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Evaluation of the phenotypic variation in a caper (*Capparis spinosa* L.) population growing in south of Tehran using multivariate analysis

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ABSTRACT

The aim of this study was to investigate the phenotypic variation among 100 caper (*Capparis spinosa* L.) plants growing naturally in south of Tehran. Plant samples were taken randomly from three different regions of southern Tehran. The number of 29 phenotypic traits were studied on selected plants. Data were subsequently subjected to multivariate analysis. Results showed that fruit yield was significantly correlated with the number of fruits per plant, fruit weight, fruit length, fruit diameter, plant canopy, the number of thorns in every 50 cm of stem, thorn length and the number of flowers per plant (NFP). Also, there was a negative correlation between fruit length and the number of branches per each main stem. Stepwise multiple linear regression revealed that the number of main stems per plant, leaf width, petiole length, peduncle length and NFP were added to the model and hence had the greatest impact on fruit yield. Principle component analysis showed that the first two components accounted for 41.1% of the total variation. The first component was related to the fruit yield and its related traits, while the second component was related to the vegetative growth and showed competition between reproductive and vegetative functions. Cluster analysis of genotypes using Ward method and squared Euclidian distance criteria classified the samples into ten different groups. The results of this study suggested that crossing between samples 17 and 81 may produce useful recombinants.

Key words: *Capparis spinosa*, cluster analysis, graphical correlation coefficients, principal component analysis, stepwise multiple regression.

Introduction

Capparis spinosa L. (CS) (Capparaceae) locally known as “Kebbar” is a common perennial shrub in the Mediterranean regions, growing both wild and cultivated, with medicinal and aromatic properties. Although its ancient habitat is thought to be the dry areas of western or central Asia, the

plant has a wide natural distribution from the Atlantic coasts of the Canary Islands and Morocco to the Black Sea to the Crimea and Armenia, and eastward to the Caspian Sea and into Iran Romeo et al. (2007).

The fruits and the root of the plant have been used in gout and also as diuretics, astringents and tonics in traditional Iranian medicine (Afsharypour

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et al. 1998). Even its flower buds have some medical uses and are taken to improve liver functions or as a kidney disinfectant.

CS is used in phytomedicine around the world as anti-oxidative (Germano et al. 2002), antifungal (Ali-Shtayeh and Abu Ghdeib 1999), antihepatotoxic (Gadgoli and Mishra 1999), anti-inflammatory (Al-Said et al. 1988) and anti-diabetic (Ziyyat et al. 1997).

CS is one of the most commonly found aromatic plants in Mediterranean cooking: the fresh aerial parts, including the fruit and the flower buds, are stored in vinegar or brined and eaten pickled

The aim of this study was to investigate the phenotypic variation existing among CS plants growing in the south of Tehran.

Materials and Methods

In this study, *in situ* characterization was carried out over 100 CS plants growing in an area of approximately 900 hectares. Plant samples were taken randomly from three different regions of southern Tehran, Iran in 2014 (Fig. 1).

After selection of plants, the number of 29 morphological traits was measured including: the number of main stems per plant (MSP), the number



Figure 1. The area (showed in the rectangular callout) selected for the study of some morphological characteristics among 100 caper (*Capparis spinosa*) samples.

(Zargari 1995).

Despite the above unique medicinal properties of CS, study of its morphological characteristics is usually associated with some difficulties due to the exclusive physical properties of this herb including: deep roots, the large number of thorns on the stems and the wide plant canopy.

of branches per each main stem (BMS), plant canopy (PA) (m²), the number of lateral branches per every 50 cm of stem (LBS), the number of flower buds per every 50 cm of stem (FBS), the number of flowers per plant (NFP), the number of leaves per every 50 cm of stem (NLS), leaf length (LL) (cm), leaf width (LW) (cm), the ratio of leaf

