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Research Article

Identification and Selection of Genetic Diversity of Some Selected Summer Squash (*Cucurbita pepo* L.)

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Abstract: *Cucurbita pepo* L., an important member of the *Cucurbitaceae* family, has a wide genetic diversity in terms of fruit and plant characteristics. The objective of this study was to select genotypes with desirable morphological characteristics and to establish a wide core collection. Selected 200 genotypes were identified in detail using 22 morphological characters. Principal component analysis (PCA) and cluster analysis were used to determine the relationships among these genotypes. As a result of the PCA, eight PC axes explained 63.8% of the cumulation variation, while according to the cluster analysis, the morphological similarity level of the selected 200 genotypes ranged from 0.72 to 22.21, and in the constellation diagram formed, six groups were defined. In addition, correlation results clearly showed the relationships between the morphological parameters. Positive and significant correlations were found between plant growth habit and parameters such as plant branching, degree of branching, stem shoot development, petiole length and thickness, leaf blade area. The information obtained from the correlation analysis was used to improve breeding efficiency and reduce the number of plants selected. According to the results of the study, a high morphological variability was found among the squash genotypes. The diverse traits of summer squash are important for improving its agronomic qualities. The data obtained will guide similar research and support sustainable plant breeding and genetic diversity conservation.

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1. Introduction

The *Cucurbita* genus belonging to this family shows great diversity in terms of morphological features (Montes Hernandez et al., 2005). *C. pepo* L., one of the most cultivated species in this genus, is reported to be a highly polymorphic species in terms of fruit characteristics such as fruit size, shape and color, a high economic value and genetic diversity (Blanca et al., 2012; Dalda-Şekerci et al., 2020).

According to 2023 data, the total world production of *Cucurbita* was 23 681 845.99 tonnes, and 573 617 tonnes of *C. pepo* L. were produced in Türkiye (FAO, 2023; TÜİK, 2023). In this case, *C. pepo*

L. is an important species produced in both open field and greenhouse conditions in the world and in Türkiye.

C. pepo L. contains a variety of organic compounds, nutrients, vitamins, and minerals that are responsible for providing substantial health benefits (Kulczyński and Gramza-Michałowska, 2019). Moreover, it is considered a very good source of carotenoids and a food that contributes to human nutrition and health due to their important anti-inflammatory and antioxidant content (Fageria et al., 2012; Deppe, 2015; Miljić et al., 2021).

Population richness of local species is preserved in regions where genetic diversity is cultivated (Priori et al., 2018). Part of this diversity has been collected by researchers and stored in active germplasm banks of various institutions (Silva et al., 2024). Given the diversity found in active germplasm banks, the collection, characterization, and evaluation of these resources are crucial for preserving genetic diversity (Ribeiro et al., 2022; Silva et al., 2024). Determining morpho-agronomic characteristics in these genetic resources is important for breeding programs (Darrudi et al., 2018; Şener and Kaya, 2022).

Available quantitative or qualitative descriptive groups for the identification of plant genetic resources can help identify the characteristics of each genotype, allowing differences between or within inheritances to be determined (Barbieri and Castro, 2015; Dal et al., 2017; Gomes et al., 2020; Seymen, 2020). Thus, the existing diversity identified in plant genetic resources enables plant breeders to develop new varieties with desirable characteristics (Govindaraj et al., 2015). In plant breeding, sufficient genetic variation is a condition for the selection of agronomically desirable genotypes, especially for traits that are highly correlated with each other (Khan et al., 2017). Therefore, during the selection process, having information about the relationships between traits of interest is important to determine the relationships between phenotypic characters. In this regard, squash cultivars have been identified according to their fruit and plant traits in Türkiye and worldwide, and the existing morphological variations have been revealed in detail (Naik et al., 2015; Borges et al., 2019; Sajid et al., 2022; Şavkan and Türkmen, 2023; Silva et al., 2024).

The aim of this study is to evaluate the quantitative and qualitative morpho-agronomic characteristics of the gene collection consisting of genotypes of *C. pepo* L. by examining in detail the complex relationship between genotypes through multivariate analyses.

2. Material and Methods

Plant material: The study, 700 promising S1 level summer squash genotypes from the previous year's breeding studies were used as genetic material. In this study, a large core collection was established based on a limited number of parameters due to the high number of genotypes, creating starting materials for future breeding studies. Accordingly, 200 genotypes were selected based on preliminary observations.

