than 52% of changes with the components of plant height, tiller number, canopy diameter, flowering shoot yield and total shoot yield. Results of stepwise analysis showed that sodium, calcium, magnesium and soluble sugars were entered into table, respectively and had 0.9735 of explanation coefficients. The trait was justified more than 97% of essential oil changes. Essential oil and traits of sodium, calcium, magnesium and soluble sugars were considered as the resultant and causal variables, respectively. Sodium trait had direct and positive effect on oil percent (4.483). This trait had indirect effect on oil percent through calcium (-1.653), magnesium (-1.928) and soluble sugars (-0.157) traits.

Keywords: *Artemisia sieberi*, elements, correlation, essential oil, proline.

Introduction

Nowadays, study and evaluation of landraces and wild species as genetic resources in plant breeding have particular importance. Among these medicinal and pasture plants are taken in consideration because of their role in raw supply of medicinal herbs, animal fodder, protection of soil and water resources, botany aspects, ecology, best cultivation and breeding. Correlation studies, using of factor analysis, stepwise regression and path analysis as multivariate statistical techniques make it possible to investigate the relationship between yield and morphological and physiological traits. Several studies have been performed on the correlation between traits and path analysis of different plants (Zhao *et al*., 1991; Dofing and Knight, 1992; Tabaie-Aghdaie and Babaie, 2003; Chen, 2004; Farhangeian-Kashani *et al*., 2005). Although the relationship between main traits is important but correlation coefficient calculation does not indicate the nature of the relationship between traits. And using path analysis allows identifying direct and indirect effect of traits on the organs. Therefore, plant breeding specialists consider path analysis as a tool for determination the important of effective traits on used organs.

Using of correlation between morphological and biochemical traits is one of the important tools that has been used in some of *Mentha piperita* and *Mentha pulegium* species (Kukreja *et al*., 1992). According to Mirzei-Nodoshan and his colleagues on *Mentha longifolia* var. *amphilema* L., amount of leaf oil had significant positive correlation with stem length and stem diameter. Leaf oil percent had the highest direct effect on flower yield. Also, leaf length had high direct effect on flower oil percent. Leaf length and stem diameter are appropriate indicators for the best selection of species and local colognes of *Mentha longifolia* (L.) Huds. Var. *amphilema* L. (Mirzei-Nodoshan *et al*., 2001). According to Mirzaie-Nadushan and his colleagues reported on the path analysis of effective traits on increasing essential oil of three *Thymus vulgaris* varieties, stomata number and leaf length had the highest direct effect on oil amount (Mirzaie-Nadushan *et al*., 2006). Abbaszadeh and his colleagues reported that total oil yield had significant positive correlation with flower yield, flower oil percent, flower oil yield, leaf yield, leaf oil percent and leaf oil yield in *Mentha longifolia* (L.) Huds. Var. *amphilema* L. Flowering shoot yield showed significant positive correlation with plant height and sub stem number ($\alpha \leq 0.05$), and with flower yield, flower oil percent, flower oil yield, leaf yield, leaf oil percent, leaf oil yield and total oil yield ($\alpha \leq 0.01$). According to their results, leaf oil had the highest positive and direct effect on total oil yield. And after that trait, flower oil percent had the highest direct effect on total oil production (Abbaszadeh *et al*., 2011).

Thirty-four species of *Artemisia* genus have been reported in Iran that *Artemisia sieberi* Besser scattered in desert areas. Two species of it, *A. kermanensis* and *A. melaolepis*, is just for Iran (Mozaffarian, 1998). *Artemisia sieberi* is known as the main plant of plain and steppe rangelands that its geographical distribution is northwest, west, central part, south, southeast and northeast (Ghahraman, 1987). Its intestinal worms infection, malaria, fungal infections cure and anti-parasite effect has been observed (Giao *et al*., 2001; Negahbana, 2006). Several important combinations have been identified such as Bisabolene derivatives and Salsolene keton. Its antioxidant properties have been confirmed in many species (Ponser *et al*., 1995; Meshnick *et al*., 1995; Cazelles *et al*., 2002; Asgari *et al*., 2005).

In this project, study and identify morphological and physiological patterns effective in flowering shoot yield and its oil percent were performed by simple correlation and stepwise regression methods. Also examine the relationship between traits has been done by path analysis.

