Sci., Tech. and Dev., 32 (1): 7-15, 2013

GENETIC DIVERGENCE IN INDIGENOUS SPINACH GENETIC RESOURCES FOR AGRONOMIC PERFORMANCE AND IMPLICATION OF MULTIVARIATE ANALYSES FOR FUTURE SELECTION CRITERIA

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Abstract

A study was conducted during winter 2010 at National Agricultural Research Center (NARC) Islamabad, (33.40˚N and 73.07˚E), to assess diversity profile of indigenous spinach germplasm collected from different parts of the country. The evaluation was based on 20 qualitative and quantitative parameters following the botanical descriptors developed by Bioversity International. A high variation was observed for plant height, earliness of bolting and days taken to flowering whereas low medium variability was observed for remaining parameters. Trait association based on correlation analysis reflected significant and positive relationship among different attributes that could be useful in devising further crop improvement strategy in spinach. Principal component analysis (PCA) displayed the scattered distribution on the basis of agronomic performance. The cumulative contribution of the first three principal components (PCs) reflected by principal component analysis was 58.4%. Cluster analysis grouped the spinach genotypes into four major clusters regardless of collection origin which yielded useful information of the genotypic relationship among genotypes. In spite of high variability, observed in various traits, low variance for leaf related characters (which are of prime importance) was observed in the investigated germplasm, which restricts the scope of selection for these traits. This advocated the need to acquire more diverse germplasm including local and exotic genotypes for devising better crop improvement strategy in spinach.

Keywords: Spinach accessions, Variability, Correlation.

Introduction

Spinach (*Spinacia oleracea*) is one of the most important leafy vegetables and used predominantly for edible green leaves. Initially, common spinach (*Spinacia oleracea*) has long been considered member of the family Chenopodiaceae. However, in 2003, due to reclassification, the family Chenopodiaceae was combined with Amaranthaceae family under the family name 'Amaranthaceae' (Kadereit et al., 2003). It is native to the central and south-western Asia (Bihter-Avsar, 2011). Its green leaves grow to a height of upto 30 cm, whereas at maturity, the plant attains a height of 1 to 2m. Spinach can survive at low temperature of temperate regions (Bihter-Avsar, 2011). Depending upon the usage, the spinach leaves are generally grouped into three types, including: (i) savoy type (used for fresh market purpose), (ii) processing type (smooth leaf type with flat, unwrinkled and spade-shaped leaves), and (iii) baby spinach, which is preferably used in salads, due to its taste and delicate structure (Bihter-Avsar, 2011). The two main available seed types are either spiny or have a smooth surface.

Among green leafy vegetables, spinach is characterised with more delicate texture and nutrients, particularly, with bone-supportive minerals, like, calcium and magnesium (Kawashima and Soares, 2003). In addition, it contains phosphorus, iron, potassium and vitamins A, B, C, E, and K. Spinach is also enriched with anti-oxidants which scavenge free radicals and adjust blood pressure when

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consumed fresh, steamed or quickly boiled. Spinach contains high content of beta carotene (provitamin A), lutein, folate, vitamin C, calcium, iron, phosphorous and potassium (Morelock and Correll, 2008). In addition, it is also known to have a greater amount of oxalic acid which is formed as a secondary metabolite of vitamin C (Hodgkinson, 1977). With the advent of advanced computer technologies, it has become possible to study the complex relationship among genotypes through multivariate analyses which provides better understanding of the structure, particularly of the large germplasm collections (Martinez-Calvo et al., 2008). In this regard, cluster analysis and principal component analysis (PCA) has been widely used in various studies for delineating the variability profile in large group genotypes of many species (Veasey et al., 2001; Naghavi and Jahansouz, 2005; Žáková and Benková, 2006; Tucak et al., 2009). The current study was initiated to investigate the extent and pattern of variability in the indigenous spinach germplasm collected from diverse agro-climatic conditions of Pakistan.

