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Genetic diversity of spinach (*Spinacia oleracea* L.) landraces collected in Iran using some morphological traits

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ABSTRACT

Spinach has become an important vegetable crop in most regions of the world and remarkable changes in production amounts have occurred in the past decades due to demand increase in many countries. Fifty-four spinach landraces collected from diverse geographical regions of Iran were evaluated for several qualitative and quantitative traits. Landraces indicated a high variability for measured morphologic characteristics regarding results of variance analysis and descriptive statistics. The first three factors of factors analysis explained 76.8% of variation of spinach landraces. The first extracted factor can be regarded as a leaf property vector; the extracted second factor could be named as yield vector and the third factor was female plants percent vector. The dendrogram of cluster analysis generated from genotypes distance matrices showed that in a distance linkage of 800, the 54 spinach landraces could be agglomerated into sixteen clusters. The number of clusters was verified by multivariate analysis of variance test through Wilks' Lambda statistics. Some spinach landraces such as G10 G13, G38 and G41 were individual cluster and were not similar to the other collected genotypes while some of the spinach landraces were similar to each other and grouped as one cluster such as cluster 9 (C9). The cluster C14 (landrace Karaj 2) was the most favorable genotype due to good performance for most measured quantitative traits. This landrace could be recommended for commercial release after complementary experiments. Also, landraces G1 (Arak) and G3 (Urmia) indicate good potential regarding the measured traits. These landraces could be used directly as commercial cultivars or introduced in spinach breeding programs.

Key words: germplasm, morphological variation, multivariate analysis, spinach

IZVLEČEK

GENETSKA RAZNOLIKOST AKCESIJ ŠPINAČE (*Spinacia oleracea* L.) ZBRANIH V IRANU, DOLOČENA Z NEKATERIMI MORFOLOŠKIMI ZNAKI

Špinača je postala pomembna zelenjadnica v večjem delu sveta in znaten porast njene pridelave se je pojavil zaradi vse večjega povpraševanja v mnogih državah. 54 akcesij špinače, nabranih v različnih delih Irana, je bilo ovrednotenih na osnovi številnih kvalitativnih in kvantitativnih znakov. Akcesije so pokazale veliko variabilnost v merjenih morfoloških znakih glede na rezultate analize variance in opisne statistike. Prvi trije faktorji faktorske analize so pojasnili 76.8 % variabilnosti akcesij špinače. Prvi faktor od teh je bil povezan z lastnostmi listov, drugi s pridelkom in tretji z deležem ženskih rastlin. Dendrogram klasterske analize, generiran na osnovi izračunanih distanc med genotipi je pokazal, da lahko na osnovi distančne povezave 800, 54 akcesij špinače združimo v 16 skupin. Število skupin je bilo potrjeno z multivariatno analizo variance s pomočjo Wilks' Lambda statistke. Nekatere akcesije kot na primer G10 G13, G38 in G41 so bile samostojne skupine in niso bile podobne drugim zbranim genotipom, med tem ko so si bile druge akcesije podobne in so se uvrstile v eno skupino, npr. skupino 9 (C9). Skupina C14 (akcesija Karaj 2) je bila najboljši genotip glede na dobre vrednosti za večino merjenih kvantitativnih znakov. To akcesijo bi lahko priporočili za komercialno uporabo po dopolnih preizkusih. Tudi akcesiji G1 (Arak) in G3 (Urmia) kažeta dober potencial glede na merjene znake. Ti akcesiji bi bili lahko neposredno uporabljeni kot komercialni sorti ali vključeni v žlahtniteljski program špinače.

Ključne besede: genski material, morfološka variabilnost, multivariatna analiza, špinača

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1 INTRODUCTION

Spinach (*Spinacia oleracea L.*) is an edible and annual plant that grows rapidly and has the ability to survive over moderate winter. It is versatile which is used as a salad, a cooked vegetable or as a component of many other cooked meat and vegetable dishes (Sensoy et al., 2011). Leafy vegetables are an important part in the human diet and spinach is one of the dark green leafy vegetables which contains high beta carotene and folate, and is also a good source of vitamin C, calcium, iron phosphorous, sodium and potassium (Dicoteau, 2000; Avsar, 2011). Spinach as dioecious specie with both male and female plants is an herbaceous leafy vegetable in the family of *Amaranthaceae* (Salk et al., 2008) and its leaves are alternate, simple, from ovate to triangular-based, with larger leaves at the base of the plant and small leaves higher on the flowering stem (Vural et al., 2000).

