KSÜ Tarım ve Doğa Derg 29 (5), 1217-1231, 2026
KSU J. Agric Nat 29 (5), 1217-1231, 2026
DOI:10.18016/ksutarimdoga.vi.1665306

Analysis of Genetıc Diversity of Some Spinach (*Spinacia oleracea* L.) Genotypes

Gülbanu GAYRETLI[1]✉, Önder TÜRKMEN[2], Ali Tevfik UNCU[3]
[1]Institute of Science, Department of Horticulture, Konya, Türkiye, [2]Selcuk University, Horticulture Department of Agriculture Faculty, Konya,Türkiye, [3]Necmettin Erbakan University, Necmettin Erbakan University, Faculty of Science, Konya,Türkiye
[1]https://orcid.org/0000-0002-7693-7663, [2]https://orcid.org/ 0000-0003-3218-6551, [3]https://orcid.org/ 0000-0003-4729-5750
✉: gulbanu.kiymaci96@gmail.com

ABSTRACT
The aim of this study was to determine some morphological characteristics of 64 spinach genotypes, 4 S6 and 60 S7 generation obtained from some local and commercial spinach genotypes, and to reveal the possible parental potential for the development of local hybrid F1 spinach varieties from these materials. This study, which examined some morphological data of genotypes in S6 and S7 level, was carried out in Selçuk University Faculty of Agriculture Department of Horticulture greenhouses in the autumn of 2022 and spring of 2023 vegetation periods. In the study, observations were taken on the intensity of green color in the leaf blade, blistering in the leaf blade, lobing in the leaf blade, petiole attitude, petiole length, leaf blade attitude, leaf blade shape (except basal lobes), leaf anthocyanin coloration, shape of the apex in the leaf blade, leaf brightness, longitudinal section shape of the leaf blade, leaf number, leaf brittleness, time of start of bolting in genotypes. In the first period study (S6-S7); leaf blade shape of 19 genotypes was broad oval, 21 genotypes were medium oval, 7 genotypes were triangular, 16 genotypes were medium elliptical, and 1 genotype was broad elliptical. In the second period study (S7-S8), leaf blade shape of 10 genotypes was broad oval, 23 genotypes were medium oval, 2 genotypes were triangular, 28 genotypes were medium elliptical, and 2 genotypes were broad elliptical. In the study, it was revealed that the green color density of the leaf blade taken in the first (S6-S7) and second (S7-S8) vegetation periods changed from light green to dark green among the genotypes. Based on the observations, it was also examined with the principal component analysis (PCA) method in order to examine more than one trait at the same time in spinach genotypes. As a result of this analysis, six independent principal component axes were obtained. These axes represented 69.09% of the total variation. As a result of the PCA analysis, the first component (PC1) explained 16.72% of the study and the parameters of anthocyanin presence in the leaf, leaf blade blistering, leaf blade lobing, petiole attitude, leaf blade attitude, bolting time came to the fore. As a result of morphological observations, it was observed that there was a high degree of diversity among the genotypes.

Horticulture

Research Article

Article History
Received : 26.03.2025
Accepted : 09.01.2026

Keywords
Breeding line
Spinach oleracea
Variation

Bazı Ispanak (Spinacia oleracea L.) Genotiplerinin Genetik Çeşitlilik Analizi

ÖZET
Bu araştırmada bazı yerel ve ticari ıspanak genotiplerinden elde edilen 4 adet S6 ve 60 adet S7 ıslah kademesindeki 64 ıspanak genotipinin bazı morfolojik özelliklerini belirlemek ve bu materyallerden yerli hibrit F1 ıspanak çeşitlerinin geliştirilebilmesi için olası ebeveyn potansiyelin ortaya koymak amaçlanmıştır. Genotiplerin S6 ve S7 kademedeki bazı morfolojik verilerinin incelendiği bu çalışma 2022 yılı sonbahar ve 2023 yılı ilkbahar vejetasyon dönemlerinde Selçuk üniversitesi ziraat fakültesi bahçe bitkileri bölümü seralarında gerçekleştirilmiştir. Çalışmada genotiplerde yaprakta antosiyanin varlığı, yaprak ayası yeşil renk

Bahçe Bitkileri

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 26.03.2026
Kabul Tarihi : 09.01.2026

Anahtar Kelimeler
Çeşitlilik
Ebeveyn hat
Ispanak

yoğunluğu, yaprak ayası kabarcıklanma, yaprak ayasında dilimlenme, yaprak sapı durumu, yaprak sapı uzunluğu, yaprak ayası duruşu, yaprak ayası şekli, yaprak ayası apeks şekli, yaprak ayası boyuna kesit şekli, sapa kalkma eğilimi, yaprak parlaklığı ve yaprak sayısı gözlemleri alınmıştır. İlk dönem çalışmasında (S6-S7); 19 genotipin yaprak ayası şekli geniş oval iken 21 genotipte orta oval, 7 genotipte üçgen, 16 genotipte orta eliptik, 1 genotipte geniş eliptik olarak tanımlanmıştır. İkinci dönem (S7-S8) çalışmasında ise 10 genotipin yaprak ayası şekli geniş oval iken, 23 genotipte orta oval, 2 genotipte üçgen, 28 genotipte orta eliptik, 2 genotipte geniş eliptik olarak tanımlanmıştır. Çalışmada ilk dönem(S6-S7) ve ikinci dönem (S7-S8) vejetasyon döneminde alınan yaprak ayası yeşil renk yoğunluğu ise genotipler arasında açık yeşilden koyu yeşile doğru değiştiği ortaya konulmuştur. Gözlemlere dayalı olarak ıspanak genotiplerinde birden fazla özelliğin aynı anda incelenmesi amacıyla temel bileşen analizi (TBA) yöntemi ile de incelenmiştir. Bu analiz sonucunda altı bağımsız temel bileşen ekseni elde edilmiştir. Bu eksenler toplam varyasyonun %69,09'unu temsil etmiştir. TBA analizi sonucunda ilk bileşen (TB1) çalışmanın %16,72'sini açıklamış olup yaprakta antosiyanin varlığı, yaprak ayası kabarcıklanması, yaprak ayası loblanması, yaprak sapı durumu, yaprak ayası duruşu, çiçeklenme zamanı parametreleri ön plana çıkmıştır. Morfolojik gözlemler sonucunda genotipler arasında yüksek derecede çeşitlilik olduğu gözlenmiştir.

Atıf İçin : Gayretli, G., Türkmen, Ö., & Uncu, A. T. (2026). Analysis of genetic diversity of some spinach (*Spinacia oleracea* L.) genotypes. *KSÜ Tarım ve Doğa Dergisi*, 29 (5), 1217-1231. https://doi.org/10.18016/ ksutarimdoga.vi. 1665306

To Cite: Gayretli, G., Türkmen, Ö., & Uncu, A. T. (2026). Analysis of genetic diversity of some spinach (*Spinacia oleracea* L.) genotypes. *KSU J. Agric Nat 29* (5), 1217-1231. https://doi.org/10.18016/ksutarimdoga.vi.1665306

INTRODUCTION

Spinach is a vegetable with high economic value, which is commercially grown and consumed in all kinds of ecology, especially in the autumn and spring periods, and has an important place in human nutrition (Caparrotta et al., 2019). While approximately 32.3 million tons of spinach are produced in the world, Turkey ranks third in the world with 15.447 hectares of land and 232.692 tons of production (FAO, 2023). Spinach is a food product that should be consumed regularly with its high fiber, vitamin, and antioxidant content (Seymen,2021; Wang et al.,2023). Spinach is a food rich in glucuronic acid derivatives and p-coumaric acid derivatives, which show strong antioxidant activity (Xu & Leskopresent, 2015). Spinach is a vegetable species that is very important for human health with its free radical scavengers, A, B, E, and K, and high vitamin C content (Ekinci et al., 2015). The importance of spinach, which has high nutritional value, in achieving healthy, high-quality nutrition goals is becoming increasingly prominent. As in all agricultural activities, productivity is targeted with minimum cost in spinach cultivation. In this context, the availability of competitive seeds is a key issue for producers. In this respect, the development of new products that meet the demands of producers and consumers is always an important goal for spinach. The success of the breeding program depends on the genetic diversity within the population and the efficiency of the selection. Diversity in the gene pool is very important in terms of achieving cultivars with high yield and quality, as well as high adaptability and resistance to biotic and abiotic stress elements (Göktay, 2015; Rashid et al., 2020). In this context, there are many impediments to determining the characteristics of the breeding pools, to classify them, and to reveal genetic diversity (Kenneth, 2016). Morphological markers have come to the forefront, although there are drawbacks to assessing genetic diversity in plants (Bello & Olawuyi, 2015). In breeding studies, morphological characterization provides information in determining the desired traits. Phenotypic traits are widely used to assess existing genetic diversity by revealing morphological characteristics (Bajracharya et al., 2006). Although morphological studies are the easiest and fastest method for taxonomic identification of plants without the need for more complex techniques, they cannot provide definitive results, especially in yield and yield components, and can be greatly affected by ecological conditions (Smith et al., 1991). When the morphological studies were examined, Köse (2018) classified the leaf blade shape as triangular, medium oval, wide oval, and medium elliptical, while Jahan (2022) classified the leaf blade shape as elliptical, wide elliptical, oval, and wide oval. As a result of the observations he examined (Köse, 2018), it was determined that the leaf petiole position was 4 upright, 37 semi-upright, and 9 horizontal; the leaf petiole length was 6 short, 23 medium, 21 long; the leaf blade

position was 5 upright, 31 semi-upright, 13 horizontal, and 1 semi-pendulous. (Sabghnıa et al., 2014) Morphological observations on 54 spinach genotypes reported high yield diversity among the genotypes. Rashid et al. (2020) determined the presence of anthocyanin in the leaf as 81% absent in genotypes, while the leaf color in the genotypes was 55% dark green, 35.5% green, and 9.5% light. Jahan (2022) observed that leaf color was mostly green (72.55%) among genotypes. In this context, the fact that the parameters examined in morphological studies are influenced by many factors and that the features of the objects under observation are interrelated leads to many variables. To address this problem, multivariate analysis methods have been developed to examine multiple features simultaneously (Tahtalı, 2005). In characterization studies, clustering and principal component analysis are widely used to analyze similarities and differences (Karaağaç and Balkaya, 2010). Although the diversity of spinach has been studied worldwide, the morphological characterization of spinach genetic resources remains limited (Rashid et al., 2020). The use of high-performing varieties with established agronomic traits in breeding programs is crucial, and phenotypic characterization studies remain necessary (Mavromatis et al., 2010). In this context, this study aims to fill this gap and suggest potential parent candidates for future hybrid breeding programs by presenting the evaluation of local and commercial genotypes in S6-S8 breeding stages.

