

**Evaluation of Agro-Morphological Variation in Some Elite Summer Squash (*Cucurbita pepo* L.) Lines\***

Bazı Elit Yazlık Kabak (*Cucurbita pepo* L.), Hatlarında Agro-Morfolojik Varyasyonun Değerlendirilmesi


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**Abstract**

*Cucurbita pepo* L., belonging to the *Cucurbitaceae* family, is an important species with high economic value. This study was carried out within the framework of university and industry cooperation. The genetic material of the researcher consists of 113 elite summer squash (*Cucurbita pepo* L.) lines selected based on morphological characterisation studies conducted on the company's existing genetic resources. At the end of the study, hierarchical clustering, principal component, and correlation analyses were performed using data from thirty-five quantitative and qualitative traits of fifty selected elite lines to reveal the relationships among the traits. As a result of the clustering analysis, the distance range among the lines varied between 1.52 and 10.98, and the average coefficient of the resulting dendrogram (similarity average) was determined as 0.638. Additionally, it was found that the lines formed two main groups and seven subgroups. Group A includes a total of forty-two lines. In group B, there are eight lines. As a result of the basic component analysis, seven independent principal component axes among the plants were obtained, and these axes explain 70.23% of the total variation in the pumpkin population. When examining the values of the principal component axes, it was observed that the first principal component axis accounts for 16.81% of the total variation, the second principal component axis accounts for 14.33%, and the third principal component axis accounts for approximately 10.90%. The correlation results obtained indicate clear models and strong correlations showing significant relationships between the morphological and genetic features among the early maturing genotypes and the different groups. These findings provide strategic decisions and valuable information for plant breeding and genetic diversity studies, and they are also useful data for agricultural production and breeding efforts in line with the analyses.

**Keywords:** Cluster analysis, Correlation, Elite lines, Morphological characterisation, Principal component analyses, Selection, Summer squash

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## Öz

*Cucurbitaceae* familyasına ait olan *Cucurbita pepo* L., yüksek ekonomik değere sahip önemli bir türdür. Bu çalışma, üniversite ve sanayi işbirliği çerçevesinde gerçekleştirilmiştir. Araştırmanın genetik materyali, şirketin mevcut gen kaynakları üzerinde yapılan morfolojik karakterizasyon çalışmaları sonucunda seçilen 113 elit yaz kabağı (*Cucurbita pepo* L.) hattından oluşmaktadır. Çalışmanın sonunda, seçilen 50 elit hattın otuz beş nicel ve nitel verisi kullanılarak, hatlar arasındaki özellikler arasındaki ilişkileri ortaya koymak amacıyla hiyerarşik kümeleme, ana bileşen ve korelasyon analizleri gerçekleştirilmiştir. Kümeleme analizi sonucunda, hatlar arasındaki mesafe aralığı 1.52 ile 10.98 arasında değişmiş ve oluşan dendrogramın katsayı ortalaması (benzerlik ortalaması) 0.638 olarak belirlenmiştir. Ayrıca, hatların iki ana grup ve yedi alt grup oluşturduğu tespit edilmiştir. Grup A toplamda kırk iki hat içerirken, grup B ise sekiz hattan oluşmaktadır. Temel bileşen analizi sonucunda, bitkiler arasında yedi bağımsız temel bileşen eksen elde edilmiş ve bu eksenler, kabak popülasyonundaki toplam varyasyonun %70.23'ünü açıklamaktadır. Ana bileşen eksenlerinin değerleri incelendiğinde, birinci ana bileşen ekseninin toplam varyasyonun %16.81'ini, ikinci ana bileşen ekseninin varyasyonun %14.33'ünü ve üçüncü ana bileşen ekseninin toplam varyasyonun yaklaşık %10.90'ını açıkladığı gözlemlenmiştir. Elde edilen korelasyon sonuçları, yaz kabağı genotipleri arasındaki morfolojik ve genetik özellikler arasında belirli değişken grupları arasında önemli ilişkiler olduğunu gösteren net modeller ve güçlü korelasyonlar göstermektedir. Bu bulgular, bitki ıslahı ve genetik çeşitlilik çalışmaları için stratejik kararlar ve değerli bilgiler sunmakta olup, analizlerle uyumlu olarak tarımsal üretim ve ıslah çalışmaları için de faydalı veriler sağlamaktadır. Ayrıca, yeni yüksek verimli çeşitlerin geliştirilmesi, genetik kaynakların korunması ve sürdürülebilir tarım uygulamaları ile tarımsal üretim süreçlerinin optimizasyonu için ıslah çalışmalarının önemli katkılar sağlaması beklenmektedir.