Today, China, the United States, Indonesia, Japan and Turkey are among the largest commercial producers of spinach (FAO, 2011). Iran is the one of the spinach producers with about 105 thousand tons per year based on FAO statistics. The average yield of spinach in Iran is 2096 kg ha^{-1} while world's average yield is 2420 kg ha^{-1} (FAO, 2011). Also, the average yield of spinach in China is 2768 kg ha^{-1}, in the United States is 2360 kg ha^{-1}, Indonesia is 3424 kg ha^{-1}, Japan is 12471 kg ha^{-1}, and Turkey is 9249 kg ha^{-1} (FAO, 2011). Spinach is native to southwest Asia and commonly thought to have originated in Iran (Nonnicke, 1989; Swiader and Ware, 2002) and was first mentioned by the Chinese as the herb of Persia. It was first cultivated in North Africa, came to northern Europe by way of Spain, documented in Germany and then was a common garden vegetable by 1500 in England and France (Dicoteau, 2000; Swiader and Ware, 2002)

Although, hybrids cultivars of spinach were introduced in the 1950's and they have become the major type of spinach cultivars (Morelock and Correll, 2008), but Iranian farmers currently use native spinach landraces, which have good adaptability to different local conditions. The yield performance of these landraces is very low (typically about 2000 kg ha^{-1}) compared with the highest global yields (12471 kg ha^{-1}, produced in Japan; FAO 2011). Therefore, it is essential for Iran to has had spinach breeding program for increasing the genetic potential of yield as well as other important traits. Since Iran is a centre of genetic diversity of many cultivated plants, including wheat, alfalfa, spinach and etc, it is essential to conserve these important resources. Most of the spinach accessions are landraces which are highly adapted to specific environmental conditions and are useful sources of genetic variation (Asadi and Hasandokht, 2007). However, utilization of the genetic potential of different germplasms needs detailed knowledge about these genetic collections (Morelock and Correll, 2008), including characterization, evaluation and classification.

Multivariate procedures are useful for characterization, evaluation and classification of germplasm collections when a large number of accessions are to be assessed for several traits. The usefulness of multivariate procedures for handling morphological variation in plant genetic resources has been proved in many crops; wheat (Damania et al., 1996; Sorghum (Ayana and Bekele, 1999). The generated information of multivariate procedures can be useful for identifying different accessions that have explained traits for crossing, for planning efficient plant improvement program. Also, it is possible to establish core collections for revealing the structure of variation in plant genetic resources and for investigating some aspects of crop evolution (Perry and McIntosh, 1991; Ayana and Bekele, 1999).

Some investigations have been performed in the past on Iranian spinach germplasm collections, but most of them studies are limited with either using only univariate statistics or studying samples from a limited geographical range (Benedictos, 1999; Asadi and Hasandokht, 2007; Eftekhari et al., 2010). The objective of this investigation was to determine the structure of distribution of morphological variation for 10 quantitative traits and 9 qualitative traits in 54 accessions of native Iranian spinach germplasm collections sampled from a wide geographical range of Iran and identify groups of accessions with similar quantitative traits.

2 MATERIALS AND METHODS

2.1 Trial protocol

Fifty-four native Iranian spinach germplasm collections were collected as seed in Iran, and then evaluated in the field in a randomized complete block design (RCBD) replicated four times. Each spinach germplasm was collected as seed multiplied by the farmers. The geographical properties of the 54 sites of the collected spinach landraces are given in Table 1. Field soil was calcareous, loamy structure, low organic matter, and low salt content. Also, it had poor nitrogen and phosphorous and adequate potassium. Fertilization was carried out by spreading 80 kg N ha^{-1} (half of N at sowing stage and half of N at seedling emergence). Sowing was done manually at the rate of 50 kg seed ha^{-1}. Each plot contained six 3 m long rows with 25 cm between rows and plot size was 4.5 m^2. Control by hand weeding was carried out twice when the weed density was high, in the pre-flowering and post-flowering stages. The harvested plot size was 2.5 m^2 (four 2.5 m rows at the center of each plot).

Several quantitative traits consist on leaf length (LL), leaf width (LW), petiole length (PL), petiole diameter (PD), leaf area (LA), leaf numbers in flowering (LN), days to flowering (DF), female plants percent (FP), fresh yield (FY) and dry yield (DY) were measured. Also, various qualitative traits consist on leaf texture (LT, 1=smooth, 2=slight crinkled, 3= crinkled), seed type (ST, 1=smooth, 2=prickly), stem anthocyanin (SA, 1=very low, 3=low, 5=intermediate, 7=high, 9=very high), petiole attitude (PA, 1=erect, 2=semi-spared, 3= spared), vegetative leaf shape (VL, 1=elliptic, 2=broad elliptic, 3=circular, 4=ovate, 5=broad ovate, 6=triangular), reproductive leaf shape (RL, 1=smooth, 2=pointy); leaf edge (LE, 1=smooth, 2= rippler); leaf color (LC, 1=yellow-green, 2=grey-green, 3=blue-green); seed color (SC, 1=yellow-green, 2=grey-green, 3=blue-green) were measured.