Materials and Methods

The study was conducted to determine the variability pattern and correlation among 35 different accessions of spinach for various traits. Experiment was conducted in an augmented design under the field conditions of National Agricultural Research Centre, Islamabad, during 2010. The seed of spinach genotypes was obtained from National Genebank, Institute of Agri-Biotechnology and Genetic Resources (IABGR), NARC, Islamabad. Table 1 enlists all the accessions alongwith passport information. Two rows 3m long for each accession were planted with 10cm and 75cm plant spacing and row spacing, respectively. Cultural practices recommended for spinach were followed throughout the crop season. Data for the qualitative as well as quantitative traits was recorded. Quantitative characters, like, number of leaves per plant, leaf length (cm), leaf width (cm), plant height (cm), petiole length (cm), earliness of bolting, number of tillers, days to flowering and days to harvesting were recorded. Trait selection and measurement techniques were based on descriptors developed by Bioversity International (former IPGRI). Averaged data was analysed statistically for means, standard deviation, frequency distribution and simple correlation coefficients using computer programme STATISTICA for Windows. Ward's method was used to construct the phenogram and to calculate Euclidean distances among the accessions as an estimate of the genetic distances.

Table1. List of germplasm and passport information of spinach genotypes

S.No.	Accession No.	Local Name	Collecting Organisation	Collection No.	District	City/Village/Town
1.	28442	Palak	PARC/PGRI	003074(01)	Attock	Hasan Abdal
2.	28443	Palak	PARC/PGRI	003075(01)	Attock	Hasan Abdal
3.	28444	Palak	PARC/PGRI	003076(01)	Attock	Hasan Abdal
4.	28445	Palak	PARC/PGRI	003077(01)	Rawalpindi	Taxila
5.	28446	Palak	PARC/PGRI	003078(01)	Rawalpindi	Taxila
6.	28447	Palak	PARC/PGRI	003079(01)	Rawalpindi	Taxila
7.	28448	Palak	PARC/PGRI	003080(01)	Rawalpindi	Losar Sharfoo
8.	28449	Palak	PARC/PGRI	003081(01)	Rawalpindi	Losar Sharfoo
9.	28450	Palak	PARC/PGRI	003082(01)	Attock	Fatehjang
10.	28451	Palak	PARC/PGRI	003083(01)	Attock	Fatehjang
11.	28452	Palak	PARC/PGRI	003084(01)	Attock	Bahter
12.	28453	Palak	PARC/PGRI	003085(01)	Attock	Bahter
13.	28454	Palak	PARC/PGRI	003086(01)	Rawalpindi	Naswari Bazar

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S.No.	Accession No.	Local Name	Collecting Organisation	Collection No.	District	City/Village/Town
14.	28455	Palak	PARC/PGRI	003086(02)	Rawalpindi	Naswari Bazar
15.	28456	Palak	PARC-JICA	004501(03)	Gujranwala	Gujranwala
16.	28457	Desi Palak	PARC-JICA	004503(08)	Sialkot	Sialkot
17.	28458	Desi Palak	PARC-JICA	004503(12)	Sialkot	Sialkot
18.	28459	Desi Palak	PGRI/NARC	004507(02)	Hafizabad	Vaneki
19.	28460	Palak	PGRI/NARC	004508(01)	Khushab	Jauharabad
20.	28461	Palak	PGRI/NARC	004520(02)	D.G. Khan	Paritewala
21.	28462	Palak	PGRI/NARC	004525(01)	Vehari	Vehari
22.	28463	Desi Palak	PGRI/NARC	004525(02)	Vehari	Vehari
23.	28464	Palak	PGRI/NARC	004529(01)	Lodhran	Khanewal
24.	28465	Kanta Palak	PGRI/NARC	004533(04)	Bahawalpur	HasilPur
25.	28466	Palak	PGRI/NARC	004546(01)	Dir	Taimargarah
26.	28467	Palak	PGRI/NARC	004800(02)	Dhader	Dhader
27.	28468	Kanta Palak	PGRI/NARC	004800(03)	Dhader	Dhader
28.	28469	Dhari Palak	PGRI/NARC	004800(04)	Dhader	Dhader
29.	28470	Kanta Palak	PGRI/NARC	004864(01)	Kohlu	Rakhni
30.	28471	Palak	PGRI/NARC	004865(02)	Kohlu	Rakhn
31.	28472	Palak	PGRI/NARC	004868(03)	Loralai	Loralai
32.	28473	Palak	PGRI/NARC	004870(02)	Ziarat	Canjawi
33.	28474	Palak	PGRI/NARC	004872(02)	Mustung	Mustung
34.	28475	Palak	PGRI/NARC	004877(01)	Kalat	Kalat
35.	28476	Palak	PGRI/NARC	004885(03)	Quetta	Sariab

