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RESEARCH ARTICLE

Morphological characterization and molecular marker-assisted selection for resistance to *Tobacco mosaic virus* (TMV) and *Tomato spotted wilt virus* (TSWV) in S2 population of capia pepper (*Capsicum annuum* L.)

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Abstract TMV (*Tobacco mosaic virus*) and TSWV (*Tomato spotted wilt virus*) are the most common virus diseases that causes loss of productivity in pepper cultivation. The most effective method in the fight against viral diseases is the use of resistant cultivars. This study was conducted to determine the similarities and differences of 120 Capia pepper lines in the S2 stage, consisted of local populations, standard and hybrid cultivars, in terms of morphological variation and to determine the resistance levels of the lines to TSWV and TMV. As a result of molecular analysis, genotypes 34, 35, 36, 46, 47, 48, 84, and 85 were found to be homozygous resistant to *L4* allele and the *Tsw* gene. Cluster analysis and principal component analysis (PCA) were applied to determine the relationship between the lines determined as a result of single plant selection. A dendrogram was prepared to evaluate morphological similarity between the lines. In the cluster analysis, 10 groups were identified based on 25 variables. The PCA explained 69.9% of the total variation based on 10 PC axes. At the end of the study, morphological variability was found high among the pepper lines. This evaluation of plant trait variability can assist geneticists and breeders to identify populations with desirable characteristics for inclusion in pepper breeding programs. In addition, the levels of resistance to these two diseases are crucial for breeding programs aimed at developing cultivars resistant to diseases.

Keywords Capia pepper · Characterization · Cluster analysis · Genetic sources · TSWV · TMV

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Introduction

Capsicum annuum, a member of the *Solanaceae* family, has a rich genetic diversity in terms of fruit characteristics. Much of this diversity has been used to develop sweet, blocky peppers that are most popular in commercial production and are typically harvested at the green (immature) stage (Prohens et al. 2008).

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Recently, a class of sweet pepper species harvested at the mature stage (full color) has gained popularity in the international retail market. Colorful sweet peppers are known for their visual appeal, sweet taste, antioxidant activity, nutritional carotenoids, and anti-inflammatory compounds (Sun et al. 2007; Park et al. 2012; Vilarinho et al. 2015). In addition to fresh consumption of peppers produced in Turkey, Vural et al. (2000) reported that they are used in different areas of the industry such as pickles, tomato paste, dried and powdered peppers, and roasted peppers. Capia pepper is a member of the pepper group that is essential for both producers and consumers during the cultivation period and post-harvest processing. The number of genotypes in plant genetic resources increase day by day due to genotypes with different plant and fruit structures formed in local pepper populations through selection and natural hybridization over time (Haussmann et al. 2004; Zhang et al. 2016). In breeding, selecting plants with the desired characteristics from a heterogeneous gene pool makes it easier to achieve the goal. In this regard, as in many plant species, numerous researchers have carried out agronomic and morphological characterization of pepper genotypes obtained from different sources (Zewdie et al. 2004; Mutlu et al. 2009; Bozokalfa and Eşiyok 2010; Olatunji & Afolayan 2018; Başak 2019; Çetin 2023).

Genetic variation created by genetic collection richness and agronomic characteristics is significant in vegetable breeding research and is considered the basis for F1 cultivar breeding. From this perspective, correct identification and classification of botanical species is a crucial step in efficiently managing germplasm collections. Because this stage forms the basis of developing any plant species. Because this stage forms the basis of developing any plant species. In addition, many researchers have stressed the importance of morphological characterization as a fundamental step towards resolving taxonomic conflicts in many plant species (Ranjit et al. 2013; Gerrano et al. 2017; Olatunji & Afolayan 2018). Cultivation of pepper has increased scientific research on this species. These investigations, which started with the selection of the types taken from the gene sources and their use in breeding, have focused on more and more specific issues together with the innovations in science and technology. In addition to morphological characterization research, there are various investigations on the detection of different diseases and pests that significantly limit pepper production. Every year, approximately 15% yield losses are observed in plant production due to diseases, and 30% of these losses are due to viral diseases (Islam et al. 2018).

Tobacco mosaic virus (TMV) is a plant virus that can infect a wide range of plants, including peppers. It is a highly contagious virus that is primarily spread through contact, including mechanical transmission from contaminated hands, tools, equipment, pollen or seeds. It can also be transmitted by sap-feeding insects, though this is less common. If pepper plants are infected with TMV, they might display the following symptoms: mosaic patterns and yellowing in leaves, curling and distortion, stunted growth, necrosis, and reduced fruit quality. TMV symptoms are quite similar to other viral infections and physiological disorders. Therefore, accurate diagnosis is critical. Preventing TMV involves practicing good sanitation and hygiene measures to avoid virus spread. This includes washing hands, equipment, and tools regularly before working with plants, using virus-free seeds and seedlings, and removing and destroying infected plants to prevent further spread of resistant pepper cultivars if available (DPV 2000).

