Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 May; 6 (5s): 1051-1057

Examining Genetic Diversity in Spinach (Spinacia oleracea L.) through the Analysis of Select Morphological Traits.

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ABSTRACT

Spinach, a leafy green vegetable cherished for its nutritional and medicinal benefits, is globally cultivated. This study assessed the genetic diversity of fifty-one Spinacia oleracea L. germplasms from diverse regions in Bangladesh, utilizing both qualitative and quantitative traits. Variation was observed across all traits, except for Inflorescence color and seed type. Leaf shapes included elliptic, broad elliptic, ovate, and broad ovate, with elliptic being predominant. Maximum variation was noted in edible leaf weight per plant, followed by petiole length and the number of lateral branches. Specific germplasms exhibited longer and broader leaves, with RC-139 having the highest number of lateral branches. Bolting time, with NQ-68 requiring the most days, proved to be a crucial trait for spinach. The dendrogram grouped thirty-six germplasms into Cluster I, while Cluster IV contained a solitary germplasm. Cluster II showed the highest mean values for leaf length, leaf width, and edible leaf weight per plant. Principal Component Analysis revealed that leaf length, leaf width, and petiole length were pivotal contributors to germplasm diversity. Selecting for leaf traits and bolting time, germplasms TRMR-136, TRMR-144, R-342, RNF-26, NQ-68, and RC-139 are recommended for future breeding programs based on their distinctive characteristics.

Keywords: Germplasm, Morphological variation, Diversity, Cluster analysis, Principal component analysis

1. INTRODUCTION

Spinach (*Spinacia oleracea* L.) is an edible vegetable belonging to the family *Amaranthaceae*. It was originated from Iran and first declared as an herb of Persia by the Chinese [1]. Currently, China, United States Turkey, Japan and Indonesia are considered the largest producers of spinach and in Bikaner spinach production quantity was 59400 tons in 2020 FAOSTAT [2]. Leafy vegetables are an important part in the human diet and spinach is one of the dark green leafy vegetables among them. It has versatile used such as salad, a cooked vegetable or as a component of many other cooked meat and vegetable dishes [3]. Spinach is dioecious species with both male and female plants 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 [4]. A large number of nutrients have been reported in spinach including vitamins, minerals, antioxidants, carotenoids, steroids, apocyanin, flavonoids and p-coumaric acid [5]. Spinach contains highly active antioxidants that work against cancer and diabetes, nitrates for maintaining blood pressure and vitamin K1 for blood clotting [6]. On the basis of its medicinal properties, a herbal product, namely Appethyl (Green Leaf Medical, Stockholm, Sweden), has also been designed specifically to suppress appetite and reduce weight [7].

In order to develop new genetically improved cultivars, a preliminary characterization and estimation of the genetic diversity among local spinach germplasm for agronomic performance could be useful for exploitation of this genetic material for breeding programs. Characterization of germplasm acts as a starting point for the establishment of "a core collection" where the wholevariability in the collection is represented in a small subset of accessions [8]. PGRC collected spinach germplasm across the country. The value of germplasm collection depends not only on the number of accessions but also on the genetic diversity present in those accessions. Diversity in the gene pool of a species may affect the adaptability to a new environment as well as to develop new varieties more tolerant to stress and pathogens [9]. Hence, characterization of the collected germplasm is essential for easy and rapid evaluation of germplasm. Morphological traits are considered as the simplest marker for assessing the genetic diversity of species. Study of morphological diversity is mandatory before biochemical and molecular characterization [10]. 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 [1] Sabaghnia, *et al.,* 2014). Information generated by multivariate analysis can be useful for different germplasm identification which has explained traits for crossing, for planning efficient crop improvement program. Therefore, this study has sought to afford the opportunity to assess the genetic diversity of spinach germplasm by using agro-morphological traits and to detect groups of genetically distinct morphological types among the collected germplasm.