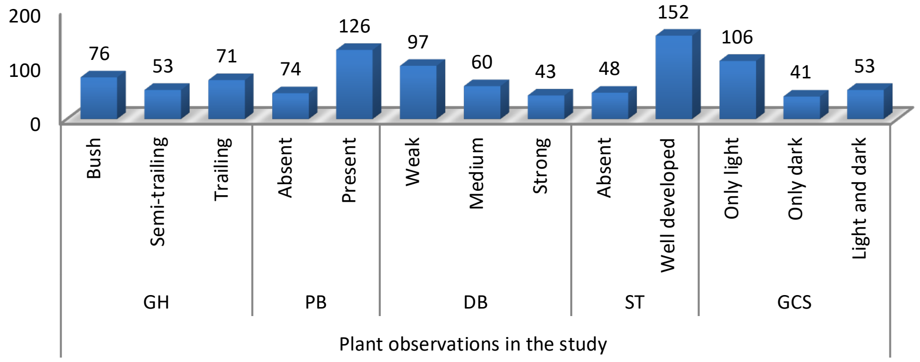
Phenotypic characters: Plant measurements and observations involve the following characteristics: growth habit (bush, semi-trailing, trailing), branching (absent, present), degree of branching (weak, medium, strong), attitude of petiole (excluding lower external leaves) (erect, semi-erect, horizontal), green color on the stem (only light, only dark, partly light and partly dark), tendrils on the stem (absent to rudimentary, well-developed), incisions on the leaf blade (absent or very shallow, shallow, medium, deep, very deep), green color of upper surface of leaf (light, medium, dark), leaf blade marbling (absent, present), leaf blade surface area (very small, small, medium, large, very large), petiole length (short, medium, long), petiole thickness (small, medium, large), the shape of the cross section of petiole (round, triangular), the number of the prickles of petiole (few, medium, many) and the silvering of leaf blade (absent, present). The observations on the immature fruit relate to the curvature of the neck in fruit (absent, present), the length of fruit (short, medium, long), the thickness of fruit (small, medium, large), the number of main colours in fruit (one, two, three), the main colours in immature fruit (whitish, cream, yellow, orange, green, whitish and yellow), the general shape of the fruit (transverse elliptical, globular, top-shaped), the intensity of the main colours in unripe fruit (light, medium, dark), and the general shape of the fruit (transverse elliptical, globular, crown-shaped, broad elliptical, ovate, elliptical, cylindrical, conical, pear-shaped, bottle-shaped, club-shaped) (UPOV, 2006).

Data analysis: All observations were made in the greenhouse and at normal harvest time. The data were analysed according to the identification form developed by UPOV for *Cucurbita* species with reference to TG/119/3 (UPOV, 2006). Using the multivariate procedure applied in JMP (JMP 17, 2017), the correlation analysis, PCA and cluster analysis based on 22 morphological characters were performed by scoring the data obtained from the materials selected according to the UPOV scale values, to identify the models of variation within the accession groups of *C. pepo* L.

3. Results and Discussion

At the end of the study, 200 genotypes at S2 stage were selected and the data of these genotypes were used in this article.

Phenotypic Traits: According to herbal observations made in the study, in terms of the growth habit of the plants, 76 genotypes were observed as bush (76), semi-trailing (53) and 71 genotypes were trailing. While 74 of the genotypes did not show branching, 126 genotypes were found to branch. Their branching degrees were determined as low 97 genotypes, medium in 60 genotypes and strong in 43 genotypes. Shoot development on the tendrils was absent to rudimentary in 48 genotypes, and shoot were well-developed in 152 genotypes. It was also observed that 53 genotypes had light and dark stem colour, 41 genotypes were only dark colour and 106 plants were only light colour (Figure 1). The distribution of plants according to their growth patterns sheds light on the genetic diversity in the study materials and their adaptation to the environment, and this is supported by the results of previous studies on squash (Gomes et al., 2020; Villanueva-Verduzco et al., 2020). In addition, the presence or absence of branching in plants indicates the diversity in their growth strategies (Christie et al., 2014; Kebrom, 2017). The evaluation of stem development in the study provides information about the strength and health of the plants. It is thought that plants with well-developed shoots will exhibit a strong growth performance. The classification of stem color in observed plants provides information regarding potential adaptations to light availability and environmental conditions (Cober and Morrison, 2010; Repinski et al., 2012). As a result, these findings provide valuable information about plant morphology and growth habits, and contribute significantly to the literature. Additionally, these findings highlight the importance of considering plant branching behaviors when examining plant growth habits and environmental adaptations.

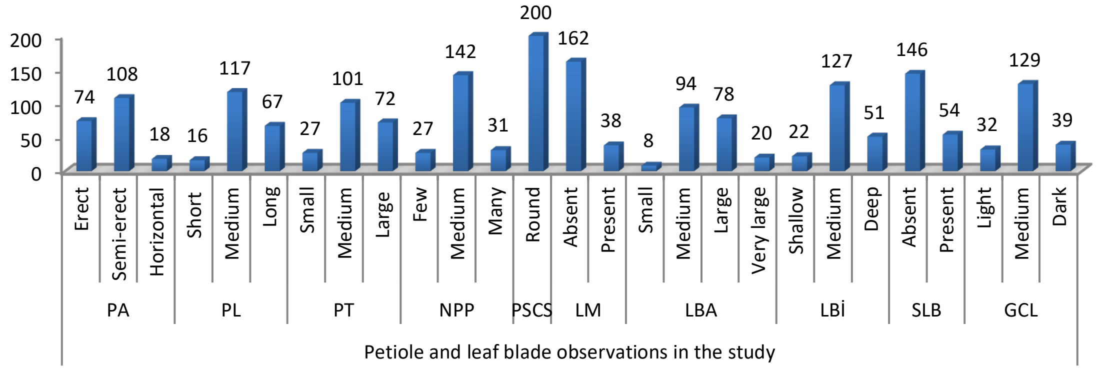


Explanation: Growth habit (GH), Plant branching (PB), Degree of branching(DB), Stem tendrils (ST), Green color on stem (GCS).

