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Multivariate and univariate analysis of genetic variation in Iranian summer savory (*Satureja hortensis* L.) accessions based on morphological traits

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Abstract

In order to evaluate the genetic variation in Iranian summer savory accessions, different accessions were analyzed using multivariate and univariate analysis. Results indicated that there were significant differences in some traits. The mean comparison analysis using least significant difference (LSD) test revealed significant differences among the accessions understudy. In this regard, the highest significant means for traits were related to Mashhad, Tabriz 1, Tabriz 2, and Kermanshah accessions. Results of correlation analysis showed high positive correlation between total fresh weight and stem fresh weight and negative correlations between the number of leaflet per main stem and distance of internodes. This can be effective for investigating the interactions between traits. Based on the results of factor analysis, significant traits in the first factor, including total fresh weight, stem fresh weight, root fresh weight as well as total dry weight, stem dry weight, and root dry weight, were highly correlated with each other and were independent of the other factors. Therefore, the first factor was considered as the weight factor due to its high value of "communality" in the traits related to the weight. According to the cluster analysis, 29 accessions clustered into four groups, with those having similar phenotypes clustering into the same group. The discriminant analysis confirmed the accuracy of the grouping to be 97.2%. Considering the grouping, no relationship was found between the genetic variation and geographical distance of the accessions. However, the accessions in separate groups can be selected for breeding programs. The results of the present study can be considered useful for identifying and managing the germplasm of the Iranian summer savory.

Key words: Correlation analysis, Cluster analysis, Discriminant analysis, Factor analysis.

INTRODUCTION

Iran is very rich in medical and aromatic plants. Most of the medicinal and aromatic plants belong to the family *Lamiaceae* such as *Salvia*, *Satureja*, *Sideritis*, *Thymus*, *Origanum*, *Macromedia*, and these are used as herbal teas (Zargari, 1970). *Satureja* belongs to the tribe *Mentheae* within the subfamily *Nepetoideae* and includes many species in the world (Nixon, 2006). This genus is native to the Mediterranean region but also distributed at different regions in the world with dark green or grey greenish leaves which grow in the arid, sunny, stony and rocky environments (Momtaz and Abdollahi, 2008; Irani *et al.,* 2014). Nine members of this genus (*S. macrosiphonia*, *S. bachtiarica*, *S. rechingeri*, *S. isophylla*, *S. atropatana*, *S. sahendica*, *S. khuzistanica*, *S. intermedia* and *S. edmondi*) are endemic to Iran in Iranian flora (Jamzad, 2009). *S. hortensis* a well-known, annual, herbaceous aromatic plant, is used as a spice and traditional herb in Iran (Hadian *et al.,* 2008; Hadian *et al.,* 2010). Extracts of this plant have been used in traditional medicine for the treatment of several diseases and also have several properties such as antitumor (Ramezani *et al.,* 2016), antifungal (Kim *et al.,* 2019), antioxidant (Kumburovic *et al.,* 2019; Popovici *et al.,* 2019), antibacterial (Sharifi *et al.,* 2017), antigelatinolytic activities (Zeidán-Chuliá *et al.,* 2013), anti-ulcer and anti-inflammatory (Hajhashemi *et al.,* 2002), antispasmodic and anti-diarrhea (Hajhashemi *et al.,* 2000), antimicrobial activity (Popovici *et al.,* 2019), insecticidal activity (Magierowicz *et al.,* 2019), and anti-*Trichomonas vaginalis* activity (Mirzaei *et al.,* 2019). The expansion of the extinction of medicinal plants due to various factors may eliminate plant germplasm and have irreparable consequences. Therefore, appropriate characterization and identification of plant samples is essential for the successful management and conservation of plant material and to ensure their sustainable use (Arif *et al.,* 2010). Knowledge of genetic distance among individuals and populations and analyzing the genetic relationships of the target species in breeding programs would allow germplasm organization and effective genotyping (Abdmishani and Shahnejat-Bushehri, 1992). Multivariate analysis (MuA) help to discover structure and objective summary of the primary features of the data for easier comprehension in genetic variation of different accessions based on target traits. Factor analysis (FA) and principal component analysis (PCA) are used for data reduction in MuA. Correlated variables are grouped together and separated from other variables with low or no correlation. Patterns of correlations are identified and either used as descriptive (PCA) or as indicative of underlying theory (FA). Clustering is a straightforward method to show association data, however, the confidence of the nodes are highly dependent on data quality, and levels of similarity for cluster nodes is dependent on the similarity index used (Zare-Chahouki, 2011).

The main purpose of this study was to investigate the genetic variation of Iranian summer savory accessions and their classification based on morphological traits using multivariate analysis. The obtained analysis can be effective in investigating the effective traits in breeding programs.

MATERIALS AND METHODS

Plant materials

Seeds of twenty-nine Iranian summer savory accessions were collected from different parts of Iran with different geographical characteristics (Table 1). The field experiment was carried out in the form of randomized complete block design (RCBD) with three replications. The seeds were sown on four rows with 20 cm spacing and 20 cm distance between plants. In order to carry out morphological evaluation, 5 plants of central row from each plot were harvested individually at the flowering stage and some morphological traits were characterized among accessions.

Morphological evaluation

The selected accessions were evaluated in terms of 28 morphological traits (Table 2). For this purpose, monitoring the accessions took place at several stages including time of flowering, and also at harvesting time from the target areas. The branch and root weight was measured using an electronic balance with 0.1 g precision. The dimensions for plant height, branch height, root height, leaf length, and leaf width were measured using a ruler and the dimensions for chlorophyll content were measured using a chlorophyll meter.

