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THE USE OF SOME MORPHOLOGICAL TRAITS FOR THE ASSESSMENT OF GENETIC DIVERSITY IN SPINACH (*SPINACIA OLERACEA* L.) LANDRACES

ABSTRACT

Investigation of native accessions of spinach (*Spinacia oleracea* L.) would be aid in the development of new genetically improved varieties, so in this research 121 spinach landraces, collected from the various spinach growing areas of Iran, were evaluated to determine their diversity using several agro-morphological traits. High coefficients of variation (CV) were recorded in fresh yield, leaf area and dry yield. Using principal component (PC) analysis, the first three PCs with eigenvalues more than 0.9 contributed 80.56% of the variability among accessions. The first PC was related to leaf yield performance (fresh and dry yields, leaf numbers at flowering and lateral branches) while the PC2 was related to leaf characteristic (leaf width, petiole length, petiole diameter and leaf area). The third PC was related to seed characteristic (seed yield and 1000-seed weight) and was named as seed property component. The 121 spinach landraces were grouped into six clusters using cluster analysis. Each cluster had some specific characteristics of its own and the clusters I and II were clearly separated from clusters III and V and also from clusters IV and VI. The studied accessions are an important resource for the generation of a core collection of spinach in the world. The results of present research will support tasks of conservation and utilization of landraces in spinach breeding programs.

Key words breeding, cluster analysis, diversity . landraces, principal component analysis

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INTRODUCTION

Spinach (*Spinacia oleracea* L.) is an important leafy vegetable, of which the leaves are consumed fresh or processed. Leafy vegetables are important part of the human diet and spinach as the dark green leafy vegetable contains high beta carotene and folate, and is also a good source of calcium, iron, phosphorous and potassium (Dicoteau 2000; Avsar 2011). Spinach is native to central Asia, most probably Iran; it was introduced into Spain by the Moors in the eleventh century (Scheewe and Reimann-Philipp 1986). Spinach belongs to the family of *Amaranthaceae* (Salk *et al.* 2008) and has two types, prickly-seeded and smooth-seeded. It produces rosettes of fleshy leaves, later the stem elongates and forms flower stalks during the reproductive phase. The male and female plants are identified according to carbohydrates and pigment contents which are higher in female than in male plants (Swiader and Ware 2002). The spinach is the most important leafy vegetable crop in various environments of Iran and comprises more than 30% of the total area sown to leafy vegetables. Iran is the seventh largest commercial producers of spinach in world following to China, the United States, Japan, Turkey, Indonesia and France (FAOSTAT 2012). Based on the FAO database, Iran produced about 105 thousand tons with average yield 2000 kg × ha^{-1} while the world's average yield is 2400 kg × ha^{-1} and the average yield of China is about 2800 kg × ha^{-1} (FAOSTAT 2012).

There are several genetic resources for spinach breeding and among them; landraces have been the preferred ones due to their ease of crossing and their usual characteristics. The characterization of landraces is essential for exploiting the variability of important traits such as yield potential and resistance to biotic and abiotic stresses. The diversity of spinach has been studied worldwide (Hu *et al.* 2007; Avsar 2011; Kuwahara *et al.* 2013) but most of them used only molecular markers and have little or no information about the agro-morphological traits. Also, the genetic diversity of Iranian spinach landraces has been studied (Benedictos 1999; Asadi and Hasandokht 2007; Eftekhari *et al.* 2010; Sabaghnia *et al.* 2013) using both molecular markers and agro-morphological traits but there are still some regions that have little or no information available regarding the important agro-morphological traits of their spinach genotypes. Although, above studies are reported from Iran, but the number of collected accessions was restricted, their information is not available for international readers and little is known about the diversity of these accessions.

In order to develop new genetically improved cultivars, a preliminary characterization and evaluation of the genetic variability among local spinach landraces for agronomic performance could be useful for exploitation of this genetic material for breeding programs. Characterization of local landraces for important traits will facilitate efficient synthesis of breeding populations that are designed to accomplish specific objectives (Mavromatis *et al.* 2010). The evaluation of agro-morphological traits is a useful method of evolutionary and pedigree relationship determination. Diversity in genetic resources can be identified by agro-morphological traits screening. Agro-morphological traits have been studied for the determination of genetic diversity and selection criteria in spinach breeding (Avsar 2011; Sabaghnia *et al.* 2013). Evaluation of genetic diversity acts as a starting point for the establishment of “a core collection” where the whole variability in the collection is represented in a small subset of accessions. Local adapted genotypes presents interesting adapted traits, thus information on diversity among spinach accessions are critical in breeding programs and for conservation of genetic resources.