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length to leaf width (LLTLW), petiole length (PL) (cm), the number of thorns in every 50 cm of stem (NTS), length of the thorn (cm) per every 50 cm of stem (LTPS), flower bud weight (FBW) (g), peduncle length (PeL) (cm), flower bud length (FBL) (mm), flower bud diameter (FBD) (mm), the ratio of flower bud length to flower bud diameter (FBLTFBD), the number of fruits per plant (FPP), fruit weight (FW) (g), fruit length (FL) (mm), fruit diameter (FD) (mm), the ratio of fruit length to fruit diameter (FLTFD), fruit stalk length (FSL) (cm), fruit stalk weight (FSW) (g), number of seeds per fruit (SPF), seed weight per fruit (SWPF) (g), thousand grain weight (TGW) (g), fruit yield per plant (Yield) (g). Statistical analyses were applied using MINITAB, SPSS and Microsoft Excel software. Visualization of the correlation coefficient matrix was carried out using the correplot package in the R system. Geographic map was drawn using Google Earth (2015).

Results

Interrelationships among traits

Correlations among studied traits have been presented graphically in figure 2. Graphical method is useful to present information derived from a large number of variables. In this figure, each correlation has been shown as a small colored circle. Also, only those correlations that were statistically significant ($\alpha=0.05$) were shown and thus, blank squares indicate non-significant correlations. Moreover, different colors determine the direction of the correlations (blue for positive correlations and red for negative correlations). Intensity of the colors used indicate the intensity of the correlations (the darker blue the closer to +1 and the darker red the closer to -1). In addition to the color intensity, the diameter of the circles drawn in squares represents the strength of the correlations (the larger the circle, the stronger the

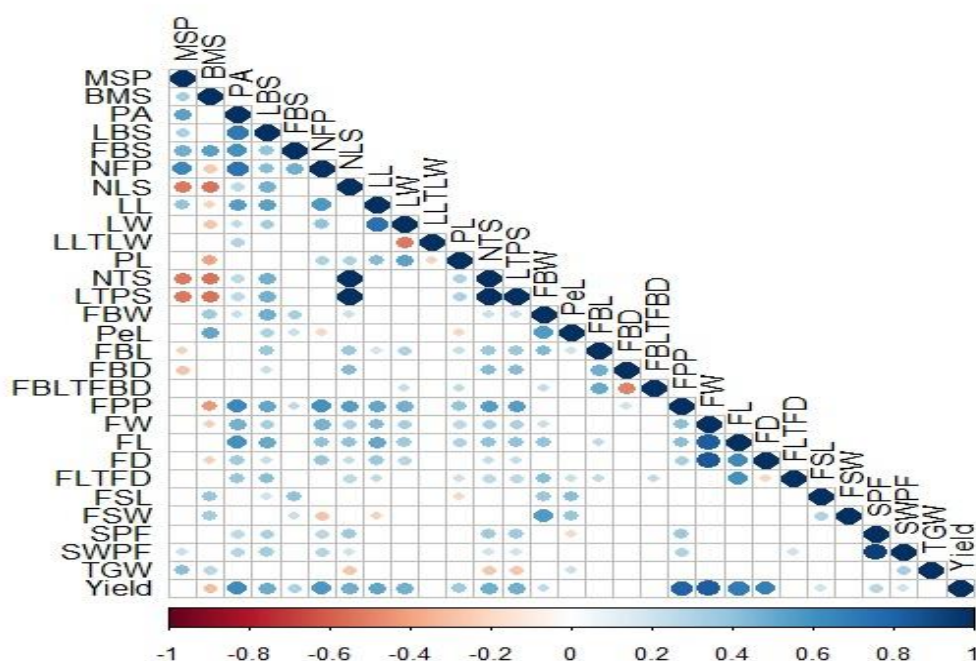


Figure 2. Correlations among characteristics studied over a population comprising of 100 carper (*Capparis spinosa* L.) samples collected from south of Tehran.

Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients.

For traits abbreviations refer to text.

correlation).

According to the above explanations, the strongest positive correlations were existed between NTS-NLS, LTPS-NLS and LTPS-NTS. Such strong correlations are likely due to pleiotropy or linkage. However, determining which of the pleiotropy or linkage was responsible of the observed mentioned correlations can only be resolved through QTL mapping. Although, elimination of the thorns “through genetic engineering” converts the plant into a suitable source for feeding animals however, such an action may have a negative effect on the number of leaves with regard to the fact that there was a strong relationship between the number of leaves and number and length of thorns, resulting in decreased fruit yield (Figure 2). Also, there were positive strong correlations between pair of traits SWPF-SPF, FW-FL, FW-FD. Fruit yield per plant showed positive correlation with traits FPP, FW, FL, FD, PA, NTS, LTPS and NFP. There was a negative correlation between Yield and BMS. Also, the observed correlations between MSB as also BMS and traits NLS, NTS and LTPS were strongly negative (Figure 2).