Statistical analysis

All evaluated traits, descriptive statistics, coefficient of variation (CV), and standard deviation (SD) are listed in Table 2. Geometric mean was used for traits that were recorded as percentage (%) and arithmetic mean was used for other traits. The analysis of variance (ANOVA) was carried out using the GLM procedure in SAS version 9.1 software. The assumptions of ANOVA were tested by insuring that the residuals were random and homogenous. The significance of differences among treatment means was tested using the least significant difference (LSD) test. Correlation of quantitative traits with Pearson correlation coefficients, factor analysis, KMO-Bartlett’s test, cluster analysis using Ward’s method based on squared Euclidean distance (SED), and discriminant analysis (DA) were carried out in SPSS version 16 software. Factor analysis was performed using principal component analysis (PCoA) and Varimax rotation of factors to better understand the internal relationships of traits. Also, the KMO statistic and the Bartlett test confirmed the accuracy of factor analysis. Factor loading values higher than 0.6 were considered significant in main and independent factors.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) and mean comparison

Analysis of variance showed significant differences between accessions for root fresh weight (RFW), total plant height (TPH), stem length (SL), distance of internodes (DI), number of leaflet per main stem (NLL), length of the middle leaf on main stem (LMLM), length of the first leaf on lateral stem (LFLL), and length of

Table 1. Geographical characteristics of Iranian summer savory (*Satureja hortensis* L.) accessions in this study.

Number	Collection site	Latitude (N)	Longitude (E)	Altitude (m)
1	Shush	32˚11' 44"	48˚15'14"	84
2	Tabriz 1	38˚06'14"	46˚16'20"	1361
3	Ardabil	38˚15'24"	48˚17'44"	1339
4	Razan 1	35˚23'29"	49˚02'00"	1854
5	Mashhad	36˚15'36"	59˚36'44"	1022
6	Sistan and Baluchestan	27˚35'06"	60˚35'01"	1530
7	Tonekabon 2	36˚48'58"	50˚52'19"	-18
8	Shiraz	39˚35'34"	52˚34'53"	1519
9	Qom	34˚38'36"	50˚52'22"	965
10	Kermanshah	34˚19'49"	47˚04'38"	1330
11	Sanandaj	35˚19'30"	46˚58'59"	1577
12	Parsabad	39˚37'16"	47˚54'18"	61
13	Sardasht	36˚09'31"	45˚28'31"	1528
14	Tuserkan 2	34˚33'02"	48˚27'08"	1864
15	Hamedan	34˚48'01"	48˚30'48"	1841
16	Tabriz 2	38˚06'14"	46˚16'20"	1361
17	Isfahan	32˚39'28"	51˚39'54"	1601
18	Qazvin	36˚16'24"	49˚59'52"	1307
19	Tuserkan 1	34˚33'02"	48˚27'08"	1864
20	Ilam	33˚38'39"	46˚22'56"	1364
21	Borujerd	34˚01'51"	48˚44'35"	1963
22	Kelachay	37˚05'08"	50˚21'22"	-13
23	Arak	34˚05'46"	49˚42'04"	1737
24	Dezful	32˚23'00"	48˚25'22"	150
25	Razan 2	35˚23'29"	49˚02'00"	1854
26	Tehran	35˚41'51"	51˚23'00"	1187
27	Tonekabon 1	36˚48'58"	50˚52'19"	-18
28	Sarab	37˚56'33"	47˚32'16"	1682
29	Qeshm	26˚48'35"	55˚53'24"	79

the middle leaf lateral main stem (LMLL) with SL, DI, NLL, and LFLL at 0.001, RFW and LMLM at 0.01, and TPH and LMLL at 0.05 probability level (Table 3). The mean comparison analysis of investigated traits using Least Significant Difference (LSD) test indicated that traits with the highest mean are important in terms of diversity and differences among accessions based on morphological traits (Table 4). For example, three traits in Tabriz 1 (LMLM, LFLL, and LMLL) and Tabriz 2 (TPH, SL, and DI), and one trait in Kermanshah (NLL) and Mashhad (RFW) accessions were the most effective traits in revealing differences between these accessions.

Correlation analysis

The results of correlation analysis between studied traits are presented in Table 5. The results showed high positive and negative correlations between traits, *e.g*. TFW had the highest positive correlation with SFW (0.995), RFW (0.819), TDW (0.948) and SDW (0.973) traits. The highest negative correlations were observed between SL with NLL (-0.706), and DI with NLL (-0.814) at 1% probability level. Correlation of traits is used to investigate and establish a logical and meaningful relationship between traits. Creating relationships between several traits can make measurements easy for identifying traits that may be difficult to measure. It is also possible to select traits that have significant correlations when the trait emerges at a particular time or needs identification and accurate measurement. Therefore, in some cases where the measurement of a trait is costly, complex, and time-consuming, other traits that have a significant and high correlation with it can be used for indirect measurement. Thus, if there is a high correlation between two traits, whether positive or negative, by measuring one trait we can find out the status of the other traits (Ayob-Nezhadghan and Hassanpour, 2018).

Factor analysis

One of the statistical methods for analyzing the data set is the factor analysis method. Factor analysis is the expansion of the principal component analysis. Both methods attempt to estimate the covariance matrix,

Table 2. Descriptive statistics of traits in Iranian summer savory (*Satureja hortensis* L.) accessions in the present study.