The aim of this research was to screen several Iranian spinach landraces collected from highly diverse ecological regions in order to determine genetic variability and relationship using agro-morphological traits and to detect groups of similar and specific morphological types using thirteen agro-morphological traits.

MATERIALS AND METHODS

The plant materials comprised 120 spinach (*Spinacia oleracea* L.) accessions collected from the major spinach growing areas of Iran. These accessions and one control (check) cultivar (Varamin) were evaluated in a simple lattice design (11 × 11) with four replications. Sowing was performed manually in six rows (25 cm) with depth around 2 cm at the rate of 40 kg seed × ha^{-1}. The size of each plot was 3 m^2 and the harvested area was 2.5 m^2 (four 2.5 rows at the center of each plot). Soil tillage was done by shallow ploughing (30 cm) followed by rotary harrowing. Soil was calcareous and poor for organic matter and had a loamy structure and was slightly alkaline and had poor nitrogen and phosphorous contents and adequate potassium content. Fertilization was carried out by spreading 4 t ha^{-1} of manure, 100 kg N × ha^{-1} and 100 kg P_2O_5 × ha^{-1}. Data on days to flowering starting (days from sowing to appearance of 10% flowers), were recorded as a single value for each plot. Leaf length (cm), leaf width (cm), petiole length (cm), petiole diameter (mm), leaf area (cm^{-2}), leaf/plant at flowering (numbers), female plants (%) and lateral branches/plant (numbers), were recorded from 10 plants that had been randomly chosen in each row and the means of the quantitative data sets were used for analysis. In addition, 1000 mature seeds randomly chosen were used to record weight (g). Also, Fresh yield of harvested leaves at flowering starting (kg × ha^{-1}), dry yield (kg × ha^{-1}) and seed yield were measured.

The datasets were first tested for normality by Anderson and Darling normality test using MINITAB version 16 (2010) statistical software. The data recorded were analyzed for simple descriptive statistics (i.e. mean, coefficient of variation, minimum, maximum and range). Analysis of variance was performed to evaluate differences in measured traits using appropriate models via PROC LATTICE procedure of SAS version 6.12 (SAS 1996). Because the efficiency of the siple lattice design compared with a randomized complete block design (RCBD) was less than 105% for most of measured traits, the data were analyzed as RCBD, assuming blocks as a random effect. Then, dataset was subjected for numerical taxonomic techniques using the procedure of principal component (PC) analysis and cluster analysis using computer software STATISTICA 12.0 (Statsoft 2013) and IBM-SPSS Statistics 22.0 (SPSS 2013). As the various spinach traits were recorded via different scales, the mean observations of traits were standardized to ignore scale differences. PC analysis was performed to explore the importance of different traits in describing multivariate polymorphism (Naghavi and Jahansouz 2005). Cluster analysis was conducted on the basis the measure of dissimilarity as Euclidean distance and the clustering method was un-weighted pair group method using centroids or UPGMC. The number of clusters was determined using multivariate ANOVA via Wilks' lambda statistics. About 30% of landraces was taken at random within each of the four clusters and plotted according to their first two PC scores.

RESULTS

All of the measured traits had normal distribution, and no transformation was applied for traits. Some descriptive statistics for 13 characters in the 121 spinach accessions are presented in Table 1. High coefficients of variation (CV) were recorded in fresh yield (58.94%), leaf area (49.96%) and dry yield (47.72%) revealing a high level of diversity among the Iranian accessions of spinach. Small CV values were observed for the some other traits including days to flowering (7.04%), female plants percent (18.02%) and seed yield (18.46%) characters. The minimum fresh yield was 7397.97 kg × ha^{-1}, the maximum fresh yield was 36580.82 kg × ha^{-1} and the average fresh yield of studied accessions was 22289.46 kg × ha^{-1} (Table 1). The maximum leaf area was 97.60 cm^2; the minimum leaf area was 15.48 cm^2 and the average leaf area was 55.13 cm^2. Such similar information could be obtained from Table 1. The development of most agro-morphological traits of spinach is a highly coordinated process and so for having an effective breeding program; it is necessary to considering all of the important traits which influencing on yield performance.