2. MATERIALS AND METHODS

The study was done in the field area of RNB Global University-Bikaner. The soil in the test field was silty clay with a pH of 6. Fifty-one germplasm of spinach were used in this study. Seeds of fifty-one germplasm were obtained from seed gene bank of PGRC were arranged in a randomized complete block design. Line sowing was done in 3m long of 2 rows plot. Row to row distance was 50 cm and plot to plot distance was 100 cm. Direct seeding was completed with inside the well-organized plot on 22th November, 2021. Well-decomposed farmyard manure (FYM) at 10 t/ha and urea 180 kg/ha, TSP 125 kg/ha, MP 125kg/ha, gypsum 110 kg/ha, zinc 3 kg/ha and boron 1.5 kg/ha were applied [11]. Whole amount of farmyard manure (FYM), TSP and half amount of urea and MP were applied during land preparation.Urea and MP were applied in three equal installments at 10, 30 and 45 days after sowing. Plants were isolated with nylon net to control cross pollination and the seeds were collected from these plants. Normal cultivation techniques and standard intercultural operations were followed to have a good crop. Twenty-one observations on qualitative (13) and quantitative (8) characters were recorded in different stage of life cycle as per European Cooperative Program for Plant Genetic Resources (ECPGR) descriptors [12].Qualitative data were represented in the form of frequency distribution. By simple descriptive statistics including mean, variance, standard deviation and coefficient of variation quantitative data were analyzed. Cluster analyses and Principle component analysis were performed by computerized software STAR 2.0.1[13].

3. RESULTS AND DISCUSSION

The agro-morphological study is the simplest and easiest way for the taxonomic description of plants [14]. The description of agro-morphological traits is an important prerequisite for efficientutilization of plant materials in every plant breeding program [15]. The characterization of landraces is essential for exploiting the variability of important traits such as yield potential and resistance to biotic and abiotic stresses [8]. In this study, fifty-one germplasm of spinach were characterized morphologically based on thirteen (13) qualitative and (8) quantitative traits. Twenty-one (21) morphological traits including six (6) quantitative and fifteen (15) qualitative of200 spinach accessions were recorded by [9]. Fifty-four spinach landraces collected from diversegeographical regions of Iran were evaluated for ten (10) qualitative and nine (9) quantitative characters [1]. Divergence of forty-four spinach were estimated using twenty-one (21) morphological characters by [16]. Variation were observed in respect of different morphological traits which shown the possibility to get desirable plant characters in selective accessions, in order to fulfill the demand of a plant breeder [8].

QUALITATIVE CHARACTERS

Qualitative characters are considered as important markers in describing the physical appearance of a plant [17]. In this study a total of thirteen (13) qualitative characters including early plant vigor, stem anthocyanin content, leaf shape excluding basal lobes, leaf color, leaf blistering, leaf texture, leaf edge, petiole attitude, plant growth habit, inflorescence color, inflorescence shape, seed type and seed colour were evaluated to know the variability of the germplasm. [1] also recorded various qualitative traits consist on leaf texture, seed type, stem anthocyanin, petiole attitude and vegetative leaf shape. Qualitative characteristics of different germplasm of spinach are presented in Table 1. Considerable variation was found in studied characters except inflorescence color and seed type. Considerable variation was also observed among leaf features of accessions like anthocyanin, blistering and lobing by [9]. Poor, good and very good type earlyplant vigor were recorded as per descriptor states of which 25.49% of the germplasm had poor vigor, 43.14% germplasm had medium vigor and very good vigor in 31.37% germplasm. Amongthe fifty-one germplasm, only 1.96% germplasm had strong and medium anthocyanin content in stem rest 96.08% germplasm had no coloration in stem. Stem anthocyanin content was also recorded by [1] in two types i.e very low and strong. Leaf shape exhibited as elliptic, broad elliptic, ovate and broad ovate where elliptic shape was found in maximum germplasm (64.71%) where as 11.76% had broad elliptic leaf shape and 13.73% had ovate leaf shape. Leaf color was found in two categories such as yellow green (27.45%) and green (72.55%). Two types of leaf blistering i.e. weak, medium and strong were observed. 45.10% germplasm had weak leaf blistering, 29.41% had medium and 25.49% had strong leaf blistering. Leaf texture of the germplasm exhibited as smooth (90.20%) and slight crinkled (9.80%). The edge of leaf was observed in two categories. Leaf edge was smooth in58.82% germplasm and ripple in 41.18% germplasm. Variation was not found in petiole attitude. Three types petiole attitude were found i.e. Erect (35.29%), semi spared (45.10%) and spared (19.61%). [9] also found variation in case of petiole attitude, most of the accessions were erect including the germplasm of India, Plant growth habit was categorized in erect, semi spared and spared types. Inflorescence color was yellow in all germplasm. In case of inflorescence shape, 47.06% germplasm had globose shape, 45.10% had semi drooping and only 7.84% had completely drooping type. All the germplasm showed prickly type seed. Two types of seed color i.e. yellow (35.29%) and gray (64.71%) were observed among the accession.