**Anahtar Kelimeler:** Elit hatlar, Korelasyon, Küme analizi, Morfolojik karakterizasyon, Temel bileşen analizi, Seleksiyon, Yazlık kabak

## 1. Introduction

The genus *Cucurbita* belonging to the family *Cucurbitaceae* shows a great diversity in terms of morphological characteristics (Montes-Hernandez et al., 2005). Summer squash (*C. pepo* L.), one of the most cultivated species in this genus, is a highly polymorphic species with high economic value and genetic diversity in terms of fruit characteristics such as fruit size, shape, and colour (Blanca et al., 2012). When we look at the production values of this species with high economic value in 2021; it is seen that while the world summer squash production is 27 962 481 tonnes in total, 609.622 tonnes of gum (summer squash) squash is produced in Türkiye (FAO, 2022).

Summer squash (*C. pepo* L.) is a widely cultivated and consumed vegetable globally, offering high nutritional value and economic benefits. It has great potential for productivity and quality in Türkiye. However, to fully realize this potential, it requires high genetic performance and effective cultivation techniques (Nacar et al., 2017; Şavkan and Türkmen, 2023). Breeding studies aiming to develop plants with desirable traits rely on genetic diversity and its effective utilization through selection to maximize the genetic yield potential of plants (Ribaut et al., 2002; Genç and Yağbasanlar, 2018; Begna, 2021). Plant breeding, both historically and today, primarily relies on the selection method. However, in the selection breeding method, breeders are not able to create genetic variations. Instead, they select plants that are suitable for breeding purposes by using existing variations that occur naturally (Şahiner Öylek, 2022). In progeny-controlled single selection, individual plants are first selected, and the seeds of the selected plants are grown in separate rows or plots to observe and control the offspring (Baydar, 2020). This process helps reveal the mode of transmission of the selected elite plants' characteristics to their offspring and the degree of heritability. It also helps determine whether the superior qualities in plants are due to genotype or environmental conditions, allowing for the selection of superior genotypes (Genç and Yağbasanlar, 2018). Developing and conserving elite lines identified through this process plays a crucial role in breeding new and resistant plant varieties.

Evaluating agro-morphological variations in elite lines, which consist of plant genotypes with superior traits selected for use in breeding programs, is critical for increasing yield and quality in agricultural production by revealing genetic diversity and superior traits (Channa et al., 2016; Tian et al., 2017). Agro-morphological variation, which is an important research topic in plant breeding and agricultural production, refers to the variation observed in morphological and agronomic characteristics of plants. This variation is caused by the combination of various factors such as genetic factors, environmental conditions, and cultivation techniques. Morphological traits include the visible characteristics of the plant such as leaf, stem, flower, and fruit structure, while agronomic traits include traits that affect the agricultural performance of the plant such as yield, disease resistance, and ripening time. In many species and genus, variety descriptions have been made by many researchers in our country and the world according to fruit and plant characteristics and existing morphological variations have been revealed in detail (Akışcan and Yaman, 2018; Mavi et al., 2021; Özer et al., 2021; Aktaş and Durmaz, 2023; Yaşar et al., 2025).

Moreover, in breeding studies, morphological or agro-morphological traits are the main markers to determine and estimate genetic diversity among genotypes (Al-Aysh et al., 2012; Şavkan and Türkmen, 2023). This is because morphological markers are simpler, more direct and cheaper than molecular and biochemical markers (Bernousi et al., 2011; Islam et al., 2021). In addition, heritability and genetic advance (GA) are also important indicators for the breeding plan. A significant amount of GA together with high heritability is required for effective selection in breeding programmes (Johnson et al., 1955). In addition, analysis of correlation coefficients between characters is also useful for selection of important yield-related traits. Therefore, the collective information of lines in the gene accession can help to formulate a comprehensive breeding plan for their improvement (Narolia and Reddy, 2010). In addition, previous researchers working with germplasm of different vegetable species have suggested the use of multivariate analysis (e.g. correlation and principal component analysis) and cluster as a valid tool to deal with germplasm collection and characterization and evaluation of their genetic relationships (Islam et al., 2022; Şavkan et al., 2024).

In view of the above, the aim of this study was to determine the variation in qualitative and quantitative traits in 50 selected elite squash lines. This is essential for conserving plant genetic resources and promoting sustainable agricultural practices.

## 2. Materials and Methods

### 2.1. Plant material and experiment design

The research was conducted in the R&D greenhouse of Beta Tohumculuk A.Ş. The material of our study consisted of 113 elite summer squash lines at the S4 level, consisting of local populations, standard and hybrid varieties in Turkey at S4 level selected from previous breeding studies. Seeds were planted in vials filled with a peat-perlite (2:1) mixture, with 5 plants from each line. After preparing the soil in the greenhouse, the seedlings were grown in the vials until they were ready for transplanting. Transplanting was done when the seedlings reached the appropriate size. The seedlings were planted next to the drippers above rows. Regular irrigation and maintenance procedures were carried out.