Principal components analysis (PCA)

Figure 3 shows the loading plot obtained from PCA. The first and second principal components accounted for 27 and 14 percent of the total variance, respectively. According to the results, Yield, FPP, FL, LBS and PA had a large positive loadings on component 1, so we labeled this component as Fruit Yield and its related traits (reproductive function). Also, MSP and BMS had the largest positive loadings on component 2, so we labeled this component as vegetative growth (vegetative functions). There was a competition between vegetative and reproductive functions with regard to the large negative loading of BMS on component 1. In agreement with this result, some researchers have similarly reported competition between vegetative and reproductive

functions (Fotokian and Agahi 2014, Gilliam 2014).

Stepwise multiple linear regression

Result derived from SMLR analysis of traits over fruit yield has been presented in table 1. According to this table, MSP, LW, PL, PeL and NFP were added to the model and hence had the greatest impact on fruit yield. According to the results the most appropriate estimated model was as follow:

$$\text{Yield} = -505.958 + 71.601 \times \text{MSP} + 5.963 \times \text{LW} + 22.814 \times \text{PL} + 24.297 \times \text{PeL} + (-4.284) \times \text{NFPP}$$

The standardized coefficients “which are equal to the path coefficients” revealed that amongst predictors, MSP and LW with 0.660 and 0.569 had the highest direct effect on fruit yield, respectively.

The Collinearity Statistics revealed that all tolerance statistics were higher than 0.1 and at the same time, all VIF values were lower than 10. Accordingly, there was no problem concerning multicollinearity and thus, no further investigation was needed for the results. Moreover, the value of the Durbin-Watson statistic suggested that the residuals were not correlated (Table 1).

Cluster analysis

Cluster analysis (CA) was carried out using WARD method (as the procedure for combining clusters) based on squared Euclidian distance. Dendrogram cut off was carried out according to the first sudden jump in coefficients printed out in the agglomeration schedule in SPSS software. Discriminant analysis using cross-validation revealed that about 97.0% of original grouped cases were correctly classified (data are not shown).

Based on the results, CA divided population into 10 different groups. The minimum distance (3.97) was observed between clusters 6 and 1 while clusters 7 and 1 had the highest distance (9.87) relative to each other.

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Also, evaluation of the relevant distance matrix revealed that the minimum distance (3.51) was multiple linear regression showed that MSP, LW, PL, PeL and NFP had the largest impact over yield.