MATERIAL and METHOD

The study material consisted of 4 S6-Generation and 60 S7-generation spinach genotypes available in the Department of Horticulture, Faculty of Agriculture, Selcuk University. Since spinach is a dioecious plant, inbreeding was not possible to obtain pure lines; this was achieved through full-sibling hybridization. The first year of the study involved 64 genotypes. However, due to the nature of spinach (dioecious flower structure) and the sources of the genotypes (high-heterozygosity F1 hybrid varieties), it can exhibit greater variation than many other plant species. In this context, off-type individuals can sometimes be observed in the S6-7 generations. These off-type individuals may be identified as separate genotypes and potentially used in future breeding programs. Therefore, the first year's study, conducted with 64 genotypes, was continued with 65 genotypes in the second year.

The first period of the study began with seed sowing on 10.10.2022, with 2g/m2 calculated for plots prepared as 1x1 m2 in the Selcuk University research and application greenhouse. Step-by-step progress and morphological measurements of seed multiplication were recorded, adhering to the isolation methods. The second period of the study was carried out on 29.08.2023 in accordance with the procedure, on the plots prepared in the greenhouse with a plot width of 1x1.4 m^2 for each genotype from the seeds we obtained. To ensure the ideal development of the plants, the necessary climate and soil conditions were kept at optimal levels, and the necessary cultural and maintenance procedures were applied on time. After the spinach genotypes in S6 and S7 levels completed their development, observations were made using UPOV parameters (UPOV TG/55/7) to define their morphological characteristics. The intensity of green color in the leaf blade, blistering in the leaf blade, lobing in the leaf blade, petiole attitude, petiole length, leaf blade attitude, leaf blade shape (except basal lobes), leaf anthocyanin coloration, shape of the apex in the leaf blade, leaf brightness, longitudinal section shape of the leaf blade, leaf number, leaf brittleness, time of start of bolting observations were taken. Parameters were observed from an average of 5 plants of each spinach genotype that had completed development. Based on these observations, spinach genotypes were also analyzed using Principal Component Analysis (PCA). The present study aimed to interpret the morphological parameters together. Therefore, Principal Component Analysis (PCA) was used for multivariate statistical analysis, which provided a significant reduction in the number of parameters required to explain most of the data. In this way, the values of the obtained features were subjected to PCA separately using the JMP 10 statistical program.

RESULTS and DISCUSSION

In the first period of the study, observations were taken from genotypes at the S6-S7 level according to UPOV parameters. In the first period of the study (S6-S7), anthocyanin coloration in the leaf was observed in 4 genotypes, while it was not observed in 60 genotypes. Leaf blade green color intensity was determined as very dark in 6 genotypes, medium in 24 genotypes, and dark in 34 genotypes (Table 1,2). In the second period of the study (S7-S8), anthocyanin coloration in the leaf was observed in 4 genotypes, whereas it was absent in 61 genotypes. The green color intensity of the leaf blade was determined as very dark in 17 genotypes, medium in 20 genotypes, dark in 18 genotypes, and light in 10 genotypes (Table 3). Köse (2018) observed leaf blade green color intensity as 9 (light), 28 (medium), 12 (dark), and 1 genotype as very dark among 50 spinach genotypes. Sabaghnıa et al. (2014) reported high yield diversity among genotypes based on morphological observations. Rashid et al. (2020) did not observe 81% anthocyanin presence in genotypes, while leaf color among genotypes was mostly dark green. Jahan (2022) observed that most leaves were green (72.55%). Leaf quality and appearance are very important marketing characteristics for vegetables whose leaves are consumed. Leaf color in spinach is also an important quality element in terms of marketability of the product (Ferrante et al., 2004). In the present study, it was revealed that

leaf color diversity ranged from light green to dark green among genotypes. It is known that chlorophylls play an important role as the primary photosynthetic pigment, capturing light energy from the sun in plants with abundant vegetative parts. In this context, the dark green color of spinach is a desired characteristic because chlorophylls dominate over carotenoids (Dutta et al., 2005). Leaf color, in terms of darkness and brightness, is associated with chlorophyll pigments and is a desired feature in spinach consumed fresh and frozen (Rashid et al., 2020). According to studies and the current study's results, the green color of spinach leaf blades varies among genotypes. However, it is predicted that color is influenced by many factors, including plant nutrition and environmental conditions. In spinach cultivation, factors such as the growing season and bolting are thought to affect the performance and quality of fresh spinach. It is known that high temperatures and long days generally cause the plant to transition from the vegetative to the generative phase. It is known that heat and long days generally trigger a shift from vegetative to generative growth, resulting in decreased yield (Chitwood et al., 2016). In all genotypes, leaf blade blistering in the first period was determined as 26 medium, 6 very weak, 9 strong, 15 weak, and absent in 8 genotypes. Leaf blade lobing was observed as strong in 13 genotypes, very weak in 6 genotypes, weak in 9 genotypes, and moderate in 24 genotypes (Table 1). In the next period of the study (S7-S8), leaf blade blistering was determined as 22 medium, 4 very weak, 10 strong, 17 weak, and absent in 12 genotypes. While leaf blade lobing was not observed in 1 genotype, it was strong in 31 genotypes, weak in 3 genotypes, and medium in 30 genotypes (Table 3). In the studies conducted, leaf blade blistering was 14 very weak, 21 weak, 12 moderate, and 3 strong; 43.5% weak and 32% absent, 45.10% weak, 29.41% moderate, and 25.49% strong, respectively (Köse, 2018; Rashid et al., 2020; Jahan, 2022). In other studies, leaf blade lobing was classified as: Köse (2018), 45% weak, 29% medium, 17% strong; Arif et al. (2013), slightly deep (91.7%) and medium deep (5.6%). It is known that spinach, which shows blistering, accumulates high levels of nitrate (Arshi, 2000). In the first period of the study (S6-S7), leaf petiole attitude was determined as 26 horizontal, 29 semi-erect, 9 upright, and leaf petiole length was determined as 24 medium, 13 short, 27 long. When leaf blade attitude was examined across genotypes, it was found to be 34 horizontal, 24 semi-erect, and 6 vertical (Table 1). In the second period (S7-S8) of the study, leaf petiole attitude was determined as 24 horizontal, 19 semi-erect, 22 vertical; leaf petiole length was determined as 27 medium, 19 short, 19 long. When leaf blade attitude was examined across genotypes, 28 were horizontal, 24 semi-erect, 7 vertical, and 6 semi-drooping (Table 3). As a result of morphological observations, leaf petiole attitude was determined as 4 vertical, 37 semi-erect, and 9 horizontal; leaf petiole length was determined as 6 short, 23 medium, and 21 long; leaf blade attitude was determined as 5 vertical, 31 semi-vertical, 13 horizontal, and 1 semi-drooping (Köse,2018). Rashid et al. (2020) leaf petiole attitude among genotypes was grouped as 52.5% erect, 39.5% semi-erect, 8% horizontal; as erect, semi-erect and horizontal; Arif et al. (2013) as 50% horizontal and 33.3% erect; Rafiei et al. (2021) classified the local diversity in terms of leaf petiole length as short, medium and long; Arif et al. (2013) as 8.9% short and 91.1% medium. Based on morphological observations by Jahan (2022), no diversity was observed among genotypes in leaf petiole attitude. In addition to the leaf blade shape, other leaf morphological characteristics such as the shape of the leaf blade apex and the longitudinal section shape of the leaf blade were also examined. In the first period study (S6-S7), the leaf blade apex shape was obtuse in 35 genotypes, rounded in 18, and acute in 11; the longitudinal section shape was convex in 54, flat in 7, and concave in 3 (Table 2). In the same period, the time of start of bolting was late in 10 genotypes, early in 17, and medium in 37. Furthermore, while the proportion of monoecious plants was almost absent in most genotypes, differences were observed among genotypes in the ratios of female and male plants (Table 2). In spinach cultivation, leaf blade shape is an important parameter for product marketability, the fresh market, and cultural practices. Petiole length in spinach is known to be an important commercial factor for machine harvesting and canned production (Sabaghnıa et al., 2014). However, as stem length increases, nitrate accumulation in the leaf blades occurs, which can be considered a disadvantage in commercial cultivation (Lorenz, 1978). As a result of the morphological observations made in the first period of the present study (S6-S7), the leaf blade shape was defined as 19 genotypes as wide oval, 21 genotypes as medium oval, 7 genotypes as triangular, 16 genotypes as medium elliptical, and 1 genotype as wide elliptical (Table 1). As a result of the observations made in the second period of the study (S7-S8), the leaf blade shape was defined as 10 genotypes as wide oval, 23 genotypes as medium oval, 2 genotypes as triangular, 28 genotypes as medium elliptical, and 2 genotypes as wide elliptical (Table 4). When the studies were examined, Köse (2018) classified the leaf blade shape into different groups as triangular, medium oval, wide oval, medium elliptical; Jahan (2022) classified it as elliptical, oval, and wide oval; Rafiei et al. (2021) classified it as oval and deltoid. In the study, it is thought that differences in leaf blade shape between the two periods are due to differences in growing seasons. It has been shown that stress (such as cold and excessive humidity) during the growing season affects the dry weight of spinach (Abolghasemi et al., 2019). In the study, the leaf blade apex shape was 35 blunt, 18 round, 11 pointed, and the leaf blade longitudinal sectional shape was 54 convex, 7 flat, 3 concave (Table 2). As a result of the observations taken in the second period (S7-S8), the leaf blade apex shape was 45 blunt, 5 round, 15 pointed, and the leaf blade longitudinal sectional shape was 47 convex, 8 flat, 10 concave (Table 4).