Figure 1. Some vegetative observation data in the study.

When the observations on the petiole are examined, the petiole attitude in the plant was determined as erect in 74 genotypes, semi-erect in 108 genotypes, and horizontal in 18 genotypes. Petiole length was determined as short (16 genotypes) and, long (67 genotypes), and most of them were medium (117 genotypes). The petiole thickness was determined as small (27 genotypes), medium (72 genotypes), and large (101 genotypes). When looking at the number of prickles on petiole, while 31 genotypes were observed to have many prickles, 142 genotypes had a medium number of prickles, and

27 genotypes had few prickles. The cross-sectional shape of the petiole was found to be round in all the genotypes (Figure 2).

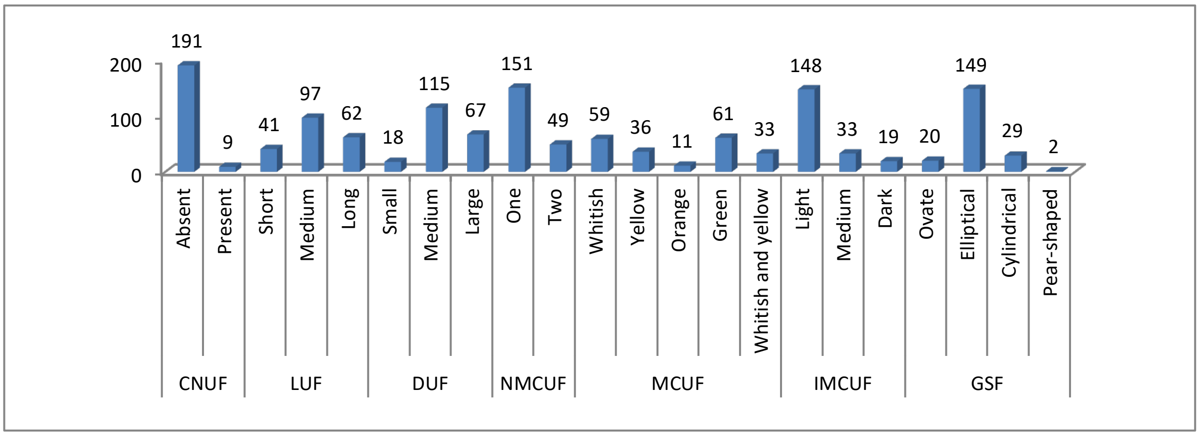


Explanation: Petiole attitude (PA), Petiole lenght (PL), Petiole thickness (PT), Number of prickles on petiole (NPP), Petiole shape of cross section (PSCS), Leaf marbling (LM), Leaf blade area (LBA), Leaf blade incisions (LBİ), Silvering of leaf blade (SLB), Green color of upper leaf (GCL).

Figure 2. Petiole and leaf observation data in the study.

According to the observations on leaf blades, while there were 38 genotypes with marbling on the leaf blade, no marbling was detected in 162 genotypes. Incisions on the leaf blade was observed shallow in 22 genotypes , medium in 127 genotypes, and very deeply in 51 genotypes. The leaf blade area was determined to be small in 8 genotypes, medium in 94 genotypes, large in 78 genotypes, and very large in 20 genotypes. While the presence of silvering of leaf blade was absent in 146 genotypes, silvering was observed in 54 genotypes. Finally, only 32 genotypes were light, 129 genotypes were medium and 39 genotypes were dark in green colour on the upper leaf blade (Figure 2). Observations made on plant leaves in studies conducted on summer squash reveal that there are significant differences in parameters such as petiole attitude, length, thickness, uprightness, the shape of cross section, and leaf blade characteristics (Dalda-Şekerci et al., 2020; Şavkan and Türkmen, 2023). In this regard, it can be understood that leaf characteristics in this study may affect plant health, growth, and overall yield, therefore, it is necessary to optimize growing practices and select squash varieties with desired leaf characteristics for a better performance.

When it comes to the curving of neck in unripe fruit, while there were curvatures in nine genotypes, no curvature was detected in 191 of them. The length in unripe fruit was found to be short in 41 genotypes, medium in 97 genotypes, and long in 62 genotypes. It was observed that the diameter of unripe fruit was small in 18 genotypes, medium in 115 genotypes, and large in 67 genotypes. Morphological characteristics such as color, fruit shape, and size are important for industrial targets. In this context, while the number of major colors in immature fruits was determined to be single in 151 of the genotypes, two colors were detected in 49 of them. In the study, there were significant differences in terms of main color in immature fruit, with samples being whitish (59 genotypes), yellow (36 genotypes), whitish and yellow (33 genotypes) orange (11 genotypes), and mostly green (61 genotypes). The intensity of the main colour was light in 148 genotypes, medium in 33 genotypes and dark in 19 genotypes when the intensity of the main colour in immature fruit was evaluated. In our study, it was observed that there were significant differences in the general shape of the fruits of the plants. When the general shape of the fruit was examined, they were found to be ovate (20 genotypes), cylindrical (29 genotypes), pear-shaped (2 genotypes), and most of them were eliptical genotypes) (Figure 3).