Table 1

Means, coefficients of variation (CV), minimum and maximum for 13 traits in 121 accessions of Iranian spinach landraces

Measured traits	Mean	CV (%)	Minimum	Maximum	Range
Leaf length [cm]	10.37	24.62	6.43	13.99	7.56
Leaf width [cm]	6.40	33.02	2.80	9.11	6.31
Petiole length [cm]	9.01	37.98	4.49	13.69	9.20
Petiole diameter [mm]	10.79	33.06	5.48	15.86	10.38
Leaf area [cm^{-2}]	55.13	49.96	15.48	97.60	82.12
Leaf numbers at flowering [No]	15.73	35.1	8.00	22.00	14.00
Days to flowering [day]	161.53	7.04	147.00	176.00	29.00
Female plants percent [%]	56.62	18.02	41.10	67.97	26.87
Fresh yield [kg × ha^{-1}]	22289.46	58.94	7397.97	36580.82	29182.85
Dry yield [kg × ha^{-1}]	2182.00	47.72	719.32	3484.24	2764.92
Seed yield [kg × ha^{-1}]	809.41	18.46	536.45	986.00	449.55
1000-seed weight [g]	9.97	36.12	5.77	14.35	8.58
Lateral branches [No]	3.22	33.5	2.00	5.00	3.00

In order to know with which combination type of agro-morphologic traits, the spinach would attain high performance, PC analysis was performed (Table 2). First three main PCs were extracted from the complicated components, the total cumulative variance of these five factors amounted to 80.56% and these components had eigen values >0.9. The PC analysis simplified the complex data by transforming the number of associated traits into a smaller number of variables as PCs. The first PC accounts for maximum variability in the data with respect to succeeding components and accounted for about 59.92% of the variation; PC2 for 11.22% and PC3 for 9.42% (Table 2). The first PC was related to leaf yield performance (fresh and dry yields) and its contributing traits such as leaf numbers at flowering and lateral branches. Thus, the PC1 could be named as leaf yield component. The PC2 was related to leaf characteristic and its contributing traits such as leaf width, petiole length, petiole diameter and leaf area. The other trait, which contributed more positively to PC2, was leaf length suggesting that this component reflected the leaf characteristics of each genotype. Thus, the PC2 could be named as leaf property component. The third PC was related to seed characteristic (seed yield and 1000-seed weight) suggesting that this component reflected the seed characteristic of each genotype. Thus, the PC3 could be named as seed property component.

Table 2

Varimax rotated principal components for 13 traits in the 121 accessions of Iranian spiach landraces

Measured traits	PC1	PC2	PC3
Leaf length	0.401	0.596	0.345
Leaf width	0.367	0.770	0.109
Petiole length	-0.004	0.846	0.100
Petiole diameter	0.327	0.794	0.320
Leaf area	0.409	0.789	0.189
Leaf numbers at flowering	0.851	0.275	0.263
Days to flowering	0.656	0.223	0.401
Female plants percent	0.496	0.376	0.469
Fresh yield	0.833	0.273	0.325
Dry yield	0.859	0.129	0.380
Seed yield	0.375	0.540	0.600
1000-seed weight	0.240	0.168	0.894
Lateral branches	0.795	0.342	-0.041
Eigenvalue	7.790	1.459	0.935
% of Variance	59.924	11.219	9.421
% of Cumulative	59.924	71.143	80.564

The all of 121 spinach landraces in this study were grouped into 6 clusters based on UPGMC and cutoff point which is verified via Wilks' lambda statistics. Cluster I consisted of 6 accessions, cluster II of 18, cluster III of 16, cluster IV of 31, cluster V of 23 and cluster VI of 27 accessions (Table 3). The mean value and the standard deviation for each cluster revealed that accessions in cluster I had low amounts of measured traits and these accessions were early flowering genotypes, whereas accessions grouped in cluster II were low in leaf numbers at flowering, days to flowering, female plants percent, fresh yield, dry yield and lateral branches traits; medium in leaf length, leaf width, petiole diameter, leaf area, seed yield and 1000-seed weight traits; and high in petiole length as relatively long petiole length (Table 3). Accessions in clusters III showed high values for all of the measured traits while accessions in clusters IV showed medium values for all of the measured traits.