Results and Discussion

Variability profile:

The spinach genotypes exhibited medium to high variability for various plants attributes investigated (Table 2). A high variance for plant height, earliness of bolting and number of days to flowering was observed whereas for rest of parameters, a low to medium variation was noted. Plant height ranged between 116.6cm (28451) to 167cm (28461). Earliness of bolting ranged between 84.8 (28468) to 129 (28458) and whereas the number of days to flowering ranged between 96.8 (28469) to 140.4 (28458). The lowest variability was observed for number of tillers per plant which ranged between 6 (28469) and 11 (28474). Spinach is mainly grown for fresh leaves and the number of leaves per plant and leaf size thus determine total yield. However, a low variance was observed for number and size of leaf in this study which depicted a narrow base of the present germplasm collection for these traits. This minimises the scope of selection for these traits in the germplasm assayed. A large scale testing of broad base germplasm by making extensive local collections as well as acquiring exotic genotypes is imperative to develop a sound breeding programme (Mahmood et al., 2010; Jan et al., 2011).

Table 2. Variability profile in spinach germplasm analysed by descriptive statistics.

Parameter	Mean ± SE	SD	Variance	Minimum	Maximum
Leaves per plant (No.)	16.81 ± 0.41	2.47	6.08	11.6	21.2
Leaf length (cm)	13.99 ± 0.54	3.23	10.41	8.6	18
Leaf width (cm)	9.07 ± 0.26	1.56	2.45	6.6	11.6
Plant height (cm)	143.59 ± 2.25	13.52	182.69	116.6	167
Petiole length (cm)	14.49 ± 1.03	6.20	38.38	6.8	25.8
Earliness of Bolting	109.44 ± 1.99	11.95	142.87	84.8	129
Tillers per plant (No.)	8.27 ± 0.21	1.27	1.60	6	11
Days to flowering (No.)	118.72 ± 1.94	11.61	134.80	96.8	140.4
Days to harvesting (No.)	198 ± 0.29	1.74	3.03	190	201

Qualitative Attributes

Various qualitative traits observed in spinach germplasm reflected diverse patterns (Table 3). The predominant plant attitude recorded in spinach was spreading type (50%) which was followed by erect type (33.3%). Majority of the spinach plants (83.3%) contained dark green leaves and few wrinkles on leaves were observed on 72.2% of genotypes. Leaf lobation was slightly deep in major fraction (91.7%) of the spinach accessions followed by deep lobation (5.6%). The wrinkle leaf shape was recorded in 94.4% spinach germplasm and leaf tip was sharp in 80.6% genotypes, which was followed by intermediate (16.7%) leaf tip. Light green colour of the proximal part of petiole was predominant (88.9%) in spinach. The colour of the midrib was light green in 94.4% genotypes. The seed shape of most of the accessions (72.2%) was mixed and it was followed by smooth to prickly (11.1%) seeds. The predominant (66.7%) colour of the main root was observed to be gray which was followed by white (27.8%). The diverse groups for morphological traits reflected high variation in plant attitude, wrinkles of leaves, shape of leaf tip, seed shape and colour of main root; therefore, for these traits, selection for various genetic markers could be utilised by the breeders. However, for rest of the traits, where low variability was observed, the accessions with distinct characters are suggested to be acquired or collected from the centers of maximum diversity.