Tomato spotted wilt virus (TSWV) is a significant emerging disease that affects various crops, including *Capsicum annuum*. TSWV is transmitted by thrips, which are small insects belonging to the order *Thysanoptera*. Thrips transmit plant viruses in the *Tospovirus* genus, which includes TSWV. Once infected at the larval stage, adult thrips usually transmit TSWV for life, and transmission to plant hosts occurs when thrips feed (Jones 2005). To develop resistance to TSWV in pepper, molecular marker-assisted selection can be employed. Several molecular markers associated with virus resistance loci in pepper and tomato have been developed to expedite marker-assisted breeding. These markers can provide information for early selection and are useful for breeding horticultural crops resistant to viruses. Marker-assisted breeding involves introgression of resistance genes into cultivars, which has been the principal route of breeding cultivars resistant to TSWV (Siddique et al. 2022). In the case of Capia pepper, the S2 population can be used for morphological characterization and molecular marker-assisted selection for resistance to TSWV.

Morphological characterization involves assessing various traits such as plant height, leaf shape,

fruit size, and disease symptoms to identify individuals with desirable characteristics (Manivannan et al. 2018). Molecular markers linked to potential resistance loci can be used to identify individuals carrying the resistance genes (Siddique et al. 2022). These markers can be identified through techniques such as genotyping-by-sequencing, which allows for genome-wide discovery of single nucleotide polymorphism (SNP) markers associated with disease resistance (Manivannan et al. 2018). In addition to marker-assisted selection, it is important to understand the genetic basis of resistance to TSWV in Capia pepper. The *Tsw* gene has been identified as the dominant resistance gene in pepper cultivars resistant to TSWV. However, naturally occurring resistance-breaking strains of TSWV have been identified, highlighting the need for further research on the genetic determinants of resistance. Understanding the specific genetic determinants that allow the virus to overcome the *Tsw* gene can aid in the development of strategies to enhance resistance in Capia pepper (Margaria et al. 2007). Overall, morphological characterization and molecular marker-assisted selection can be valuable tools for developing resistance to TSWV and TMV in the S2 population of Capia pepper. By identifying individuals with desirable traits and carrying resistance genes, breeders can select and develop cultivars that are resistant to TSWV and TMV, thereby reducing the impact of this devastating disease on pepper production.

In this study, Capia pepper lines in the S2 population were characterized by morphological observations. At the same time their resistance levels to TMV and TSWV, two harmful plant viruses that cause serious economic damage, were determined. By doing so, we aimed to create an important road map for more efficient use of pepper gene resources by determining the genetic distance and population structure between breeding materials.

Materials and methods

Plant material

The materials of our study consisted of 120 Capia pepper lines consisting of local populations, standard and hybrid cultivars at the S2 stage.

Phenotypic characters

One genotype of six plants per accession were grown in a completely randomized design double row with a spacing of 0.4 m × 0.4 m between and over rows on tubes prepared at 0.7 m intervals (Fig. 1). 120 genotypes were characterized using 25 morphological characters (13 qualitative and 12 quantitative) and quantitative characters were measured while qualitative characters were observed. Qualitative characters; anthocyanin coloration in internode (slight, moderate, excessive), length of plant (cm), width of the plant (cm), plant index (length/width), plant grown habit (upright, semi-upright, prostrate), leaf color (light green, green, dark green), leaf shape (lanceolate, ovate, broad elliptic), fruit attitude (upright, semi-upright, drooping), immature fruit color (light green, green, dark green), mature fruit color (light red, red, dark red), fruit longitudinal section shape (oblate, round, cordate, square, rectangular, trapezoid, triangular, narrow triangular, hornshaped), fruit cross-section shape (elliptic, angular, circular), fruit tip shape (very acute, moderately acute, rounded, moderately depressed, very depressed), number of fruit loculus (predominantly two, equally two and three, predominantly three, equally three and four), fruit stalk tip (absent, shallow, medium, deep, very deep) and ripe fruit pungency (sweet/hot) while quantitative values; plant length (cm), plant width (cm), plant index (p.length /p.width), stem length (cm), stem diameter (mm) fruit length (cm), fruit width (cm), fruit flesh

Fig. 1 A view of planting pepper seedlings in the greenhouse

thickness (mm), seed cavity length (cm), fruit flesh hardness (kg/N), soluble solid contents (Briks (Hanna HI 96801) (Çetin et al. 2021)) and pH.

DNA extraction

DNA extraction was performed according to a modified CTAB procedure (Doyle and Doyle 1987) in young leaves.