Material and methods

In this study, *Artemisia sieberi* were sampled in the natural habitat of Qom, Iran (latitude 53', 50^{O} and longitude 38', 34^{O}) located 140km southwest of Tehran with 163.2mm annual rainfall, average temperature of 17°C and relative humidity of 46% in full bloom stage and using plot. Nine plots (each plot area was $10m^2$) from the habitat with intervals of 100 - 500m from each other were selected and 20 – 40plants from the each plot were harvested. For selection of plot location, the first area (it had minimal vegetation cover because of high solute) was selected and the plots were located at intervals of 100m with its repetitions. The plots of second and third area were located in direction perpendicular to the first with vegetation cover changing.

Morphological trait was measured such as plant height, tiller number, big and small canopy diameter. Flowering shoot and other shoots were separately harvested and transferred to the laboratory after encoding. Eight plants with root of each plot were harvested for measuring root length and weight. Roots length and dry weight were measured after drying in the laboratory. Samples for chlorophyll measuring were separated from harvested plant and immediately transferred into ice in habitat. The other shoots of harvested bush were transferred to the laboratory and their dry weights were determined after drying. The plant elements (sodium, potassium, magnesium, calcium and iron) were measured by induced couple plasma. Plant Clare was measured with titration method and solution of silver nitrate in soil science laboratory. Proline and soluble sugars were measured with Irrigoyen method (Irrigoyen *et al*., 1992). Chlorophyll amount was measured with the following formula:

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

Chl a, Chl b and Chl a + b were containing chlorophyll a, b and a + b(mg/l) of fresh weight, respectively. a in formula belongs to extract absorption in related wavelengths. For measuring other traits, these methods were used: soil calcium and magnesium (titration method), total nitrogen of soil (Kjeldahl method), soil sulphate (Turbidometry equipment), soil organic carbon (Walcky-Black method), soil and plant Clare (titration with silver nitrate solution), soil total lime (Volumetric method), soil texture (Hydrometer method), soil sodium and potassium (Flame photometer method) and soil bicarbonate (Titrometer with Methyl orange) (Ghazan-Sahi, 1997). Oil was obtained from dried flowering shoot by using water distillation method in 3 hours. Obtained data were analysis with SAS and Path statistical program.

Results

Simple correlation results showed (Table 1) that plant height had significant positive correlation with tiller number (r=0.93**), canopy diameter 1 (r=0.91**), canopy diameter 2 (r=0.96**), flowering shoot yield (r=0.97**), aerial organs weight (r=0.80**), total dry matter weight (r=0.78*) and potassium (r=0.86**), and significant negative correlation with proline (r=-0.71*). There was significant positive correlation between root length and tiller number (r=0.69*), canopy diameter 1 (r=0.84**), canopy diameter 2 (r=0.72*) and magnesium (r=0.77*). Tiller number showed significant positive correlation with canopy diameter 1 (r=0.94**), canopy diameter 2 (r=0.93**), flowering shoot yield (r=0.85**) and potassium (r=0.71**). Canopy diameter 1 had significant correlation with canopy diameter 2 (r=0.96**), flowering shoot yield (r=0.82**) and potassium (r=0.82**). There was significant positive correlation between canopy diameter 2 and flowering shoot yield (r=0.90**), aerial organs weight (r=0.69*), total dry matter weight (r=0.68*) and potassium (r=0.86**). Flowering shoot yield had significant positive correlation with aerial organs weight (r=0.90**), total dry matter weight (r=0.89**), total chlorophyll (r=0.67*), chlorophyll a (r=0.69*) and potassium (r=0.89**), and significant negative correlation with soluble sugars (r=-0.68*) and proline (r=-0.81**). There was significant positive correlation between aerial organs weight and total dry matter weight (r=0.99**), total chlorophyll (r=0.77*), chlorophyll a (r=0.82**) and potassium (r=0.79**). Aerial organs weight had significant negative correlation with soluble sugars (r=-0.75*), proline (r=-0.85**) and Clare (r=-0.81**). Root weight showed significant positive correlation with total dry matter weight (r=0.68*) and potassium (r=0.74*). Total dry matter weight had significant positive correlation with total chlorophyll (r=0.72*), chlorophyll a (r=0.79*) and potassium (r=0.82**), and significant negative correlation with soluble sugars (r=-0.70*), proline (r=-0.85**) and Clare (r=-0.75**).there was significant positive correlation between oil percent and sodium (r=0.74*). Soluble sugars showed significant positive correlation with proline (r=0.89**) and Clare (r=0.92**), and significant negative correlation with total chlorophyll (r=-0.92**), chlorophyll a (r=-0.95**) and chlorophyll b (r=-0.82**). Proline had significant positive correlation with Clare (r=0.92**), significant negative correlation with total chlorophyll (r=-0.91**), chlorophyll a (r=-0.96**) and chlorophyll b (r=-0.79*). Total chlorophyll showed significant positive correlation with chlorophyll a (r=0.95**) and chlorophyll b (r=0.95**), and significant negative correlation with sodium (r=-0.71*) and Clare (r=-0.97**). There was significant positive correlation between chlorophyll a and chlorophyll b (r=0.83**). Chlorophyll a had significant negative correlation with sodium (r=-0.67*) and Clare (r=-0.96**). Chlorophyll b showed significant negative correlation with sodium (r=-0.68*), calcium (r=-0.67*) and Clare (r=-0.89**). There was significant positive correlation between sodium and magnesium (r=0.91**), calcium (r=0.80**) and Clare (r=0.79*). Magnesium had significant positive correlation with calcium (r=0.65*). Calcium showed significant positive correlation with Clare (r=0.72*).