Explanation**:** Curving of neck in immature fruit (CNUF), Length in immature fruit (LUF), diameter in immature fruit (UFD), Number of major colors in immature fruit (NMCUF), Major colors in immature fruit (MCUF), General shape of fruit (GSF)

Figure 3. Unripe fruit observation data in the study.

In addition to high intraspecific and interspecific variation (Ozturk et al., 2021), differences in anatomical features are also a common phenomenon among *Cucurbita* species (Balkaya et al., 2010). Therefore, intraspecific variations in fruit shape, size, and shell texture can be easily observed. Many studies have shown that diversity in morphological traits is high in the *Cucurbitaceae* family, and by examining genetic variations in immature squash fruits such as size, shape, color, and overall fruit shape, they have shed light on diversity in this crop (Çeğil & Çürük, 2019; Dalda-Şekerci et al., 2020; Yildiz et al., 2015). Accordingly, understanding these characteristics is highly significant for agricultural practices and breeding programs aiming to increase yield and fruit quality, because these findings emphasize the importance of considering various parameters to optimize the cultivation and selection of summer squash varieties in line with market demand.

The correlation results revealed that plant growth habit, growth branching and degree, petiole attitude, green color on stem, the development of stem tendrils, green color on the upper surface of the leaf , leaf area, petiole length and petiole thickness were positively and significantly correlated. Green color on the stem was positively and significantly correlated with main color, the number and intensity of the main colors in unripe fruit, while leaf blade area was positively and significantly correlated with petiole length, and petiole thickness. Unripe fruit length was positively and significantly correlated with the diameter of unripe fruit, the number of colors and main color intensity fruit, while the main color of unripe fruit was positively and significantly correlated with the number of main colors and main color intensity (Figure 4).