Nob.	Trait	Abb.	Mu	Min	Max	Mean	SD	Coefficient of variation (%)
Y1	Total fresh weight	TFW	gr	32.18	86.32	55.73	13.09	23.49
Y2	Stem fresh weight	SFW	gr	31.16	88.64	54.13	12.87	23.78
Y3	Root fresh weight	RFW	gr	1.03	3.21	1.88	0.50	26.59
Y4	Total plant height	TPH	cm	53.13	76.15	60.30	4.32	7.15
Y5	Stem length	SL	cm	36.97	61.13	43.89	5.02	11.44
Y6	Root Length	RL	cm	13.50	19.63	16.50	1.45	8.79
Y7	Number of internodes	NI	cou.	13.00	15.00	14.43	0.60	4.16
Y8	Distance of internodes	DI	cm	2.93	5.13	3.49	0.48	13.74
Y9	Chlorophyll	Ch	-	20.67	31.95	25.36	2.59	10.20
Y10	Number of branches per main stem	NB	cou.	18.00	22.00	20.07	0.87	4.32
Y11	Number of leaves on main stem	NL	cou.	12.00	16.00	13.13	0.88	6.70
Y12	Number of leaflet per main stem	NLL	cou.	5.00	33.00	25.61	7.43	29.01
Y13	Total dry weight	TDW	gr	4.83	12.44	8.63	1.93	22.35
Y14	Stem dry weight	SDW	gr	4.54	11.67	8.24	1.82	22.09
Y15	Root dry weight	RDW	gr	0.29	0.73	0.49	0.10	20.41
Y16	Length of the first leaf on main stem	LFLM	cm	4.10	9.16	4.87	0.91	18.68
Y17	Length of the middle leaf on main stem	LMLM	cm	3.60	5.03	4.12	0.34	8.24
Y18	Length of the terminal leaf on main stem	LTLM	cm	1.72	2.87	2.24	0.27	12.04
Y19	Width of the first leaf on main stem	WFLM	cm	0.56	0.90	0.65	0.06	9.23
Y20	Width of the middle leaf on main stem	WMLM	cm	0.45	0.62	0.52	0.04	7.68
Y21	Width of the terminal leaf on main stem	WTLM	cm	0.23	0.41	0.30	0.04	13.32
Y22	Length of the first leaf on lateral stem	LFLL	cm	2.61	4.54	3.77	0.041	1.09
Y23	Length of the middle leaf lateral main stem	LMLL	cm	3.49	4.60	4	0.28	7
Y24	Length of the terminal leaf on lateral stem	LTLL	cm	2.11	3.22	2.39	0.27	11.30
Y25	Width of the first leaf on lateral stem	WFLL	cm	0.51	0.88	0.60	0.07	11.67
Y26	Width of the middle leaf on lateral stem	WMLL	cm	0.49	0.63	0.56	0.03	5.36
Y27	Width of the terminal leaf on lateral stem	WTLL	cm	0.27	0.36	0.30	0.02	6.67
Y28	Essential oil yield	EOY	v/w (%)	1.94	3.45	2.41	0.30	12.45

Nob: Number, Abb: Abbreviation, Mu: Measuring unit, Min: Minimum, Max: Maximum, SD: Standard deviation, cou.: Counting.

but the factor analysis estimation is more accurate. Adequacy of sample size is determined by KMO and Bartlett test in the factor analysis. In general, this is an index option for comparing the values of simple and partial correlation coefficients on all variables. High value of KMO indicates the appropriateness of factor analysis, and the Bartlett test also tests the assumption of correlation coefficient matrix equality. If the Bartlett test is not significant, it is possible for the correlation matrix to be a single matrix and this means that the matrix is not suitable for subsequent analysis (Zare-Chahouki, 2010 and 2011). According to Table 6, the value of the KMO statistic is 0.706; so, the data are suitable for factor analysis. The results of the Bartlett test are also significant, thus confirming the opposite assumption, that there is a significant correlation between the variables. Besides KMO, "Communalities" are also effective in selecting the right number of factors in factor analysis. For example, it can be observed that 96.8% of the variance of the TFW is the variance of "Communality" (Table 7). "Initial" indicates "Communalities" before factor extraction; so all of them are equal to one. In this study, most of the "Extractions" were higher than

Table 3. Analysis of variance (mean squares) for studied traits in Iranian summer savory (*Satureja hortensis* L.) accessions.

Source of variation	DF	TFW	SFW	RFW	TPH	SL	RL	NI	DI	Ch	NB	NL	NLL	TDW	SDW
Block	2	2088.4**	2064.89**	1.181*	17.907^{ns}	16.289^{ns}	4.516^{ns}	37.497***	0.159^{ns}	21.894^{ns}	25.408***	7.372*	47.842^{ns}	65.411**	76.122***
Treat	28	514.33^{ns}	497.6^{ns}	0.753**	56.188*	75.735***	6.377^{ns}	1.108 ^{ns}	0.719***	20.259^{ns}	2.301^{ns}	2.307^{ns}	165.882***	11.226^{ns}	9.980^{ns}
Error	56	366.25	346.99	0.325	27.535	12.346	4.943	0.692	0.106	21.457	2.935	1.736	25.562	10.648	8.307
Coefficient of variation (%)	-	34.34	34.49	30.32	8.70	8.005	13.47	5.765	9.34	18.27	8.53	10.03	19.74	37.82	34.97

DF: Degree of Freedom, TFW: Total fresh weight, SFW: Stem fresh weight, RFW: Root fresh weight, TPH: Total plant height, SL: Stem length, RL: Root Length, NI: Number of internodes, DI: Distance of internodes, Ch: Chlorophyll, NB: Number of branches per main stem, NL: Number of leaves on main stem, NLL: Number of leaflet per main stem, TDW: Total dry weight, SDW: Stem dry weight, *: Significant at the 0.05 probability levels, **: Significant at the 0.01 probability levels, ***: Significant at the 0.001 probability levels, ^{ns}: Not significant.

Table 3 (Continued). Analysis of variance (mean squares) for studied traits in Iranian summer savory (*Satureja hortensis* L.) accessions.

Source of variation	DF	RDW	LFLM	LMLM	LTLM	WFLM	WMLM	WTLM	LFLL	LMLL	LTLL	WFLL	WMLL	WTLL	EOY
Block	2	0.0857^{ns}	3.491^{ns}	2.013***	1.356**	0.009^{ns}	0.025*	0.019**	1.205**	0.016	0.889**	0.094**	0.008^{ns}	0.005^{ns}	0.318*
Treat	28	0.853^{ns}	2.539^{ns}	0.349**	0.225^{ns}	0.014^{ns}	0.006^{ns}	0.005^{ns}	0.527***	0.242*	0.219^{ns}	0.015^{ns}	0.003^{ns}	0.001^{ns}	0.112^{ns}
Error	56	0.018	2.734	0.147	0.156	0.0170	0.005	0.003	0.201	0.141	0.219	0.014	0.005	0.002	0.095
Coefficient of variation (%)	-	27.82	33.93	9.311	17.60	20.020	13.91	18.47	11.88	9.391	15.65	19.71	13.24	15.74	12.74

DF: Degree of Freedom, RDW: Root dry weight, LFLM: Length of the first leaf on main stem, LMLM: Length of the middle leaf on main stem, LTLM: Length of the terminal leaf on main stem, WFLM: Width of the first leaf on main stem, WMLM: Width of the middle leaf on main stem, WTLM: Width of the terminal leaf on main stem, LFLL: Length of the first leaf on lateral stem, LMLL: Length of the middle leaf lateral main stem, LTLL: Length of the terminal leaf on lateral stem, WFLL: Width of first leaf on the lateral stem, WMLL: Width of the middle leaf on lateral stem, EOY: Essential oil yield, *: Significant at the 0.05 probability levels, **: Significant at the 0.01 probability levels, ***: Significant at the 0.001 probability levels, ^{ns}: Not significant.

