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Assessment the Effective Phytochemical and Growth Traits of Wild Collected Artemisia (*Artemisia sieberi* Besser subsp), Using Multivariate Statistical Methods

Mohammad Reza Ardakani[1], Bohloul Abbaszadeh[2] and Seyed Alireza Valadabadi[3]

[1]Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran
[2]Research Institute of Forests and Rangelands, Tehran, Iran
[3]Department of Agronomy, Shahr-e-Ghods Branch, Islamic Azad University, Shahr-e-Ghods, Iran

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ABSTRACT

*In this experiment, different multivariate statistical analysis methods were used to determine the effective traits on artemisia yield and essential oils content. After collecting artemisia from Ghom province, Iran, the morphology of plant was studied on 20-40 plants in each plot. Proline, chlorophyll, soluble sugars, sodium, potassium, calcium and magnesium contents were also measured. Results of Pearson correlation indicated that flowering shoot yield had positive significant correlation with total shoot yield (r=0.0.90**), total dry weight (r=0.89**), total chlorophyll (r=0.67*), chlorophyll a (r=0.69*) and potassium (0.89**), and negative significant correlation with soluble sugars (r=-0.68*) and proline (r=-0.81**). Essential oil percentage was significantly correlated to Na content (r=0.74*). Results of principal component analysis showed that the four first factors contributed to 52.24%, 29.93%, 8.75% and 4.08% of the variations. Stepwise analysis also indicated that Na, Ca, Mg and soluble sugars entered to the model respectively. In path analysis, essential oil yield was considered as the dependent variable. Na content had direct positive effect (4.483) on the essential oil percentage. This trait indirectly affected essential oil percentage through Ca (-1.653), Mg (-1.928) and soluble sugars (-0.157).*

Keywords: medicinal plant, path analysis, principal component analysis, stepwise analysis.

INTRODUCTION

Evaluating indigenous populations and wild species, as the genetic resources, is highly important. Among these plants, medicinal and rangeland species are under attention because they provide raw material for medications and forage for livestock, they are effective in soil conservation, they are used in plant breeding, etc.

Correlation, factor analysis, stepwise regression analysis and path analysis are the multivariate statistical analysis methods, making it possible to study the relation of different traits such as the yield, yield components, morphological and physiological traits. Many experiments have studied the relation of traits in different plants by the use of correlation and path analysis [22, 23, 26].

One of the mechanisms by which salinity affects plants is the reduction of photosynthesis which consequently reduces plant chlorophyll content and CO_2 absorption [9]. One of the plant strategies to cope with the saline conditions is to accumulate adaptation substances such as soluble sugars (sucrose, glucose, fructose, trehalose and raffinose), insoluble sugars (starch, amylose and amylopectin) and proline [16]. It has been reported that under stress conditions, ferulic acid probably changes to diferulic acids in hemicellulose, or hydroxyproline-rich glycoproteins

changes to insoluble forms and makes the cell wall tougher [21] and that is why the delayed growth caused by reduced cell elongation is considered as an indication of stressed plants [20].

The dual correlation of morphological and biochemical traits has been a method used in different *Mentha* species [2]. Mirzaei Nodoushan et al. [10] studied two clones of *Mentha longifolia* (var. amphilema) and reported that leaf essential oil percentage was significantly correlated to leaf width and length. Leaf essential oil percentage had the highest direct effect on the flower yield. Mirzaei Nodoushan et al. [11] conducted path analysis on the effective traits on essential oil enhancement in three *Thymus* species and concluded that the number of stomata and leaf length had the highest direct effect on the essential oil yield. Abbaszadeh et al. [4] reported that in *Mentha longifolia* L. var. amphilema, the total essential oil yield was significantly correlated to flower essential oil percentage and yield, leaf yield, leaf essential oil yield and percentage. Path analysis in their experiment indicated that leaf essential oil yield followed by flower essential oil percentage had the highest direct effect on the total essential oil yield.

Artemisia is reported to have 34 species in Iran; *Artemisia sieberi* is mainly distributed in arid regions [24-25]. Artemisia is effective in curing different diseases [19]. The objective of this experiment was to evaluate the salinity tolerance in artemisia of Ghom province, Iran, in order to introduce it as a salinity tolerant medicinal species.