Table 1: Qualitative variations of different characteristics in spinach germplasm

Characters	Descriptor states	No. of germplasm	Percent of germ plasm
Early plant vigor	Poor Good Very good	13 22 16	25.49 43.14 31.37
Stem anthocyanin content	Absent MediumStrong	49 1 1	96.08 1.96 1.96
Leaf shape excluding basal lobes	Elliptic Broad ellipticOvate Broad ovate	33 6 7 5	64.71 11.76 13.73 9.80
Leaf color	Yellow GreenGreen	14 37	27.45 72.55
Leaf blistering	Weak MediumStrong	23 15 13	45.10 29.41 25.49
Leaf texture	Smooth Slight crinkled	46 5	90.20 9.80
Leaf edge	SmoothRipple	30 21	58.82 41.18
Petiole attitude	Erect Semi spared Spared	18 23 10	35.29 45.10 19.61
Plant growth habit	Erect Spreading Drooping	24 23 4	47.06 45.10 7.84
Inflorescence color	Yellow	51	100
Inflorescence shape	Globose Semi drooping Completely drooping	24 23 4	47.06 45.10 7.84
Seed type	Prickly	51	100
Seed color	Yellow Grey	18 33	35.29 64.71

Quantitative characters

Quantitative characters are also important markers, especially those which are showing high variability [18] The present study was based on 8 quantitative traits including leaf length, leaf width, Petiole length, number of lateral branches, Edible leaf weight per plant, bolting time, daysfor 50% flowering and 100 seed weight. Some descriptive statistics including range, mean, standard deviation and CV% of the measured quantitative data of spinach is presented in Table 2.

[8] also recorded descriptive statistics for 13 characters in the 121 spinach accessions. The highest quantitative variation was observed in edible leaf weight per plant (CV-30.59%) followed by petiole length (CV-22.21%) and number of lateral branches (CV-19.40%). The minimum CV was found for days to 50% flowering (CV-10.34%). Percent of coefficient of variation indicated the variability among the accessions comparing within the characters. The characters showing above 20% of the coefficient of variation could be used as reliable markers for the characterization of accessions [19]. [9] also found all quantitative characters had high values of CV ranging from 30.8% (leaf length) to 66.9% (plant height).

Leaf length ranged from 7.83-16.83 cm. On an average, leaf length was 13.08 cm. [8] also found 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. Leaf width ranged from 2.62-10.21 cm with an average of 7.75 cm. Based on leaf length and leaf width, it was evident that germplasm TRMR-95, NT-34, TRMR- 136, NRI-121 had longer leaf and NRI-121, TRMR-136, RC-139, TRMR-12 and NT-33 had broader leaf. Petiole length was found minimum in germplasm KMR-332 (4.83 cm) and maximum TRMR-144 (13.30 cm). For the characters number of lateral branches, average value obtained 5.69. Germplasm RC-139 had highest number of lateral branches (9) where as SNQR- 28 had the lowest number (4). Edible leaf weight per plant ranged from 9.67-49.5 g with an average 27.55 g. Edible leaf weight per plant was found maximum in TRMR-136 (49.5 g), TRMR-144 (47.78 g) and RC-139 (41.33 g) and the minimum in M-35 (9.67 g), KAS-33 (11.30 g) and RNF-114 (13.0 g). In case of bolting time, it was observed that average time required for bolting was 61.27.

The germplasm NQ-68 required 85 days bolting followed by TRMR-136 (83 days) and RNF-126which was most important characters for spinach. SNQR-28 had lowest bolting time. [9] also confirmed that the bolting period of accessions also varied greatly, as some of the accessions bolted very early, some very late. [20] also found considerable variation among bolting periods, representing it as one of the selection criteria for the assessment of spinach germplasms.