Trait Association:

Association among various traits reflected by correlation coefficients in spinach germplasm revealed significant relationship for different traits studied (Table 4). Number of leaves per plant showed positive and significant correlation with leaf length and leaf width. For the same traits, Varalakshmi and Devaraju (2010) also observed positive correlation. Similarly, a positive and significant associated and existed between leaf length and petiole length as well as leaf width. Plant height displayed a positive relationship with earliness of bolting, tillers per plant days to flowering and days to harvesting. However, it was significant only with earliness of bolting and days to flowering. There existed a positive and significant correlation between days to flowering and days to harvesting. Earliness of bolting also remained significantly correlated with days to flowering. A negative association among several traits was also observed which was non-significant. Where association was similar, character pairs are suggested to be employed in a broad spectrum but in character pairs with different correlation magnitude. The germplasm should be handled with care for improvement through simple selection (Jatoi et al., 2011). Moreover, for the yield improvement, emphasis should be made on indirect selection through leaf characters, like, leaf number, size and weight (Varalakshmi and Devaraju, 2010). Trait association yielded a better understanding of the relationships among various parameters which could be useful for devising better crop improvement strategy in spinach. Taking into account, the positive correlation between various traits of economic values have been considered to be a better way for successful crop improvement (Saleem et al., 2006; Eivazi et al., 2007). The genotypes which matured earlier should be used in the breeding programme as they showed significant relationship with days to harvesting, which is desirable for the breeders.

Table 3. Distribution of qualitative attributes studied in spinach germplasm

Plant Trait	Description	Frequency	(%)
Plant attitude	Semi-spread	3	8.3
	Spread	18	50.0
	Erect	12	33.3
	Semi-erect	2	5.6
	highly spread	1	2.8
Leaf colour	Dark green	30	83.3
	Light green	3	8.3
	Pale green	3	8.3
Wrinkles of leaf	Few	26	72.2
	Intermediate	8	22.2
	Many	2	5.6
Lobation of leaf	Slightly deep	33	91.7
	Deep	2	5.6
	Very deep	1	2.8
Shape of leaf	Wrinkle type	34	94.4
	Star shape	2	5.6
Shape of leaf tip	Sharp	29	80.6
	Intermediate	6	16.7
	Round	1	2.8
Colour of proximal part of petiole	Light green	32	88.9
	Dark green	3	8.3
	Pink	1	2.8
Midrib colour	Light green	34	94.4
	Dark green	2	5.6
Resistance to downy mildew	Resistant	33	91.7
	Very resistant	2	5.6
	Susceptible	1	2.8
Seed shape	Mixed	26	72.2
	Smooth	3	8.3
	Prickly	2	5.6
	Smooth to prickly	4	11.1
	Prickly to smooth	1	2.8
Colour of main root	White	10	27.8
	Brown	2	5.6
	Gray	24	66.7

Table 4. Trait association among various parameters in spinach accessions

Parameter	L/P	LL	LW	pH	PL	EOB	T/P	DTF
Leaf length (cm)	0.491*							
Leaf width (cm)	0.321*	0.638*						
Plant height (cm)	-0.109	-0.079	-0.096					
Petiole length (cm)	0.006	0.335*	0.201	0.207				
Earliness of Bolting	0.100	-0.167	-0.145	0.440*	0.128			
Tillers per plant (No.)	-0.059	-0.068	-0.290	0.016	-0.035	-0.068		
Days to flowering (No.)	0.048	-0.072	-0.154	0.266*	0.013	0.409*	0.113	
Days to harvesting (No.)	0.092	-0.157	0.048	0.179	0.103	0.224	0.096	0.254*