MATERIALS AND METHODS

For this experiment, artemisia was collected from Ghom province, Iran, by plotting, at full flowering stage. In the habitat, nine plots were set, each 10 m^2 with intervals of 100-500 m, and 20-40 samples were taken from each plot. To determine the place of plots, the first row of plots including three plots (the three replications) was located right beside a lake which was more saline and had lower vegetative growth. Then, the second and the third rows of plots, each including another three replications, were located farther from the lake.

At the full flowering stage, the morphology of plant (e.g., plant height, the number of tillers and the small and large diameter of canopy) was studied first and then, flowering shoots and other shoots were separately harvested and transferred to laboratory for further measurements. Eight plants along with their roots were harvested from each plot, dried in laboratory, and root length and dry weight were measured. Samples were also harvested for chlorophyll content measurement; these samples were stored in ice soon after harvest was conducted. The remaining harvested shoots were dried in laboratory, weighted and their mineral nutrients contents (Na, K, Mg and Ca) were determined by Inductively Coupled Plasma (ICP). Chlorine content was measured by the method of Silver nitrate titration [6]. Proline and soluble sugars contents were measure by the methods of Irrigoyen et al. [13] and Bates [15], respectively. Plant pigments were evaluated by the following equations:

Chlorophyll a (mg/l) = (12.25 × $a^{\dagger}663$)-(2.79 × a647)
Chlorophyll b (mg/l) = (21.5 × a647)-(5.1 × a663)
Chlorophyll a + b (mg/l) = (7.15 × a663)+(18.71 × a647)

In these equations, $a^{\dagger}$ is amount of absorption by the extracts in different wavelengths.

Finally, data were analyzed using SAS and Path software.

RESULTS

Pearson correlation. The results of Pearson correlation (Table 1) indicated that plant height was negatively correlated to proline content (r=-0.71*). Root length was significantly correlated to the number of tillers (r=0.69*), canopy 1 (r=0.84**), canopy 2 (r=0.72*) and Mg (r=0.77*). Shoot yield was positively correlated to dry weight (r=0.99**), total chlorophyll content (r=0.77*), chlorophyll a (r=0.082**) and K (r=0.79**), and was negatively correlated to soluble sugars (r=-0.75*), proline (r=-0.85**) and chlorine (r=-0.81**). Root yield was significantly correlated to the total dry weight (r=0.68*) and K (r=0.74*). Soluble sugars content was significantly correlated to proline (r=0.89**) and chlorine (r=0.92**). Proline was significantly correlated to chlorine (r=0.92**). The total chlorophyll content was negatively correlated to Na (r=-0.71*) and chlorine (r=-0.97**). Na was significantly correlated to Mg (r=0.91**), Ca (r=0.80**) and chlorine (r=0.79*). Finally, results indicated that Mg was significantly correlated to Ca (r=0.65*), and Ca was significantly correlated to chlorine (r=0.72*).

Table 1. Results of Pearson correlation for the measured traits

	Plant height	Root length	Number of tillers	Canopy 1	Canopy 2	Stem diameter	Flowering shoot yield	Shoot weight	Root weight	Total weight	EO %	Soluble sugars	Proline	Total Chl	Chl a	Chl b	Na	K	Mg	Ca	Cl
Plant height	1																				
Root length	0.75ns	1																			
Number of tillers	0.93**	0.69*	1																		
Canopy 1	0.91**	0.84**	0.94**	1																	
Canopy 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														
Shoot 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	0.68*	0.05ns	0.89**	0.99**	0.68*	1											
EO %	-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 Chl	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							
Chl 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						
Chl 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					
Na	-0.02ns	0.61ns	0.11ns	0.3.4ns	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				
K	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			
Mg	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		
Ca	-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	
Cl	-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, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.*
Chl, chlorophyll; EO, Essential Oil;

Table 2. The steps of stepwise regression analysis for the measured traits

Entered traits to the model	Steps of stepwise regression analysis			
	Step 1	Step 2	Step 3	Step 4
The fixed number	0.29755	1.55393	2.08306	2.16469
Na	0.05224	0.09064	0.18547	0.21489
Ca	-	-0.07032	-0.09861	-0.19902
Mg	-	-	-0.14644	-0.18087
Soluble sugars	-	-	-	-0.26972
Partial R-square	0.5573	0.1692	0.2127	0.0343
Model R-square	0.5573	0.7625	0.9392	0.9735