Molecular marker analyses

In molecular research, the 189D23M SCAR dominant marker determined by Tomita et al. (2008) was used for the *L3* allele. Moreover, the 060I2END dominant marker, 087H3T7HRM and 087H3T7 CAPS (SspI used for restriction, Uncut restriction profile: RR, Homozygous digestion profile: rr) codominant SNP marker determined by Yang et al. (2009) were used for the *L4* allele. In testing the resistance against *Tsw,* the CAPS marker named SCAC568 was used (Moury et al. 2000). The markers used are given in Table 1. PCR amplifications and PCR conditions were conducted as described by Moury et al. (2000), Tomita et al. (2008), and Yang et al. (2009) with some modifications. *L* gene was mapped to the pepper chromosome 11 whereas *Tsw* gene was located at chromosome 10.

Data analysis

The obtained PCR products were evaluated in capillary electrophoresis and the evaluation of the data was performed manually. Evaluations were made according to the values of allele lengths given by the results of the marker used for each line in the capillary electropherogram views for *L3* allele. Evaluations were carried out according to the allele lengths obtained by markers used in each line in electropherogram images and clustering of HRM melting curves for *L4* allele. Evaluations were carried out based on the values of allele lengths shown in capillary electropherogram images of the restriction of the marker used for each line with the Xbal enzyme for *Tsw* allele.

All characters were measured in the greenhouse and at normal harvest time. Characters were analyzed according to the identification form developed by UPOV for *Capsicum* species with reference TG/76/8 (UPOV 2006). Fruit character analyses were performed on three fruits of each plant. These characters were described according to the principles of numerical taxonomy (Sneath and Sokal 1973), so that similarity or dissimilarity coefficients between cultivars could be determined. Using the multivariate procedure applied in Minitab (MINITAB 16, 2016), the principal component analysis (PCA) and cluster analysis based on 25 morphological features were performed by scoring the data obtained from the materials selected after selection according to the UPOV

Table 1 List of primers used in disease testing

Marker name	Marker sequences (5–3)	PCR product (bp)	Locus	Restriction enzyme	References
SCAC568 (CAPS)	GTGCCAGAGGAGGATTTAT GCGAGGTGGACACTGATACT	568 bp	*Tsw*	XbaI (Codominant)	Moury et al. (2000)
189D23M (SCAR)	ATTGTCAGAGTCGGGAAG CA AACGACAAGGGTTTATTG TATGC	783 bp	*L3*	Dominant	Tomita et al. (2008)
060I2END (SCAR)	GCACATCAGCAGGTTTAG TACG CCAACTGTCAAACCTCGGTT	751 bp	*L4*	Dominant	Yang et al. (2009)
087H3T7HRM (SNP)	CATGATTACATTTTATGTTGC AAAAGGAAGGTTCTCATT GTT	150 bp	*L4*/(HRM)	Codominant	Yang et al. (2009)
087H3T7 SNP CAPS	CCTTTGCCTGCATTATTC TTG GCCCAAATTTATTCCCAA ATGC	440 bp	*L4*/CAPS	SspI (Codominant)	Yang et al. (2009)

scale values, to identify the variation models within the Capia pepper accession groups. In addition, SPSS 21.0 statistical software (IBM. Chicago. IL. USA) was used for correlation analysis (SPSS 2021).

Results and discussion

Phenotypic traits

A wide variation was observed in the study in terms of plant and fruit characteristics. The development of anthocyanin in the internode was not observed in 27 lines (22.5%), while a slight development was observed in 62 lines (51.67%), a moderate development was observed in 25 lines (20.83%), and an excessive development was observed in six lines (5%).

The lengths of plants that become thinner varied between 46.9 and 200 cm. The smallest plant length was 46.9 cm in line 114, while the most considerable length was measured in line 45 as 200 cm. The widths of the plants varied between 20 and 67 cm. The smallest width was detected in line 90 (20 cm), whereas the greatest was in line 119 (67 cm). Plant index values were determined using the length and width values of plants. These index values were found to be 1.03–5.70. Line 114 was the line with the lowest length/width index. It is considered that the plant width and plant index (length/width) values will contribute to the determination of species suitable for greenhouse cultivation/open field conditions and appropriate planting distances for the promising lines to be selected.

When fruit characteristics were investigated, fruit attitude was upright in four lines (3.33%), semi-upright in nine lines (7.50%), and drooping in 107 lines (89.17%). A high number of variations were also observed in the longitudinal section parameters of the fruit. These are cordate (0.83%), trapezoidal (0.83%), rectangular (1.67%), acute-triangular (30.83%), and, in more than half of the lines, triangular (66.67%). Fruit shapes other than triangles and acute triangles were detected in bell peppers, three-nose peppers, and ornamental peppers, which are characterized as non-type plants. The smallest plant length was observed as 4.80 cm in line 9, while the biggest length was measured in line 77 as 21.10 cm. Apart from the non-type plants, the lengths of fruits varied between 9.30 and 21.10 cm. The smallest fruit length was detected in ornamental pepper, which was line 9. When the fruit width values were examined, the smallest width was detected in line 81 (2.50 cm), whereas the greatest width was in line 120 (6.10 cm). The smallest thickness of flesh in fruits was observed as 1.42 mm in line 32, while the greatest thickness was measured in line 119 as 6.90 mm. The shortest length of the seed cavity was measured as 15.90 mm in line 10, while the highest length was measured as 90.27 mm in line 48.