### 2.2. Agro-morphological parameters

Morphological parameters of each plant were examined, and selfing was performed to ensure the progress of the breeding stage in the existing lines for squash selection breeding. The morphological characteristics of the lines were determined by taking measurements and observations throughout vegetation. The study used the UPOV descriptor list. The morphological data of 50 lines selected at the end of the study are given here. Qualitative data were observed, and quantitative data were measured on 5 marketable squash fruits from each line. The measurements and observations made are as follows (UPOV, 2006). Quantitative data collected from observation includes the following characteristics of the lines: growth habit (bush, semi-trailing, trailing), branching (absent, present), degree of branching (weak, medium, strong), attitude of petiole (excluding lower external leaves) (erect, semi-erect, horizontal), green colour on the stem (only light, only dark, , partly light and partly dark), tendrils on the stem (absent to rudimentary, well-developed), leaf blade size (small, medium, large), incisions on the leaf blade (light, medium, strong), leaf blade marbling (absent, present), green colour of upper surface of leaf (light, medium, dark), leaf blade surface area (very small, small, medium, large, very large), petiole length (short, medium, long), petiole thickness (small, medium, large), petiole cross-sectional shape (round, triangular), the number of the prickles of petiole (few, medium, many) and the silvering of leaf blade (absent, present). The observations regarding fruits are related to presence of neck in immature fruit (absent, present), the curving of the neck in unripe fruit (absent, present), length in unripe fruit (short, medium, long), major colours in unripe fruit (white, cream, yellow, orange, green, partly white and partly yellow, partly green and partly yellow), the intensity of major colour in unripe fruit (light, medium, dark), shape of the cross-section in unripe fruit (round, grooved, angular, lobed), presence of wart in unripe fruit (absent, present), gloss in unripe fruit (light, medium, strong), and a comparison of vein colour with the colour of the other part of the unripe fruit (same, different), colour of linear bands on the vein in immature fruit (whitish, yellow, orange, green), presence of mottling in immature fruit (present, absent), type of mottling in immature fruit (scattered, scattered and fragmented, scattered and banded, scattered, fragmented and banded), colour of mottling in immature fruit (whitish, yellow, orange, green), conspicuousness of the spots on the skin of the immature fruit (weak, medium, strong), size of the blossom mark on the immature fruit (small, medium, large), protrusion of the blossom mark on the immature fruit (present, absent), general shape of the fruit (transverse elliptical, globular, top-shaped, broadly elliptical, ovate, elliptical, cylindrical, tapered cylindrical, pear-shaped, bottle-shaped, club-shaped). Quantitative data measured includes fruit diameter (mm), fruit length (cm), and fruit weight (g).

### 2.3. Statistical analyses

Statistical analysis and heatmap distribution graphs were conducted using JMP Statistical Software (JMP 18.0). Using the multivariate procedure applied in JMP Statistical Software (JMP 18.0), cluster, correlation and principal component analysis (PCA) based on morphological features were performed by scoring the data obtained from the materials selected after selection according to the UPOV scale values, to identify the variation models within the squash accession.

## 3. Results and Discussion

### 3.1. Agro-morphological analyses

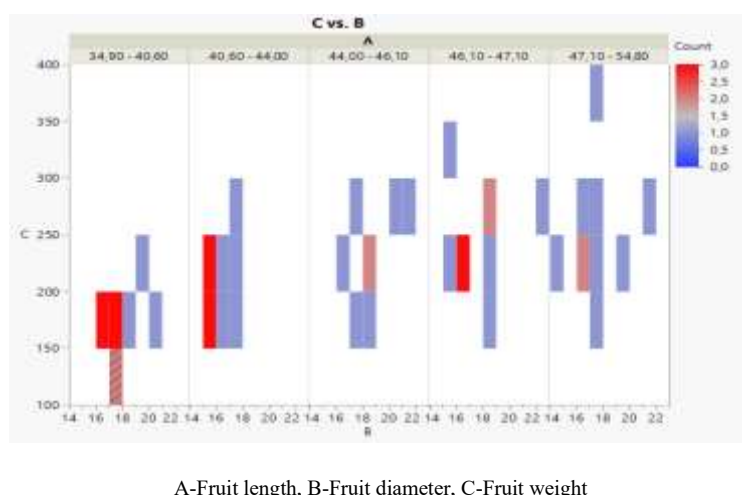
When the characteristics related to the plant were analysed; in terms of growth habit, it was found that 100% of the plants were semi-trailing; in terms of plant branching, 4% were absent, 96% were present; in terms of the

degree of branching, 8% were weak, 52% were medium and 40% were strong; in terms of petiole attitude, 4% were erect and 96% were semi-erect. When the characteristics related to the stem were examined; in terms of green colour, 48% of the stems were only light, 18% were only dark, and 34% were light and dark; in terms of tendrils on the stem, the majority, 42%, were absent or poorly developed, 58% had well-developed shoots.