Table 4. Mean comparison of significant traits in studied Iranian summer savory (*Satureja hortensis* L.) accessions.

Accession	RFW	TPH	SL	DI	NLL	LMLM	LFLL	LMLL
Shush	1.486^{defgh}	57.7^{cde}	41.1^{cdefg}	3.22^{efg}	26.133^{abcdef}	3.911^{cdefg}	3.888^{abcde}	3.488^{h}
Tabriz 1	1.523^{defgh}	64.883^{bc}	53.633^{b}	4.413^{bc}	5.467^{g}	5.033^{a}	4.544^{a}	4.6^{a}
Ardabil	1.998^{bcdefg}	57.833^{cde}	40.067^{defg}	2.993^{fg}	30.4^{abcde}	3.8^{defg}	3.655^{defg}	3.7^{efgh}
Razan 1	2.411^{abcd}	62.1^{bcd}	42.467^{cdefg}	3.34^{efg}	29.733^{abcde}	4.311^{cdef}	3.144^{fgh}	3.766^{efgh}
Mashhad	3.213^{a}	53.133^{e}	36.967^{g}	3.146^{efg}	24.933^{bcdef}	4.2^{cdefg}	3.877^{abcde}	3.877^{cdefgh}
Sistan and Baluchestan	$1.826^{bcdefgh}$	61.472^{bcd}	45.017^{cde}	3.253^{efg}	28.533^{abcdef}	4.3^{cdef}	4^{abcde}	4.566^{ab}
Tonekabon 2	2.606^{ab}	58.367^{cde}	41.133^{cdefg}	3.106^{efg}	29.267^{abcde}	4.011^{cdefg}	3.733^{cdef}	$3.977^{bcdefgh}$
Shiraz	$1.844^{bcdefgh}$	59.706^{cde}	43.217^{cdef}	3.478^{def}	30.967^{abcd}	3.866^{cdefg}	3.888^{abcde}	3.833^{defgh}
Qom	2.173^{bcdef}	55.767^{de}	38.067^{fg}	2.925^{g}	29.1^{abcde}	4.077^{cdefg}	4.033^{abcde}	$3.977^{bcdefgh}$
Kermanshah	2.190^{bcdef}	60.2^{cde}	43.867^{cde}	3.433^{efg}	33.333^{a}	3.844^{defg}	2.983^{gh}	3.911^{cdefgh}
Sanandaj	1.738^{efgh}	60.75^{bcde}	46.35^{c}	3.97^{cd}	30.4^{abcde}	4.4^{bcd}	3.55^{defg}	4.233^{abcdef}
Parsabad	2.586^{ab}	59.333^{cde}	41.2^{cdefg}	3.41^{efg}	29.6^{abcde}	4.033^{cdefg}	3.477^{defg}	$4.066^{abcdefgh}$
Sardasht	$1.765^{bcdefgh}$	69.239^{ab}	54.361^{b}	4.733^{ab}	6.133^{g}	5^{ab}	4.466^{ab}	4.388^{abcd}
Tuserkan 2	$1.715^{bcdefgh}$	59.528^{cde}	42.556^{cdefg}	3.423^{efg}	26^{abcdef}	3.6^{g}	3.755^{bcdef}	3.6^{gh}
Hamedan	$1.918^{bcdefgh}$	61.967^{bcd}	46.067^{c}	3.233^{efg}	32.667^{ab}	3.977^{cdefg}	4.122^{abcd}	4.266^{abcde}
Tabriz 2	1.268^{fgh}	76.150^{a}	61.133^{a}	5.126^{a}	6.667^{g}	4.3^{cdef}	4.444^{abc}	4.466^{abc}
Isfahan	1.306^{fgh}	61.2^{bcde}	42.167^{cdefg}	3.186^{efg}	23.467^{def}	4.222^{cdefg}	3.7^{defg}	3.911^{cdefgh}
Qazvin	1.2^{gh}	59.967^{cde}	44.4^{cde}	3.313^{efg}	27.867^{abcdef}	3.755^{fg}	3.666^{defg}	3.666^{efgh}
Tuserkan 1	1.026^{h}	56.533^{cde}	42.267^{cdefg}	3.6^{de}	20.378^{f}	4.344^{cdef}	3.388^{efg}	3.866^{cdefgh}
Ilam	1.306^{fgh}	59.667^{cde}	43.4^{cdef}	3.386^{efg}	22.533^{f}	4.077^{cdefg}	3.855^{abcdef}	3.95^{cdefgh}
Borujerd	1.626^{cdefgh}	59.667^{cde}	43.2^{cdef}	3.313^{efg}	27.733^{abcdef}	3.788^{defg}	3.422^{defg}	3.844^{defgh}
Kelachay	2.053^{bcdefg}	56.242^{de}	39.3^{efg}	3.353^{efg}	24.267^{cdef}	4.294^{cdef}	4.083^{abcde}	4.277^{abcde}
Arak	1.560^{defgh}	62.6^{bcd}	46.367^{c}	3.36^{efg}	30.133^{abcde}	4.483^{abc}	3.977^{abcde}	$4.122^{abcdefg}$
Dezful	$1.746^{bcdefgh}$	58.567^{cde}	41.533^{cdefg}	3.293^{efg}	25.333^{abcdef}	3.855^{defg}	2.605^{h}	3.644^{fgh}
Razan 2	2.293^{abcde}	56.067^{de}	40.133^{defg}	3.226^{efg}	28.133^{abcdef}	3.844^{defg}	3.633^{defg}	$4.011^{abcdefgh}$
Tehran	2.505^{abc}	63.033^{bcd}	44.9^{cde}	3.346^{efg}	30^{abcde}	3.88^{cdefg}	3.622^{defg}	3.766^{efgh}
Tonekabon 1	2.156^{bcdef}	58^{cde}	40.867^{cdefg}	3.493^{def}	23.333^{def}	4.388^{bcde}	3.844^{abcdef}	$4.111^{abcdefg}$
Sarab	2.134^{bcdef}	58.856^{cde}	41.9^{cdefg}	3.53^{de}	32^{abc}	3.766^{efg}	3.877^{abcde}	3.888^{cdefgh}
Qeshm	$1.730^{bcdefgh}$	59.911^{cde}	45.3^{cd}	3.46^{def}	28.317^{abcdef}	4.066^{cdefg}	4.077^{abcde}	$4.077^{abcdefgh}$