Table 2: Quantitative variation of different characteristics in spinachgermplasm

Characters	Range	Mean	SD	CV%
Leaf length (cm)	7.83-16.83	13.08	2.09	16.00
Leaf width (cm)	2.62-10.21	7.75	1.45	18.74
Petiole length (cm)	4.83-13.30	8.26	1.83	22.21
Number of lateral branches	4-9	5.69	1.10	19.40
Edible leaf weight per plant (g)	9.67-49.5	27.55	8.43	30.59
Bolting time	49-85	61.38	7.70	12.57
Days to 50% flowering	58-93	72.27	7.47	10.34
100 seed weight (g)	1.16-3.8	2.39	0.56	23.21

Germplasm distribution and dendrogram

Cluster analysis is a tool for classifying the materials into groups. On the basis of multiple variable grouping of a large number of accessions, is a reliable technique to determine the similarities and extent of differences among them [21]. The cluster analysis indicates the extent of variability that could be useful for future breeding programs [22]. Several authors have used this tool for morphological characterization of different species representing their intra specific relationship [8]; [20];[23]; [24]. On the basis of eight quantitative characters, fifty-one spinach germplasm were grouped into five clusters. The dendrogram (Fig.1) shown that the maximum thirty-six germplasm were grouped into cluster I indicating overall genetic similarity among them, followed by nine germplasm in cluster III. Cluster II and V composed of only 3 and 2 of each, respectively. Cluster IV was occupied by only one germplasm. Divergence of forty-four spinach landraces were estimated by [16]. Based upon cluster analysis, landraces were classifiedinto four groups each with 14, 4, 19 and 7 landraces.[8] also reported 6 clusters for 121 spinach landraces.

Table 3: Distribution of fifty-onegermplasmin five different clusters

Cluster	Number ofgermplasm	Germplasm included in different clusters
I	36	NT-11, NT-34, NT-48, NT-67, NT-73, N-35, N-156, N-205, N-250, TRMR-12 TRMR-95, R-14, R-148, RC-126, SSR-33, SSR-49, SSR-88, RNF-109, SA-83, SA-96, SA-119, NRI-24, NRI-29, NRI-121, NRI-160, NRI-205, MRA-33, MRA-42, MRA-71, SNQR-56, SRS-73, SRS-81, NQR-26, NSR-108, NTR-33, KAS-33
II	3	TRMR-136, TRMR-144, R-342
III	9	R-86, RC-144, RNF-114, SNQR-28, NQ-24 , KMR-332, MI-35, SU-24,M-35
IV	1	RC-139
V	2	RNF-26, NQ-68