When the leaf-related characters were examined; in terms of leaf blade size, 20% were medium and 80% were large; in terms of leaf blade incisions, 14% were light, 48% were medium and 36% were strong; in terms of leaf blade upper surface green colour, 8% were light, 54% were medium and 38% were dark; in terms of marbling, 72% were absent and 28% were present. In terms of leaf blade surface area, 18 % had medium, 72% had large and 10% had very large. Petiole observations; in terms of petiole length, 8 % had short, 44% had medium and 48 % had long petioles; In terms of petiole thickness, 16% were thin, 74% were medium and 10 % were thick; in terms of petiole cross-sectional shape, all of them, i.e. 100%, were round; in terms of the number of petiole prickles, 66% were few, 32% were medium and 2% were many. In addition, when silvering was analysed in 50 lines, it was determined that 96% had no silvering and 4% had silvering.

When the immature fruit characters were analysed, it was found that the majority (74%) of the immature fruit had no neck, 26 of them had a neck; the majority (98%) of the immature fruit had no neck, 2% of them had a neck curvature. In terms of the number of main colours in the fruit, 18% were whitish, 78% were green and 4% were green-yellow; in terms of the intensity of the main colour, 60% were light, 18% were medium and 22% were dark. In terms of the shape of the cross-section of the fruit, 28% were round, 70% were angular and 2% were lobed; in terms of warts, almost all (94%) were absent and 6% had warts; in terms of fruit brightness, 34% were light, 54% were medium and 12% were strong; in terms of vein colour and main colour of the fruit, all (100%) were the same; in terms of the colour of the linear bands on the vein, 16% were whitish and 84% were green; in terms of spotting on the fruit, 6% were absent and 94% had spotted. In terms of the type of mottling, 100% were scattered (mottling-type observations were taken from 49 genotypes and the ratios were calculated according to 49). In terms of the colour of the spots, 82% were whitish and 14% were green; in terms of the conspicuousness of the spots on the peel, 52% were weak, 42% were medium and 2% were strong; in terms of the size of the flower mark, 84% were small, 14% were medium and 2% were large; in terms of the flower mark protrusion, 84% were absent and 16% were present (flower mark protrusion observation was taken from 49 genotypes and calculated according to 49). When the general shape of the fruit was analysed, it was determined that 18% of the fruits were elliptical, 2% of them were oval-shaped, 60% of them were cylindrical and 20% of them were golf club-shaped.

As a result of the quantitative measurements of the fruits, 31 coded line 54.83 mm maximum fruit diameter, 8 coded line 34.9 mm minimum fruit diameter, 10 coded line 22.2 cm maximum fruit length, 17 coded line 14.9 cm minimum fruit length, 31 coded line 385 g maximum fruit weight and 13 coded line 145 g minimum fruit weight. Heat-map-distribution graphs and statistical analysis results of quantitative values are given in *Figure 1* and *Table 1*.



**Figure 1: Heatmap- distribution graphs analysis of quantitative properties**

**Table 1: Statistical values calculated for quantitative properties**

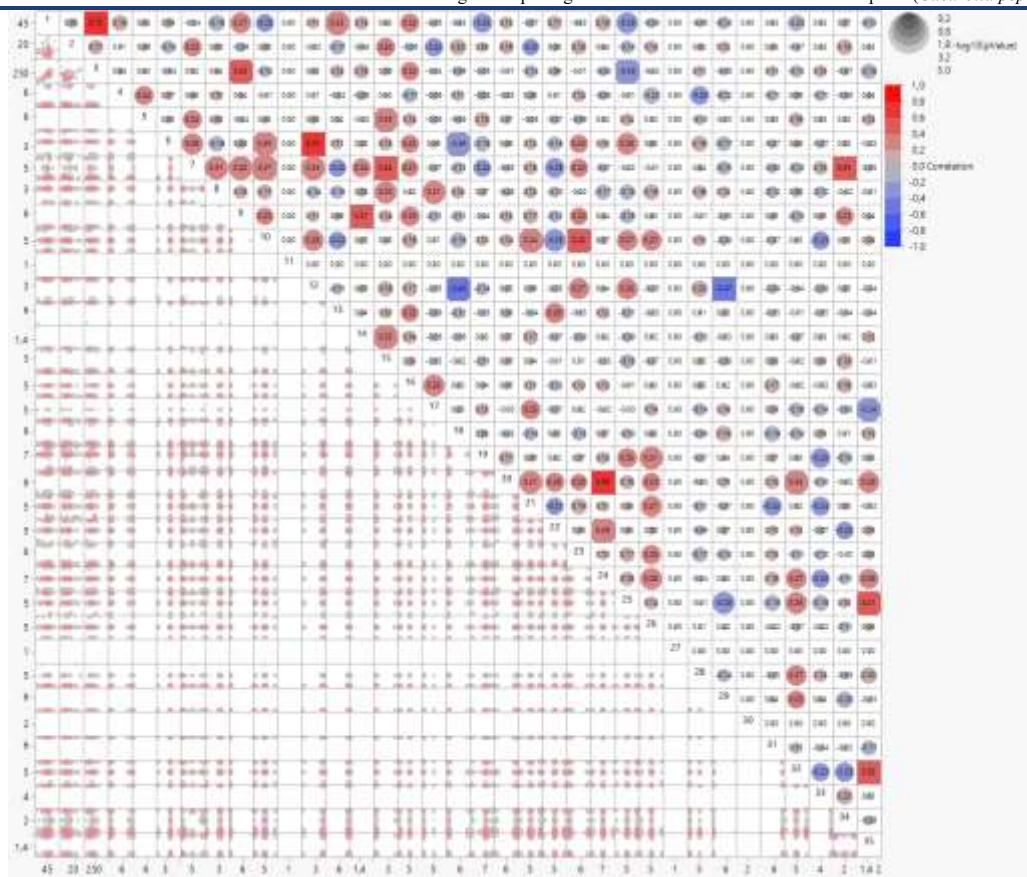
Parameters	Max	Median	Min	Mean	Std Dev.	Std Er. Mean
<b>A</b> (Fruit length/cm)	54.8	44.75	34.9	44.08	4.27	0.603
<b>B</b> (Fruit diameter/mm)	22.2	17.05	14.9	17.41	1.68	0.238
<b>C</b> (Fruit weight/gr)	385	217	145	220.26	47.01	6.68