Relationship among Genotypes

The dendrogram generated through cluster analysis divided the spinach accessions into four main clusters which were further classified into sub clusters (Fig. 1). Cluster-I was the largest, comprising of 17 genotypes, cluster-II of 7 accessions, cluster-III 4 genotypes while cluster-IV contained 7 genotypes (Table 5). Inter-cluster variability pattern among spinach accessions rendered useful information (Table 6). The germplasm grouped in cluster-I were of medium plant height, late flowering, more tillers and late in bolting. The germplasm contained in cluster-II were characterised with more leaf per plant, longer leaves and the highest plant height. Spinach accessions grouped in cluster-III had high number of leaves per plant, larger leaf length and more plant height, while the germplasm in cluster-IV reflected the highest number of leaves per plant as well as leaf length and width, moderate plant height, moderate maturity and moderate petiole length. Results for principal components analysis presented in Table 7 indicated the worth of first four components with Eigen value greater than unity. These could be considered important for determining almost two-thirds of the variability among 35 accessions. The populations contributing the first PC were late in maturity bearing better leaves and leaf area, whereas the PC_2 contributed for the populations with better vegetative components other than leaves. Tillering capacity contributed by the populations in PC_3 and PC_4 contributed negligible economic importance for explaining variability among the spinach germplasm. Based on the first two PCs, all the genotypes were plotted in as scattered diagram (Fig. 2). The germpalsm were more or less scattered that indicated the worth of genetic dissimilarities for future use in spinach improvement programme.