2.2 Statistical analysis

The datasets were first tested for normality by Anderson and Darling normality test using MINITAB version 16 (2010) statistical software. Analysis of variance was performed to evaluate differences among measured quantitative traits and the accessions were compared by LSD (least significant differences) criteria. The factor analysis (Cattell, 1965), which consisted of the reduction of a large number of correlated variables to a much smaller number of groups of variables called factors. After extraction, the matrix of factor loading was submitted to a varimax orthogonal rotation, as applied by Kaiser (1958). The array of communality, the amount of variance of a variable accounted by the common factors together, was estimated by the highest correlation coefficient in each array as suggested by Seiller and Stafford (1985). The 54 spinach accessions were clustered using the SPSS 16 (SPSS, 2008), which grouped the accessions into different clusters. The measure of dissimilarity was Euclidean distance and the clustering method was un-weighted pair group method using centroids or UPGMC (Sneath and Sokal, 1973). The number of clusters was determined using multivariate ANOVA via Wilks' lambda statistics.

Table 1: Geographical properties of the 54 locations where spinach landraces are collected

No.	Name	Longitude	Latitude	Altitude (meter)	No.	Name	Longitude	Latitude	Altitude (meter)
1	Arak	49° 41′ E	34 ° 05′ N	1755	28	Qum	50° 53 ′ E	34 ° 38′ N	930
2	Ardestan	52° 22′ E	33 ° 23′ N	1205	29	Gochan	58° 30 ′ E	37 ° 06′ N	1240
3	Urmia	45° 04′ E	37 ° 33′ N	1340	30	Kashan	51° 27 ′ E	33 ° 59′ N	950
4	Esfahan 1	52° 02′ E	32 ° 32′ N	1525	31	Karaj 1	50° 97 ′ E	35 ° 82′ N	1300
5	Esfahan 2	51° 35 ′ E	33 ° 10′ N	1570	32	Karaj 2	50° 85 ′ E	35 ° 80′ N	1350
6	Bojnord	57° 19 ′ E	37 ° 28′ N	1070	33	Karaj 3	50° 87 ′ E	35 ° 86′ N	1230
7	Brojerd	48° 45 ′ E	33 ° 53′ N	1580	34	Kerman	57° 05 ′ E	30 ° 17′ N	1775
8	Beenab	46° 05 ′ E	37 ° 53′ N	1290	35	Kermanshah	47° 65 ′ E	34 ° 31′ N	1400
9	Birjand	59° 21 ′ E	32 ° 87′ N	1491	36	Lahijan 1	50° 14 ′ E	37 ° 26′ N	-11
10	Tabriz	46° 18 ′ E	38 ° 04′ N	1366	37	Lahijan 2	50° 11 ′ E	37 ° 16′ N	-10
11	Chamkahriz	51° 18 ′ E	32 ° 18′ N	1685	38	Langrood	50° 14 ′ E	37 ° 19′ N	-25
12	Khoramabad	48° 21 ′ E	33 ° 29′ N	1200	39	Mako	44° 55 ′ E	39 ° 28′ N	1182
13	Drood	48° 70 ′ E	33 ° 40′ N	1326	40	Mobarake	51° 30 ′ E	32 ° 21′ N	1900
14	Rahimabad	51° 57 ′ E	32 ° 28′ N	1550	41	Maragheh 1	46° 16 ′ E	37 ° 21′ N	1477
15	Rahnan 1	51° 36 ′ E	32 ° 41′ N	1545	42	Maragheh 2	46° 20 ′ E	37 ° 24′ N	1485
16	Rahnan 2	51° 40 ′ E	32 ° 42′ N	1525	43	Mashahad	59° 36 ′ E	36 ° 18′ N	979
17	Zabol	61° 29 ′ E	31 ° 01′ N	475	44	Malekan 1	46° 06 ′ E	37 ° 08′ N	1302
18	Zanjan	48° 40 ′ E	36 ° 40′ N	1650	45	Malekan 2	46° 09 ′ E	37 ° 03′ N	1291
19	Saveh	50° 05 ′ E	35 ° 10′ N	998	46	Minandab	46° 06 ′ E	36 ° 57′ N	1314
20	Salmas	44° 76 ′ E	36 ° 19′ N	1398	47	Mianeh	47° 72 ′ E	37 ° 41′ N	1100
21	Sanandaj	46° 89 ′ E	35 ° 31′ N	1518	48	Hamadan	48° 31 ′ E	34 ° 48′ N	1850
22	Sirjan	55° 40 ′ E	29 ° 27′ N	1735	49	Varamin 1	51° 39 ′ E	35 ° 19′ N	915
23	Shiraz 1	52° 22 ′ E	29 ° 37′ N	1540	50	Varamin 2	51° 38 ′ E	35 ° 11′ N	911
24	Shiraz 2	52° 12 ′ E	29 ° 17′ N	1320	51	Varamin 3	51° 28 ′ E	35 ° 19′ N	923
25	Shirvan	57° 92 ′ E	37 ° 40′ N	1492	52	Varamin 4	51° 38 ′ E	35 ° 23′ N	918
26	Salehabad	50° 57 ′ E	34 ° 31′ N	970	53	Varamin 5	51° 35 ′ E	35 ° 19′ N	905
27	Ajabsher	45° 55 ′ E	37 ° 28′ N	1330	54	Yazd	54° 21 ′ E	31 ° 53′ N	1215