Among the types of pumpkin, there is a high level of diversity especially in terms of fruit characteristics and a significant variation in anatomical features. This results in noticeable differences in fruit shape, size, and skin texture among different plants of the same species (Şavkan and Türkmen, 2023; Şavkan et al., 2025). Numerous studies have highlighted the extensive diversity in agro-morphological traits within the *Cucurbitaceae* family by examining genetic variation (Çeğil and Çürük, 2019; Dalda-Şekerci et al., 2020; Silva et al., 2024; Yaşar et al., 2025). Understanding these traits is crucial for agricultural practices and breeding programs aiming to enhance yield and fruit quality. These findings underscore the importance of considering various parameters to optimize the breeding and selection of summer squash cultivars by market demand.

### 3.2. Correlation analyses

According to the correlation results, fruit length showed a strong positive significant correlation with fruit weight (0.72), which may provide a strategic advantage for breeders who want to obtain large and heavy fruits in plant breeding to produce productive and attractive fruits by considering these two traits together. The main colour in unripe fruit has a strong positive significant correlation with the colour of the linear bands on the vein (0.75) in unripe fruit, indicating that the overall colour of the fruit and the colour bands on the veins change together. Wart in unripe fruit showed a positive significant correlation with the type of spots in unripe fruit (0.57), while the intensity of the main colour in unripe fruit showed a positive significant correlation with the colour of spots in unripe fruit (0.54). The intensity of the main colour in unripe fruit showed a positive significant relationship with the green colour of the stalk (0.53). In other words, these relationships with main fruit colour can be used to identify genotypes and may help to develop desired fruit colour combinations according to consumer preferences. Glossiness of immature fruit showed a positive significant relationship with leaf blade swelling (0.42), while petiole length showed a positive significant relationship with stem shoot development (0.41). Leaf size showed a strong positive significant relationship with leaf blade area (0.69), which is important for photosynthetic efficiency. It can therefore increase plant growth and productivity. The fact that the degree of branching in the plant showed a strong negative correlation with branching development (0.58) can be interpreted as a decrease in shoot development as the degree of branching increases. This information can therefore be used in plant structure design and pruning strategies (Figure 2). In this context, PCA analysis was performed on the morphological parameters with high correlation values, and the parameters coded 2-4-5-8-11-13-16-17-19-22-27-28-30-31-33 with low correlation were not included in the analysis. The correlation matrix shows clear patterns and strong correlations, indicating significant relationships among certain variable groups in the analysis of summer squash genotypes morphological characteristics. These findings provide important insights for plant breeding and genetic diversity studies. The positive and negative correlations between variables highlight crucial points to consider in genotype selection and breeding program planning. This information can be utilized to make strategic decisions in genetic diversity studies and contribute to the development of hybrid varieties when determining heterotic groups. Furthermore, these results align with and support findings in existing literature (Soltani et al., 2016; Khan et al., 2017; Şavkan et al., 2025b).





**Figure 2: Correlation coefficients among parameters**

1(fruit length), 2(fruit diameter), 3(fruit weight), 4(curvature of the neck in immature fruit), 5(presence of neck in immature fruit), 6(main colour in immature fruit), 7(intensity of the main colour in immature fruit), 8(shape of the cross-section in immature fruit), 9 (warts on unripe fruit), 10 (shine on unripe fruit), 11(unripe fruit: Comparison of vein colour with the colour of the other part of the fruit), 12(colour of linear bands above the vein on unripe fruit), 13(mottling on unripe fruit), 14(type of mottling on unripe fruit), 15(colour of mottling on unripe fruit), 16(conspicuousness of mottling on the peel of immature fruit), 17(size of flower mark on immature fruit), 18(protrusion of flower mark on immature fruit), 19(general shape of fruit), 20(size of leaf blade), 21(incisions of leaf blade), 22(green colour of upper surface of leaf blade), 23(leaf blade swelling), 24(leaf blade area), 25(petiole length), 26(petiole thickness), 27(petiole cross sectional shape), 28(number of prickles on the petiole), 29(silvering on leaf), 30(growing in habit), 31(branching on the plant), 32(degree of branching on the plant), 33(attitude of the petiole on the plant), 34(green colour on the stem), 35(stem tendrils)