Table 1. In the first period (S6-S7), Anthocyanin coloration in the leaf (A), İntensity of green color in the leaf blade (B), Blistering in the leaf blade (C), Lobing in the leaf blade (D), Petiole retention (E), Petiole length (F), Leaf blade attitude (G), Leaf blade shape (excluding basal lobes) (H), Leaf brittleness (I) characteristics of genotypes

Çizelge 1. İlk dönem (S6-S7) genotiplerin yapraktaki antosiyanin renklenmesi (A), yaprak ayasında yeşil rengin yoğunluğu (B), yaprak ayasında kabarcıklanma (C), yaprak ayasında lob oluşumu (D), yaprak sapı tutumu (E), yaprak sapı uzunluğu (F), yaprak ayası tutumu (G), yaprak ayası şekli (bazal loblar hariç) (H), yaprak kırılganlığı (I) özellikleri

Genotypes	A	B	C	D	E	F	G	H	I
1	Present	Very dark	Very weak	Strong	Horizontal	Medium	Semi-erect	Medium elliptic	Medium
2	Present	Very dark	Very weak	Strong	Semi-erect	Medium	Horizontal	Medium elliptic	Low
3	Absent	Medium	Absent	Absent	Horizontal	Medium	Horizontal	Broad ovate	Medium
4	Absent	Medium	Absent	Absent	Horizontal	Medium	Erect	Broad ovate	Low
5	Absent	Dark	Medium	Absent	Erect	Short	Semi-erect	Medium ovate	Low
6	Absent	Very dark	Medium	Wery weak	Semi-erect	Short	Horizontal	Medium ovate	Medium
7	Absent	Medium	Absent	Absent	Semi-erect	Short	Semi-erect	Medium ovate	Low
8a	Present	Dark	Medium	Weak	Horizontal	Medium	Horizontal	Broad ovate	High
9	Present	Very dark	Medium	Medium	Semi-erect	Medium	Horizontal	Broad ovate	High
10	Absent	Medium	Medium	Weak	Horizontal	Long	Horizontal	Broad ovate	Medium
11	Absent	Very dark	Strong	Absent	Semi-erect	Short	Semi-erect	Medium elliptic	High
12	Absent	Very dark	Strong	Wery weak	Erect	Short	Semi-erect	Broad ovate	Medium
13	Absent	Dark	Medium	Medium	Erect	Medium	Semi-erect	Medium ovate	High
14	Absent	Dark	Strong	Medium	Erect	Long	Semi-erect	Broad ovate	High
15	Absent	Medium	Weak	Wery weak	Horizontal	Short	Horizontal	Medium elliptic	Medium
16	Absent	Dark	Medium	Absent	Semi-erect	Short	Erect	Broad ovate	Low
17	Absent	Medium	Very weak	Weak	Horizontal	Medium	Horizontal	Broad ovate	Medium
18	Absent	Medium	Medium	Medium	Horizontal	Medium	Horizontal	Broad ovate	Low
19	Absent	Medium	Weak	Absent	Semi-erect	Long	Horizontal	Medium elliptic	Medium
20	Absent	Medium	Weak	Medium	Horizontal	Medium	Horizontal	Broad ovate	Medium
21	Absent	Medium	Very weak	Weak	Semi-erect	Long	Semi-erect	Medium elliptic	Medium
22	Absent	Medium	Medium	Medium	Semi-erect	Medium	Semi-erect	Broad ovate	High
23	Absent	Medium	Weak	Very weak	Semi-erect	Short	Horizontal	Medium elliptic	Medium
24	Absent	Medium	Medium	Weak	Erect	Medium	Erect	Medium ovate	High
25	Absent	Medium	Weak	Medium	Semi-erect	Medium	Semi-erect	Medium ovate	Medium
26	Absent	Medium	Weak	Medium	Horizontal	Medium	Horizontal	Medium eliptik	Medium
27	Absent	Medium	Medium	Weak	Horizontal	Medium	Semi-erect	Medium ovate	Low
28	Absent	Medium	Weak	Medium	Horizontal	Long	Horizontal	Medium ovate	Medium
29	Absent	Dark	Weak	Weak	Horizontal	Long	Horizontal	Medium elliptic	Low
30	Absent	Dark	Medium	Medium	Semi-erect	Long	Horizontal	Triangular	Medium
31	Absent	Dark	Weak	Absent	Semi-erect	Medium	Erect	Medium elliptic	Low
32	Absent	Dark	Absent	Medium	Horizontal	Medium	Semi-erect	Triangular	Low
33	Absent	Medium	Absent	Absent	Horizontal	Long	Horizontal	Medium elliptic	Low
34	Absent	Dark	Weak	Very weak	Horizontal	Long	Horizontal	Triangular	Low
35	Absent	Dark	Medium	Very weak	Horizontal	Long	Horizontal	Triangular	Low
36	Absent	Medium	Medium	Medium	Semi-erect	Long	Horizontal	Broad elliptic	Low
37	Absent	Medium	Very weak	Absent	Semi-erect	Medium	Semi-erect	Medium elliptic	Low
38	Absent	Medium	Weak	Strong	Horizontal	Long	Horizontal	Broad ovate	Medium
39	Absent	Dark	Medium	Medium	Erect	Long	Horizontal	Medium ovate	Low
40	Absent	Medium	Weak	Absent	Erect	Medium	Erect	Medium elliptic	Medium
41	Absent	Dark	Absent	Strong	Semi-erect	Long	Horizontal	Triangular	Medium
42	Absent	Medium	Weak	Strong	Horizontal	Short	Semi-erect	Medium elliptic	Low
43	Absent	Dark	Very weak	Strong	Horizontal	Medium	Horizontal	Broad ovate	Medium
44	Absent	Dark	Medium	Medium	Semi-erect	Short	Horizontal	Medium ovate	Low
45	Absent	Dark	Medium	Medium	Erect	Long	Erect	Triangular	Medium
46	Absent	Dark	Medium	Medium	Horizontal	Medium	Horizontal	Broad ovate	Medium
47	Absent	Dark	Medium	Medium	Semi-erect	Short	Semi-erect	Medium ovate	Medium
48	Absent	Dark	Strong	Strong	Erect	Long	Semi-erect	Medium ovate	Medium
49	Absent	Dark	Medium	Medium	Semi-erect	Long	Horizontal	Medium ovate	High
50	Absent	Dark	Weak	Weak	Semi-erect	Long	Horizontal	Medium elliptic	Medium
51	Absent	Dark	Medium	Medium	Horizontal	Medium	Semi-erect	Medium ovate	Low
52	Absent	Medium	Medium	Medium	Semi-erect	Long	Horizontal	Medium ovate	Medium
53	Absent	Dark	Medium	Strong	Semi-erect	Long	Horizontal	Broad ovate	Low
54	Absent	Dark	Medium	Medium	Semi-erect	Long	Semi-erect	Broad ovate	Low
55	Absent	Dark	Medium	Medium	Horizontal	Short	Horizontal	Medium ovate	Low
56	Absent	Dark	Strong	Strong	Horizontal	Medium	Horizontal	Medium ovate	Medium
57	Absent	Dark	Weak	Strong	Semi-erect	Long	Semi-erect	Broad ovate l	Medium
58	Absent	Dark	Medium	Strong	Semi-erect	Long	Semi-erect	Medium ovate	Low
59	Absent	Dark	Strong	Strong	Semi-erect	Long	Semi-erect	Triangular	Low
60	Absent	Dark	Strong	Weak	Horizontal	Medium	Horizontal	Medium ovate	Low
61	Absent	Dark	Absent	Strong	Horizontal	Long	Horizontal	Broad ovate	Low
62	Absent	Dark	Strong	Medium	Semi-erect	Long	Semi-erect	Medium elliptic	High
63	Absent	Dark	Absent	Absent	Semi-erect	Long	Semi-erect	Medium ovate	Low
64	Absent	Dark	Strong	Medium	Semi-erect	Short	Semi-erect	Medium ovate	Low

Table 2. In the first period (S6-S7), Shape of the apex in the leaf blade (J), Longitudinal section shape of the leaf blade(K), Leaf brightness (L), Leaf number (M), Time of start of bolting (N), Monoecious plant ratio (O), Female plant ratio (P), Male plant ratio (R) characteristics of genotypes

Çizelge 2. Ilk dönem (S6-S7) genotiplerin yaprak ayası apex şekli (J), yaprak ayasının boyuna kesit şekli (K), yaprak parlaklığı (L), yaprak sayısı (M), çiçekleme başlama zamanı (N), monoecious bitki oranı (O), dişi bitki oranı (P), erkek bitki oranı (R) özellikleri