The study also investigated the properties of capia peppers, such as soluble solid contents and pH, as these peppers are consumed fresh and also used to produce paste in the industry. The lowest pH level in fruit was measured as 4.60 in line 105, and the highest level was determined as 5.34 in line 63. The lowest soluble solid content was detected in line 30 (5.40%), and the highest soluble solid content was measured in line 10 (12.90%). In addition fruit flesh hardness, the highest value was measured in line 34 with − 1.23 kg/N and the lowest hardness value was measured in line 9 with − 3.58 kg/N.

Correlation results revealed that plant height was positively correlated with stem diameter, stem length, anthocyanin in the internode and plant index. Stem diameter was positively correlated with plant width and fruit width. Plant attitude showed negative and significant correlations with fruit attitude, immature fruit colour and fruit flesh thickness. Leaf colour showed a positive significant correlation with immature fruit colour. Fruit attitude was positively and significantly correlated with immature fruit colour, stem and fruit length, fruit flesh thickness, seed cavity length and fruit longitudinal section shape. Plant index was positively correlated with stem length and pH, but negatively and significantly correlated with plant width. Fruit width showed a positive significant correlation with fruit flesh thickness. Fruit length was positively correlated with fruit flesh thickness, seed cavity length and fruit longitudinal section shape, while it was negatively and significantly correlated with S.C.C. Seed cavity length showed a positive and significant relationship with fruit longitudinal section shape and a negative and significant relationship with stalk tip. Fruit longitudinal section shape showed a negative significant correlation with fruit tip shape, while fruit cross-sectional shape showed a positive significant correlation with stalk tip. Fruit tip shape was positively correlated with fruit lobe

number and fruit lobes number was positively correlated with stalk tip (Table 2). Correlation results clearly showed the relationship between morphological parameters. For example, the positive and significant correlation of plant index with plant height, the negative and significant correlation of plant width with plant length, the negative and significant correlation of fruit flesh thickness with fruit flesh hardness, the positive and significant correlation of seed cavity length with fruit longitudinal section shape, and the negative and significant correlation of seed cavity length with fruit longitudinal section shape show that the measurements taken support the accuracy of the measurements.

At the end of the study, the degree of genetic relationship among 120 capia pepper lines was determined using phenotypic indicators. Some indicators highlight the distinction between pepper genotypes in phenotypic terms. This is because more variations were observed in these genotypes, which led to significant differences. Consequently, the phenotypic variation of lines was identified that could be used in pepper breeding programs in the future, and it was determined that phenotypic features would be beneficial in the distinction of pepper genotypes.

Principal component analysis (PCA)

Principal component analysis was performed in order to reveal genetic diversity and relatedness between capia pepper lines to group lines based on 25 morphological characteristics and to investigate their relationships. A substantial variation was obtained in the PCA analysis. In the PCA analysis performed based on the correlation matrix, eigenvalue, and variance percentages, the variance values of the first three principal components were calculated for all morphologic characteristics. According to the PCA, 25 independent principal component axes were obtained in pepper lines. When the values of the principal component axis were examined, the variation values of the first three principal components were 13.9%, 10.9%, and 7.7%, respectively, and the cumulative value represented 32.5% (Table 3). It is considered that cumulative variance was lower than in previous research because the study materials were in the S2 stage.

The properties that contributed to the variation in the first three components were as follows: fruit attitude (0.328), longitudinal section (0.323), fruit length (0.369), the thickness of flesh (0.339), and the length of seed cavity (0.315) in the PC-1 axis; anthocyanin coloration in the internode (−0.326), plant length (−0.453), plant width (0.309), and plant index (−0.539) in the PC-2 axis; and the shape of apex (0.356), plant width (0.375), the thickness of stem (0.441), and fruit width (0.367) in the PC-3 axis (Table 3). Accordingly, it was determined that fruit characteristics were more effective in distinguishing pepper lines in the first component of PCA. In contrast, plant characteristics were more dominant in the second component, and plant characteristics, as well as specific fruit characteristics, were effective in the third component. Figure 2 presents the graphical representation of the score plot analyse to show the relationship between pepper lines.

At the end of the morphological characterization analysis performed in plants in the S2 stage, 10 autonomous PC axes with eigenvalues greater than 1.0 were detected, and it was determined that they represented 69,9% of the cumulative variation. There are numerous investigations in the literature that were carried out to determine the existing variation levels of populations in many *Capsicum* species. According to the principal component analysis performed in 47 pepper genotypes of the *C. chinense* species, Luitel et al. (2018) determined that the total variation in the first two axes was 89.42%. Taş (2020) reported that in a population of 75 *C. chinense* pepper genotypes, the first six component axes explained 70.99% of the total variation. Velázquez-Ventura et al. (2018) reported that 131 pepper genotypes from *C. annuum* and *C. frutescens* L. species had a variation of 65.2%. Principal component analysis was applied in another study on pepper. A total of 17 principal component axes were obtained. 75.82% of the total variance was accounted for by these axes (Başak 2019).