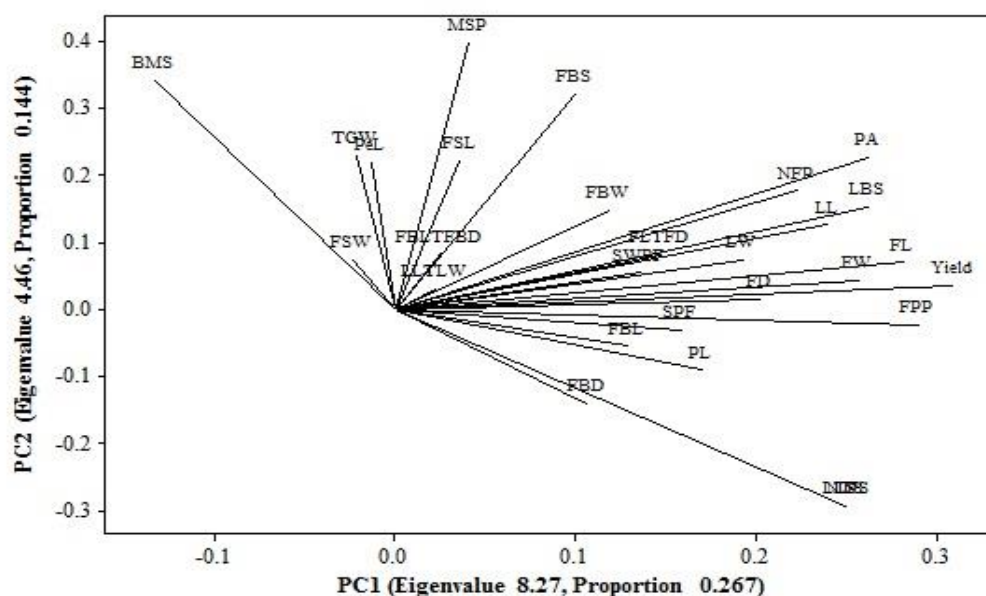


Figure 3. Loading plot of 29 traits studied over a population comprising of 100 carper (*Capparis spinosa L.*) samples collected from south of Tehran.

existed between carper plants of 21 and 41 while, plants 17 and 81 had the highest distance (199.11) relative to each other. Clusters 8 and 10 with 21 and 3

members had the highest and lowest number of members, respectively. Properties of clusters (mean \pm standard deviation) are presented in Table 2. In comparison with the overall mean, cluster 7 had a higher mean in terms of 23 out of the 29 studied traits. Thus, samples with high-performances were grouped in cluster 7 (Table 2). Clusters 4 and 5 had also a relatively high mean performances.

Discussion

Assessment of morphological characteristics is a step required for analysis of biodiversity in natural populations as well as an important aspect of genetic, taxonomic and biodiversity studies (Saadaoui et al. 2013). In this study relationships among traits were investigated using correlation coefficients, stepwise multiple linear regression and principal components analysis. Stepwise

Therefore, these traits can be used in future carper breeding programs.

Assessment of morphological variation revealed that the studied carper population was highly adapted to arid and semi-arid environmental conditions and hence could be a valuable source for researches concerning genetic resistance to drought. The distribution of genetic diversity within and among populations is a function of the rate of gene flow between populations. The extent of gene flow in a species depends on the distribution of the habitats it occupies, on the size and degree of isolation of its populations, and on the movement of pollen and seeds between populations. In the landraces as a whole, each population proved to be heterogeneous at a large number of loci. This result can be explained considering the high rate of free pollinations within families in any allogamous species such as carper, a low gene flow due to local isolation of the populations, and/or large effective population sizes. Therefore, each sampled landrace is actually a mixture of a large number of distinct genotypes

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that casually intercross at each generation sharing a common gene pool, which belongs to the landraces because of local adaptation, which may be ascribed to a combination of climatic conditions and agronomic practices (Hartings et al. 2008). Thus, the population used in this study could potentially be a rich source of genes that have adapted to the environmental conditions over the years.

A cross between those plant samples that showed a high genetic distance may lead to valuable new recombinants. Results obtained from cluster analysis showed that plant samples 17 and 81 had the highest distance (199.11) relative to each other.

Table 1. Stepwise multiple linear regression results carried out on 29 traits studied over a population comprising of 100 carper (*Capparis spinosa* L.) samples collected from south of Tehran.

Model	Unstandardized Coefficients		Standardized Coefficients			Collinearity Statistics	
	B	Std. Error	Beta	t	Sig.	Tolerance	VIF
Constant	-505.958	59.167		-8.551	0.000		
MSP	71.601	4.084	0.660	17.531	0.000	0.306	3.273
LW	5.963	0.243	0.569	24.502	0.000	0.803	1.245
PL	22.814	5.545	0.091	4.114	0.000	0.891	1.122
PeL	24.297	6.883	0.078	3.530	0.001	0.884	1.131
NFP	-4.284	1.641	-0.099	-2.610	0.011	0.299	3.343

Dependent Variable: fruit yield per plant

VIF: variance inflation factor

Durbin-Watson 1.949

For traits abbreviations refer to text

Table 2: Properties of clusters (mean \pm standard deviation) obtained from cluster analysis of a population comprising of 100 carper (*Capparis spinosa* L.) samples collected from south of Tehran.