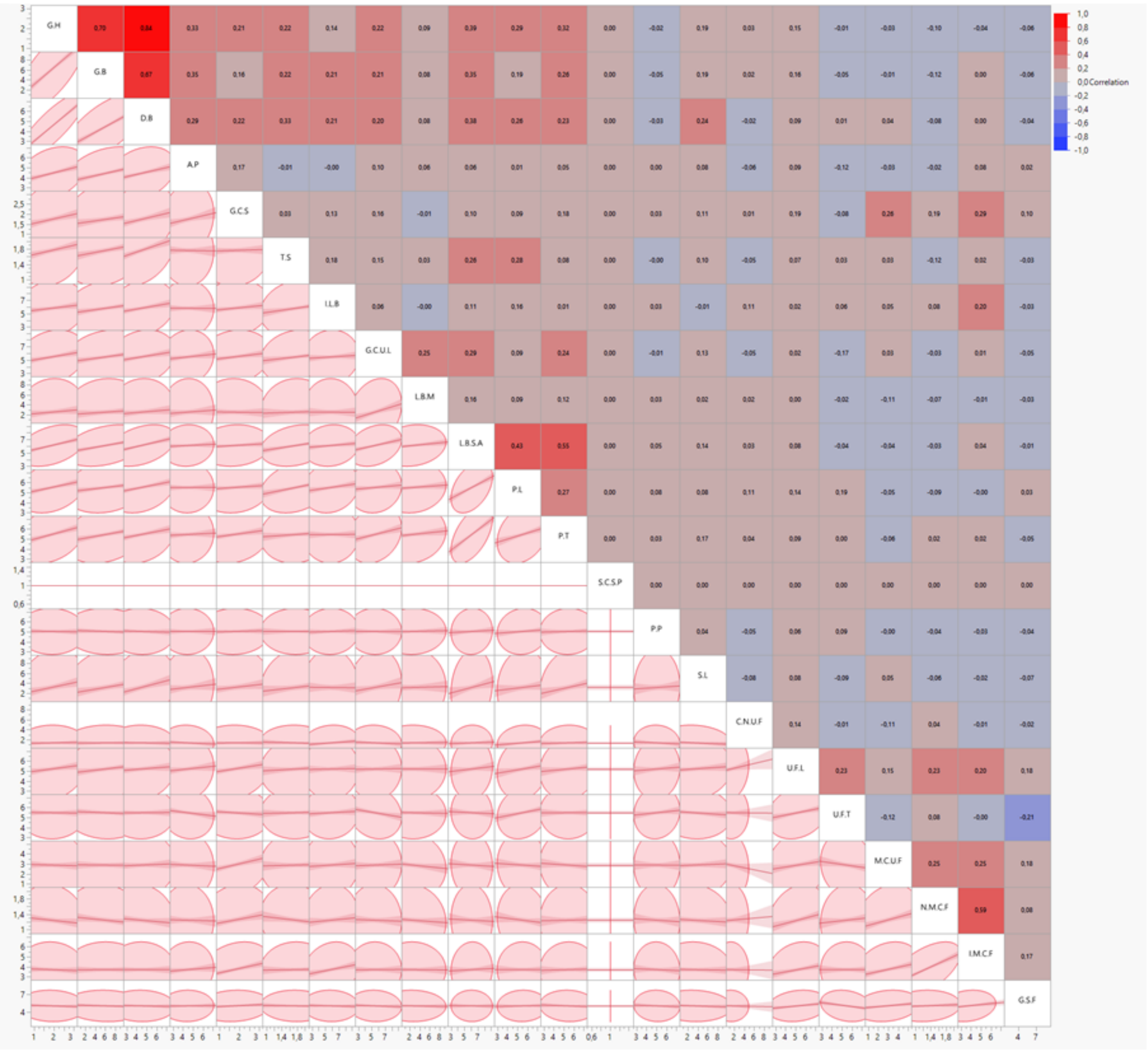


Figure 4. Correlation coefficients among morphological traits.

Positive and significant correlations between plant growth habit and plant branching, degree of branching, stem shoot development, petiole length and thickness, leaf blade area clearly showed the relationship between morphological parameters. Correlations were also found between the green colour of the stem and the green colour of the upper leaf surface. Correlations were also observed between main colour, number of main colours, intensity of main colours and immature fruit length and immature fruit diameter, number of colours and intensity of main colours. These correlations support the validity of measurements such as overall fruit shape. In summary, this information has been evaluated to improve breeding perspectives and to be used in plant selection. In addition, the cross-sectional shape of petiole was not included in the principal component analysis because no correlation data were found in the analysis. This clearly shows the details that need to be taken into account when analyzing the relationships between certain morphological parameters. Information obtained from correlation analysis has been reported to be used to improve breeding efficiency and reduce plant populations in selection (Soltani et al., 2016). Although a few studies have reported the use of correlation analysis on squash (Borges et al., 2019), the information obtained based on correlation analysis in studies conducted by various researchers on different species, especially squash, has provided insight into genetic diversity among genetic accessions, relationships among varieties, and potential breeding studies (Naik et al., 2015; Khan et al., 2017; Aydın et al., 2022; Moon et al., 2023; Şavkan et al., 2024).

3.1. Principal component analysis (PCA)

Principal component analysis aims to describe the variability in the data and can help to clearly distinguish between different groups. This analysis is a useful technique for identifying relationships and similarities between samples (Abdi & Williams, 2010). In this direction, principal component analysis is considered to be a useful method for identifying groups of plant and fruit characters and contributes to the understanding of relationships between populations. Eight eigenvectors were obtained from the PCA, explaining 63.8% of the variation in the squash accession(Table 1). Literature reports that PCA axes with eigenvalues > 1 are reliable (Kanal & Balkaya, 2021; Ateş, 2022). In this study, the eigenvalue coefficients of the first eight components varied between 3.78 and 1.01.The first PC axis explained 18% of the variation, the second 10.5% and the third 0.08% (Table 1). In the study, the criteria analyzed in the principal component analysis are considered to have significant values if the weight coefficient values in the components are 0.3 and above (Taş, 2020). Accordingly, the characteristics with high coefficients of 0.3 and above on the PC-1 axis were plant growth habit (0.429), plant branching (0.395), the degree of branching (0.422), and leaf blade area (0.338), and those on the PC-2 axis were green color of the stem (-0.345), immature fruit length (-0.301), the main color in immature fruit (-0.385), the number of colors in immature fruit (-0.504), and the intensity of the main color (-0.527) (Table 1). Figure 5 presents the graphical representation of the score plot analyses that show the relationship between squash genotypes.

Table 1. Factor groups and corresponding principal component axes according to PCA of morphological traits