Table 3

Mean and standard deviation for 6 clusters analysis based on 13 traits in the 121 accessions of Iranian spinach landraces

Measured traits	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V	Cluster-VI
No. of accessions	6	18	16	31	23	27
Leaf length	7.77 ± 0.65	9.41 ± 0.26	11.34 ± 0.29	10.03 ± 0.14	11.19 ± 0.19	10.70 ± 0.12
Leaf width	4.61 ± 0.40	5.81 ± 0.18	7.11 ± 0.37	6.08 ± 0.14	6.66 ± 0.16	6.90 ± 0.13
Petiole length	6.86 ± 0.81	8.95 ± 0.43	9.29 ± 0.40	8.46 ± 0.25	9.66 ± 0.38	9.43 ± 0.28
Petiole diameter	7.47 ± 0.66	9.87 ± 0.36	12.42 ± 0.34	10.14 ± 0.21	11.77 ± 0.32	11.09 ± 0.26
Leaf area	32.56 ± 4.27	46.17 ± 2.46	67.99 ± 4.57	50.21 ± 1.51	61.01 ± 1.96	59.15 ± 1.75
Leaf numbers at flowering	11.33 ± 0.95	13.44 ± 0.47	20.50 ± 0.38	14.55 ± 0.24	16.96 ± 0.32	15.70 ± 0.20
Days to flowering	151.17 ± 1.64	156.33 ± 0.81	168.31 ± 1.14	160.39 ± 0.76	163.52 ± 0.94	162.89 ± 0.61
Female plants percent	50.61 ± 2.47	52.89 ± 1.10	61.40 ± 1.07	54.29 ± 0.63	60.10 ± 0.63	57.34 ± 0.77
Fresh yield	8884.97 ± 612.58	14351.52 ± 347.78	33600.49 ± 433.31	19496.10 ± 267.05	26702.40 ± 200.90	23305.37 ± 178.12
Dry yield	1227.18 ± 225.41	1692.00 ± 79.31	3078.45 ± 87.55	1988.21 ± 27.50	2431.18 ± 42.04	2199.87 ± 23.89
Seed yield	701.34 ± 37.60	764.87 ± 15.94	873.54 ± 15.61	781.54 ± 12.28	842.17 ± 11.97	829.22 ± 8.18
1000-seed weight	7.24 ± 0.43	8.69 ± 0.22	11.20 ± 0.41	9.73 ± 0.31	10.49 ± 0.37	10.55 ± 0.28
Lateral branches	2.50 ± 0.22	2.94 ± 0.13	3.88 ± 0.13	3.00 ± 0.00	3.52 ± 0.11	3.19 ± 0.08

Cluster V had high amounts of measured traits expect leaf numbers at flowering and dry yield traits which were medium, whereas accessions grouped in cluster VI were high for all measured traits expect leaf numbers at flowering, female plants percent, fresh yield, dry yield and lateral branches traits which had medium values (Table 3). In general, accessions in clusters I and II showed low values for most of spinach traits, whereas accessions in clusters II and V indicated medium values for most measured traits of spinach; and accessions in clusters III and VI indicated high values for most of the spinach measured traits. These useful information on the clusters to which particular accessions with traits of interest belong will aid in researching more accessions with similar characteristics in breeding programs (Naghavi and Jahansouz 2005). There are 31 accessions in cluster IV, including Varamin cultivar, and theoretically, these accessions may be similar to the check cultivar that are grown in Iran.