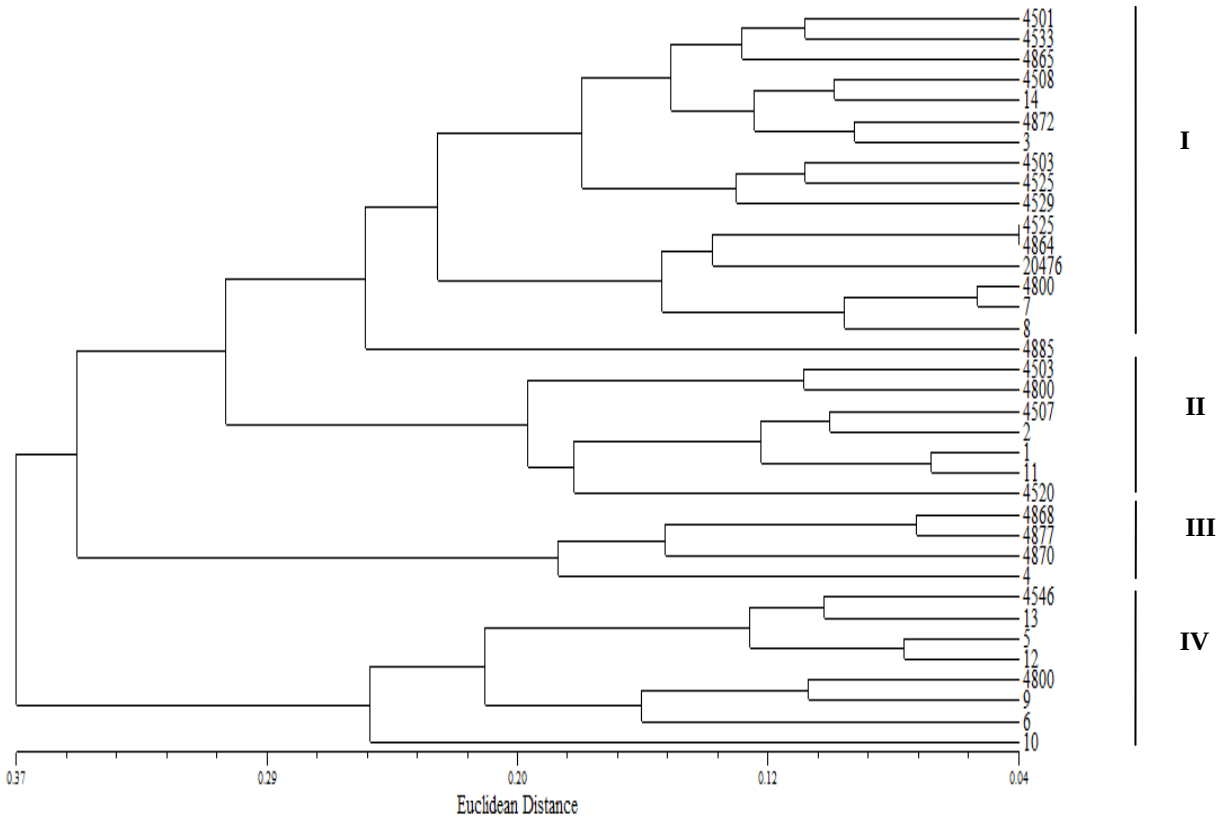

Fig. 1. The relationship among spinach accessions reflected in cluster analysis.

Table 5. Clustering pattern of spinach genotypes into different clusters.

Main Group	Sub Group	No. of Accessions	Accession Number
1	1	3	4501, 4533, 4865
	2	4	4508, 14, 4872, 3
	3	3	4503, 4525, 4529
	4	6	4525, 4864, 20476, 4800, 7, 8,
	5	1	4885
2	1	2	4503, 4800
	2	5	4507, 2, 1, 11, 4520
3	1	4	4868, 4877, 4870, 4
4	1	4	4546, 13, 5, 12
	2	3	4800, 9, 6
	3	1	10

Table 6. Inter-cluster variation for various parameters in spinach accessions.

Parameters	Cluster-1	Cluster-2	Cluster-3	Cluster-4
Leaves per plant (No.)	16.4±3.0	17.2±3.0	16.4±1.19	17.6±0.86
Leaf length (cm)	13.4±3.5	14.2±3.2	14.6±3.64	15.3±2.32
Leaf width (cm)	8.5±1.5	8.7±2.0	9.7±1.68	10.2±0.57
Plant height (cm)	139.6±6.7	157.7±14.6	157.2±4.57	133.0±12.12
Petiole length (cm)	13.4±5.7	17.2±7.3	11.7±6.74	15.9±5.96
Earliness of Bolting	113.5±6.7	116.9±15.0	97.9±2.73	100.1±12.11
Tillers per plant (No.)	8.3±1.4	9.0±1.0	7.7±1.10	7.9±1.19
Days to flowering (No.)	121.5±9.2	128.8±9.2	113.2±6.81	106.7±9.20
Days to harvesting (No.)	197.8±2.3	198.4±1.9	198.0±0	198.1±0.35

Table 7. Principal component analysis for Spinach germplasm based on ten agronomic traits.

		PC_1	PC_2	PC_3	PC_4
		2.2	1.9	1.1	1.0
		25.0	21.0	12.4	11.4
Traits	Communality	25.0	46.0	58.4	69.8
Leaves per plant (No.)	0.756	0.489	0.412	0.536	-0.244
Leaf length (cm)	0.825	0.802	0.369	0.082	0.201
Leaf width (cm)	0.705	0.765	0.331	-0.063	-0.078
Plant height (cm)	0.631	-0.417	0.561	-0.362	0.107
Petiole length (cm)	0.797	0.205	0.504	-0.444	0.551
Earliness of Bolting	0.703	-0.461	0.638	-0.079	-0.280
Tillers per plant (No.)	0.903	-0.291	-0.105	0.539	0.719
Days to flowering (No.)	0.569	-0.441	0.522	0.306	-0.092
Days to harvesting (No.)	0.387	-0.273	0.465	0.309	0.037