	Eigenanalysis of the Correlation Matrix							
Eigenvalue	3.783	2.195	1.581	1.384	1.18	1.163	1.113	1.006
Proportion	0.18	0.105	0.076	0.066	0.056	0.055	0.053	0.048
Cumulative	0.18	0.285	0.36	0.426	0.482	0.538	0.591	0.638
	Eigenvectors							
Parameters	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
G.H	0.429	0.06	0.111	-0.232	0.091	0.019	0.031	0.04
G.B	0.395	0.042	0.156	-0.246	0.087	-0.034	-0.021	0.004
D.B	0.422	0.033	0.122	-0.257	-0.06	-0.031	-0.021	0.019
A.P	0.186	-0.043	0.344	-0.209	0.319	0.171	-0.058	-0.248
G.C.S	0.174	-0.345	0.118	0.063	0	0.101	-0.037	-0.016
T.S	0.215	0.051	-0.148	-0.029	-0.497	-0.205	0.039	-0.1
L.B.I	0.141	-0.134	-0.174	-0.188	-0.22	-0.44	-0.299	-0.219
G.C.U.S.L	0.211	0.02	0.14	0.43	0.01	-0.068	-0.239	-0.142
L.B.M	0.104	0.103	-0.029	0.358	0.274	-0.044	-0.21	-0.491
L. B. S. A	0.338	0.046	-0.198	0.337	-0.012	-0.047	0.083	0.147
P.L	0.248	0.042	-0.408	0.079	-0.122	-0.048	0.248	-0.078
P.T	0.27	0.021	-0.167	0.384	0.165	0.13	0.03	0.345
P.P	0.01	0.022	-0.182	0.061	-0.162	0.475	-0.07	-0.503
S.L	0.168	0.021	0.135	0.093	-0.189	0.351	-0.007	0.303
C. N. U. F	0.018	-0.019	-0.25	-0.079	0.453	-0.381	0.214	0.048
U.F.L	0.117	-0.301	-0.229	-0.136	0.195	0.292	0.313	-0.117
U.F.T	-0.013	0.012	-0.527	-0.312	0.03	0.295	-0.17	0.01
M.C.U.F	0.01	-0.385	0.164	0.048	-0.361	0.055	0.08	0.04
N.M.C.U.F	-0.041	-0.504	-0.116	0.037	0.159	0.003	-0.262	0.177
I.M.C.U.F	0.034	-0.527	-0.055	0.048	0.039	-0.103	-0.232	-0.007
G.S.F	-0.023	-0.255	0.15	0.121	-0.045	-0.118	0.653	-0.276

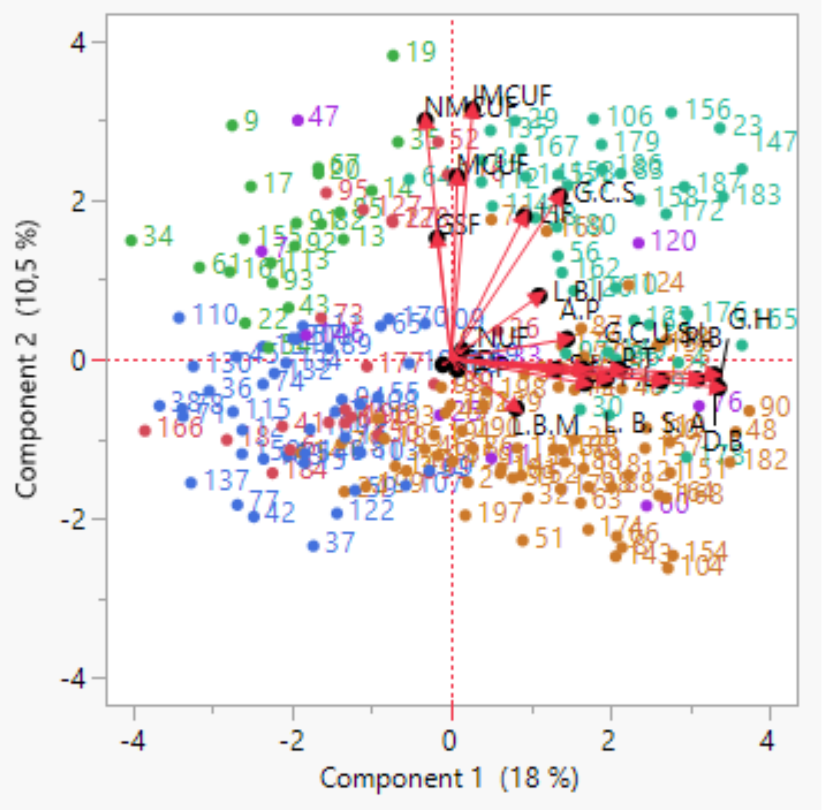


Figure 5. Score plot analyses obtained through the PCA formed with morphological characteristics.

Many studies have been reported to determine the level of variation in populations of *C. pepo* L. species. Martins et al. (2015) described *Cucurbita* populations collected in central and northern Portugal for 20 morphological characters. They reported that the three species were separated by PCA, with a cumulative variation of 52.5%. In a study analyzing the diversity of 64 genotypes of *C. pepo* L. using morphological markers, as a result of PCA, it was reported that the first three PC axes accounted for 42.58% of the cumulative variation (Yunli et al., 2020). Şavkan and Türkmen (2023) evaluated 59 summer squash genotypes with 24 plant and fruit characteristics and reported that 10 PC axes explained 94.3% of the cumulative variation. The results obtained in our study provide useful information to understand the genetic diversity and relationships in the squash population, and when the literature data are evaluated, it is understood that the variation richness is at a sufficient level. In addition, the principal component analysis performed in this study represents an important step to understand the genetic diversity and relationships among summer squash crops, and such analyses are considered to play an important role in plant breeding and genetic studies.