The study results indicated that the specified variation level was, in general, consistent with the literatures. Furthermore, this high variation level can be used in both pepper breeding programs and the mapping of resistance to diseases and pests, yield, and some quality criteria. In future breeding programs, these populations will be supported with molecular characterization research, and the results obtained will be used more effectively. Also, thanks to the cluster analysis performed based on the specified agronomic characteristics in both periods in order to

Table 2 Correlation coefficients among anthocyanin coloration in internode (ACI), plant grown habit (GH), leaf color (LC), leaf shape (LS), fruit attitude (FA), immature fruit colour (IFC), mature fruit colour (MFC), fruit longitudinal section shape (FLS), fruit cross-section shape (FCS), fruit tip shape (FTS), number of fruit loculus(NFL), fruit stalk tip (FST), plant length (PL), plant width (PW), plant index (PI), stem length (SL), stem diameter (SD), fruit length (FL), fruit width (FW), fruit flesh thickness (FFT), seed cavity length (SCL), fruit flesh hardness (FFH), soluble solid contents (SSC), pH of capia pepper lines

	PL	SD	ACI	GH	LC	LS	FA	IFC	MFC	PI	SL	FL	FW	FFT	SCL	FFH	SSC	pH	PW	FLS	FCS	FTS	NFL	FST
PL	1	**,481****	**,330****	,055	,144	−,072	−,073	,101	,136	**,720****	**,363****	,049	**,234****	,126	,088	,011	,005	,167*	,002	−,024	−,051	−,067	,078	,094
SD		1	,059	,017	,201*	−,140	,070	,080	,211*	,080	,078	,163*	**,217****	,180*	,196*	,068	−,112	−,015	**,363****	,107	−,161*	,103	,062	−,052
ACI			1	−,050	,077	−,014	,062	,075	−,070	**,352****	,144	,083	,051	,056	,070	,006	,059	,161*	−,207*	−,019	,078	−,060	,006	**,306****
GH				1	−,001	,005	**−,336****	**−,237****	,005	,076	−,204*	−,176*	−,094	**−,332****	−,146	−,072	−,007	,109	,033	−,211*	−,005	,017	,078	,135
LC					1	,168*	,033	**,374****	,126	,077	,150	,007	−,140	−,067	,004	,196*	,137	,000	−,001	,022	−,049	−,108	−,016	−,033
LS						1	,044	−,017	,036	,017	−,020	−,091	−,038	−,071	−,087	,057	,100	,003	−,124	−,002	,071	−,059	,069	−,063
FA							1	**,252****	,013	−,094	**,261****	**,427****	,063	**,321****	**,217****	−,003	−,027	−,026	,002	**,353****	,036	−,087	−,185*	−,108
IFC								1	,097	,047	,151*	,171*	,029	**,264****	,061	−,138	−,064	,022	,032	**,265****	,118	**−,241****	−,181*	−,128
MFC									1	,032	,185*	,051	−,010	−,052	,042	−,005	,085	−,077	,118	,015	−,019	,010	−,004	,095
PI										1	**,322****	,039	,184*	−,034	−,053	,123	,021	**,270****	**−,633****	,003	,000	−,147	,156*	,195*
SL											1	,102	,077	**,235****	,204*	−,053	,065	,078	−,080	,027	,005	−,098	−,081	−,173*
FL												1	,159*	**,262****	**,456****	−,003	**−,245****	−,108	,033	**,646****	−,087	−,201*	−,205*	−,052
FW													1	**,456****	,103	−,141	**−,239****	−,036	−,023	,023	,014	,182*	,159*	,093
FFT														1	**,289****	**−,282****	−,167*	,067	,145	,207*	,022	−,094	−,169*	−,122
SCL															1	−,030	−,063	−,103	,183*	**,319****	−,161*	,201*	−,191*	**−,274****
FFH																1	,141	,019	−,142	,080	−,117	,173*	**,260****	,139
SSC																	1	,122	−,085	**−,225****	−,074	−,015	,086	,025
pH																		1	−,176*	−,064	−,049	−,017	,053	,086
PW																			1	−,005	−,053	,100	−,146	−,209*
FLS																				1	−,101	**−,347****	−,053	−,112
FCS																					1	−,025	−,167*	**,220****
FTS																						1	**,214****	,192*
NFL																							1	**,294****
FST																								1

Correlation is significant at the 0.05 level(*), correlation is significant at the 0.01 level (**)

Table 3 Factor groups based on the principal component analysis of the characteristics examined in the study and the corresponding principal component axes