Principle components analysis

Results indicated (Table 2) that the four first indicators were stated 52.24, 29.93, 8.57 and 4.08%, respectively and in total more than 94% of total variances changes. The first indicator was justified more than 52% of changes with aerial organs yield (plant height, tiller number, canopy diameter 2, flowering shoot yield and total shoot yield). The second indicator was stated more than 29% of changes with effective traits in water absorption from the soil (root length, sodium and magnesium absorption amount). The third indicator was explained more than 8% of changes with effective traits in development of plant terrestrial organs (root weight and absorbed calcium). The fourth indicator was stated more than 4% of changes with effective traits in photosynthesis (total chlorophyll, chlorophyll a, magnesium, sodium and calcium).

Stepwise analysis

Stepwise regression results indicated (Table 3) that sodium, calcium, magnesium and soluble sugars was entered into the table, respectively. Obtained equation was as follow:

$$y= 2.1649 + 0.21489x - 0.18087x_2 - 0.9902x_3 - 0.26972x_4$$

In this equation, y, x_1, x_2, x_3 and x_4 shows oil percent, sodium, calcium, magnesium and soluble sugars amount, respectively. The model has 0.9735 explanation coefficient. It means that these traits were justified more than 97% of oil percent changes. Sodium amount in the plant body that entered into the model in the first step showed the highest correlation with oil percent (r=0.74655) (Table 1). This trait had 0.5573 of single coefficient (Table 3). Calcium, the second trait that entered into the model, showed 0.1692 of single coefficient and 0.7265 of total coefficient. Plant body magnesium as the third trait had 0.2127 of single explanation coefficient and 0.9392 of total coefficient. And soluble sugars as the fourth trait was entered into the model that had the highest role in oil percent with 0.0343 of single explanation coefficient and 0.9735 of total explanation coefficient.

Path analysis

The direct and indirect effect of each trait that was entered into the stepwise analysis was calculated based on their correlation coefficients. Oil percent and sodium, calcium, magnesium and soluble sugars were considered as the resultant and causal variables, respectively. Sodium trait had positive and direct effect (4.483) on oil percent (Table 4). This trait indirectly affected oil percent through calcium (-1.653), magnesium (-1.928) and soluble sugars (-.0157) traits. Plant body calcium was the second trait that had negative direct effect (-2.065) on oil percent. This trait indirectly affected oil percent through sodium, magnesium and soluble sugars with 3.586, -1.374 and -0.149, respectively. Magnesium with -2.1 of explanation coefficient had negative direct effect on oil percent and indirectly affected it through sodium (4.115), calcium (-1.351) and soluble sugars (-0.097). Soluble sugars was the forth trait that entered into the stepwise regression model and had negative direct effect (-0.276) on oil percent. The trait indirectly affected oil percent through sodium (2.551), calcium (-1.112) and magnesium (-0.738).