The outlier analysis performed on our research data is also crucial for identifying outliers that contain extreme readings. Mahalanobis distance is an important statistical approach used to identify outliers. This distance calculates the distance between two points by calculating the covariance matrix computed from the data and considering the behavior of other points. In this way, the distance obtained is expressed in standard deviation and is a data-dependent value (Anonymous, 2024). Mahalanobis distance is used to identify outliers in the gene accessions, helping to identify atypical genotypes.

In the study, some genotypes (4, 7, 25, 33, 76, 85, 120, 146, and 176) with a Mahalanobis distance exceeding 5.825 were defined as outliers (Figure 6). Also, the 6th group, which includes some of these genotypes, is shown in the constellation diagram (Figure 7). The detection of outliers is essential to obtain reliable results in data analysis and to avoid misleading effects. Therefore, statistical methods such as Mahalanobis distance are valuable for identifying outliers in the data set and understanding their impact on the analysis. Accurate identification of outliers can improve the reliability of data analysis and help make the right decisions.

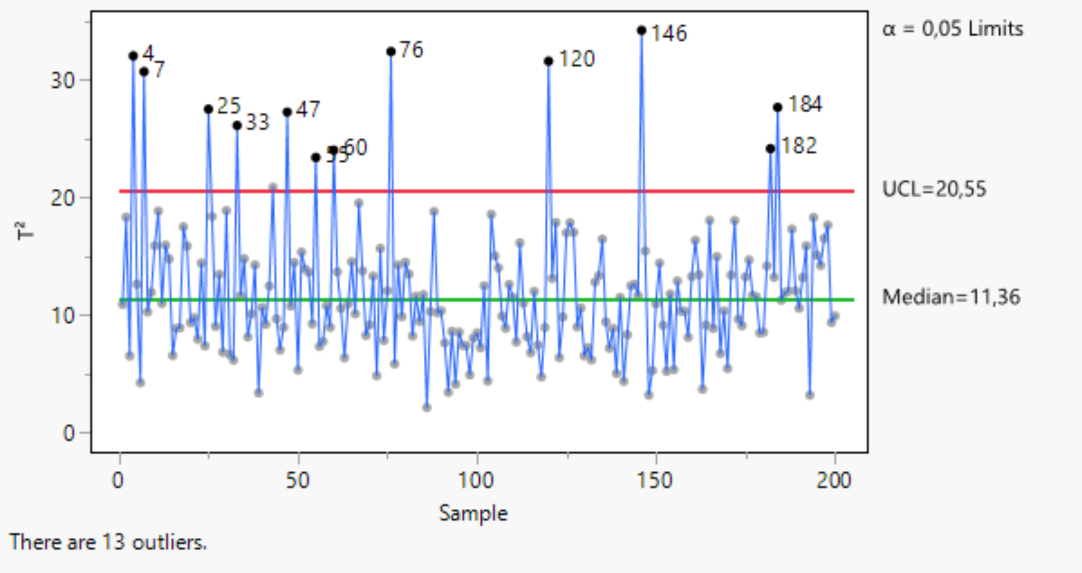


Figure 6. Outlier Plot Analysis formed with morphological characteristics.

3.2. Cluster analysis

Cluster analysis that uses data from characterization studies is an important method for identifying relationships between populations and measuring the utility of identifying these plant and fruit characteristics. Clustering is more reliable to analyze when the first two or three PC axes explain 25% or more of the cumulative variation in PCA (Mohammadi and Prassana, 2003). According to the cluster analysis, the morphological similarity level of the 200 selected genotypes ranged from 0.72 to 22.21 and the genotypes were categorized into six groups with 47.9% similarity level in the diagram (Table 2). In addition, Figure 7 shows the constellation diagram, which shows the resulting groups more easily. Several studies in squash have identified distinctive groups within genetic accessions based on similarity dendrograms between genetic accessions (Borges et al., 2011; Ferreira et al., 2016; Uddain et al., 2019; Gomes et al., 2020; Villanueva-Verduzco et al., 2020; Sajid et al., 2022). According to these results, it was possible to evaluate the variation observed among the squash genotypes, to group the genotypes, and to guide future breeding programs. When evaluated with the reported literature data, it is concluded that the richness of variation is at a sufficient level. In addition, dendrograms obtained as a result of such analyses will help to identify highly dissimilar genotypes and achieve high heterosis through cross-breeding programs, thus saving labor and time in breeding programs. It is also of significant value for plant breeding and genetic improvement conservation, providing valuable information to expand genetic diversity, distributing different genotypes, and structuring breeding programs.