Eigenanalysis of the correlation matrix										
Eigen value	3,4653	2,7301	1,928	1,7714	1,6094	1,4845	1,3028	1,1015	1,0527	1,0001
Proportion	0,139	0,109	0,077	0,071	0,064	0,059	0,052	0,044	0,042	0,041
Cumulative	0,139	0,248	0,325	0,396	0,46	0,52	0,572	0,616	0,658	0,699
Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**
ACI	0,064	−0,326	−0,076	0,108	0,014	−0,073	−0,129	−0,373	0,224	−0,185
GH	–0,236	−0,048	0,125	−0,102	0,006	−0,377	−0,164	0,035	−0,101	0,397
LC	0,064	−0,123	−0,162	−0,448	−0,121	0,13	−0,262	0,216	0,276	−0,124
LS	−0,051	−0,02	−0,244	−0,065	−0,051	0,201	−0,009	0,484	−0,207	0,05
FA	0,328	0,057	−0,167	0,094	0,021	0,345	0,048	−0,186	−0,011	0,196
IFC	0,266	−0,033	−0,196	−0,084	−0,238	0,071	−0,309	0,207	0,311	0,009
MFC	0,054	−0,063	0,105	−0,300	−0,122	0,113	−0,242	−0,088	−0,522	−0,082
FLSS	0,323	0,073	−0,149	−0,039	0,428	−0,049	−0,15	0,087	0,004	0,147
FCSS	−0,029	−0,008	−0,141	0,298	−0,271	0,038	−0,459	−0,109	−0,123	−0,128
FTS	−0,183	0,004	0,356	0,088	0,003	0,392	0,053	−0,104	0,073	−0,127
NFL	−0,178	−0,194	0,169	−0,009	0,27	0,307	0,075	0,248	0,013	0,149
FST	−0,162	−0,243	0,066	0,220	0,149	0,186	−0,421	−0,28	0,01	0,048
RFP	−0,177	0,07	0,014	−0,146	0,206	−0,363	−0,085	0,079	0,121	−0,591
PL	0,141	−0,453	0,223	−0,143	−0,114	−0,162	0,003	0,005	−0,052	0,045
PW	0,091	0,309	0,375	−0,233	−0,209	0,018	−0,101	−0,145	0,127	0,133
PI	0,039	−0,539	−0,086	0,05	0,074	−0,172	0,066	0,11	−0,153	0,004
SL	0,233	−0,227	−0,068	−0,083	−0,259	0,077	0,277	−0,095	−0,295	−0,103
SD	0,185	−0,125	0,441	−0,307	−0,007	0,029	−0,124	0	0,127	0,114
FL	0,369	0,045	−0,03	0,024	0,377	−0,063	−0,137	−0,127	−0,097	0,064
FW	0,155	−0,138	0,367	0,319	0,035	0,079	0,018	0,347	−0,024	−0,222
FFT	0,339	0,013	0,152	0,247	−0,149	0,017	0,115	0,144	0,216	−0,218
SCL	0,315	0,078	0,068	−0,129	0,158	−0,158	0,199	−0,184	−0,108	−0,141
FFH	−0,105	−0,117	−0,067	−0,27	0,409	0,318	0,019	−0,08	0,119	−0,153
SSC	−0,139	−0,086	−0,201	−0,255	−0,153	0,167	0,277	−0,271	0,047	−0,139
pH	−0,04	−0,233	−0,086	0,053	−0,078	−0,116	0,224	−0,061	0,431	0,332

ACI anthocyanin coloration in internode, *GH* plant grown habit, *LC* leaf color, *LS* leaf shape, *FA* fruit attitude, *IFC* immature fruit color, *MFC* mature fruit color, *FLSS* fruit longitudinal section shape, *FCSS* fruit cross− section shape, *FTS* fruit tip shape, *NFL* number of fruit loculus, *FST* fruit stalk tip, *RFP* ripe fruit pungency, *PL* plant lenght, *PW* plant width, *PI* plant index, *SL* stem length, *SD* stem diameter, *FL* fruit length, *FW* fruit width, *FFT* fruit flesh thickness, *SCL* seed cavity length, *FFH* fruit flesh hardness, SSC soluble solid contents, pH

differentiate plant accessions and determine group accessions, it becomes more likely to determine heterotic effects that will come from parents in the expansion population and to obtain new superior genotypes.

Cluster analysis

Using the data obtained in characterisation research, cluster analysis has been used by several researchers to identify and differentiate accessions from each other and to group plant accessions based on their similarities (Tyagi et al. 2014; Dikshita and Sivarajb 2015; Olatunji and Afolayan, 2019). It was reported that if 25% or more of the total variation can be explained in the PCA by the first two or three principal component axes, the cluster analysis to be performed will be more reliable (Mohammadi and Prassana 2003). In the dendrogram constructed from the cluster analysis performed according to the correlation matrix, the lines were identified as 10 groups