### 3.3. Principal component analyses

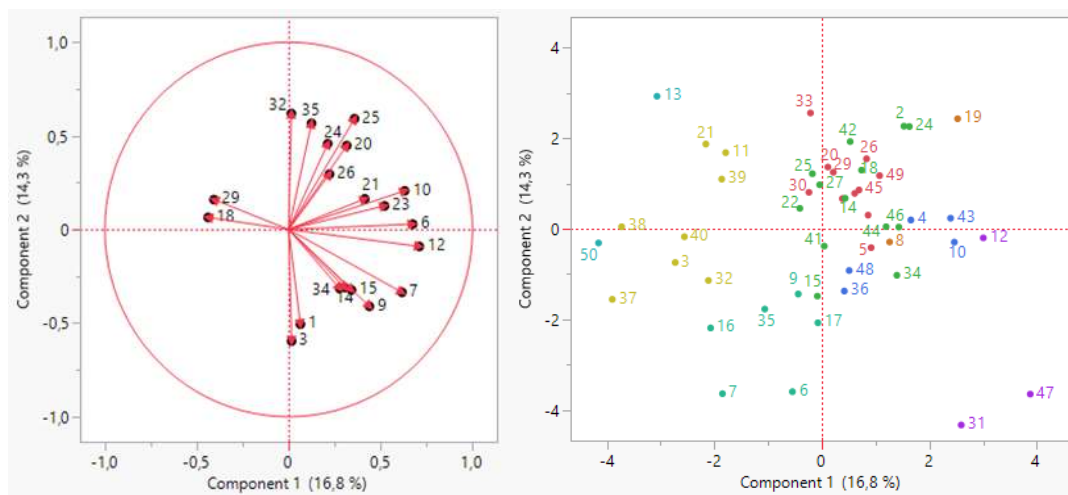
The primary purpose of principal component analysis is to capture the variability within a dataset and effectively distinguish between different groups. This technique is valuable for identifying relationships and similarities among samples (Abdi and Williams, 2010). Specifically, principal component analysis is a helpful tool for categorizing plant and fruit characteristics and contributing to the understanding of population relationships. Notably, certain attributes such as plant growth tendency, petiole shape, and the comparison of vein and fruit colour were not considered in the analysis due to no variation observed among the lines. After the analysis, seven principal component axes were identified across the plants, accounting for 70.23% of the total variation in the squash population. In the literature, it has been noted that the principal component axes with eigenvalues greater than 1 in PCA analysis are highly reliable (Taş, 2020). In this study, the coefficients of the eigenvalues of the first twelve principal components ranged between 3.36 and 1.08. Upon analysing the values of the principal component axes, it was observed that the first principal component axis accounted for 16.81% of the total variation, the second principal component axis explained 14.33% of the variation, and the third principal component axis explained approximately 10.90% of the total variation (Table 2).

**Table 2: Eigenanalysis of the correlation matrix in PCA**

<b>Eigenvalue</b>	3,362	2,867	2,181	1,747	1,567	1,235	1,088
<b>Percent</b>	16,811	14,334	10,904	8,733	7,837	6,174	5,442
<b>Cum.Percent</b>	16,811	31,145	42,049	50,782	58,619	64,793	70,235
<b>Parameters</b>	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>	<b>PC4</b>	<b>PC5</b>	<b>PC6</b>	<b>PC7</b>
1	0,0348	-0,2988	<b>0,3602</b>	<b>-0,3603</b>	0,0167	0,2286	0,0509
3	0,0087	<b>-0,3520</b>	<b>0,3356</b>	-0,2839	-0,0296	-0,0288	-0,1495
6	<b>0,3679</b>	0,0168	-0,1463	-0,2812	0,0713	-0,1668	0,1880
7	<b>0,3364</b>	-0,1982	-0,1037	<b>0,3385</b>	0,1306	0,0452	0,1945
9	0,2396	-0,2426	<b>0,3082</b>	0,1549	0,0093	-0,0705	<b>-0,3777</b>
10	<b>0,3444</b>	0,1218	-0,0799	0,1529	-0,2439	-0,1733	-0,1366
12	<b>0,3875</b>	-0,0535	-0,1867	<b>-0,3451</b>	0,1138	-0,0207	0,0904
14	0,1663	-0,1836	0,2719	0,2348	0,2235	-0,2031	-0,2987
15	0,1864	-0,1907	0,0366	0,2846	<b>0,3122</b>	-0,0853	<b>0,3560</b>
18	-0,2390	0,0381	0,0999	0,2902	-0,0271	<b>0,3832</b>	-0,0384
20	0,1723	0,2646	<b>0,4211</b>	-0,0164	-0,0129	0,1218	<b>0,3038</b>
21	0,2259	0,0964	0,1038	0,1990	-0,2923	-0,1392	0,0455
23	0,2845	0,0743	-0,0052	-0,0098	<b>-0,3408</b>	0,0334	-0,1410
24	0,1160	0,2704	<b>0,3881</b>	-0,0979	-0,0135	0,2589	<b>0,3213</b>
25	0,1955	<b>0,3492</b>	-0,1978	-0,0801	0,1387	0,2609	-0,2618
26	0,1211	0,1745	0,1587	0,1045	<b>-0,4544</b>	0,0720	-0,0409
29	-0,2215	0,0941	0,1868	0,2179	-0,0621	<b>-0,4354</b>	0,2585
32	0,0081	<b>0,3657</b>	0,1452	-0,0606	<b>0,4049</b>	-0,2698	0,0181
34	0,1513	-0,1854	-0,1325	0,2897	0,1114	<b>0,4844</b>	0,1366
35	0,0678	<b>0,3346</b>	0,1655	0,0851	<b>0,3849</b>	0,1170	<b>-0,3762</b>