RFW: Root fresh weight, TPH: Total plant height, SL: Stem length, DI: Distance of internodes, NLL: Number of leaflet per main stem, LMLM: Length of the middle leaf on main stem, LFLL: Length of the first leaf on lateral stem, LMLL: Length of the middle leaf lateral main stem. Means followed by the same letters in each column are not significantly different.

5%, indicating the ability of the specified factors to explain the variance of the variables. In general, the "Communality" of most traits was high, showing that the number of factors selected was appropriate and that the selected factors were able to justify the variation of traits optimally.

Results of factor analysis for morphological traits showed 8 main and independent factors accounted for 87.705% of total variation (Table 7). The first factor with high eigenvalue (6.169) explained for 22.033% of the total variance and included the TFW, SFW, RFW, NB, TDW, SDW and RDW traits. Significant traits in the first factor were highly correlated with each other and were independent of the other factors. Therefore, this factor was called the weight factor due to its high value of "Communality" in the mentioned trait. TPH, SL, NI, DI, and NLL were the traits constituted the second factor with 4.875 eigenvalues. This factor accounts for 17.411% of the total variation and is reported as stem length with a high SL "Communality" (0.965). The third factor with 13.279% of the total variance and 3.718 eigenvalues was called leaf length and width factor on the main stem and LMLM, LTLM, and WMLM traits were included in this factor. The fourth factor was assigned as the factor for the length of the first leaf on the lateral stem, accounting for 9.276% of the total variance with eigenvalues of 2.597. Furthermore, WFLM, WTLL, LFLM, and NL traits were identified as influential traits in five, six, seven and eight factors, respectively, due to their importance and the high value of "Communality" in related factors. Also, in the factors with positively significant traits indicate that the traits have a positive and highly significant correlation, which is a reason to be considered as an independent factor. Figure 1 shows the changes in the eigenvalues with the factors. This graph is used for the optimal number of components.

Table 5. Correlation coefficients of traits in Iranian summer savory (*Satureja hortensis* L.) accessions.

Traits	TFW	SFW	RFW	TPH	SL	SL	NI	DI	Ch	NB	NL	NLL	TDW	SDW
TFW	1													
SFW	0.995**	1												
RFW	0.819**	0.817**	1											
TPH	-0.092	-0.103	-0.370*	1										
SL	-0.133	-0.135	-0.460*	0.951**	1									
RL	0.264	0.226	0.516**	-0.377*	-0.601**	1								
NI	-0.024	-0.038	-0.434*	0.616**	0.610**	-0.301	1							
DI	-0.210	-0.202	-0.369*	0.828**	0.906**	-0.621**	0.325	1						
Ch	0.151	0.163	0.532**	-0.159	-0.268	0.354	-0.581**	-0.105	1					
NB	0.613**	0.609**	0.408*	0.105	0.048	0.198	0.252	-0.041	-0.048	1				
NL	0.054	0.058	-0.085	-0.096	-0.108	0.148	0.274	-0.284	-0.247	-0.154	1			
NLL	0.221	0.218	0.356	-0.610**	-0.706**	0.449*	-0.219	-0.814**	0.158	0.159	0.219	1		
TDW	0.948**	0.937**	0.792**	0.043	-0.024	0.315	0.005	-0.079	0.204	0.657**	0.037	0.121	1	
SDW	0.973**	0.966**	0.769**	0.071	0.025	0.186	0.087	-0.055	0.166	0.610**	0.027	0.119	0.958**	1
RDW	0.798**	0.808**	0.893**	-0.383*	-0.473**	0.494**	-0.321	-0.438*	0.402*	0.479**	0.014	0.457*	0.774**	0.750**
LFLM	0.137	0.129	0.115	0.000	0.249	-0.041	0.061	0.304	0.291	0.057	-0.021	-0.242	0.210	0.208
LMLM	0.055	0.056	-0.169	0.044*	0.528**	-0.312	0.133	0.608**	-0.250	0.114	-0.092	-0.699**	0.091	0.099
LTLM	-0.167	-0.179	-0.208	0.000*	0.522**	-0.300	-0.065	0.672**	0.036	-0.090	-0.424*	-0.636**	-0.095	-0.092
WFLM	0.224	0.241	0.242	0.000	-0.091	0.137	-0.309	-0.043	0.420*	0.031	-0.221	-0.230	0.231	0.195
WMLM	0.082	0.096	0.047	0.411	0.168	-0.033	-0.273	0.294	0.148	0.155	-0.250	-0.289	0.112	0.076
WTLM	0.056	0.047	0.201	0.589	0.013	0.171	-0.381*	0.137	0.529**	-0.019	-0.403*	-0.142	0.102	0.073
LFLL	0.055	0.069	-0.146	0.622*	0.501**	-0.404*	0.284	0.472**	-0.382*	0.038	-0.058	-0.534**	0.041	0.096
LMLL	0.225	0.221	-0.091	0.000**	0.602**	-0.419*	0.330	0.581**	0.131	0.433*	-0.202	-0.499**	0.224	0.264
LTLL	0.074	0.067	-0.127	0.824**	0.615**	-0.282	0.188	0.657**	-0.056	0.113	-0.292	-0.812**	0.130	0.149
WFLL	0.064	0.071	0.129	-0.172	-0.127	-0.032	-0.345	-0.109	0.434*	-0.068	-0.278	-0.020	0.043	0.014
WMLL	0.155	0.176	0.283	-0.078	-0.056	0.032	-0.309	0.084	0.531**	0.279	-0.359	0.012	0.211	0.148
WTLL	0.218	0.235	0.213	0.392*	0.389*	-0.159	-0.247	0.513**	0.361	-0.011	-0.241	-0.497**	0.280	0.282
EOY	-0.243	-0.263	0.041	-0.281	-0.368*	0.354	-0.383*	-0.354	0.277	-0.246	-0.97	0.374*	-0.228	-0.281