Genotip	J	K	L	M	N	O	P	R
1	Obtuse	Convex	Medium	High	Late	Absent	High	Medium
2	Obtuse	Convex	Medium	High	Late	Absent	Medium	Medium
3	Obtuse	Convex	Medium	High	Early	Absent	Low	Low
4	Obtuse	Convex	Medium	Medium	Late	Absent	High	Medium
5	Rounded	Flat	Medium	Low	Late	Absent	Medium	Medium
6	Obtuse	Convex	Strong	Medium	Medium	Absent	High	High
7	Acute	Flat	Medium	Medium	Early	Medium	Low	Low
8a	Obtuse	Convex	Medium	High	Late	Medium	Low	Low
9	Rounded	Convex	Strong	High	Late	Absent	Low	Medium
10	Obtuse	Convex	Strong	Medium	Medium	Absent	Medium	Medium
11	Obtuse	Flat	Medium	Low	Medium	Absent	Low	Low
12	Rounded	Flat	Medium	High	Medium	Absent	Medium	Medium
13	Obtuse	Convex	Strong	Medium	Late	Absent	Medium	Medium
14	Obtuse	Concave	Medium	High	Late	Absent	Medium	Medium
15	Obtuse	Convex	Medium	Medium	Medium	Absent	Medium	Medium
16	Rounded	Concave	Medium	Medium	Early	Absent	High	Medium
17	Obtuse	Convex	Medium	Medium	Medium	Absent	Low	Low
18	Obtuse	Convex	Medium	Medium	Medium	Absent	Low	Medium
19	Acute	Convex	Medium	High	Medium	Absent	Medium	Medium
20	Obtuse	Concave	Medium	Medium	Medium	Absent	Medium	Medium
21	Obtuse	Convex	Medium	Medium	Medium	Absent	Medium	Medium
22	Obtuse	Convex	Medium	Medium	Medium	Absent	Medium	Medium
23	Obtuse	Convex	Medium	High	Early	Absent	Medium	Medium
24	Obtuse	Flat	Medium	Medium	Medium	Absent	High	Medium
25	Obtuse	Flat	Medium	Medium	Medium	Absent	Low	Low
26	Acute	Convex	Weak	Medium	Late	Absent	High	Low
27	Acute	Convex	Weak	High	Medium	Absent	Medium	Medium
28	Obtuse	Convex	Medium	High	Medium	Absent	Low	Medium
29	Obtuse	Convex	Strong	High	Medium	Absent	Medium	Medium
30	Acute	Convex	Medium	High	Early	Absent	Medium	Medium
31	Obtuse	Convex	Medium	High	Medium	Absent	Medium	Low
32	Acute	Convex	Weak	High	Medium	Absent	Low	Low
33	Obtuse	Convex	Medium	Medium	Late	Absent	Low	Low
34	Acute	Convex	Medium	Medium	Medium	Absent	Medium	Medium
35	Acute	Convex	Medium	Medium	Medium	Absent	Low	High
36	Rounded	Convex	Medium	Medium	Medium	Absent	Medium	Medium
37	Obtuse	Convex	Medium	Low	Medium	Absent	Medium	Medium
38	Obtuse	Convex	Medium	Low	Medium	Absent	Medium	Medium
39	Obtuse	Convex	Medium	Medium	Medium	Absent	Medium	Medium
40	Obtuse	Convex	Medium	Medium	Early	Absent	High	Medium
41	Acute	Convex	Medium	Medium	Early	Absent	High	High
42	Obtuse	Flat	Medium	Low	Early	Absent	Low	Low
43	Rounded	Convex	Medium	Medium	Early	Absent	Medium	Medium
44	Rounded	Convex	Medium	Medium	Medium	Absent	Medium	Medium
45	Acute	Convex	Medium	Medium	Medium	Absent	Medium	Medium
46	Rounded	Convex	Medium	Medium	Early	Absent	Medium	Medium
47	Rounded	Convex	Medium	Medium	Early	Absent	Medium	Medium
48	Obtuse	Convex	Medium	Medium	Early	Absent	Medium	Medium
49	Obtuse	Convex	Medium	Medium	Medium	Absent	Medium	Medium
50	Obtuse	Convex	Medium	Low	Medium	Absent	Medium	Medium
51	Obtuse	Convex	Medium	Low	Early	Absent	Medium	Medium
52	Rounded	Convex	Strong	Medium	Early	Absent	High	Medium
53	Obtuse	Convex	Strong	Low	Medium	Absent	Low	Medium
54	Rounded	Convex	Strong	High	Early	Absent	Medium	Medium
55	Obtuse	Convex	Medium	Medium	Medium	Absent	Low	Medium
56	Rounded	Convex	Medium	Low	Medium	Absent	Medium	Medium
57	Rounded	Convex	Medium	Low	Early	Absent	Medium	Medium
58	Rounded	Convex	Medium	Medium	Early	Absent	High	High
59	Acute	Convex	Medium	Medium	Medium	Absent	Medium	Medium
60	Rounded	Convex	Medium	High	Medium	Absent	High	High
61	Rounded	Convex	Medium	Medium	Medium	Absent	High	High
62	Obtuse	Convex	Medium	Medium	Medium	Absent	Medium	Medium
63	Rounded	Convex	Medium	Medium	Medium	Absent	Medium	Medium
64	Rounded	Convex	Medium	High	Medium	Absent	High	High

Table 3. In the second period (S7-S8), Anthocyanin coloration in the leaf (A), İntensity of green color in the leaf blade (B), Blistering in the leaf blade (C), Lobing in the leaf blade (D), petiole retention (E), Petiole length (F), Leaf blade attitude (G) characteristics of the genotypes

Çizelge 3. İkinci dönem(S7-S8) genotiplerin yapraktaki antosiyanin renklenmesi (A), yaprak ayasında yeşil rengin yoğunluğu (B), yaprak ayasında kabarcıklanma (C), yaprak ayasında lob oluşumu (D), yaprak sapı tutumu (E), yaprak sapı uzunluğu (F), yaprak ayası tutumu (G)özellikleri

Genotypes	A	B	C	D	E	F	G
1	Present	Very dark	Very weak	Strong	Semi-erect	Long	Semi-erect
2	Present	Very dark	Very weak	Medium	Erect	Long	Semi-erect
3	Absent	Medium	Weak	Medium	Erect	Medium	Semi-erect
4	Absent	Very dark	Medium	Medium	Erect	Medium	Erect
5	Absent	Very dark	Strong	Strong	Erect	Short	Erect
6	Absent	Very dark	Very weak	Weak	Erect	Short	Semi-erect
7	Absent	Medium	Medium	Strong	Erect	Long	Erect
8a	Present	Medium	Absent	Medium	Erect	Long	Semi-erect
8b	Absent	Very dark	Strong	Strong	Horizontal	Medium	Semi-pendulous
9	Present	Light	Weak	Strong	Semi-erect	Long	Horizontal
10	Absent	Very dark	Strong	Strong	Erect	Long	Semi-erect
11	Absent	Very dark	Very weak	Weak	Erect	Medium	Horizontal
12	Absent	Very dark	Weak	Absent	Erect	Long	Erect
13	Absent	Medium	Strong	Medium	Erect	Short	Horizontal
14	Absent	Medium	Weak	Strong	Erect	Long	Horizontal
15	Absent	Light	Absent	Medium	Horizontal	Medium	Semi-pendulous
16	Absent	Light	Medium	Medium	Horizontal	Medium	Erect
17	Absent	Light	Absent	Strong	Semi-erect	Short	Semi-erect
18	Absent	Light	Absent	Strong	Semi-erect	Short	Erect
19	Absent	Light	Medium	Medium	Horizontal	Medium	Semi-pendulous
20	Absent	Dark	Medium	Medium	Horizontal	Short	Semi-pendulous
21	Absent	Dark	Weak	Strong	Erect	Long	Erect
22	Absent	Medium	Medium	Medium	Horizontal	Short	Semi-pendulous
23	Absent	Very dark	Absent	Medium	Horizontal	Short	Horizontal
24	Absent	Light	Weak	Strong	Erect	Long	Semi-erect
25	Absent	Medium	Weak	Strong	Horizontal	Medium	Horizontal
26	Absent	Medium	Absent	Strong	Horizontal	Medium	Horizontal
27	Absent	Medium	Absent	Weak	Erect	Medium	Semi-erect
28	Absent	Medium	Absent	Strong	Horizontal	Long	Horizontal
29	Absent	Medium	Absent	Strong	Erect	Long	Horizontal
30	Absent	Medium	Weak	Strong	Erect	Long	Semi-erect
31	Absent	Dark	Medium	Medium	Semi-erect	Long	Horizontal
32	Absent	Medium	Weak	Medium	Erect	Medium	Semi-erect
33	Absent	Medium	Medium	Strong	Horizontal	Long	Horizontal
34	Absent	Dark	Medium	Medium	Horizontal	Short	Semi-pendulous
35	Absent	Medium	Medium	Strong	Horizontal	Short	Semi-erect
36	Absent	Very dark	Medium	Strong	Semi-erect	Long	Horizontal
37	Absent	Medium	Weak	Strong	Horizontal	Short	Horizontal
38	Absent	Dark	Medium	Strong	Semi-erect	Short	Horizontal
39	Absent	Dark	Medium	Medium	Erect	Long	Horizontal
40	Absent	Medium	Medium	Strong	Semi-erect	Medium	Horizontal
41	Absent	Light	Absent	Strong	Semi-erect	Medium	Semi-erect
42	Absent	Light	Weak	Medium	Semi-erect	Short	Horizontal
43	Absent	Very dark	Strong	Strong	Horizontal	Long	Horizontal
44	Absent	Light	Weak	Strong	Semi-erect	Medium	Semi-erect
45	Absent	Very dark	Medium	Medium	Semi-erect	Medium	Horizontal
46	Absent	Medium	Weak	Medium	Horizontal	Medium	Horizontal
47	Absent	Dark	Medium	Medium	Horizontal	Short	Semi-erect
48	Absent	Dark	Strong	Strong	Semi-erect	Short	Semi-erect
49	Absent	Very dark	Strong	Medium	Horizontal	Medium	Semi-erect
50	Absent	Dark	Strong	Medium	Semi-erect	Medium	Horizontal
51	Absent	Dark	Medium	Medium	Horizontal	Long	Horizontal
52	Absent	Very dark	Strong	Strong	Semi-erect	Medium	Horizontal
53	Absent	Dark	Strong	Strong	Horizontal	Medium	Horizontal
54	Absent	Medium	Strong	Medium	Erect	Medium	Semi-erect
55	Absent	Dark	Medium	Strong	Horizontal	Short	Horizontal
56	Absent	Medium	Weak	Strong	Horizontal	Short	Semi-erect
57	Absent	Dark	Medium	Strong	Semi-erect	Short	Semi-erect
58	Absent	Dark	Absent	Medium	Horizontal	Short	Semi-erect
59	Absent	Dark	Medium	Medium	Semi-erect	Medium	Semi-erect
60	Absent	Very dark	Medium	Medium	Horizontal	Medium	Horizontal
61	Absent	Dark	Medium	Medium	Semi-erect	Medium	Horizontal
62	Absent	Dark	Weak	Medium	Semi-erect	Medium	Horizontal
63	Absent	Dark	Weak	Medium	Semi-erect	Medium	Semi-erect
64	Absent	Very dark	Absent	Medium	Semi-erect	Medium	Semi-erect

Table 4. In the second period (S7-S8), Leaf blade shape (excluding basal lobes) (H), Leaf brittleness (I) Shape of the apex in the leaf blade (J), Longitudinal section shape of the leaf blade (K), Leaf brightness (L), Leaf number (M), Time of start of bolting (N) characteristics of the genotypes

Çizelge 4. İkinci dönem (S7-S8) genotiplerin yaprak ayası şekli (bazal loblar hariç) (H), Yaprak kırılganlığı (I) yaprak ayası apex şekli (J), yaprak ayasının boyuna kesit şekli (K), yaprak parlaklığı (L), yaprak sayısı (M), sapa kalkma eğilimi (N) özellikleri