**Explanation:** 1(fruit length), 3(fruit weight), 6(main colour in immature fruit), 7(intensity of the main colour in immature fruit), 9 (warts on unripe fruit), 10 (shine on unripe fruit), 12(colour of linear bands above the vein on unripe fruit), 14(type of mottling on unripe fruit), 15(colour of mottling on unripe fruit), 18(protrusion of flower mark on immature fruit), 20(size of leaf blade), 21(incisions of leaf blade), 23(leaf blade swelling), 24(leaf blade area), 25(petiole length), 26(petiole thickness), 29(silvering on leaf), 32(degree of branching on the plant), 34(green colour on the stem), 35(stem tendrils)

The three-dimensional score plot and the three-dimensional scatterplot analyses of the lines along the PC1, PC2 and PC3 axes show the current variability of this research collection (*Figure 3*). In the study, in terms of the criteria examined in the principal component analysis, the main colour of the fruit (0.368), the intensity of the main colour (0.336), the shininess of the immature fruit (0.344) and the colour of the linear bands on the veining (0.388) on the PC-1 axis, and the fruit weight (0.352), petiole length(0.349), degree of plant branching (0.366) and stem tendrils(0.335) on PC-2 axis, and the PC-3 axis, fruit length (0.360), fruit weight (0.336), immature fruit warts (0.308), leaf blade size (0.421), and leaf blade area (0.388) parameters were positively significant (*Table 2, Figure 3*).

**Figure 3: Score plot and scatterplot analyses**



### 3.4. Outlier analyses

Outlier analysis is an extremely important statistical approach used in our research to detect outliers, including extreme measurement values (Çetin, 2023; Şavkan et al., 2024). This analysis helps identify atypical lines in the gene accession. During the outlier analysis, all parameters were evaluated together without any distinction between parametric and non-parametric variables. The identification of outliers was based on exceeding the Upper Control Limit (UCL = 19.03). This approach was chosen to maintain consistency across all measured characteristics and the same threshold was applied to all lines regardless of data distribution characteristics. In this study, the lines (47, 50, 13, 31) above the UCL (19.03) value were defined as outliers (Figure 4). Identifying outliers through statistical methods is crucial for understanding their effects in data analysis. Correctly identifying outliers can increase the reliability of data analysis and aid in making informed decisions. In this case, the separation of outlier lines 50 and 13 from the other lines in the 2nd subgroup of group A is important for the reliability of the analysis and for selecting lines in crossbreeding based on PCA and cluster analysis (Figure 3; Figure 5). Selecting lines that are far from each other is desirable for the heterosis effect, which is important in crossbreeding.