TFW: Total fresh weight, SFW: Stem fresh weight, RFW: Root fresh weight, TPH: Total plant height, SL: Stem length, RL: Root Length, NI: Number of internodes, DI: Distance of internodes, Ch: Chlorophyll, NB: Number of branches per main stem, NL: Number of leaves on main stem, NLL: Number of leaflet per main stem, TDW: Total dry weight, SDW: Stem dry weight, *, **: significant at 0.05 and 0.01 probability level, respectively.

Table 5 (Continued). Correlation coefficients of traits in Iranian summer savory (*Satureja hortensis* L.) accessions.

Traits	RDW	LFLM	LMLM	LTLM	WFLM	WMLM	WTLM	LFLL	LMLL	LTLL	WFLL	WMLL	WTLL	EOY
RDW	1													
LFLM	0.163	1												
LMLM	-0.174	0.263	1											
LTLM	-0.268	0.293	0.714**	1										
WFLM	0.232	-0.016	0.234	0.161	1									
WMLM	0.019	0.041	0.678**	0.581**	0.488**	1								
WTLM	0.145	0.127	0.208	0.539**	0.553**	0.599**	1							
LFLL	-0.121	0.207	0.504**	0.233	-0.111	0.027	-0.341	1						
LMLL	-0.103	0.369*	0.723**	0.528**	0.004	0.333	0.024	0.623**	1					
LTLL	-0.168	0.300	0.712**	0.683**	0.379*	0.393*	0.353	0.519**	0.718**	1				
WFLL	0.062	-0.002	-0.020	-0.039	0.760**	0.221	0.304	-0.107	0.016	0.171	1			
WMLL	0.258	0.382*	0.138	0.214	0.376*	0.516**	0.339	-0.148	0.100	0.059	0.456*	1		
WTLL	0.169	0.257	0.389*	0.511**	0.320	0.449*	0.490**	0.305	0.313	0.621**	0.131	0.244	1	
EOY	0.061	-0.162	-0.315	0.128	-0.047	-0.005	0.279	-0.287	-0.390*	-0.240	0.066	0.047	-0.035	1

RDW: Root dry weight, LFLM: Length of the first leaf on main stem, LMLM: Length of the middle leaf on main stem, LTLM: Length of the terminal leaf on main stem, WFLM: Width of the first leaf on main stem, WMLM: Width of the middle leaf on main stem, WTLM: Width of the terminal leaf on main stem, LFLL: Length of the first leaf on lateral stem, LMLL: Length of the middle leaf lateral main stem, LTLL: Length of the terminal leaf on lateral stem, WFLL: Width of first leaf on the lateral stem, WMLL: Width of the middle leaf on lateral stem, EOY: Essential oil yield, *, **: significant at 0.05 and 0.01 probability level, respectively.

Table 6. KMO and Bartlett's test results.

Test		Result
KMO measure of sampling adequacy.		0.706
Bartlett's test of Sphericity	Approx. Chi-Square	1.798E3
	df	190
	Sig.	0.000

KMO: Kaiser-Meyer-Olkin, df: Degree of freedom, Sig.: Significancy.

According to this diagram, it can be seen that the first, second and third factors have the highest eigenvalues and the eigenvalues decrease from the eighth factor. Therefore, three factors can be identified as important factors that play the most critical role in explaining data variance. Figure 2 also shows the rotated three-dimensional graph and illustrates the distributions of the investigated variables for the first, second and the third factors. Seven, five, and three traits were included respectively in the first, second, and the third factors with 52.723% of the total variance and showed differences in accessions.

Cluster analysis

In plant and agricultural science, cluster analysis is a statistical method for grouping different populations,

Table 7. Rotated component matrix of 28 traits obtained from summer savory (*Satureja hortensis* L.) accessions.