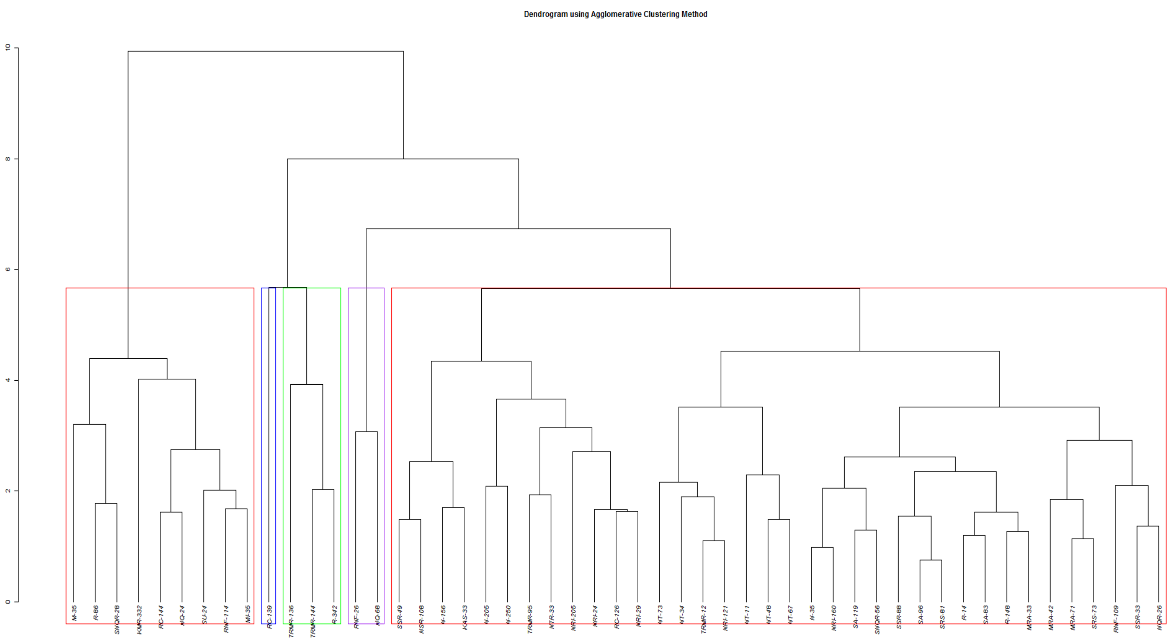

Fig. 1: Cluster tree of fifty-one germplasm of spinach based on quantitative characters

Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 May; 6 (5s): 1051-1057

Cluster mean

The genetic differences between clusters were reflected by cluster means. Eight character's meanvalue studied in spinach germplasm for five clusters are presented in Table 4. Variations were observed in cluster mean for all characters. The maximum cluster mean value were observed in cluster II for the characters leaf length (cm), leaf width (cm) and edible leaf weight per plant (g).[1] also recorded the cluster C14 had the largest leaf width and the cluster C1 had the smallest leaf width. Both leaf length and leaf width are important characters for yield performance in spinach [25]. So, the germplasm belong to this cluster may be selected for leafy character. In cluster II petiole length was maximum. The long petiole length is essential for machinery harvesting and genetic improving for having long petiole length which is one of the breeding targets of spinach [16].

Number of lateral branches was highest in cluster IV. Bolting time and days to fifty percent flowering time observed maximum in cluster V, so the germplasm in this cluster is desirable for spinach due to longer bolting time and late flowering. These useful information on the clusters to which particular accessions with traits of interest belong will aid inresearching more accessions with similar characteristics in breeding programs [26] and grouping of the germplasm to different clusters gives an opportunity to select germplasm to develop high yielding and good quality varieties.

Table 4: Cluster means for eight characters of fifty-one spinach germplasm

Characters	I	II	III	IV	V
Leaf length (cm)	13.77	14.61	10.02	13.56	11.93
Leaf width (cm)	8.16	9.06	5.73	9.43	6.67
Petiole length (cm)	8.33	12.63	6.42	7.55	8.84
Number of lateral branches	5.53	6.33	5.67	9.00	6.00
Edible leaf weight perplant (g)	27.42	45.85	21.65	41.33	22.10
Bolting time	60.67	71.33	55.44	61.00	83.50
Days to 50% flowering	71.67	80.67	67.44	72.00	92.50
100 seed weight (g)	2.51	1.75	1.99	2.40	3.05

Principal component analysis (PCA)

Principle component analysis (PCA) is a reliable multivariate technique that converts a large dataset in the form of a few factors which include several inter-correlated variables [27]. The analysis was carried out to determine the main differentiating characters that cause variation among fifty- one spinach germplasm by calculating the first two principal components which accounted for 60.00 % of the total divergence (Fig. 2). The overall data were reduced to five factors or principal components (PC) representing about 92.37% of the total variability.

The first PC explained 37.10% of the total variability and the second PC explained 22.90% of the variation among fifty-one spinach germplasm. Thus, the present study was revealed that the first PC was more important than the second PC for explaining the variability among the germplasm based on studied traits. Usually, the first component contributes toward maximum variance while the rest of the factors justify the remaining amount of variance [28]. The spinach germplasmRNF-26, NQ-68, TRMR-136, TRMR-144, R-342 and RC-139were separately isolated from the others and they were away from centroid. This result showed the uniqueness and divergence of the germplasm. Each factor reflected the PC values of different traits, showing their importance towards the overall variability [9]. The eigen values and vectors of eight characters for a PCA of important traits for the first fifth principal components in fifty-one spinach germplasm was shown in Table 5.