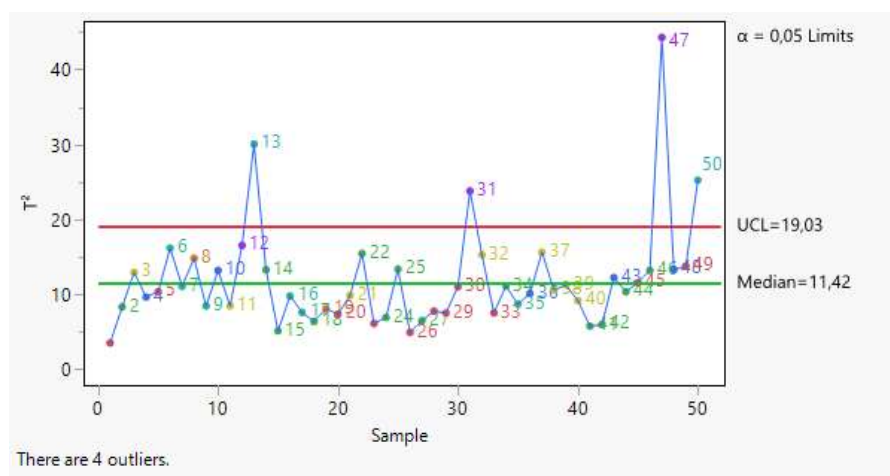


Figure 4: Outlier analysis

### 3.5. Cluster analyses

The study performed a 'Hierarchical Clustering' analysis to explore the relationship among the fifty elite squash lines. Clustering analysis revealed that the morphological similarity level of the lines ranged between 1.52 and 10.98, and the variation rate explained by clustering was 0.638. It was found that the lines were mainly grouped into two categories, labelled as Group A and Group B (Figure 5). Group A was further divided into five subgroups, while Group B constituted a single main group. Group A contained forty-two lines in total, while Group B had eight lines. The subgroup lists can be found in Table 3. In several squash studies, different genetic groups have been identified based on the similarity of dendrogram among the genetic accessions (Sajid et al., 2022; Şavkan et al., 2025a). These results enable the assessment of the observed variation among squash genotypes, a

Table 3: Subgroups identified through cluster analysis

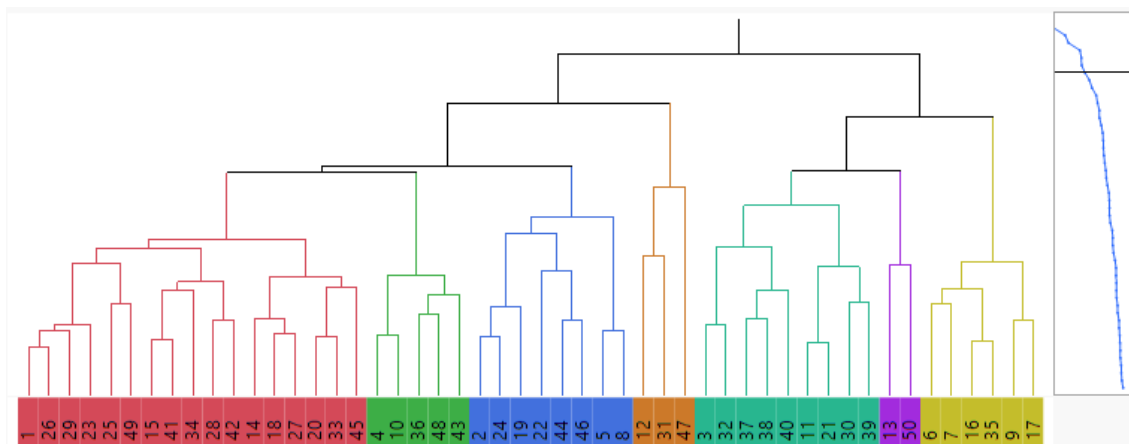
Cluster	Number of Members	Most Representative Variable	Cluster Proportion of Variation Explained	Total Proportion of Variation Explained	
1	4	12	0,559	0,112	
3	3	3	0,65	0,098	
5	4	10	0,471	0,094	
4	3	7	0,618	0,093	
2	2	24	0,844	0,084	
7	2	35	0,788	0,079	
6	2	14	0,783	0,078	

Proportion of variation explained by clustering: 0,638

Genotypes distance range by clustering: 1,517-10,981

grouping of genotypes, and the planning of future breeding programs. The dendrograms resulting from these analyses can help identify dissimilar genotypes, allowing for the generation of high heterosis through

crossbreeding programs while saving time and labour. This information is valuable for plant breeding, genetic improvement, and conservation efforts, providing insights into genetic diversity and guiding breeding programs.



**Figure 5:** Dendrogram created using the traits examined in squash genotypes at the S4 level.

#### 4. Conclusions

Summer squash is a widely produced and consumed type of vegetable. Similar to other vegetable types, the varieties of summer squash demanded by producers and consumers change over time. The supply of seeds is subject to international competition, and new varieties are continuously being introduced to the market. After the study, fifty elite lines were selected based on morphological observations, and data on these lines were presented. We investigated thirty-five agro-morphological characters in these lines and performed cluster and principal component analyses to understand the relationships between them. Clustering analysis revealed that the morphological similarity level of the lines ranged between 1.52 and 10.98 and formed two main groups, and the variation rate explained by clustering was 0.638. The principal component analysis revealed that the seven independent principal component axes represented 70.23% of the total variation in the squash population. The correlation results obtained showed that there are significant relationships between morphological and genetic characteristics of summer squash genotypes and provide important clues for strategic decisions in plant breeding and genetic diversity studies. In addition, such analyses provide important data to increase productivity in agricultural production and to be used in plant breeding studies. Based on this data, we believe that these lines can be used as intermediate breeding material to develop disease-resistant  $F_1$  hybrid summer squash varieties with high yield and quality.

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#### Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

#### Conflicts of Interest

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

#### Authorship Contribution Statement

Concept: Türkmen, Ö., Yaşar, A.; Design: Türkmen, Ö., Yaşar, A.; Data Collection or Processing: Yaşar, A.; Statistical Analyses: Şavkan, A.; Literature Search: Şavkan, A. N.; Writing, Review and Editing: Şavkan, A. N., Türkmen, Ö.

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