Genotypes	H	I	J	K	L	M	N
1	Medium elliptic	Low	Obtuse	Convex	Medium	Medium	Medium
2	Medium elliptic	Low	Obtuse	Convex	Medium	Medium	Medium
3	Medium ovate	Low	Obtuse	Convex	Medium	Medium	Early
4	Broad ovate	Low	Rounded	Flat	Medium	Very high	Early
5	Medium elliptic	Low	Obtuse	Flat	Strong	Medium	Medium
6	Medium elliptic	Medium	Obtuse	Flat	Medium	Medium	Early
7	Medium ovate	Low	Obtuse	Convex	Medium	Medium	Early
8a	Medium ovate	Low	Rounded	Flat	Medium	High	Early
8b	Broad ovate	Low	Obtuse	Convex	Strong	Low	Medium
9	Broad elliptic	Medium	Rounded	Convex	Weak	Medium	Early
10	Medium Ovate	Low	Obtuse	Concave	Medium	High	Early
11	Medium elliptic	Low	Obtuse	Concave	Medium	Medium	Early
12	Medium elliptic	Low	Obtuse	Flat	Strong	High	Early
13	Medium elliptic	Low	Obtuse	Convex	Medium	Medium	Medium
14	Medium ovate	Low	Obtuse	Convex	Medium	Medium	Early
15	Medium ovate	High	Obtuse	Convex	Medium	Medium	Early
16	Medium elliptic	Medium	Rounded	Concave	Medium	High	Late
17	Medium elliptic	Low	Acute	Convex	Medium	High	Early
18	Medium elliptic	Low	Obtuse	Convex	Medium	Medium	Early
19	Medium elliptic	Low	Obtuse	Convex	Medium	Medium	Medium
20	Medium ovate	Low	Obtuse	Convex	Medium	High	Medium
21	Triangular	Low	Acute	Convex	Medium	Very high	Early
22	Broad ovate	Low	Obtuse	Convex	Medium	Very high	Late
23	Medium elliptic	Medium	Acute	Convex	Medium	Medium	Medium
24	Medium ovate	Low	Acute	Convex	Medium	High	Medium
25	Medium elliptic	Medium	Acute	Convex	Weak	Medium	Early
26	Medium elliptic	Medium	Acute	Convex	Weak	Medium	Medium
27	Medium elliptic	Low	Acute	Convex	Weak	High	Early
28	Medium ovate	Medium	Obtuse	Convex	Medium	High	Medium
29	Medium ovate	Low	Acute	Convex	Medium	High	Medium
30	Triangular	Medium	Acute	Convex	Medium	High	Medium
31	Medium ovate	Low	Obtuse	Concave	Medium	High	Medium
32	Medium ovate	Low	Obtuse	Concave	Medium	High	Late
33	Medium ovate	Low	Acute	Convex	Medium	Medium	Medium
34	Broad ovate	Low	Rounded	Convex	Medium	Medium	Early
35	Medium Oval	Low	Obtuse	Concave	Medium	Medium	Medium
36	Broad ovate	Low	Acute	Convex	Medium	High	Early
37	Medium ovate	Medium	Obtuse	Convex	Medium	Medium	Medium
38	Medium elliptic	Low	Acute	Convex	Medium	Medium	Medium
39	Broad ovate	Low	Obtuse	Convex	Medium	High	Medium
40	Medium elliptic	Medium	Obtuse	Convex	Medium	Medium	Early
41	Medium elliptic	Low	Obtuse	Flat	Medium	Medium	Early
42	Medium ovate	Low	Obtuse	Convex	Medium	Medium	Early
43	Broad ovate	Low	Obtuse	Convex	Medium	High	Medium
44	Medium elliptic	Low	Obtuse	Convex	Strong	Medium	Late
45	Broad ovate	Low	Obtuse	Flat	Medium	Medium	Early
46	Medium ovate	Low	Obtuse	Convex	Medium	High	Early
47	Medium elliptic	Low	Obtuse	Convex	Medium	High	Early
48	Medium ovate	Low	Acute	Convex	Medium	High	Medium
49	Broad ovate	Low	Obtuse	Convex	Medium	Medium	Early
50	Medium ovate	Low	Obtuse	Convex	Strong	High	Early
51	Broad ovate	Low	Obtuse	Convex	Medium	Medium	Early
52	Medium elliptic	Low	Obtuse	Convex	Medium	Low	Late
53	Medium elliptic	Low	Obtuse	Convex	Medium	Low	Late
54	Medium ovate	Low	Obtuse	Convex	Medium	Low	Late
55	Medium ovate	Medium	Obtuse	Convex	Medium	Low	Late
56	Medium elliptic	Low	Obtuse	Convex	Medium	Low	Late
57	Medium elliptic	Low	Obtuse	Concave	Medium	Low	Medium
58	Medium elliptic	Low	Acute	Concave	Medium	Medium	Late
59	Medium elliptic	Low	Obtuse	Concave	Strong	Medium	Late
60	Broad elliptic	Low	Obtuse	Concave	Medium	Medium	Late
61	Medium ovate	Low	Acute	Convex	Medium	Medium	Medium
62	Medium ovate	Low	Obtuse	Convex	Medium	Medium	Medium
63	Medium elliptic	Medium	Obtuse	Convex	Medium	Medium	Late
64	Medium elliptic	Low	Obtuse	Convex	Strong	High	Medium

Köse (2018) reported that the longitudinal sectional shape of the leaf blade was 46 convex and 4 flat. When the apex shape of the leaf blade was examined in studies, 37% of them were round Rashid et al., 2020; Rafiei et al., 2021), grouped them as round and pointed.

In spinach, it was determined that the longitudinal section shape of the leaf blade was an important factor in yield, along with leaf width and leaf number (Abolghasemi et al., 2019). When leaf brittleness was examined during the first period of the study, 27 genotypes were classified as medium, 28 as low, and 9 as high. Leaf brightness was determined as strong for 8 genotypes, medium for 53 genotypes, and weak for 3 genotypes. The number of leaves was determined as 18 genotypes high, 36 genotypes medium, and 10 genotypes low (Table 1). The bolting time of the genotypes was determined as late in 10 genotypes, early in 17 genotypes, and moderate in 37 genotypes. Among the genotypes, the proportion of monoecious plants was absent in 62 genotypes and medium in 2 genotypes. The ratio of female plants was determined as 13 high, 16 low, and 35 medium. The ratio of genotypes to male plants was 46 genotypes medium, 11 genotypes less, and 7 genotypes high (Table 2). In the second period of the study (S7-S8), leaf brightness was determined as strong for 7 genotypes, medium for 54 genotypes, and weak for 4 genotypes. The number of leaves was determined as 21 genotypes high, 3 genotypes very high, 34 genotypes medium, and 7 genotypes low. The bolting time of the genotypes was determined as late in 13 genotypes, early in 27 genotypes, and medium in 25 genotypes (Table 4). Köse (2018) determined the leaf brightness status as 38 (medium) and 11 (strong). Another strong factor was the leaf brightness status, with weak (56%), medium (30%), and strong (13.5%) (Rashid et al., 2020). The bolting time in the genotypes was determined as 80% medium (Rashid et al., 2020).

It has been shown that phenotypic diversity in plants (leaf shape, seed type, leaf color) affects plant growth and development, as well as biochemical properties (Abolghasemi et al., 2022). Parameters such as leaf blade shape, leaf morphology, leaf color, presence of anthocyanins in the leaf, bolting start time, and increased yield potential are among the most important criteria in commercial spinach cultivation and breeding programs (Ma et al., 2016). In this context, diversity was observed in agro-morphological measurements and observations made in both periods of the study.

Principal Component Analysis

Principal component analysis (PCA) is a reliable multivariate technique to transform a large data set into several factors containing several interrelated variables (Pachauri et al., 2017). This analysis can capture the extent of diversity across a large number of genotypes by reducing data volume (Pachauri et al., 2017; Fereidoonfar et al., 2018; Shen et al., 2019). Therefore, PCA was performed to identify different traits that cause diversity among spinach genotypes. According to the specified parameters, it is desired that the Eigenvalue be greater than 1 to define the PC value for each factor. In other words, it has been stated that the larger the eigenvalue, the more data that component represents (Avşar, 2011). Principal component (PC) axes, eigenvalues, variation and cumulative variation rates, and the weights of the principal components obtained from the Principal Component Analysis (PCA) were considered to have significant weights if the weight values of the principal components in the PCA analysis were above 0.3 (Brown, 1991). When interpreting PCA results, the threshold for component loadings was set according to that reported by Brown (1991). In the study, eigenvalues were found to be between 2.34 and 1.17 (Table 5). Eigenvalues greater than 1 indicate that the main component's weights are reliable (Mohammadi & Prasanna, 2003). The morphological observations in spinach were subjected to principal components analysis (PCA). Considering the components, the first six components in the PCA explained 69.092% of the study's variance. Other studies have reported that the first two components of a PCA should explain more than 25% of the variation (Seymen ,2021; Kayak,2024). PC1 represented leaf petiole attitude and leaf blade attitude as the main loading factors with eigenvectors in the range of 0.38–0.51 and 16% overall contribution rate. PC2 contributed 15% with green color intensity, leaf blade blistering, and leaf brightness as the higher loading factors (0.37–0.43). The contribution of PC3 was up to 11%, mainly due to anthocyanin presence in the leaf, leaf blade blistering (0.34-0.39 eigenvector). (Table 5). PCA could help identify the best representative traits responsible for the observed genotypic variability. PCA of the present results indicated that leaf and anthocyanin-related traits were the main factors contributing to the variability. When the studies were examined, it was reported that the first six components explained 67.229% of the study, and as a result of PCA Analysis, the first component (PC1) explained 16.729% of the study, and the second component (PC2) explained 12.618% of the study (Rashid et al., 2020). Sabaghnia et al. (2014) reported that leaf traits are the most important factor contributing to the variability of spinach germplasm. In the study conducted by Jahan (2022), it was reported that the first PC explained 37.10% of the total variability, the second PC explained 22.90% of the variation, and the eigenvalues were between 0.42 and 2.97. In another study, the first five components explained 79% of the variance, with the first component (PC1) accounting for 30% and the second (PC2) for 17% (Mohebodini et al., 2017). As a result of the PCA analysis conducted by Avşar (2011), it was reported that the eigenvalues of the first, second and third components were 25.38%, 18.33% and 11.89% of the variance, respectively, and these first three components explained 55.60% of

the variance. When the studies were examined, it was revealed that the results were similar to the current study.