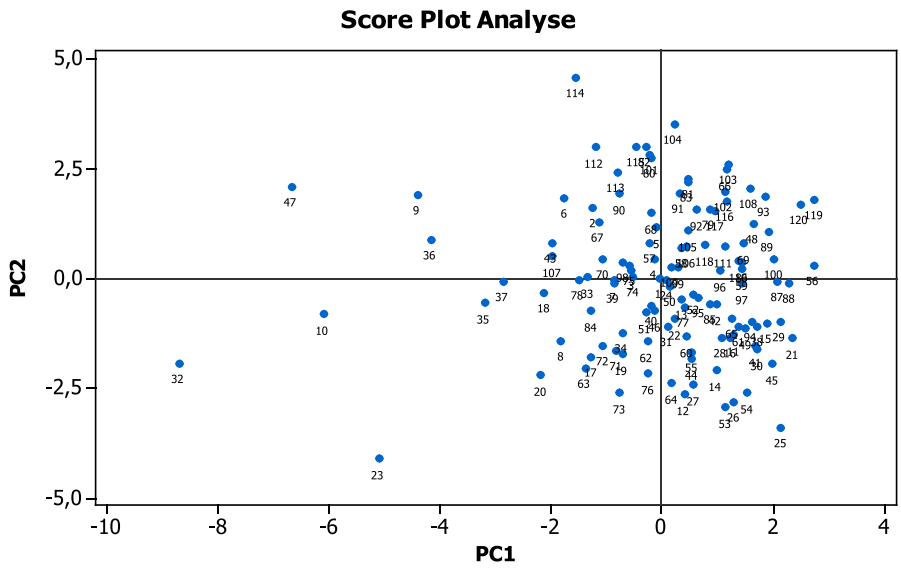

Fig. 2 Two-dimensional graphic obtained through the principal component analysis performed with morphological data

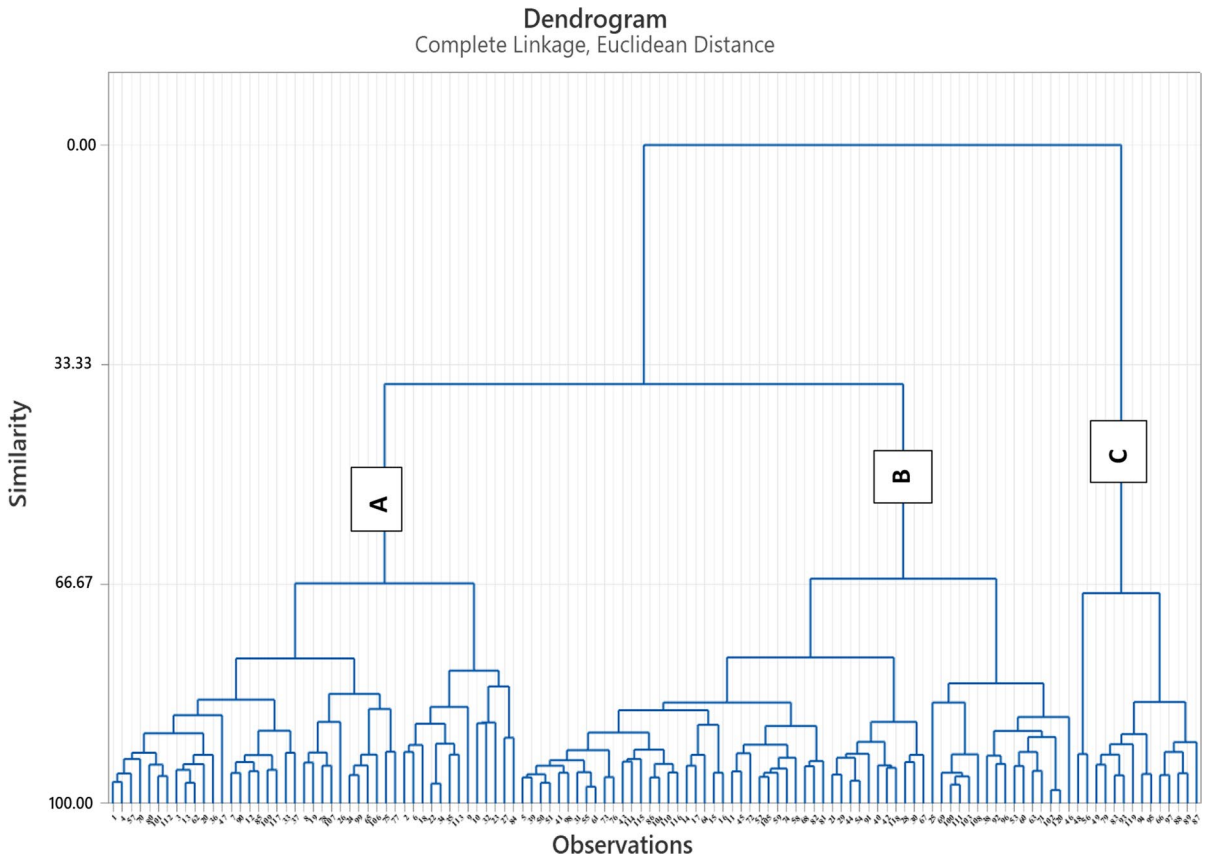

Fig. 3 Dendrogram obtained at the end of the cluster analysis performed with morphological data

(Fig. 3). However, the dendrogram consisted of three main groups. It was seen that the first and second main groups involved four subgroups each, and the third leading group consisted of two subgroups. The lines in the groups obtained in the dendrogram are presented in Table 4.