Table 2. Clusters based on highly correlated variables in cluster analysis

Cluster	**Number of Members**	**Most Representative Variable**	**Cluster Proportion of Variation Explained**	**Total Proportion of Variation Explained**
1	6	G.H	0.47	0.134
2	3	I.M.C.U.F	0.58	0.083
3	3	P.T	0.538	0.077
4	3	S.T	0.473	0.068
6	3	G.S.F	0.447	0.064
5	3	P.P	0.37	0.053

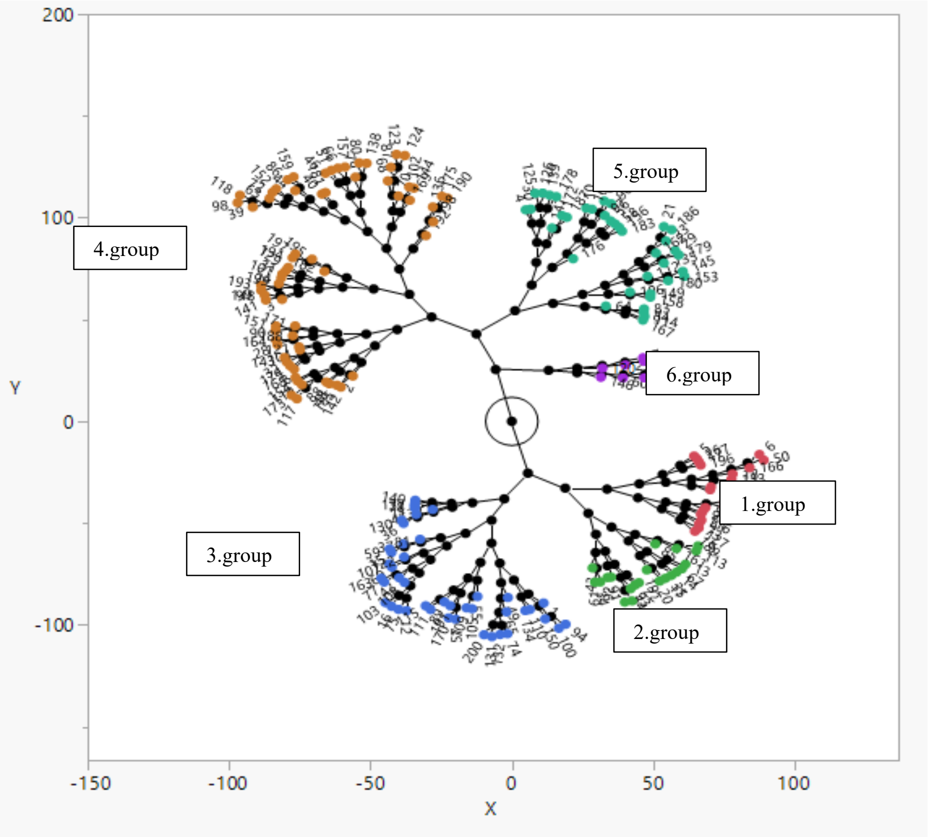


Figure 7. Constellation diagram obtained from clustering analysis.

Conclusion

This study identifyed to the existing material in order to develop a new varieties F1 hybrid variety for summer squash, which is a good alternative crop in greenhouse agriculture. According to the findings, a high level of morphological variation was found among the squash genotypes. In the principal component analysis performed at the end of the study, the parameters of plant growth habit, plant branching, branching degree, leaf blade area, green color on stem, unripe fruit length, the main color in unripe fruit, unripe fruit, the number of the main color, the intensity of the main color, unripe fruit diameter, petiole length, and petiole attitude effectively determined the level of variation. According to the cluster analysis, the morphological similarity level of the selected 200 genotypes varied from 0.72 to 22.21, and in the constellation diagram created, the genotypes were classified into into six groups with 47.9% similarity level.

The aim of the study was to develop a good genetic accession by including the selected genotypes in future breeding studies. When selection is applied to a population, the population average is expected to increase regarding the traits considered. In breeding studies, morphologically and genetically distant individuals are selected as parents to create heterosis. At the end of the study, it was concluded that it would be useful to include the selected genotypes in the gene accessions to be evaluated in breeding programs for variety development. In fact, according to the variations we obtained as a result of the selection, the desired criteria for the traits analyzed were achieved to a great extent and generally supported the literature.

In addition, this study reveals the differences between the genotypes on fruit traits in detail. The determination of traits such as fruit size, diameter, color, color intensity, and general shape provide valuable information on plant cultivation and fruit quality. Such analyses provide important data to increase productivity in agricultural production and to be used in plant breeding studies. The correlation results clearly showed the relationships between the morphological parameters. For example, positive and significant correlations were found between plant growth habit and parameters such as plant branching, degree of branching, stem tendril growth, petiole length and thickness, and leaf blade area. It has been reported that the information obtained from correlation analysis can be used to improve breeding efficiency and reduce the number of plants selected. Further research could explore genetic and physiological mechanisms underlying the observed differences in growth habits and traits between fruits and plants.

Ethical Statement

Ethical approval is not required for this study because this article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest

The author declares that he has no known competing financial interests or personal relationships that could have influenced the work reported in this article.

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Author Contributions

The contributions of all the authors are defined as follows: study concept and design: A. N. Şavkan, O. Turkmen; data collection: A. N. Şavkan Y. Dal-Canbar; analysis and interpretation of results: A. N. Şavkan, Y. Dal-Canbar, O. Turkmen; preparing a draft text: A. N. Şavkan, Y. Dal-Canbar, O. Turkmen. All the authors reviewed the results and approved the final version of the article.

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