Table 5. Principal Component Analysis (PCA) of spinach genotypes at S7- S8 generation
Çizelge 5. S7- S8 kademesindeki ıspanak genotiplerinin Principal Component Analizi (PCA)

Eigen value	2.34	2.14	1.67	1.32	1.17	1.00
Variance%	16.72	15.31	11.95	9.47	8.42	7.19
Total Variance %	16.72	32.04	43.99	53.47	61.89	69.09
Trait	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Leaf anthocyanin coloration	0,19847	0,17482	0,39010	-0,55006	0,01284	0,14232
Leaf blade intensity of green color	-0,12218	0,37195	0,02897	0,09170	0,44140	-0,40772
Leaf blade: blistering	0,17766	0,36513	0,34545	0,29923	0,13054	0,09923
Leaf blade: lobing	0,24748	-0,13278	0,29173	0,41862	-0,46002	0,24748
Petiole: attitude	0,51478	0,00173	-0,05782	0,00095	0,16188	0,16100
Petiole: length	-0,33813	-0,21017	0,14548	0,42666	0,04014	-0,04299
Leaf blade: attitude	0,38292	-0,13719	0,08031	0,10601	0,53491	0,07708
Leaf blade: shape	0,11453	0,19695	-0,54094	-0,04207	-0,02743	-0,38314
Leaf brittleness	0,22228	-0,32805	-0,26659	-0,11719	-0,02789	0,24561
Leaf blade: shape of apex	-0,07832	0,19871	-0,34369	0,37329	0,17066	0,61948
Leaf blade: shape in longitudinal section	0,17542	-0,37194	0,21493	0,15657	0,23319	-0,30227
Leaf brightness	-0,11062	0,43165	0,17872	0,02019	-0,02626	0,12932
Number of leaves	-0,36767	-0,15476	0,22723	-0,20803	0,10464	0,25944
Time of start of bolting	0,28294	0,28746	0,00233	0,08900	-0,40708	-0,03466

It has been reported that if the angle between the vectors in the created graph is < 90 °, there is a positive relationship, and if it is > 90 °, there is a negative relationship (Yan & Kang, 2002). In the graph showing the interaction between the features in the second period of the study, it is seen that the total of the PC1 (16.7%) (the first Principal Component- 1st Main Component) and PC2 (the second Principal Component- 2nd Main Component) (15.3%) values is calculated as 32%. In biplot plots, it is desirable that the PC1% and PC2% values are as high as possible. Because this total percentage is high, it indicates that interaction is important in the subjects discussed. Therefore, it has been shown that more than 25% of the variance in PC1 and PC2 components should be explained (Mozafari et al., 2019). The graph showed a strong positive correlation between the presence of anthocyanin in the leaf and bolting time, whereas these parameters showed a strong negative correlation with petiole length and leaf number. It was also found that leaf blade lobing and leaf blade attitude were positively related. On the other hand, a negative relationship was observed between leaf blade apex shape, leaf brightness, and leaf blade green color intensity, whereas these variables showed a strong positive relationship among themselves (Figure 1). The graph was examined, and there was a strong positive correlation between the time of start of bolting and petiole attitude. It was also found that there was a positive relationship between leaf petiole attitude and Leaf blade attitude. Additionally, for the traits declared as significant in PC2, a strong positive correlation was observed between leaf brightness and leaf blade green color intensity. A positive correlation of traits represents that the improvement of one can improve the other trait too. This study was conducted to identify the key distinguishing traits that drive variation in 65 spinach genotypes. It is believed that future breeding programs will be useful in examining the relationships between these traits. The resulting PCA components can be used in breeding programs for parent selection, variation determination, and the identification of targeted trait combinations. Morphological characters that contribute significantly to PC1 and PC2, which explain a large portion of the variance, can be used as selection criteria in spinach breeding programs. Since traits showing high variation among these characters are effective in determining the genetic distance between genotypes, they can be evaluated in breeding programs for planning hybridization combinations and determining selection criteria. Furthermore, widely separated genotypes can form combinations with high heterosis potential in breeding programs. Thus, PCA is known to contribute to a more efficient selection process by reducing multiple morphological data to a smaller number of components (Taş & Balkaya, 2021).The reasons for the diversity of morphological traits in spinach germ plasm are thought to be related to adaptation to environmental conditions in which it has been grown for many years and the intense selection that comes with it (Sabaghnıa et al., 2014). In order to explain the mutual relationships between agro-morphological traits in different studies, (Arif et al., 2013) and (Sabaghnıa et al., 2014). were conducted. In the study, score plot graphs of different spinach genotypes were created using PC1 and PC2

components (Figure 2).

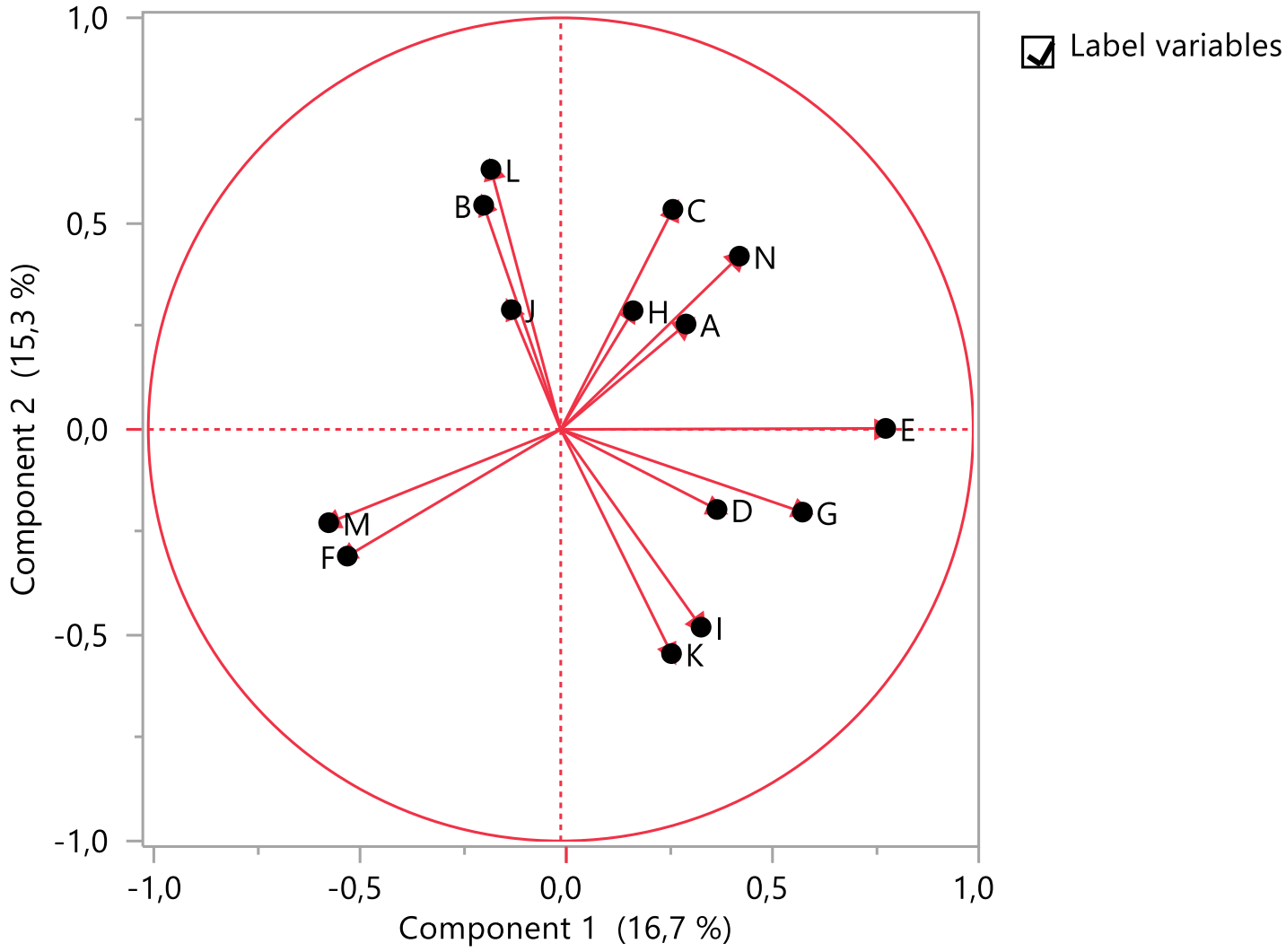

Figure 1. Loading plot graph of PC 1 and 2 obtained from PCA of spinach genotypes according to agro-morphological characteristics

Şekil 1. Ispanak genotiplerinin Agro-morfolojik özelliklere göre PCA 'dan elde edilen PC 1 ve 2'ye ait Loading plot grafiği

A: Leaf anthocyanin coloration, B: Leaf blade intensity of green color, C: Leaf blade blistering, D Leaf blade lobing, E: Petiole attitude, F: Petiole length, G: Leaf blade attitude, H: Leaf blade shape, I: Leaf brittleness J: Leaf blade shape of apex, K: Leaf blade shape in longitudinal section ,L: Leaf brightness , M: Number of leaves, N: Time of start of bolting

Cluster Analysis According to Morphological Parameters in S7 and S8 Generation Genotypes

After the spinach genotypes completed their development in the S7-S8 (second period) generation, morphological characterization was performed by taking observations in line with UPOV parameters. The obtained results were evaluated in the JMP statistical program and a dendrogram was created using hierarchical cluster analysis.