Populations with high scores for the first eigenvectors are leaf length (0.4860),leaf width (0.4827) and petiole length (0.4792) and overall variation 37.10% these traits were themost important contributors towards diversity of the germplasm in PC1. Similar to this, [1] also reported leaf trait as the most important factor contributing towards variability of spinach germplasm. The second eigen vector was mostly connected with scores of edible leaf weight per plant (0.3889) and number of lateral branches (0.1000), these traits were the second most important contributors among the eight characters for fifty-one germplasm. The plant breeders might select the combination of morphological characters through PCA and then by plotting the main components in a single plot, germplasms closed to the ideal could be indicated [29].

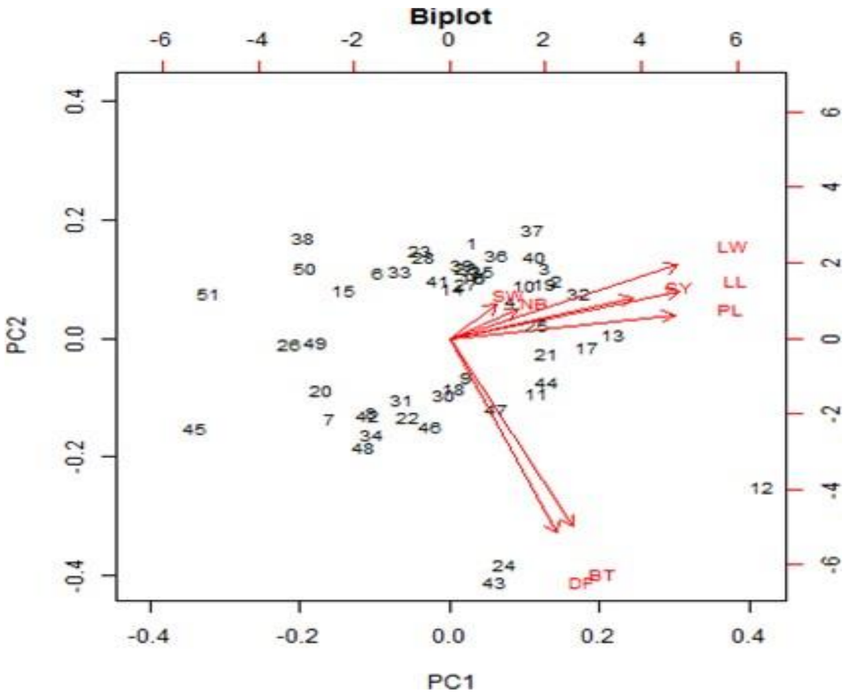

Fig. 2: The plot of the first two PCA 1 and PCA 2 for spinachgermplasm

Table 5: Principal Components for eight traits of spinach germplasm

Characters	PC1	PC2	PC3	PC4	PC5
Leaf length (cm)	0.4860	0.1608	0.2498	0.1398	0.1077
Leaf width (cm)	0.4827	0.2534	0.1207	0.0493	0.1730
Petiole length (cm)	0.4792	0.0783	0.1145	0.1616	0.2582
Number of lateral branches	0.1447	0.1000	-0.7036	-0.4856	0.4690
Edible leaf weight perplant (g)	0.3889	0.1395	-0.4592	0.0518	-0.7780
Bolting time	0.2605	-0.6454	0.0303	-0.0756	0.0581
Days to 50% flowering	0.2259	-0.6662	-0.0317	-0.0663	-0.0696
100 seed weight (g)	0.1006	0.1183	0.4496	-0.8387	-0.2415
Eigenvalue	2.97	1.83	1.21	0.95	0.42
Variability (%)	37.10	22.90	15.08	11.93	5.30
Cumulative (%)	37.10	60.00	75.08	87.01	92.37

4. CONCLUSION

Characterization of fifty-one germplasm of spinach based on the qualitative and quantitative characters was suitable to assess the genetic diversity among collected spinach germplasm. Most of the qualitative traits showed distinct variations among the germplasm. Qualitatively, the maximum variation was observed in leaf shape, petiole attitude and leaf shape. According to PCA, leaf characters were found to be the important characters in contributing towards divergence. The present findings have a great genetic potential for the studied germplasm. In spinach breeding program these genetically diverse germplasm will be useful for screening the desirable traits. Concurrently, the promising germplasm identified in this present study might be used in future utilization to create new varieties with desirable traits for spinach improvement.

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