3 RESULTS AND DISCUSSION

All of the quantitative dataset was normal according to Anderson and Darling normality test, and so no transformation was applied for traits (data not shown). Some descriptive statistics including minimum value, maximum value, arithmetic mean and coefficient of variation (CV) for all measured traits (variables) of 54 spinach genotypes are presented in Table 2. For example, the minimum amount of fresh yield was 5949.60 kg ha^{-1}, the maximum amount of fresh yield was 44957.00 kg ha^{-1} and the average fresh yield of studied genotypes was 22151.82 kg ha^{-1}. The maximum leaf length was 15.98 cm; the minimum leaf length was 5.87 cm and the average leaf length was 10.16 cm. The maximum, minimum and average leaf numbers at flowering time were 24, 12 and 16.93, respectively. The maximum percent of female plants was 84 %, the minimum percent of female plants was 20 %, and the average percent of female plants was 54.88 %. Such information can be derived for the other traits from Table 2. Regarding CV values which ranges from 6 (in days to flowering) to 40 % (in fresh yield) in quantitative traits and ranges from 32 (in leaf edge) to 46 % (in vegetative leaf shape) in quantitative traits, indicates remarkable variation among 54 spinach landraces (Table 2).

The results of factor analysis are given in Table 3. When fitting the factor analysis model, the first three factors explained 76.8 % of variation for spinach landraces. The first factor extracted can be regarded as a leaf property vector (Table 3). It has high loadings for five traits as leaf length, leaf width, petiole length, leaf area and leaf numbers in flowering, which all of them were the related to leaf characteristics. This factor accounted for 50.6 % of the total variation in spinach landraces data set. The extracted second factor could be named as yield vector and accounted for 15.4 % of the total data variability. It has high loadings for days to flowering, petiole diameter, fresh yield and dry yield traits, which petiole diameter, fresh yield and dry yield were the related to yield performance. The third factor is a female plants percent vector (Table 3) which shows this trait had high loadings in this factor and accounted for 10.7 % of the total data variability. It seems that leaf property vector as the most important factor and yield vector are more influent characteristics among nine measured quantitative traits.

Table 2: Descriptive statistics of the measured traits in 54 spinach landraces

Traits	Max.	Min.	Average	CV
Leaf length (cm)	15.98	5.87	10.16	0.17
Leaf width (cm)	10.50	2.61	6.31	0.24
Petiole length (cm)	13.3	4	8.40	0.22
Petiole diameter (mm)	14.6	6	10.62	0.15
Leaf area (cm^2)	118.8	11.2	53.88	0.36
Leaf numbers in flowering	24	12	16.93	0.14
Days to flowering	183	137	162.36	0.06
Female plants percent	84	20	54.88	0.20
Fresh yield (kg ha^{-1})	44957.00	5949.60	22151.82	0.40
Dry yield (kg ha^{-1})	4286.90	526.00	2161.66	0.38
Leaf texture	3	1	1.74	0.43
Seed type	2	1	1.24	0.35
Stem anthocyanin content	9	1	1.52	0.46
Petiole attitude	3	1	1.85	0.37
Vegetative leaf shape	6	1	1.65	0.46
Reproductive leaf shape	2	1	2.31	0.44
Leaf edge	2	1	1.57	0.32
Leaf color	3	1	2.02	0.39
Seed color	3	1	2.39	0.45

LT, Leaf texture (1=smooth, 2=slight crinkled, 3= crinkled); Seed type (1=smooth, 2=prickly); SA, Stem anthocyanin (1=very low, 3=low, 5=intermediate, 7=high, 9=very high); PA, Petiole attitude (1=erect, 2=semi-spared, 3= spared); VL, Vegetative leaf shape (1=elliptic, 2=broad elliptic, 3=circular, 4=ovate, 5=broad ovate, 6=triangular); RL, Reproductive leaf shape (1=smooth, 2=Pointy); LE, Leaf edge (1=smooth, 2= Rippler); LC, Leaf color (1=yellow-green, 2=grey-green, 3=blue-green); SC, Seed color (1=yellow-green, 2=grey-green, 3=blue-green).