Traits	Component								Comunalities	
	1	2	3	4	5	6	7	8	Initial	Extraction
TFW	**0.977**	-0.050	0.022	-0.063	0.065	-0.030	-0.003	-0.049	1	0.968
SFW	**0.972**	-0.058	0.016	-0.087	0.089	-0.026	0.007	-0.045	1	0.966
RFW	**0.842**	-0.353	-0.059	0.138	0.077	0.220	0.140	0.109	1	0.941
TPH	-0.034	**0.938**	0.152	-0.029	-0.130	0.022	0.048	0.018	1	0.925
SL	-0.110	**0.926**	0.206	-0.211	-0.070	-0.003	0.046	0.040	1	0.965
RL	0.331	-0.454	-0.003	0.539	-0.089	0.052	-0.004	-0.208	1	0.661
NI	-0.006	**0.677**	-0.159	-0.142	-0.240	-0.470	-0.135	-0.282	1	0.879
DI	-0.172	**0.798**	0.327	-0.240	-0.045	0.160	0.166	0.185	1	0.921
Ch	0.200	-0.137	-0.087	0.487	0.377	0.412	0.480	0.223	1	0.896
NB	**0.680**	0.110	0.131	0.008	-0.088	-0.558	0.023	0.229	1	0.865
NL	0.015	-0.089	-0.166	0.008	-0.177	-0.064	-0.022	**-0.893**	1	0.868
NLL	0.211	**-0.610**	-0.443	0.362	-0.156	-0.293	-0.019	0.027	1	0.854
TDW	**0.966**	0.074	0.052	0.035	0.044	-0.014	0.069	-0.048	1	0.952
SDW	**0.969**	0.124	0.009	-0.038	0.032	0.000	0.038	-0.037	1	0.960
RDW	**0.839**	-0.377	-0.059	0.126	0.017	0.115	0.152	0.028	1	0.902
LFLM	0.139	0.217	0.118	-0.114	-0.088	0.135	**0.860**	-0.112	1	0.872
LMLM	0.021	0.29	**0.840**	-0.366	0.023	-0.012	0.072	-0.111	1	0.947
LTLM	-0.169	0.354	**0.755**	0.012	-0.103	0.255	0.135	0.292	1	0.903
WFLM	0.190	-0.036	0.335	0.143	**0.858**	0.138	-0.058	-0.026	1	0.929
WMLM	0.054	-0.003	**0.862**	0.137	0.264	-0.072	0.081	0.109	1	0.859
WTLM	0.076	0.067	0.527	0.574	0.301	0.289	0.059	0.247	1	0.855
LFLL	0.062	0.286	0.180	**-0.794**	-0.152	0.194	-0.002	0.027	1	0.810
LMLL	0.213	0.430	0.506	-0.529	-0.075	-0.180	0.139	0.156	1	0.848
LTLL	0.090	0.541	0.580	-0.287	0.193	0.266	-0.012	0.076	1	0.834
WFLL	-0.003	-0.114	0.001	-0.013	**0.898**	0.031	0.099	0.189	1	0.866
WMLL	0.142	-0.108	0.254	0.151	0.389	-0.203	**0.656**	0.348	1	0.863
WTLL	0.262	0.357	0.385	-0.016	0.149	**0.630**	0.127	0.168	1	0.807
EOY	-0.218	-0.394	0.043	0.483	-0.215	0.277	-0.060	0.275	1	0.641
Eigenvalues	6.169	4.875	3.718	2.597	2.323	1.731	1.588	1.555	-	-
Variance (%)	22.033	17.411	13.279	9.276	8.298	6.182	5.672	5.553	-	-
Cumulative (%)	22.033	39.444	52.723	62	70.298	76.48	82.152	87.705	-	-

TFW: Total fresh weight, SFW: Stem fresh weight, RFW: Root fresh weight, TPH: Total plant height, SL: Stem length, RL: Root Length, NI: Number of internodes, DI: Distance of internodes, Ch: Chlorophyll, NB: Number of branches per main stem, NL: Number of leaves on main stem, NLL: Number of leaflet per main stem, TDW: Total dry weight, SDW: Stem dry weight, RDW: Root dry weight, LFLM: Length of the first leaf on main stem, LMLM: Length of the middle leaf on main stem, LTLM: Length of the terminal leaf on main stem, WFLM: Width of the first leaf on main stem, WMLM: Width of the middle leaf on main stem, WTLM: Width of the terminal leaf on main stem, LFLL: Length of the first leaf on lateral stem, LMLL: Length of the middle leaf lateral main stem, LTLL: Length of the terminal leaf on lateral stem, WFLL: Width of first leaf on the lateral stem, WMLL: Width of the middle leaf on lateral stem, EOY: Essential oil yield.

Factor loading values more than 0.6 were considered as significant, i.e. the bold numbers are the highest loading for each factors.

Extraction method is based on Principle Component Analysis (PCA).

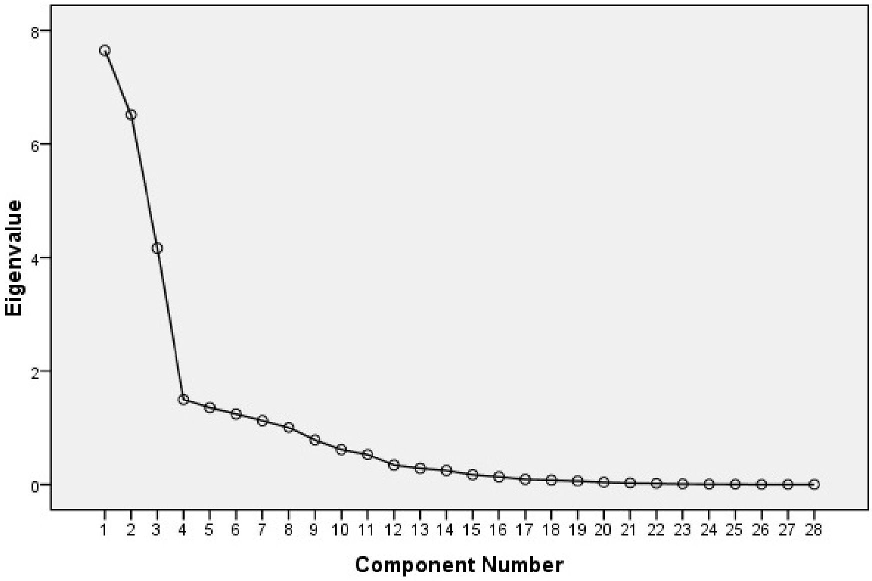

Figure 1. Scree plot to determine the number of factors in factor analysis of morphological traits in Iranian summer savory (*Satureja hortensis* L.) accessions.

germplasms, species, genotypes, ecotypes, accessions, etc., according to their similarity degrees. Through cluster analysis, the studied plants are divided into homogenous and distinct groups. Grouping plants by using morphological traits or molecular markers is an effective way to determine the relationship and the genetic distance among accessions. In this study, the accessions were divided into four main groups using Ward's method based on Squared Euclidean Distance (SED) (Figure 3). For the accuracy of grouping in the phylogenetic dendrogram, discriminant analysis (DA) was used which confirmed grouping with 97.2% (Table 8). The first group was divided into two subgroups, which comprised of thirteen accessions. The second and third groups included three and nine accessions, respectively. The fourth group, similar to the first one, included two subgroups, and in total, encompassed four accessions (Figure 3). The accessions which are in the same subgroup or group, possess the same genetic diversity but are not similar to the other subgroups or groups. Since diversity is a major component of plant selection, it is possible to combine molecular phylogenetic and morphological results using Mantle and other tests to employ for breeding programs of Iranian summer savory accessions.