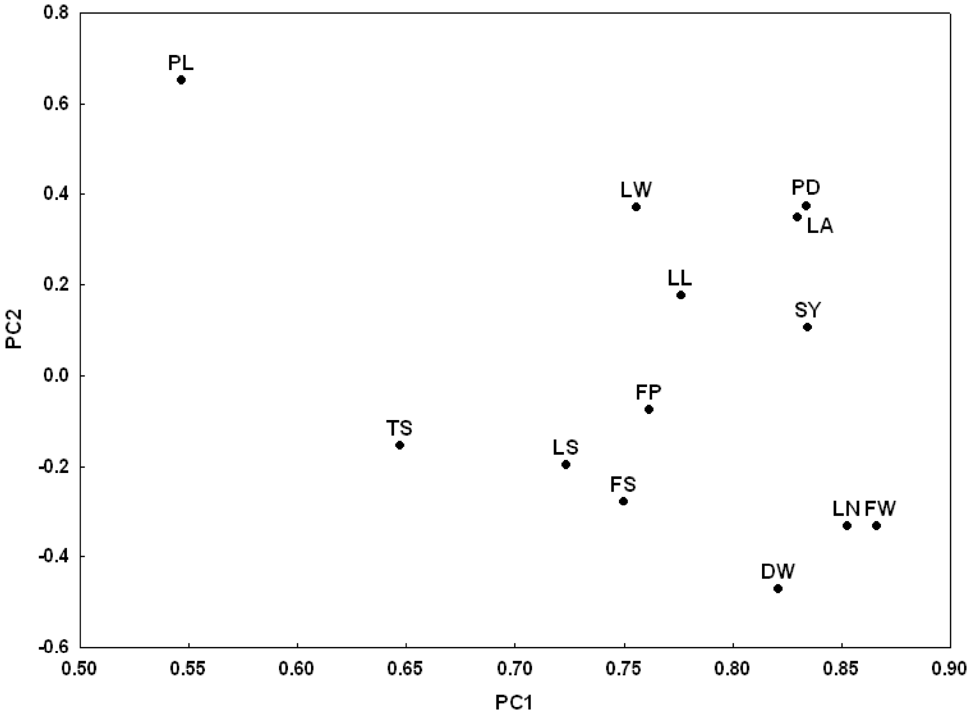

Fig. 1. Principal component analysis (original scores) for 13 traits in the 121 accessions of Iranian spinach landraces

To better understand the relationships among the spinach traits, the relationships are graphically displayed in a plot of PC1 and PC2 (Fig. 1). In this plot, the PC1 axis dose not distinguishes the measured traits, but the PC2 axis separates leaf length, leaf width, leaf area, petiole length, petiole diameter and seed yield from the other measured traits (Fig. 1). The most prominent relation by Fig. 1 is a strong positive association among leaf numbers at flowering, fresh weight and dry weight as indicated by the small obtuse angles between their vectors. Within each cluster 30% of landraces were randomly chosen and the first two PC scores were plotted to help visualization of group differences (Fig. 2). The PC1 could separate relatively accessions of clusters I and II from clusters III and V, but accessions of clusters IV and VI were not clearly separated, which may be related to a mixture of accessions with different morphological traits.

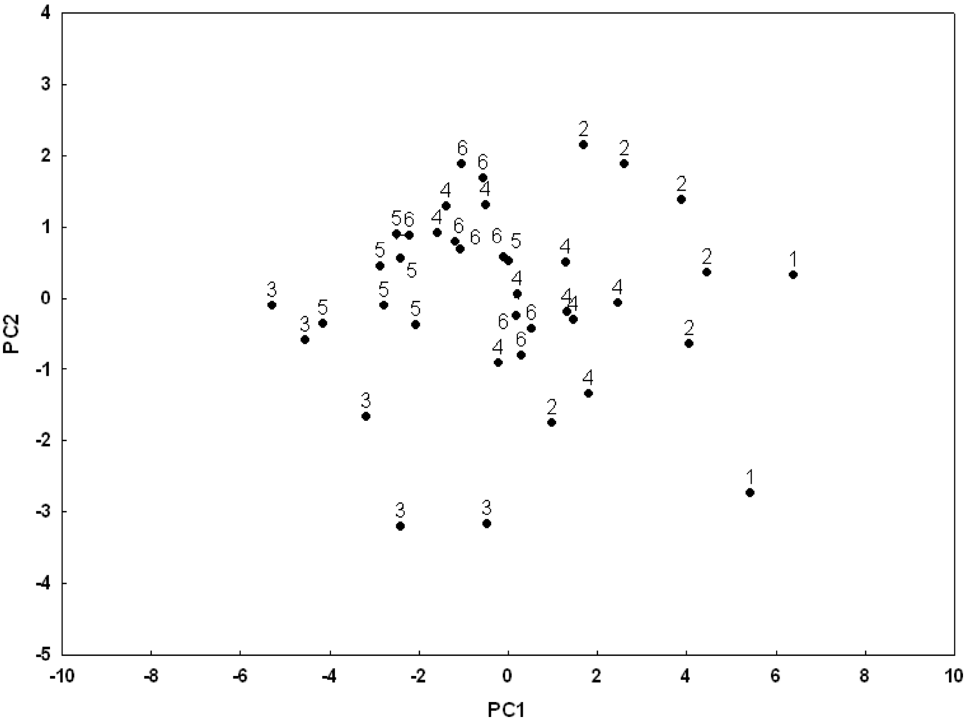

Fig. 2. Principal component analysis for 13 traits in the 121 accessions of Iranian spinach landraces. Plot is shown for 30% of accessions randomly selected within . each cluster1, 2, 3, 4, 5 and 6 represent the cluster number