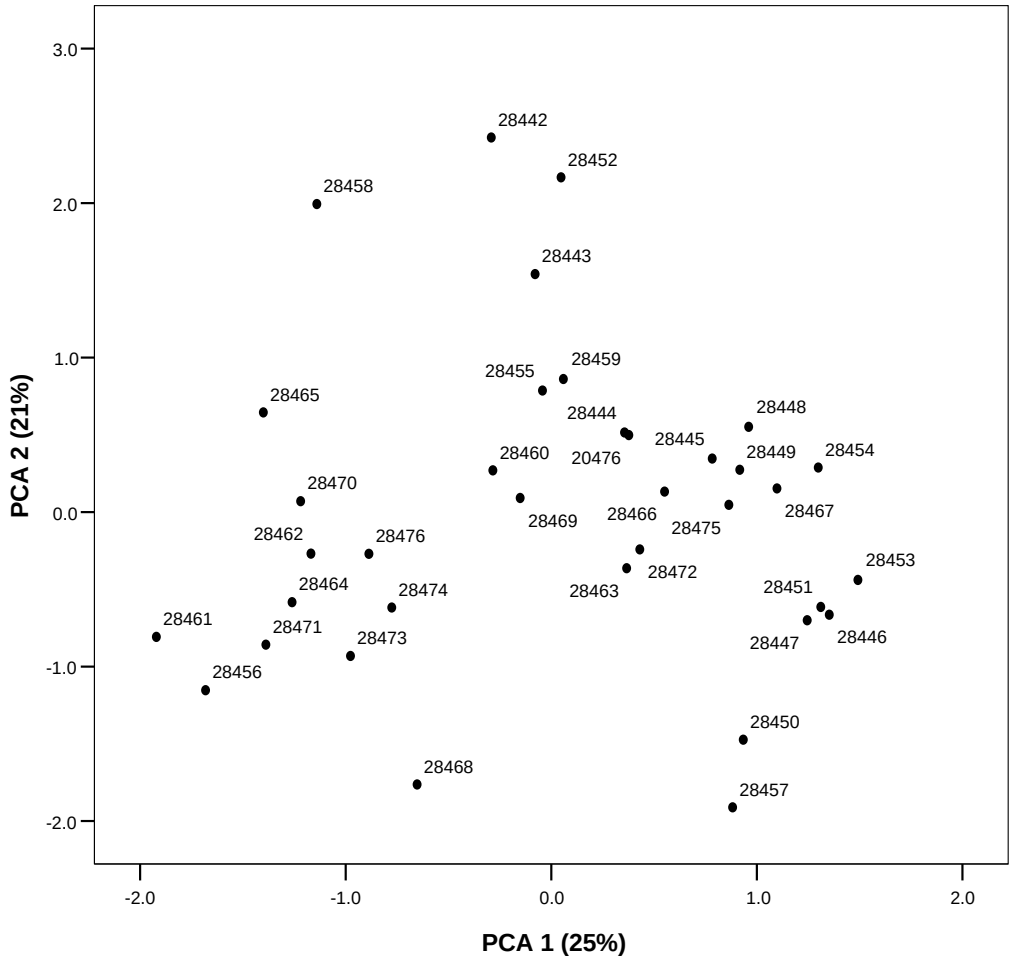

Fig. 2. Scattered diagram of spinach genetic resources for first two components contributing half of the variability for agronomic traits.

Conclusion

The current study is the first report from Pakistan which deals with the diversity profile of 35 spinach genotypes on the basis of 20 morpho-agronomic traits. The germplasm investigated represented a collection from diverse areas covering three provinces of Pakistan. The PCA displayed a scattered distribution of the genotypes on scatter plot. The cumulative variance explained by first three PCs (58.34%) reflected a diverse base of the germplasm investigated. It was also supplemented by cluster analysis where genotypes were grouped into different clusters regardless of their collection origin. However, a low variability was observed for leaf characters, which are the primary characters of high significance. Obtaining more diverse collections comprising local and exotic germplasm is suggested for future breeding in spinach.

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