Figure 3 shows a dendrogram of spinach genotype similarities. Cluster analysis is a method used to group genotypes. Hierarchical clustering methods use elements of a proximity matrix to construct a tree dendrogram. A dendrogram constructed from genotype distance matrices clearly groups genotypes. A detailed examination of Figure 3 reveals that multiple branching and distinct color groups reflect the diversity of the spinach gene pool in the study, as determined by the morphological parameters used. Knowing the similarities or differences between genotypes is very important for future breeding programs. Figure 3 demonstrates that genotypes within the same group are more homogeneous, while genotypes in distant clusters and different color groups are important for developing new varieties. An examination of the dendrogram reveals that the genotypes are divided into seven subclusters, each with its own branching pattern. The genotypes are morphologically distinct from each other, as evidenced by their placement in different-colored groups and their connection by long branches. The genotypes consisted of 4 in the red group, 7 in the blue group, 3 in the yellow group, 8 in the purple group, 10 in the green group, 24 in the orange group, and 9 in the other color groups. An examination of the figure reveals that genotypes

25-26, 1-2, 52-53, and 61-62 exhibit the shortest branching and are closest to each other across the examined morphological parameters. Furthermore, when similarities were examined across different groups, genotypes 24-29, 3-14, and 18-41 were found to be closely linked and have similar characteristics. Genotypes 1, 2, 8a, and 9 within the same group share common traits such as leaf stalk length and leaf anthocyanin presence, and are considered promising genotypes for future spinach breeding programs. Similarly, genotypes 6, 11, and 27 within the same group share common traits such as leaf blade blistering, leaf blade lobing, leaf blade shape, and bolting time. When genotypes within the orange group were examined, they were found to be dominant for leaf brittleness and leaf blade longitudinal cross-sectional shape. Furthermore, a look at the PCA analysis conducted at the S7-S8 stage (Figure 2) reveals that the results overlap with the dendrogram.

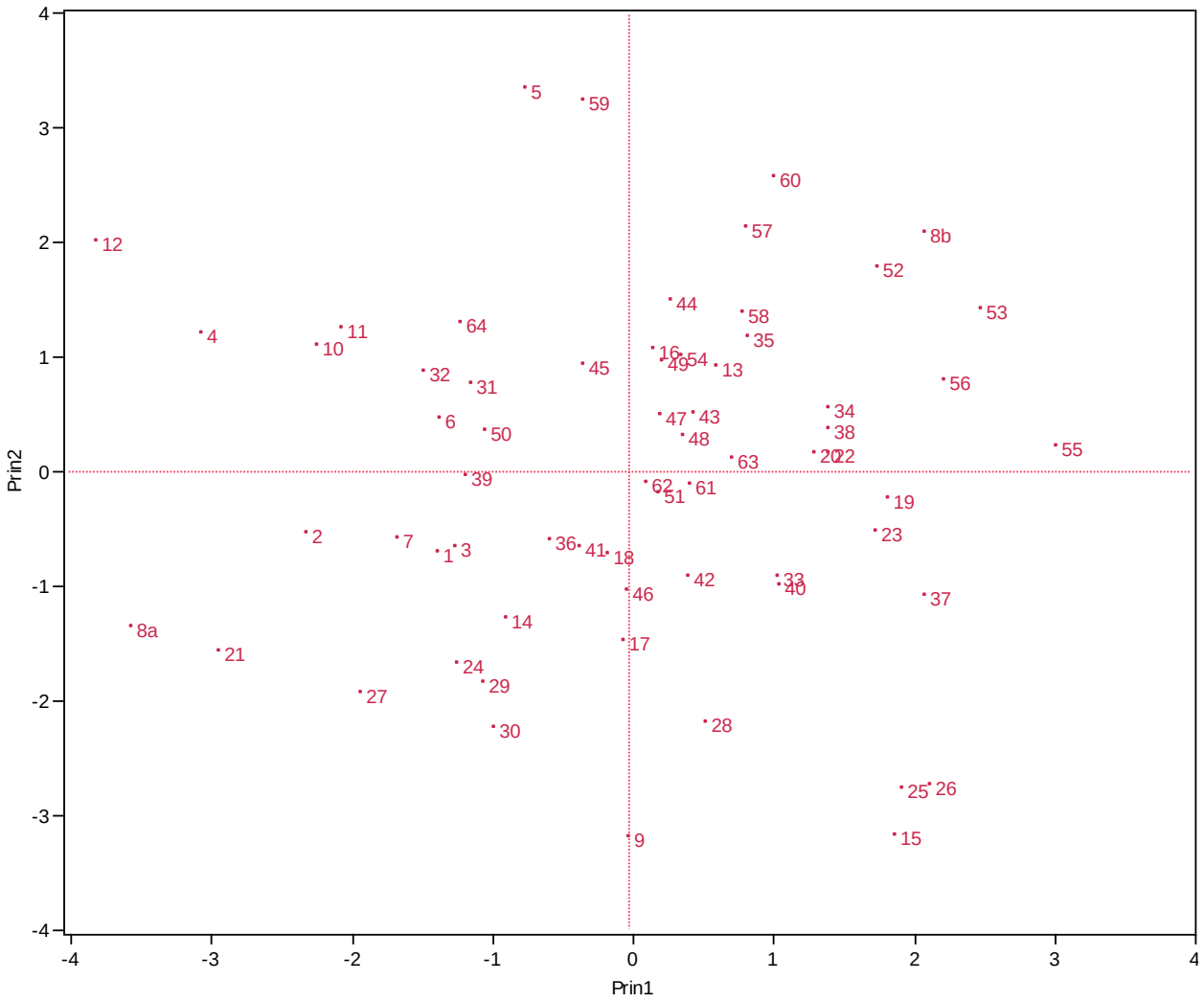

Figure 2. Score Plot graph of spinach genotypes according to agro-morphological traits obtained from PCA on PC 1 and 2.
Şekil 2. Agro-morfolojik özelliklere göre ıspanak genotiplerinin PCA 'dan elde edilen PC 1 ve 2'ye Score Plot grafiği

The separation of the spinach gene pool into clusters of different branches and colors in the thesis study demonstrates a high level of diversity. This situation reveals that materials can be used for future breeding studies. In a morphological-based cluster analysis, genotypes in distant clusters (red and green) are considered genetically distinct. Morphological differences between these genotypes indicate high genetic variation and, consequently, a wide variety of gene pools. It is known that selecting genetically distant parents in future spinach breeding programs will increase heterosis, resulting in new individuals with superior agronomic traits. Furthermore, such parental combinations increase genetic variation within the population, providing a broader basis for selection studies. Therefore, selecting genotypes from different clusters as parents in crossbreeding programs is expected to contribute to more effective development of target traits. A study examined cluster analysis of 54 spinach genotypes and reported that the genotypes were divided into 16 groups. Some spinach landraces, such as G10, G13, G38, and G41, formed separate clusters and did not resemble the other genotypes collected, whereas others were similar to each other and grouped into a single cluster (Sabaghnıa et al., 2014). Another study found that the genotypes could

be divided into 5 groups based on morphological characteristics in a dendrogram of 51 spinach genotypes. It was reported that 36 spinach germplasms were separated into groups showing genetic similarity in cluster one and 9 in cluster three (Jahan, 2022). A cluster analysis conducted to determine morphological similarities among landraces reported that the landraces were divided into 4 main groups (Rafiei et al., 2021).

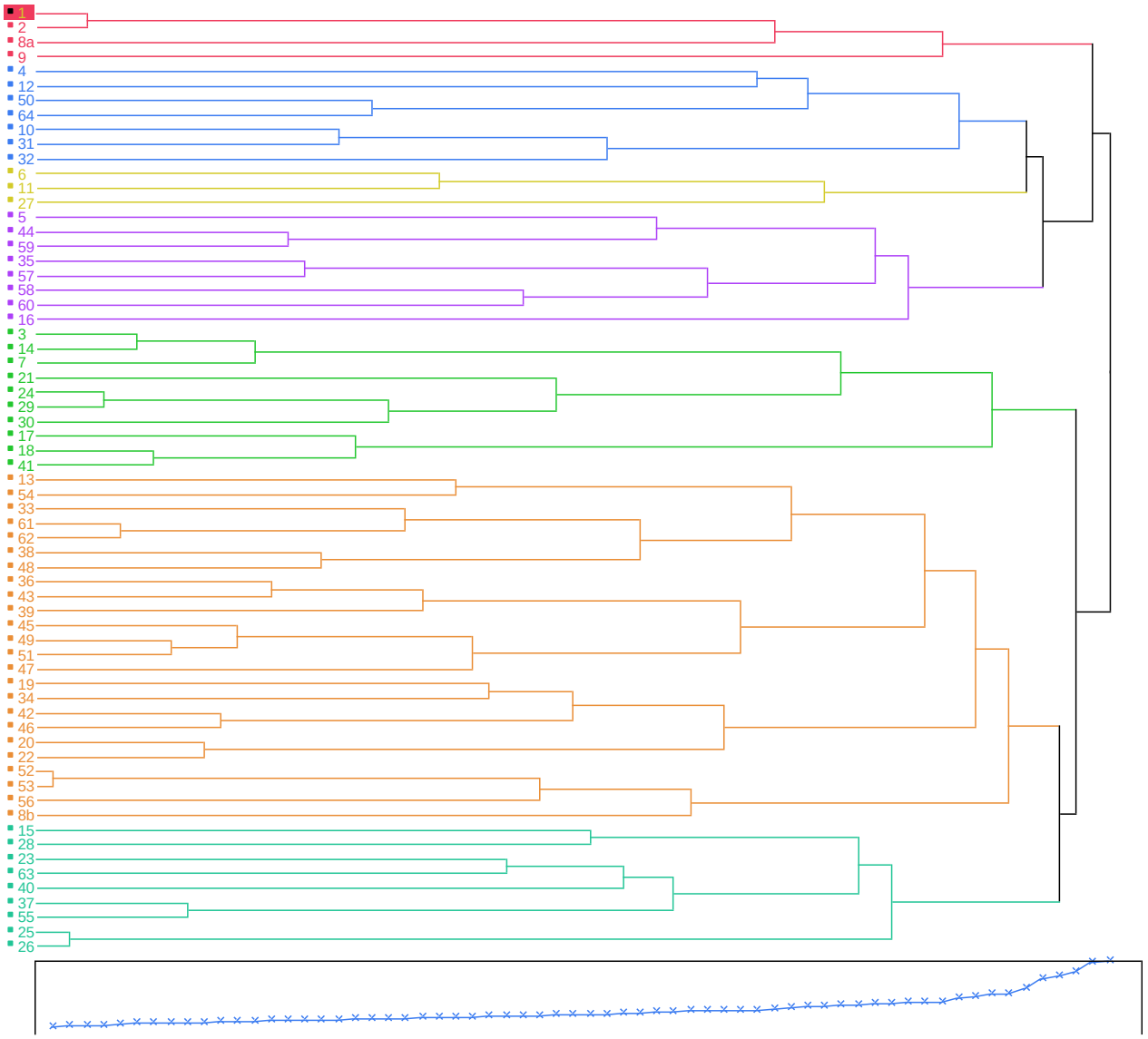

Figure 3. Dendrogram created as a result of observations taken from spinach
Şekil 3. Ispanaklardan alınan gözlemler sonucunda oluşturulan dendrogram

CONCLUSION

In the study, observations were made using UPOV parameters to determine the morphological characteristics of spinach genotypes in the S6-S7 (64 genotypes) and S7-S8 (65 genotypes) generations after development was complete. As a result of the morphological observations taken in both periods, it was determined that the leaf blade shape of the genotypes changed from wide and medium oval, to triangular, and then to wide and medium elliptical. In the study, leaf color varied from light green to dark green across genotypes. When the components are considered, the first six components, based on 14 traits, explained 69.09% of the variance in the study, as determined by PCA.PCA of the present results indicated that leaf and anthocyanin-related traits were the main factors contributing to the variability. As a result of the cluster analysis, the genotypes are divided into 7 sub-clusters. When the dendrogram was examined, genotypes 25-26, 1-2, 52-53, and 61-62 showed short branching and were the closest to each other in terms of morphological parameters. The study revealed that genotypes 9, 15, 25, 26, 8a, 21, 12, 5, 59, and 55 showed higher genetic diversity for leaf traits than other genotypes. Plant breeders can select morphological character combinations through PCA and establish selection criteria based on the main factors contributing to variability. In this context, the aim is to shed light on future breeding studies.