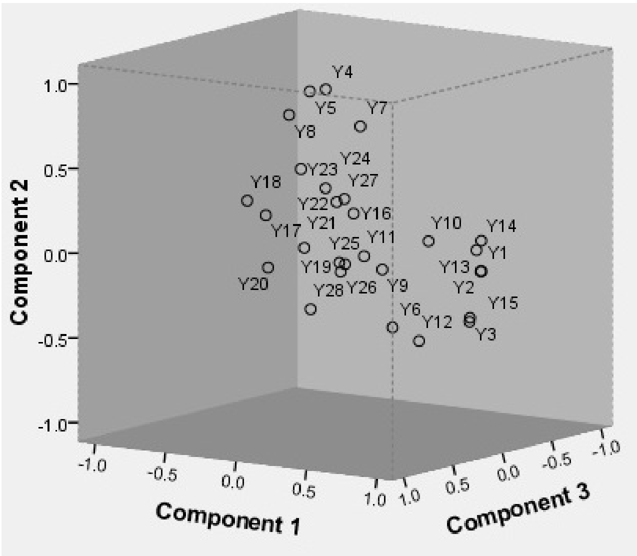

Figure 2. 3D Component in rotated space in factor analysis of morphological traits in Iranian summer savory (*Satureja hortensis* L.) accessions.

CONCLUSION

In general, the results of the present study indicated that there are considerable variations in all measured traits in the Iranian summer savory (*Satureja hortensis* L.) accessions. The results of multivariate analyses, such as cluster and discriminant analysis, were able to show this variation. Placing each accession of the Iranian summer savory in different groups indicates the genetic variation, which can be used in breeding programs. The results of the grouping revealed that there was no relationship between the genetic variation and geographical distance of the accessions.

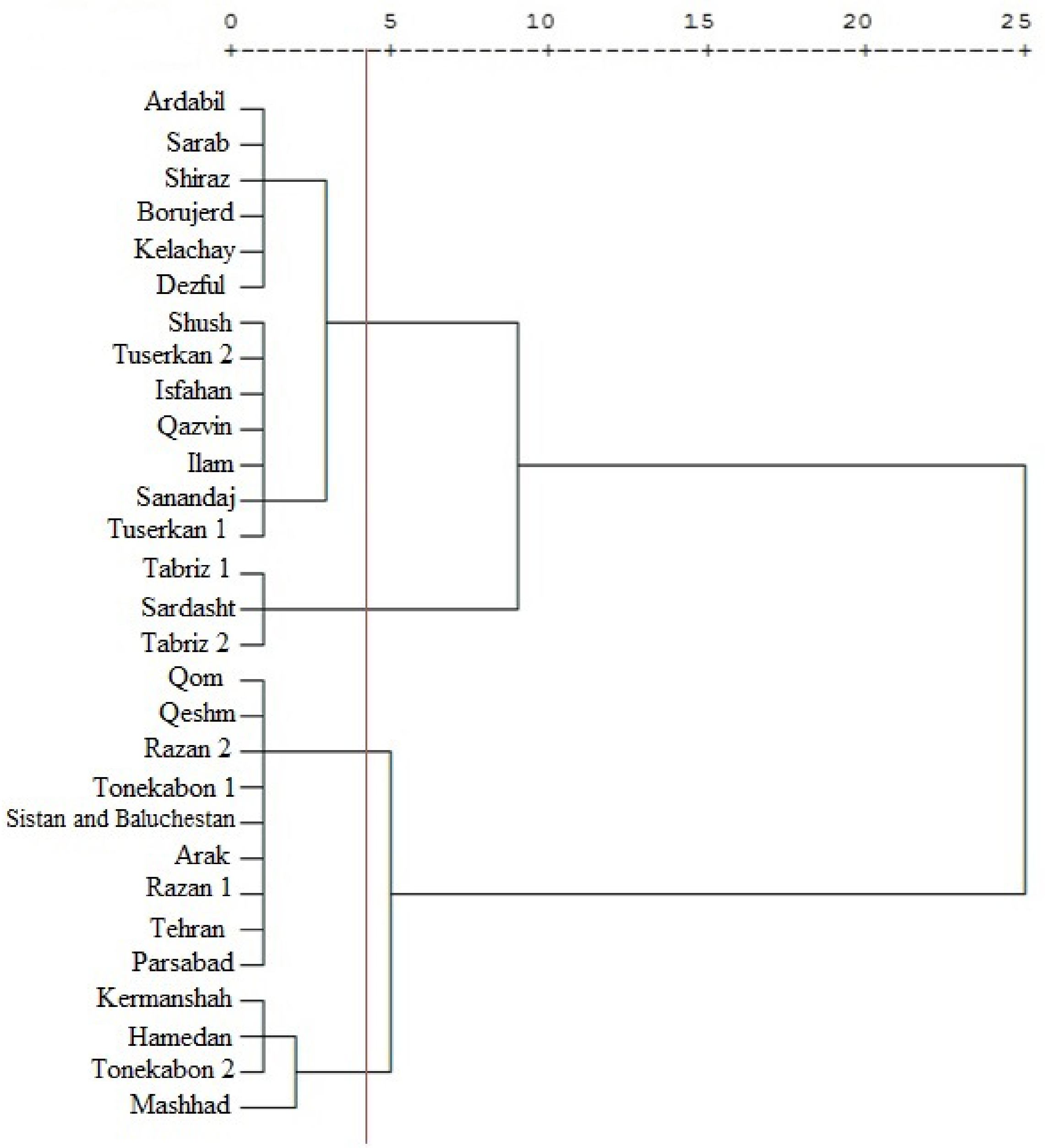

Figure 3. Phylogenetic dendrogram of morphological traits in Iranian summer savory (*Satureja hortensis* L.) accessions using the Ward's method.

Table 8. Discrimination function analysis for evaluating the accuracy of grouping in cluster analysis.

	Grouping of cluster analysis	1	2	3	4	Total
Number of Accessions	1	13	0	0	0	13
	2	0	3	0	0	3
	3	1	0	8	0	9
	4	0	0	0	4	4
Success rate (%)	1	100	0	0	0	100
	2	0	100	0	0	100
	3	11.1	0	88.9	0	100
	4	0	0	0	100	100
Mean of success (%)			**97.2**			

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