Table 3: Factor components loadings of quantitative traits obtained from 54 spinach landraces.

	F1	F2	F3
Leaf length (cm)	**0.66**	0.40	0.27
Leaf width (cm)	**0.92**	0.11	0.04
Petiole length (cm)	**0.86**	0.12	-0.08
Petiole diameter (mm)	0.21	**0.78**	0.10
Leaf area (cm^2)	**0.89**	0.29	0.13
Leaf numbers in flowering	**0.63**	0.41	-0.11
Days to flowering	0.07	**0.66**	0.12
Female plants percent	0.04	0.03	**0.97**
Fresh yield (kg ha^{-1})	0.33	**0.90**	-0.08
Dry yield (kg ha^{-1})	0.27	**0.92**	-0.11
Eigen value	5.1	1.5	1.1
% Variance	50.6	15.4	10.7
% Cumulative variance	50.6	66.0	76.8

To better understand the relationships among the quantitative traits of spinach landraces, the relationships are graphically displayed in a plot of factor 1 and factor 2 (Fig. 1). In this plot, the first factor axis mainly distinguishes the methods of leaf width from the other quantitative traits. The second factor axis separates leaf length and petiole length from the other remained quantitative traits (Fig. 1). Therefore, regarding two factors' loading scores, nine measured quantitative traits could be divided into three groups: leaf width as the first group, leaf length and petiole length as the second group, and petiole diameter, leaf area, leaf numbers in flowering, days to flowering, female plants percent, fresh yield and dry yield.

Cluster analysis is a tool for classifying objects into groups. Agglomerative hierarchical clustering methods use the elements of a proximity matrix to generate a tree diagram or dendrogram. The dendrogram generated from genotypes distance matrices showed to clearly group them (Fig. 2). In a distance linkage of 800, the examined 54 spinach landraces could be agglomerated into sixteen clusters. The number of clusters was verified by multivariate analysis of variance test through Wilks' Lambda statistics (data not shown). The related spinach landraces of each sixteen clusters and their qualitative traits are given in Table 4. Some spinach landraces such as G10 G13, G38 and G41 were individual cluster and were not similar to the other collected genotypes while some of the spinach landraces were similar to each other and grouped as one cluster such as cluster 9 (C9) which consist on G17, G12, G23, G37, G28, G34, G36, G40, and G48.

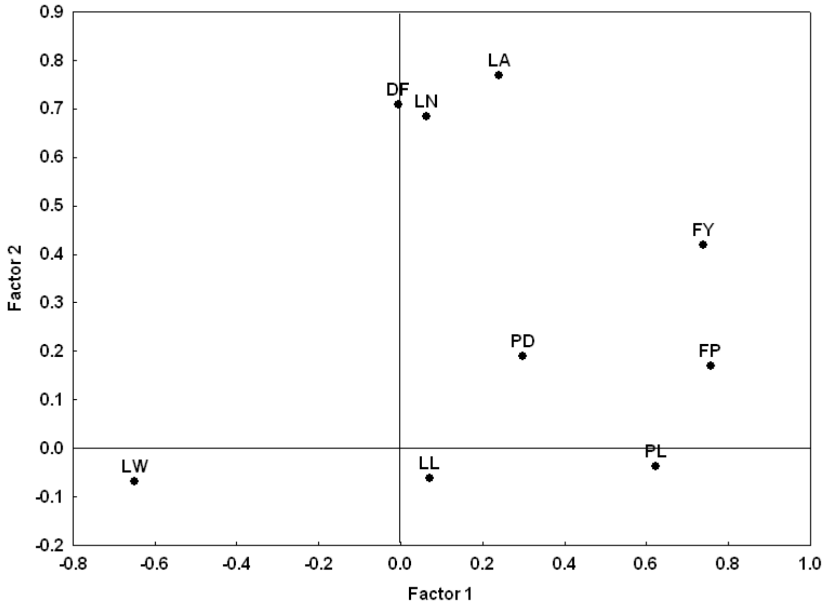

Figure 1: Plot of two first factor analysis of nine traits for the 54 spinach genotypes. LL, Leaf length (cm); LW, Leaf width (cm); PL, Petiole length (cm); PD, Petiole diameter (mm); LA, Leaf area (cm^2); LN, Leaf numbers in flowering; DF, Days to flowering; FP, Female plants percent; FY, Fresh yield (kg ha^{-1}); DY, Dry yield (kg ha^{-1}).