Traits	Cluster										Overall average
	1	2	3	4	5	6	7	8	9	10	
MSP	7.5 \pm 1.58	6.5 \pm 1.58	9.43 \pm 1.72	11.5 \pm 1.58	36.5 \pm 1.58	11.5 \pm 1.58	11.67 \pm 1.58	8.1 \pm 2.17	4.5 \pm 1.58	9.67 \pm 1.53	11.69
BMS	18.5 \pm 1.58	11.5 \pm 1.58	25.43 \pm 1.72	21.5 \pm 1.58	18.5 \pm 1.58	22.5 \pm 1.58	17.67 \pm 1.58	13.62 \pm 2.56	6.5 \pm 1.58	25.67 \pm 1.53	18.14
PA	3.15 \pm 0.16	4.15 \pm 0.16	4.14 \pm 0.17	7.65 \pm 0.16	9.15 \pm 0.16	5.15 \pm 0.16	9.17 \pm 0.16	7.95 \pm 0.38	4.15 \pm 0.16	4.17 \pm 0.15	5.88
LBS	3.7 \pm 1.34	7.5 \pm 1.58	13.43 \pm 1.72	14.5 \pm 1.58	13.5 \pm 1.58	5.5 \pm 1.58	14.67 \pm 1.58	13.48 \pm 1.81	10.5 \pm 1.58	13.67 \pm 1.53	11.05
FBS	32.5 \pm 1.58	32.5 \pm 1.58	56.86 \pm 1.35	47.5 \pm 1.58	62.5 \pm 1.58	66.5 \pm 1.58	73.44 \pm 1.67	48.76 \pm 6.3	34.5 \pm 1.58	59 \pm 1	51.41
NFP	31.5 \pm 1.58	61.5 \pm 1.58	36.86 \pm 1.35	70.5 \pm 1.58	158.5 \pm 1.58	70.5 \pm 1.58	124.44 \pm 1.67	83.57 \pm 24.01	98.5 \pm 1.58	39 \pm 1	77.49
LS	40 \pm 2.98	72 \pm 2.98	73.14 \pm 3.02	86 \pm 2.98	34 \pm 2.98	52 \pm 2.98	109.56 \pm 2.79	128.29 \pm 5.26	129 \pm 2.98	76 \pm 2	80.00
LL	2.07 \pm 0.08	2.68 \pm 0.19	2.27 \pm 0.11	2.66 \pm 0.11	2.87 \pm 0.24	2.12 \pm 0.19	2.74 \pm 0.21	2.51 \pm 0.24	2.53 \pm 0.19	2.3 \pm 0.26	2.48
LW	1.71 \pm 0.09	2.46 \pm 0.18	1.96 \pm 0.11	2.06 \pm 0.13	2.24 \pm 0.14	1.64 \pm 0.12	2.43 \pm 0.27	1.9 \pm 0.15	2.13 \pm 0.18	1.9 \pm 0.26	2.04
LLTLW	1.21 \pm 0.05	1.09 \pm 0.04	1.16 \pm 0.1	1.3 \pm 0.08	1.28 \pm 0.05	1.29 \pm 0.05	1.13 \pm 0.08	1.33 \pm 0.16	1.19 \pm 0.1	1.22 \pm 0.1	1.22
PL	0.5 \pm 0.05	0.79 \pm 0.06	0.59 \pm 0.07	0.67 \pm 0.05	0.65 \pm 0.05	0.64 \pm 0.05	0.72 \pm 0.07	0.64 \pm 0.1	0.75 \pm 0.07	0.53 \pm 0.06	0.65
NTS	80 \pm 5.96	144 \pm 5.96	146.29 \pm 6.05	172 \pm 5.96	68 \pm 5.96	104 \pm 5.96	219.11 \pm 5.58	256.57 \pm 10.53	258 \pm 5.96	152 \pm 4	160.00
LTPS	32 \pm 2.39	57.6 \pm 2.39	58.51 \pm 2.42	68.8 \pm 2.39	27.2 \pm 2.39	41.6 \pm 2.39	87.64 \pm 2.23	102.63 \pm 4.21	103.2 \pm 2.39	60.8 \pm 1.6	64.00
FBW	0.52 \pm 0.14	0.6 \pm 0.09	0.98 \pm 0.14	0.72 \pm 0.08	0.59 \pm 0.07	0.6 \pm 0.08	0.79 \pm 0.14	0.7 \pm 0.11	0.54 \pm 0.06	0.67 \pm 0.11	0.67
PeL	2.68 \pm 0.2	2.7 \pm 0.42	3.57 \pm 0.35	3.27 \pm 0.29	2.65 \pm 0.24	2.36 \pm 0.23	3.07 \pm 0.22	2.59 \pm 0.19	2.13 \pm 0.12	3.17 \pm 0.29	2.82