Discussion

Traits correlation results indicated that aerial organs had significant negative correlation with soluble sugars and proline (one of adapted solution to adverse condition). But positive correlation between root length and some of related traits to aerial organs showed that in case of root development, there was possibility of increasing water absorption and therefore aerial organs and especially tiller numbers development. Potassium absorption and increasing in plant body as one of the important elements had positive correlation with aerial organs development. Negative correlation of chlorophylls with Clare and proline indicated that chlorophylls amount are reduced in adverse condition (climatic and soil condition) and proline amount is added in this condition. Positive correlation between oil percent and sodium showed that there was possibility of oil increasing in salinity condition. So it seems that good models could be obtained by examining the relationship between absorbed elements and oil producing in habitat, field and controlled conditions. Positive relation between elements stated that in the presence of different salt types in the soil, their entering into plant is partly possible. Entering some of them enhances the others entering. Results of principle component analysis indicated that aerial organs yield was in the first priority and effective traits in water absorption were in the next place. Furthermore, the role of elements such as sodium, calcium and magnesium were more effective in increasing oil percent than the others. Sodium as one of main elements in the natural habitat and saline areas could play a role in increasing oil percent. Lack of prevent the plant water absorption and creating moisture stress are some of its important roles. And after entering, interfere in plant normal growth system and make change in photosynthesis and respiration pathways. It was observed that calcium and magnesium acted as two neutralizing elements of sodium effect and were entered into stepwise regression model with negative role and symptom. It seems that under saline conditions, some elements such as calcium and magnesium are moderated imposed stress to the plant and will reduce its oil percent. The results matched by other results on *Mentha longifolia* var. *amphilema* L. (Mirzei-Nodoshan *et al*., 2001) and path analysis of effective traits on increasing oil of three *Thymus vulgaris* species (Mirzaie-Nadushan *et al*., 2006). Also Abbaszadeh and his colleagues reported that total oil yield had significant positive correlation with flower yield, flower oil percent, flower oil yield, leaf yield, leaf oil percent and leaf oil yield ($\alpha \leq 0.01$) in *Mentha longifolia* (L.) Huds. Var. *amphilema* L. And it was consistent with our results.

Table 1. simple correlation between morphological and physiological traits of *Artemisia sieberi* of Qom, Iran

traits	Plant height	Root length	Tiller number	Canopy diameter 1	Canopy diameter 2	Stem diameter	Flowering shoot yield	Aerial organs weight	Root weight	Total weight	Oil percent	Soluble sugars	proline	Total chlorophyll	Chlorophyll a	Chlorophyll b	Sodium	Potassium	Magnesium	Calcium	Clare
Plant height	1																				
Root length	0.57ns	1																			
Tiller number	0.93**	0.69*	1																		
Canopy diameter 1	0.91**	0.84**	0.94**	1																	
Canopy diameter 2	0.96**	0.72*	0.93**	0.96**	1																
Stem diameter	0.48ns	0.64ns	0.62ns	0.63ns	0.60ns	1															
Flowering shoot yield	0.97**	0.44ns	0.85**	0.82**	0.90**	0.36ns	1														
Aerial organs weight	0.80**	0.10ns	0.57ns	0.53ns	0.69*	0.07ns	0.90**	1													
Root weight	0.39ns	0.22ns	0.12ns	0.35ns	0.41ns	-0.09ns	0.50ns	0.59ns	1												
Total weight	0.78*	0.12ns	0.53ns	0.53ns	o.68*	0.05ns	0.89**	0.99**	0.68*	1											
Oil percent	-0.12ns	0.38ns	0.04ns	0.15ns	0.01ns	0.55ns	-0.26ns	-0.47ns	-0.29ns	-0.47ns	1										
Soluble sugars	-0.61ns	0.25ns	-0.49ns	-0.27ns	-0.42ns	0.03ns	-0.68*	-0.75*	-0.08ns	-0.70*	0.42ns	1									
proline	-0.71*	0.11ns	-0.54ns	-0.39ns	-0.53ns	0.02ns	-0.81**	-0.88**	-0.35ns	-0.85**	0.60ns	0.89**	1								
Total chlorophyll	0.58ns	-0.23ns	0.45ns	0.23ns	0.36ns	-0.13ns	0.67*	0.77*	0.11ns	0.72*	-0.60ns	-0.92**	-0.91**	1							
Chlorophyll a	0.58ns	-0.31ns	0.39ns	0.21ns	0.37ns	0.17ns	0.69*	0.82**	0.25ns	0.79*	-0.63ns	-0.95**	-0.96**	0.95**	1						
Chlorophyll b	0.53ns	-0.14ns	0.47ns	0.23ns	0.32ns	0.08ns	0.60ns	0.65ns	-0.01ns	0.59ns	-0.53ns	-0.82**	-0.79*	0.95**	0.83**	1					
Sodium	-0.02ns	0.61ns	0.11ns	0.34ns	0.15ns	0.36ns	-0.18ns	-0.46ns	0.06ns	-0.41ns	0.74*	0.56ns	0.58ns	-0.71*	-0.67*	-0.68*	1				
Potassium	0.86**	0.56ns	0.71*	0.82**	0.86**	0.36ns	0.89**	0.79**	0.74*	0.82**	-0.05ns	0.42ns	-0.57ns	0.40ns	0.43ns	0.33ns	0.11ns	1			
Magnesium	0.30ns	0.77*	0.42ns	0.63ns	0.48ns	0.49ns	0.16ns	-0.16ns	0.27ns	-0.11ns	0.56ns	0.35ns	0.30ns	0.48ns	0.44ns	-0.47ns	0.91**	0.42ns	1		
Calcium	-0.31ns	0.19ns	-0.22ns	-0.02ns	-0.20ns	0.13ns	0.39ns	-0.55ns	0.09ns	-0.49ns	0.35ns	0.58ns	0.50ns	-0.63ns	-0.53ns	-0.67*	0.80**	-0.17ns	0.65*	1	
Clare	-0.55ns	0.29ns	-0.40ns	-0.17ns	-0.34ns	0.11ns	-0.67*	-0.81**	-0.12ns	-0.75*	0.63ns	0.92**	0.92**	-0.97**	-0.96**	-0.89**	0.79*	-0.36ns	0.57ns	0.72*	1