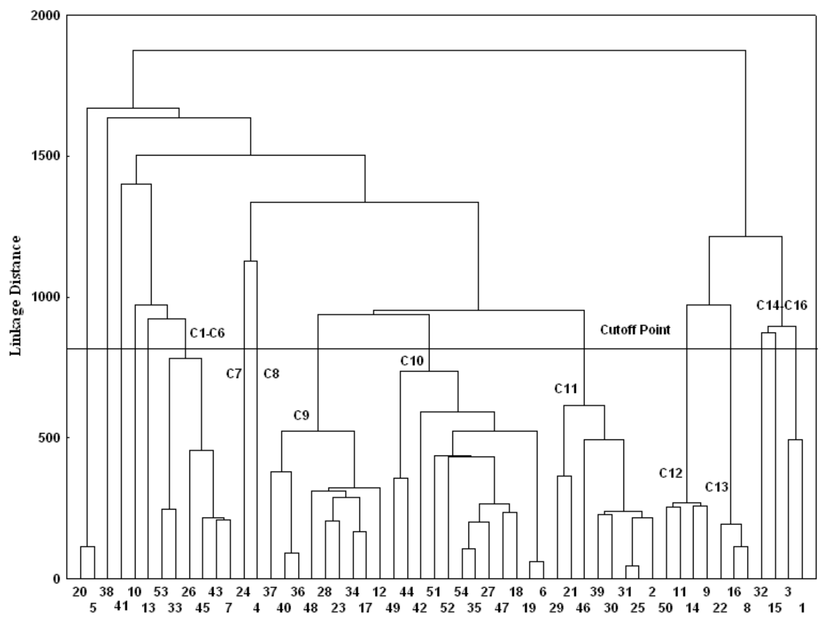

Figure 2: Hierarchical cluster analysis of the 54 spinach genotypes based on Ward's method using measured traits.

The mean and LSD (least significant differences) values of the quantitative traits of sixteen clusters are given in Table 5. The highest leaf length (LL) was belong to cluster C7 (12.57 cm) and the lowest LL was belong to cluster C2 (6.78 cm); and it is clear that, there are good variations in length of spinach landraces. According to the LSD values, sixteen clusters could be divided to six distinct groups based on leaf length. Larger leaves are found at the base of the plant and small leaves are found higher on the flowering stem (LeStrange et al., 1999; Avsar, 2011). The cluster C14 had the largest leaf width (8.18 cm) and the cluster C1 had the smallest leaf width (3.76 cm). According to the LSD values of leaf width, sixteen clusters could be divided to five distinct groups. Both leaf length and leaf width are important traits in spinach yield performance (Asadi and Hasandokht, 2007). Due to high variability of these two traits which is observed in studied landraces, it is possible to establish a breeding program to increase leaf yield in spinach.

The longest petiole length (PL) was seen in the cluster C11 (9.87 cm), while the shortest PL was seen in the cluster C1 (5.51 cm). The LSD values of petiole length divided the sixteen clusters into three distinct groups. The long petiole length is essential for machinery harvesting and genetic improving for having long petiole length is one of the breeding targets of spinach (Eftekhari et al., 2010). Also, the relative length of petiole is a commercial factor for the producing of spinach canner. The most petiole diameter (PD) as 12.81 mm was observed in the cluster C15 and the low PD as 7.5 mm was observed in the cluster C2 (Table 5). There are not any general correlations among petiole length and petiole diameter, but plants that produce the largest petioles also produce in general the thickest (Pandey and Kalloo, 1993; Avsar, 2011).

The largest leaf area (LA) was belong to cluster C14 (76 cm^2) and the smallest LA was belong to cluster C1 (24.06 cm^2). The largest size of leaf area produces the longest leaf length both in absolute length and relative to petiole length, and conversely, the shortest petioles. This would seem to show that the most of petiole length growth was made relatively in early stages, when conditions favorable for growth occurred; growth in leaf length was more rapid than growth in petiole length. The cluster C14 had the most leaf numbers in flowering time (20 leaf) while the cluster C5 had the lowest leaf numbers in flowering time (12.33 leaf). Harvest of spinach plants of marketable size is depending on leaf number and it is correlated with the length of growing period. Spinach is mainly grown for fresh leaves and both the number of leaves and leaf area determine yield performance. However, a high variance was observed for number of leaf in this study which depicted a broad base of the studied landraces for these traits. This maximizes the scope of selection for these traits in the germplasm assayed. Also, the different environmental conditions influences on leaf numbers production and it seem that leaf production per day to be highest under long-day and moderate temperature conditions (Pandey and Kalloo, 1993).