Stepwise analysis. The results of stepwise regression analysis indicated that Na, Ca, Mg and soluble sugars entered the model respectively (Table 2). The achieved model is below:

$Y = 2.1649 + 0.21489x – 0.18087x_2 – 0.09902x_3 – 0.26972x_4$

In this model:
Y, essential oils percentage
X, Na content
X_2, Ca content
X_3, Mg content
X_4, soluble sugars content

The explanation coefficient in this model was 0.9735; representing that the entered traits to the model contributed to more than 97% of the variations in essential oils percentage. Plant Na content, which was the first trait entering the model, had the highest positive correlation (r=0.74655) with the essential oils percentage (Table 1). Partial R-Square of this trait was 0.5573 (Table 2).

Path analysis. In path analysis, the direct and indirect effects of each entered trait to the stepwise regression analysis model were evaluated based on the correlation coefficients; essential oil percentage was considered as the effect and Na, Ca, Mg and soluble sugars were considered as the cause variable. Na had direct positive effect (4.483) on the essential oils percentage (Table 3). Na affected essential oils percentage through Ca (-1.653), Mg (-1.928) and soluble sugars (-0.157).

Table 3. Path analysis of the direct and indirect effect of the entered traits to the stepwise regression analysis model

	Na	Ca	Mg	Soluble sugars
Na	4.483	3.586	4.115	2.551
Ca	-1.653	-2.065	-1.351	-1.112
Mg	-1.928	-1.374	-2.1	-0.738
Soluble sugars	-0.157	-0.149	-0.97	-0.276
Total	0.746	0.001	0.568	0.427
Residual effect	-	1.017	-	-

Underlined numbers are the direct effects.

DISCUSSION

Results of this experiment generally indicated that artemisia grows well in saline and arid regions. Under salinity stress condition, plant's proline and soluble sugars content increased while shoot yield decreased. An increased root length of *Suaeda vermiculata* and *Atriplex leucoclada* under salinity stress levels (up to 200 mM induced by NaCl, $CaCl_2$, and NaCl + $CaCl_2$) was reported by Abbaszadeh [3] and Zandi Esfahan [7]. They reported that root length reduced in higher salinity stress levels.

Results of this experiment showed that root length increased in the presence of Mg. This indicates that high Na, K and Ca concentration results in the reduction of plant growth, but high Mg concentration increases plant growth. This consequently shows that artemisia, by increasing the root length, tries to absorb water from deeper soil layers which contains lower salts.

Another plant strategy to cope with the saline conditions is the accumulation of osmolites such as proline and soluble sugars. Several studies proved the accumulation of proline and soluble sugars under salinity stress conditions in balm (*Melissa officinalis* L.; [17]), *Dracocephalum moldavica* [8], *Salvia officinalis* [12], some genotypes of *Cymbopogon martini* [1] and *Petriwinkle medicina* [27]. In some other researches, the reduced shoot yield under stress condition was attributed to the reduction of turgor pressure in cells especially in stem tissues [5].

Results of the experiment indicated that in high Mg, Ca, Cl, soluble sugars and proline concentration, chlorophyll a, b and the total chlorophyll content decreased; representing that salinity stress reduces plant's photosynthetic capacity. Similar results were achieved in thyme (*Thymus vulgaris* L.; [14]) and peppermint (*Mentha piperita* L. [5]).

In this experiment, path analysis was conducted to evaluate the direct and indirect effects of the entered traits to the model of regression analysis on the essential oils percentage. The relation between different traits is important in the success of breeding programs and the selection of superior populations. Moreover, selection for agronomic traits without considering the importance of other traits may results in an unfavorable selection. So it is required to study

the correlation of the traits, and the direct and indirect effects of traits in breeding programs and populations selection. Path analysis indicated that essential oils percentage was affected by the salts of the growth environment such as Na, Ca, Mg, and also by the soluble sugars. Among these four factors, Na is of a higher importance because it is the dominant salt of saline and dry areas of Iran. Different studies have reported the effect of salinity and drought stress on the enhancement of plant proline and soluble sugars content, enhancement of plant root weight and length, and reduction of shoot yield [9, 16, 18]. These results were also achieved in our experiment.

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