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

Table 2. Eigenvectors coefficient, eigenvalues and variance percent related to each of the studied traits in principle component analysis of collected plants from Qom, Iran

	The first indicator	The second indicator	The third indicator	The forth indicator
Plant height	0.26	0.19	-0.05	0.05
Root length	0.04	0.36	-0.07	-0.12
Tiller number	0.21	0.23	-0.24	0.15
Canopy diameter 1	0.17	0.31	-0.06	0.06
Canopy diameter 2	0.21	0.26	-0.04	-0.05
Stem diameter	0.04	0.27	-0.39	-0.29
Flowering shoot yield	0.28	0.13	0.02	-0.01
Aerial organs weight	0.28	0.01	0.16	-0.17
Root weight	0.12	0.11	0.62	-0.22
Total weight	0.27	0.01	0.23	-0.18
Oil percent	-0.14	0.23	-0.26	-0.02
Soluble sugars	-0.25	0.11	0.11	-0.31
proline	-0.28	0.08	-0.04	0.15
Total chlorophyll	0.26	-0.15	-0.08	0.19
Chlorophyll a	0.26	-0.15	0.03	0.23
Chlorophyll b	0.24	0.14	-0.20	0.14
Sodium	-0.14	0.32	0.07	0.27
Potassium	0.22	0.21	0.21	-0.12
Magnesium	-0.04	0.37	0.10	0.25
Calcium	-0.17	0.16	0.29	0.59
Clare	-0.26	0.17	0.08	-0.04
Eigenvalues	10.9	6.29	1.79	0.85
Relative variance	0.52	0.299	0.085	0.9074
Cumulative variance	0.52	0.82	0.408	0.9482

Table 3. Stepwise regression of studied traits

	Regression stages	Step by step	stepwise	
Added trait to the model	1	2	3	4
Constant number	0.29755	1.55393	2.08306	2.16469
Sodium	0.05224	0.09064	0.18547	0.21489
Calcium	-	-0.07032	-0.09861	-0.19902
Magnesium	-	-	-0.14644	-0.18087
Soluble sugars	-	-	-	-0.26972
Coefficient of entered indicators into the formula	0.5573	0.1692	0.2127	0.0343
Total explanation coefficient (R^2)	0.5573	0.7625	0.9392	0.9735

Table 4. Path analysis of direct and indirect effect of the entered traits in stepwise analysis

	Sodium	Calcium	Magnesium	Soluble sugars
Sodium	4.483	3.586	4.115	2.551
Calcium	-1.653	-2.065	-1.351	-1.112
Magnesium	-1.928	-1.374	-2.1	-0.738
Soluble sugars	-0.157	-0.149	-0.097	-0.276
Total effect	0.746	0.001	0.568	0.427
	Remaining=	1.017		

Underling numbers indicate direct effect on the traits.

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