DISCUSSION

Characterization of native accessions of spinach is an important process for the evaluation and preservation of indigenous local landraces. Such genetic materials have undergone local adaptations through selection for a particular geographic region over many generations. Our study has shown considerable variations in all the agro-morphological traits except for days to flowering. Leaf area, fresh yield and dry yield showed the highest coefficient of variation compared to other traits. The observed ranges for many of the trait values were higher than those described for Iranian Spinach germplasm by Benedictos (1999), Asadi and Hasandokht (2007), Eftekhari *et al.* (2010) and Sabaghnia *et al.* (2013), showing a high degree of polymorphism present in the collected spinach accessions. This indicated that there are diverse combinations of traits at the individual genotype level, indicating possibilities for obtaining desirable trait combinations in specific accessions to meet the demands of plant breeders.

The description of agro-morphological traits is an important prerequisite for efficient utilization of plant materials in every plant breeding program (Barro-Kondombo *et al.* 2010). Evaluation of present assembled Iranian spinach have shown that there is a high level of agro-morphological diversity for most of the measured traits, which may be useful for future breeding tasks. Characters such as days to flowering, female plants percent and seed yield showed low CV values, which appears to limit the scope of selection for these traits in the present spinach accessions. Perhaps, other sources of spinach are required in order to find high variation for the mentioned traits. The observed diversity of agro-morphological traits in spinach germplasm may be associated with ecological adaptation to diverse environmental conditions where the spinach had been grown for long time, followed by intensive selection by vegetable farmers in these diverse conditions. For many centuries, spinach farmers have been selecting seeds and sharing them with other spinach producers. The introduction of landraces by farmers developed hybridization; this hybridization was essential for gene flow, which is needed for permanent incorporation of genetic information (Andersson and De Vicente, 2009).

Multivariate statistical methods, such as the PC analysis and clustering method used in the this investigation, have shown the germplasm groupings in many genetic resources such as blackgram (Ghafoor *et al.* 2001), cacti (Carmona and Casas 2005), chickpea (Naghavi and Jahansouz 2005), sorghum (Barro-Kondombo *et al.* 2010) and rice (Tripathi *et al.* 2013). In our analysis, we were able to determine clusters of accessions that were significantly different from each other for traits of interest. We found that there were no significant relationships between genetic inconsistency and geographical origins because spinach accessions which were collected from one location entered into more than one cluster. In contrast, accessions from different geographical locations were relatively unique and tend to be clustered in one part of the dendrogram which suggests that the spinach agro-morphological variation analyzed is determined not only by environmental differences but also by genetic factors.

The current 121 spinach accessions are relatively sufficient for developing a core collection and the genetic diversity of the collection can be considered for genetic improvement of most spinach traits. For improving some traits such as days to flowering, female plants percent and seed yield, the present spinach accessions are not adequate for generating a core collection and the genetic diversity of the collection can only be considered after additional germplasm is collected and characterized. The proportion of core collection into entire germplasm collection in the main field crops is about 10%, but it seems that for vegetable crops such as spinach this proportion must be enlarged due to limit number of available accessions. For example, Upadhyaya *et al.* (2001) developed a core subset of chickpea consist on 225 accessions from primary 1956 accessions. The number of registered spinach cultivars and accessions in the Institute of Plant Genetics and Crop Plant Research (IPK, 2014) is 208. Therefore, the core set of spinach accessions should be assumed very smaller comparison to major field crops. However, each core collection can only be used effectively after enrichment by additional germplasm from other resources. Therefore, the core set of accessions in all crops should be maintained as a dynamic package rather than static set and so the type and size of the core collections will change when additional accessions introduced (Mavromatis 2010).

The landraces used seems to be a promising genetic material either for cultivar release or for genetic improvement programs since they had the same or better performance in measured traits than the commercial check cultivar. The most promising landraces indicated superior values of leaf yield, seed yield and other components as compared with the commercial spinach check cultivar. The spinach accessions used in this study are a new resource that has been collected from different environmental conditions of Iran and can be used in conjunction with other plant materials from all over the world to design a complete core collection of spinach germplasm. This genetic resource is useful for screening the desirable traits in spinach breeding program. In general, the landraces seem to be different from the commercial cultivars having higher variability for the examined traits by two types of analysis carried out and such variability is desirable in breeding programs.

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