ACKNOWLEDGMENTS

This study was supported by Selcuk University Scientific Research Projects Coordination Unit (SU-BAP) (Project No: 19201088), Türkiye. This article was produced from Gülbanu Gayretli's doctoral study.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

We declare that there is no conflict of interest between us as the article authors.

REFERENCES

Abolghasemi, R., Haghighi, M., & Etemadi, N. (2022). Morphological, biochemical, and nutritional value of prickly and smooth fruit spinach. *Acta Agriculturae Slovenica*, *118*(2), 1-13.

Abolghasemi, R., Haghighi, M., Etemadi, N., Soorni, A., & Jafari, P. (2019). Screening of some native and foreign accessions of spinach for spring culture in Isfahan. *Iran Agricultural Research*, *38*(1), 87-99.

Arif, M., Jatoi, S. A., Rafique, T., & Ghafoor, A. (2013). Genetic divergence in indigenous spinach genetic resources for agronomic performance and implication of multivariate analyses for future selection criteria. *J Sci Technol Dev*, *32*(1), 7-15.

Arshi, Y. (2000). Genetic improvement of vegetable crops. *Mashhad Jihade-Daneshghahi*.

Avşar, B. (2011). *Genetic diversity of Turkish spinach cultivars (Spinacia oleracea L.)* Izmir Institute of Technology (Turkey)].

Bajracharya, J., Steele, K., Jarvis, D., Sthapit, B., & Witcombe, J. (2006). Rice landrace diversity in Nepal: variability of agro-morphological traits and SSR markers in landraces from a high-altitude site. *Field crops research*, *95*(2-3), 327-335.

Bello, O. B., & Olawuyi OJ 2015. Gene action, heterosis, correlation and regression estimates in developing hybrid cultivars in maize. *Tropical Agriculture*, *92*(2).

Brown, J. S. (1991). Principal component and cluster analysis of cotton cultivar variability a cross the U.S. cotton belt, *Crop Sci*, 31, 915-922.

Caparrotta, S., Masi, E., Atzori, G., Diamanti, I., Azzarello, E., Mancuso, S., & Pandolfi, C. (2019). Growing spinach (*Spinacia oleracea*) with different seawater concentrations: Effects on fresh, boiled and steamed leaves. *Scientia Horticulturae*, *256*, 108540.

Chitwood, J., Shi, A., Mou, B., Evans, M., Clark, J., Motes, D., Chen, P., & Hensley, D. (2016). Population structure and association analysis of bolting, plant height, and leaf erectness in spinach, *HortScience*, 51 (5), 481-486.

Dutta, D., Chaudhuri, U. R., & Chakraborty, R. (2005). Structure, health benefits, antioxidant property and processing and storage of carotenoids. *African Journal of Biotechnology*, *4*(13).

Ekinci, M., Ors, S., Sahin, U., Yildirim, E., & Dursun, A. (2015). Responses to the irrigation water amount of spinach supplemented with organic amendment in greenhouse conditions. *Communications in soil science and plant analysis*, *46*(3), 327-342.

FAO, (2023). *Food and Agriculture Organization of the United Nations*, https://www.fao.org/faostat/en/#data/QCL. Erişim tarihi 2.09.2025

Fereidoonfar, H., Salehi-Arjmand, H., Khadivi, A., & Akramian, M. (2018). Morphological variability of sumac (Rhus coriaria L.) germplasm using multivariate analysis. *Industrial Crops and Products*, *120*, 162-170.

Ferrante, A., Incrocci, L., Maggini, R., Serra, G., & Tognoni, F. (2004). Colour changes of fresh-cut leafy vegetables during storage. *J. Food Agric. Environ*, *2*(3&4), 40-44.

Göktay, M. (2015). Characterization of world spinach genetic collection by using molecular *markers. Izmir Institute of Technology* (Turkey)].

Jahan, N. (2022). Assessment of genetic diversity in spinach (*Spinacia oleracea* L.) using some morphological traits. *International Journal of Agriculture and Environmental Research*, *8 (6),* 710-722.

Karaağaç, O. and Balkaya, A. (2010). Bafra Kırmızı Biber Populasyonları (*Capsicum annuum* L. var. *conoides* (Mill.) Irish) Tanımlanması Ve Mevcut Varyasyonun Değerlendirilmesi, *Anadolu Tarım Bilimleri Dergisi*, 25 (1), 10-20.

Kayak, N. (2024). The effect on morpho-physiological and biochemical characteristics of cauliflower and cabbage harvested at different times under flooding stress conditions. *Journal of Crop Health*, 76(1), 145-159.

Kenneth, O. (2016). *Agro-morphological and nutritional characterization of tomato landraces (Lycopersicon species) in Africa* University of Nairobi].

Köse, E. (2018). Bazı ıspanak aksesyonlarının morfolojik özelliklerinin belirlenmesi [Yüksek lisans tezi, Namık Kemal Üniversitesi]. Yükseköğretim Kurulu Ulusal Tez Merkezi.

Lorenz, O. (1978). Potential levels in edible plant parts. *DR Nilsen et al*, 210-220.

Ma, J., Shi, A., Mou, B., Evans, M., Clark, J. R., Motes, D., Correll, J. C., Xiong, H., Qin, J., & Chitwood, J. (2016). Association mapping of leaf traits in spinach (*Spinacia oleracea* L.). *Plant Breeding*, *135*(3), 399-404.

Mohammadi, S. A., & Prasanna, B. M. (2003). Analysis of Genetic Diversity in Crop Plants—Salient Statistical Tools and Considerations. *Crop Sci*, *43*, 1235-1248.

Mavromatis, A., Arvanitoyannis, S., Korkovelos, A., Giakountis, A., Chatzitheodorou, V. ve Goulas, C. (2010). Genetic diversity among common bean (*Phaseolus vulgaris* L.) Greek landraces and commercial cultivars: nutritional components, RAPD and morphological markers, *Spanish Journal of Agricultural Research* (4), 986-994.

Mozafari, A., Ghaderi, N., Havas, F., & Dedejani, S. (2019). Comparative investigation of structural relationships among morpho-physiological and biochemical properties of strawberry (Fragaria× ananassa Duch.) under drought and salinity stresses: A study based on in vitro culture. *Scientia Horticulturae*, *256*, 108601.

Pachauri, A. K., Sarawgi, A., Bhandarkar, S., & Ojha, G. (2017). Agro-morphological characterization and morphological based genetic diversity analysis of Rice (*Oryza sativa* L.) germplasm. *Journal of Pharmacognosy and Phytochemistry*, *6*(6), 75-80.

Rafiei, F., Niknam, N., Shiran, B., Kohpayegani, J., & Jafari, P. (2021). Insight into morphological and molecular variations across Iranian spinach landraces. *Molecular Biology Reports*, *48*(2), 1567-1578.

Rashid, M., Yousaf, Z., Ullah, M. N., Munawar, M., Riaz, N., Younas, A., Aftab, A., & Shamsheer, B. (2020). Genetic variability assessment of worldwide spinach accessions by agro-morphological traits. *Journal of Taibah University for Science*, *14(1),* 1637-1650.

Sabaghnıa, N., Asadi-Gharneh, H., & Janmohammadı, M. (2014). Genetic diversity of spinach (Spinacia oleracea L.) landraces collected in Iran using some morphological traits. *Acta Agriculturae Slovenica*, *103*(1), 101-111.

Seymen, M. (2021). How does the flooding stress occurring in different harvest times affect the morpho-physiological and biochemical characteristics of spinach?. *Scientia Horticulturae*, *275*, 109713.

Shen, G., Girdthai, T., Liu, Z. Y., Fu, Y. H., Meng, Q. Y., & Liu, F. Z. (2019). Principal component and morphological diversity analysis of Job's-tears (Coix lacryma-jobi L.). *Chilean journal of agricultural research*, *79*(1), 131-143.

Smith, S., Al-Doss, A., & Warburton, M. (1991). Morphological and agronomic variation in North African and Arabian alfalfas. *Crop Science*, *31*(5), 1159-1163.

Tahtalı, Y. (2005).Gen dizilerinde temel bileşenler analizinin uygulanması, *Çukurova Üniversitesi*, Zootekni Anabilim Dalı.

Taş, K., & Balkaya, A. (2021). Capsicum chinense genetik kaynaklarının morfolojik varyasyonunun ana bileşen analizi ile belirlenmesi ve karakterizasyonu. *Ekin Bitki Islahı ve Genetiği Dergisi* , *7* (2), 86-105.

Wang, M., Xu, J., Ding, Z., & Xie, J. (2023). Prolong the postharvest shelf life of spinach through the antioxidative ability of melatonin. *Food Chemistry: X*, *19*, 100769.

Xu, C., & Leskopresent, D. I. (2015). Effects of A. nodosum seaweed extracts on spinach growth, physiology and nutrition value under drought stress. *Scientia Horticulturae*, *183*, 39-47.

Yan, W., & Kang, M. S. (2002). GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. *CRC press*.