The early flowering cluster was C4 with 146.67 days to flowering and the late flowering clusters were C7 and C8 with 171 days to flowering (Table 5). The higest percent of female plants (64.67%) was seen in cluster C8 and the lowest percent of female plants (46%) was seen in cluster C4. The high fresh yielding landrace was cluster C16 (36429.50 kg ha^{-1}) and the low fresh yielding landrace was cluster C1 (7452.34 kg ha^{-1}). The LSD values of fresh yield divided the sixteen clusters into nine distinct groups. Finally, the high dry yielding landrace was cluster C15 (3405.66 kg ha^{-1}) and the low dry yielding landrace was cluster C1 (727.97 kg ha^{-1}). The LSD values of dry yield divided the sixteen clusters into six distinct groups. It seems that there are remarkable variation in both fresh and dry yield of 54 spinach landraces and these genotypes could be used for increasing yield in future spinach improvement programs.

Regarding all quantitative traits, it seems that cluster C14 which contain only landrace Karaj 2 was the most favorable genotype due to good performance for most measured quantitative traits. Its leaf texture was smooth and so could accumulate low amounts of nitrate, and it had low amounts of anthocyanin (Table 4). Petiole shapeof landrace Karaj 2 is semi-spared and regarding the long petiole length simply could be used for machinery harvest. This landrace has many of good characteristics for proper performance and could be recommended for commercial release after complementary experiments. The finding of such good spinach landrace in this study at Iran as its origin indicates the high potential of native landraces in origin of plants.

After cluster C14, cluster C16 which consist on landraces G1 (Arak) and G3 (Urmia) indicate good potential regarding the measured traits. The leaf texture of these landraces was moderate (slight crinkled), and their anthocyanin content was acceptable (low). The leaf color of both Arak and Urmia landraces was grey-green which is suitable for frizzing industries (Table 4). However, these landraces could be used directly as commercial cultivars or introduced in spinach breeding programs due to high potential in most measured qualitative and quantitative characteristics. Clusters C12, C13 and C15 had good performances for some important traits such as dry yield and are useful sources of genetic variation for improving yield performance in spinach. There were landraces G8 (Beenab), G9 (Birjand), G11 (Chamkahriz), G14 (Rahimabad), G15 (Rahnan 1), G16 (Rahnan 2), G22 (Sirjan), G50 (Varamin 2) in these clusters which are collected from different geographical regions of Iran. It seems that these landraces were variable from other aspects which are not measured in this study. Finally every one of the 54 spinach landraces which is used in this investigation maybe had at least one important trait resource and could be enter to different spinach breeding program based on the breeder target(s).

Spinach is a very important source of nutrients and is dispersed throughout Iran as its origin and all over the world. Plant materials of present investigation were chosen because there are not many studies on spinach especially on landraces. A total of 54 spinach landraces were collected from different geographical regions of Iran which provided morphological data for the landraces. The dendrogram of cluster analysis for the dataset showed 16 groups. Multivariate PCA analysis of morphological data was performed for 3 parameters and the analysis showed good separation of the quantitative traits on the plot based on first two PC. This investigation provided suitable information that may be useful to plant breeders who wish to find the most distinct spinach landraces. For germplasm collections, the results of present investigation may aid to conserve more distinct accessions and to eliminate similar accessions to preparing proper spinach gene-bank in Iran. In future studies, a plant breeder may select two distinct accessions and hybridize them to create a new generation and to obtain one or more new cultivars with favorable characteristics such as resistance to biotic and abiotic stresses.

In conclusion, it was seen that characterization of spinach landraces based on the morphological traits was suitable to assess the genetic diversity among collected spinach landraces. Results of this investigation also can aid to define strategies for further collection. Since our results show that the pattern of observed variation is governed by morphological traits, future germplasm collections should aim to investigate genetic variation via different molecular markers. Also, it is essential to explore variation using more landraces which are collected geographically and climatically from different regions, instead of collecting extensively within individual regions. However, a high variability was observed for most measured traits and obtaining more diverse collections especially exotic germplasm is not needed for future breeding in spinach.