FBL	11.48 \pm 1.67	14.92 \pm 2.13	14.86 \pm 1.88	15.34 \pm 0.38	12.35 \pm 1.09	13.17 \pm 1.17	14.46 \pm 1.21	14.54 \pm 1.83	13.95 \pm 1.32	16.7 \pm 0.87	14.18
FBD	12.68 \pm 1.86	13.54 \pm 1.35	14.36 \pm 1.94	13.65 \pm 1.02	12.43 \pm 0.55	13.4 \pm 1.88	15.08 \pm 1.61	15.18 \pm 2.43	14.55 \pm 1	17.27 \pm 1.61	14.21
FBLTFBD	0.92 \pm 0.19	1.11 \pm 0.15	1.04 \pm 0.09	1.13 \pm 0.08	1 \pm 0.12	0.99 \pm 0.12	0.97 \pm 0.18	0.97 \pm 0.13	0.96 \pm 0.09	0.97 \pm 0.06	1.01
FPP	28 \pm 7.45	63 \pm 7.45	31 \pm 8.66	37 \pm 7.45	72 \pm 7.45	30 \pm 7.45	110 \pm 7.91	81.9 \pm 12.79	66 \pm 7.45	31 \pm 5	54.99
FW	3.57 \pm 1.72	4.72 \pm 1.67	3.18 \pm 1.32	5.9 \pm 1.83	6.67 \pm 1.69	5.04 \pm 2.31	8.84 \pm 3.37	6.61 \pm 1.95	6.85 \pm 3.13	1.88 \pm 0.35	5.33
FL	25.56 \pm 4.21	34.29 \pm 6.07	31 \pm 3.5	37.7 \pm 3.93	36.15 \pm 3.18	30.41 \pm 5.65	39.3 \pm 5.68	39.19 \pm 5.65	32.28 \pm 4.7	24.03 \pm 1.42	32.99
FD	16.4 \pm 2.9	17.07 \pm 2	14.21 \pm 2.56	18.36 \pm 1.87	18.97 \pm 2.1	16.93 \pm 2.75	20.86 \pm 2.68	18.28 \pm 1.68	19.59 \pm 3.26	16.9 \pm 6.41	17.76
FLTFD	1.56 \pm 0.13	2 \pm 0.24	2.21 \pm 0.25	2.06 \pm 0.19	1.92 \pm 0.19	1.8 \pm 0.18	1.88 \pm 0.11	2.14 \pm 0.24	1.66 \pm 0.14	1.54 \pm 0.48	1.88
FSL	7.45 \pm 0.37	7 \pm 0.71	8.29 \pm 0.39	7.05 \pm 0.44	7.5 \pm 0.97	6.92 \pm 0.58	8.56 \pm 0.63	7.18 \pm 0.97	6.29 \pm 0.33	8.83 \pm 0.29	7.51
FSW	3.84 \pm 0.94	2.8 \pm 0.41	6.28 \pm 0.31	2.69 \pm 0.37	2.71 \pm 0.37	3.56 \pm 1.21	4.64 \pm 0.85	3.34 \pm 0.68	3.22 \pm 0.37	2.83 \pm 0.5	3.59
SPF	166 \pm 68.55	173.8 \pm 100.63	220.29 \pm 83.09	183.2 \pm 82.07	259.4 \pm 146.17	193.4 \pm 93.2	309.89 \pm 126.06	295.19 \pm 145.56	297.2 \pm 144.88	230 \pm 133.13	232.84
SWPF	1.34 \pm 0.56	1.54 \pm 0.92	2.12 \pm 0.83	1.79 \pm 0.87	2.88 \pm 2.03	1.78 \pm 0.99	2.46 \pm 0.94	2.54 \pm 1.17	2.42 \pm 1.25	2.17 \pm 1.23	2.10
TGW	8.13 \pm 0.42	8.8 \pm 0.24	9.54 \pm 0.84	9.75 \pm 0.77	10.6 \pm 2.33	9.09 \pm 1.15	7.96 \pm 0.43	8.71 \pm 1.86	8.08 \pm 0.42	9.39 \pm 0.91	9.01
Yield	94.96\pm49.62	292.96\pm94.64	91.97\pm29.32	211.21\pm53.93	476.86\pm121.58	146.21\pm61.62	959.13\pm330.69	534.62\pm149.16	443.04\pm185.61	57.27\pm4.29	330.82
Members no.	10	10	7	10	10	10	9	21	10	3	