Table 4: The genotypes of 16 clusters and their qualitative characteristics

Class	Landraces	LT	ST	SA	PA	VL	RL	LE	LC	SC
C1	G5, G20	2	1	5	2	4	1	1	2	2
C2	G38	1	1	1	3	6	2	2	2	3
C3	G41	3	1	7	2	3	1	1	3	3
C4	G10	3	1	3	1	2	1	2	3	1
C5	G13	1	1	1	2	5	1	2	2	2
C6	G53, G33, G26, G45, G43, G7	2	1	5, 7	2	2,3	1	2	3	3
C7	G24	2	1	7	2	1	1	1	3	3
C8	G4	1	1	9	2	2	1	2	1	2
C9	G37, G40, G36, G48, G28, G23, G34, G17, G12	1	1	3,5, 9	1, 2	3	1, 2	2	2, 3	2
C10	G49, G44, G42, G51, G52, G54, G35, G27, G47, G18, G19, G6	2	1, 2	1, 5	2, 3	1, 2	1	2	2	2, 3
C11	G29, G21, G46, G39, G30, G31, G25, G2	1, 2	1	3, 7	1, 2	1	1, 2	2	1, 2	3
C12	G50, G11, G14, G9	2	2	1	2	1,3	1	2	2	2
C13	G22, G16, G8	1	1	5, 9	2	4	1	1	2	3
C14	G32	1	1	3	2	1	1	1	1	1
C15	G15	1	1	9	3	2	2	1	1	2
C16	G3, G1	2	2	3	2	1	1	1	2	1

LT, Leaf texture (1=smooth, 2=slight crinkled, 3= crinkled); Seed type (1=smooth, 2=prickly); SA, Stem anthocyanin (1=very low, 3=low, 5=intermediate, 7=high, 9=very high); PA, Petiole attitude (1=erect, 2=semi-spared, 3= spared); VL, Vegetative leaf shape (1=elliptic, 2=broad elliptic, 3=circular, 4=ovate, 5=broad ovate, 6=triangular); RL, Reproductive leaf shape (1=smooth, 2=pointy); LE, Leaf edge (1=smooth, 2= rippler); LC, Leaf color (1=yellow-green, 2=grey-green, 3=blue-green); SC, Seed color (1=yellow-green, 2=grey-green, 3=blue-green).

Table 5: The quantitative characteristics of 16 clusters of spinach landraces

Class	LL	LW	PL	PD	LA	LN	DF	FP	FY	DY
C1	7.20	3.76	5.51	8.67	24.06	14.33	150.50	53.17	7452.34	727.97
C2	6.78	4.64	8.05	7.50	31.67	16.67	162.00	53.33	9158.45	973.10
C3	10.73	5.54	7.63	10.63	47.84	18.33	147.67	59.67	10787.81	1109.33
C4	8.89	4.94	7.13	10.43	38.59	14.00	146.67	46.00	15783.36	1534.02
C5	8.08	5.78	7.05	9.50	35.17	12.33	169.33	52.33	14811.47	1546.44
C6	10.01	5.66	7.95	10.07	47.75	15.83	152.72	54.50	13111.50	1365.14
C7	12.57	6.57	8.30	12.57	69.97	17.33	171.00	62.33	28633.95	2729.43
C8	12.50	6.08	8.77	12.77	68.93	18.00	171.00	64.67	27509.96	2669.30
C9	10.64	7.02	8.93	10.51	58.65	17.37	165.27	55.93	23377.59	2302.94
C10	9.98	6.24	8.20	10.37	52.58	16.58	160.94	53.71	20891.73	2057.99
C11	10.54	7.58	9.87	9.97	61.37	16.83	168.00	64.67	24355.39	2131.18
C12	10.45	6.86	9.51	11.67	58.17	19.33	170.33	48.78	33168.25	3240.43
C13	10.90	6.32	9.39	12.20	59.79	18.00	169.56	57.89	31702.52	3103.02
C14	10.63	8.18	9.79	11.81	76.00	20.00	166.00	46.33	35384.93	3167.40
C15	11.01	6.12	9.01	12.81	60.31	17.00	170.67	61.33	34545.36	3405.66
C16	10.88	7.05	7.83	12.29	68.36	19.50	170.17	54.50	36429.54	3291.19
LSD	0.88	0.86	1.03	0.79	11.00	1.34	5.56	7.05	3395.16	314.65

LL, Leaf length (cm); LW, Leaf width (cm); PL, Petiole length (cm); PD, Petiole diameter (mm); LA, Leaf area (cm^2); LN, Leaf numbers in flowering; DF, Days to flowering; FP, Female plants percent; FY, Fresh yield (kg ha^{-1}); DY, Dry yield (kg ha^{-1}).

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