Fruit yield contributing traits have been highlighted

Our study showed that the morphological diversity in the studied population was very significant.

Thus, crossing between these samples may produce useful recombinants.

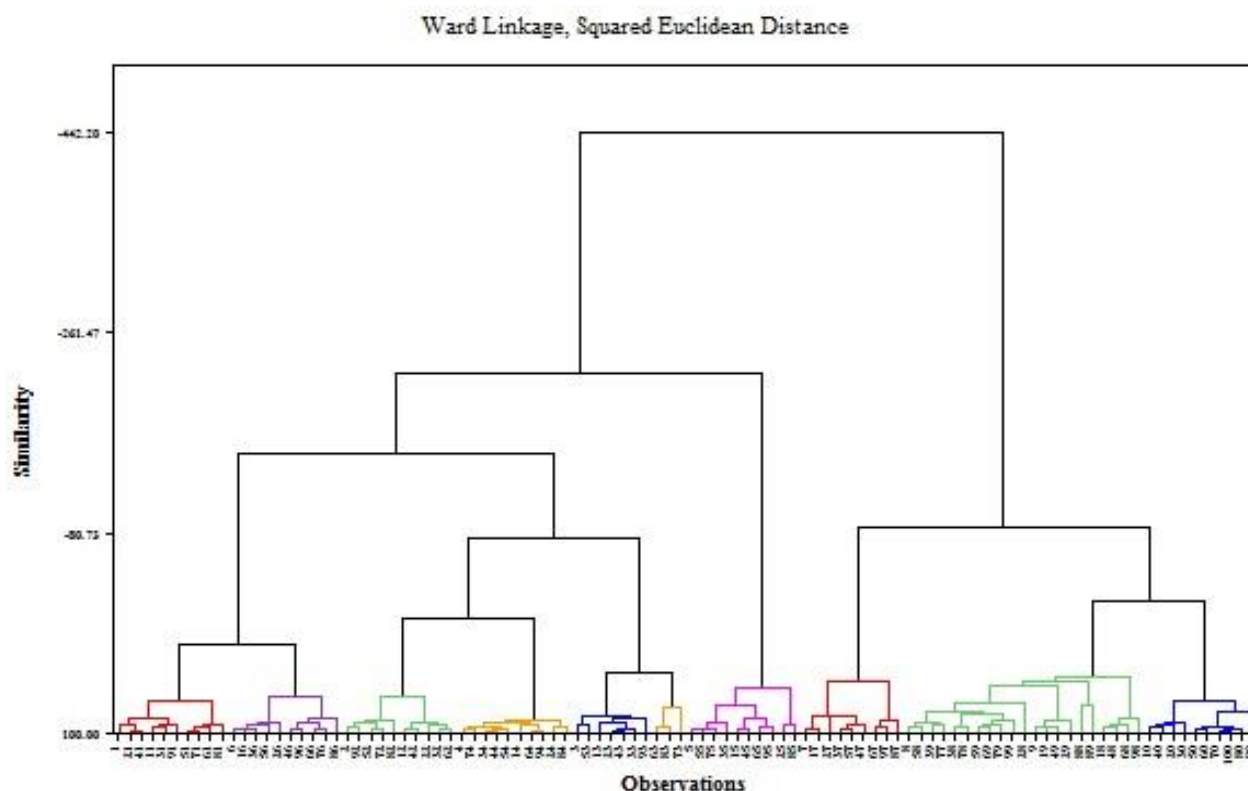


Figure 4. Dendrogram obtained from cluster analysis of a population comprising of 100 carper (*Capparis spinosa* L.) samples collected from south of Tehran.

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