The common characteristics of capia pepper lines in three main groups are detailed below.

Group A: It is the group that has the biggest number of pepper lines (45 lines) after Group B among the groups that were obtained in the dendrogram. In this group, which consists of four subgroups, the longitudinal section of the fruit had a triangle/acute triangle shape, the taste of the fruit was sweet, and the color of the ripe fruit was primarily dark red. The mean plant height in the pepper lines in this group was measured as 121.5 cm, plant width as 37.05 cm, plant index as 3.39, stem height as 28.80 cm, and stem diameter as 14.10 mm. In the pomological measurements, the mean fruit height was 11.41 cm, fruit width was 4.33 cm, length of seed cavity was 29.53 mm, thickness of flesh was 3.80 mm, soluble solid contents was 8.83%, and pH was 4.84.

Group B: It was the group in which the biggest number of genetic materials clustered, with 61 lines among the groups that were obtained at the end of cluster analysis (Table 4). In this group, which consists of four subgroups, plant attitude was found as upright/semi-upright, the color of ripe fruits mainly was dark red, the shape of the apex was pointy/very pointy, and there was no or slight stalk cavity. The mean plant height of the lines in this group was measured as 140.85 cm, the plant width as 38.85 cm, the plant index as 3.85, the stem height as 31.67 cm, and the stem diameter as 15.29 mm. In pomological measurements, on the other hand, fruit height was 13.48 cm, fruit width was 4.32 cm, the length of seed cavity was 48.50 mm, thickness of flesh was 4.32 mm, soluble solid contents was 8.20%, and pH was 4.93.

Group C: It was the group in which the least genetic material clustered, with 14 lines among the groups that were obtained at the end of cluster analysis (Table 4). In this group, which consists of two subgroups, plant attitude was mostly upright, leaf color was dark green, leaf shape was oval, fruit attitude was drooping, the color of ripe fruit was dark red, shape of the apex was pointy, there were two lobs in fruit, the shape of the cross-section was oval, there was no stalk cavity, and the taste of the fruit was sweet. The mean plant height of the lines in the group was detected as 122.15 cm, the plant width as 40.71 cm, the plant index as 2.89, the stem height as 32.72 cm, and the stem diameter as 14.13 mm. In pomological measurements, fruit height was 13.58 cm, fruit width was 4.27 cm, the length of seed cavity was 68.96 mm, thickness of flesh was 4.42 mm, soluble solid contents was 8.24%, and pH was 4.55.

According to the cluster analysis results, it was observed that lines 55–61 and 102–120 in Group B were remarkably similar, while lines 120–15 and 55–25 were the most distant lines to one another (Fig. 3). These results will help eliminate genotypes highly similar to one another and save labor force and time in breeding programs. Furthermore, the most distant genotypes will make it possible to achieve a high heterosis rate due to hybridization programs between these lines. The cluster analysis performed in the study to assess the variability and relatedness among four cultivars of Capsicum species in West

Table 4 The groups of capia pepper lines obtained through the cluster analysis

Group	Lines	Total lines (number)
A	1. subgroup (1, 4, 57, 70, 80, 101, 112, 3, 13, 62, 20, 36, 47, 7, 90, 12, 85, 109, 117, 33, 37), 2. subgroup (8, 19, 78, 107, 26, 24, 99, 65, 106, 75, 77), 3. subgroup (2, 6, 18, 22, 34, 35, 113, 9), 4. subgroup (10, 32, 23, 27, 84)	45
B	1. subgroup (5, 39, 50, 51, 41, 98, 31, 55, 61, 73, 76, 43, 114, 115, 86, 104, 110, 116, 14, 17, 64, 15, 16, 11, 45, 72, 52, 105, 59, 74, 58, 68, 82, 81), 2. subgroup (21, 29, 44, 54, 91, 40, 42, 118, 28, 30, 67), 3. subgroup (25, 69, 100, 111, 103, 108), 4. subgroup (38, 92, 96, 53, 60, 63, 71, 102, 120, 46)	61
C	1. subgroup (48, 56), 2. subgroup (49, 79, 83, 93, 119, 94, 95, 66, 97, 88, 89, 87)	14

Africa showed two distinct clusters, with cultivars with close phenotypic similarities grouped in different clusters (Olatunji and Afolayan 2019). Similarly, in a study carried out in Turkey, fifteen different groups were created based on the similarity dendrogram between pepper groups (Başak 2019). These results made it possible to evaluate the variation observed between pepper genotypes in breeding research. This has been reported by many researchers (Ince et al. 2009; Saleh et al. 2016; Başak 2019; Çetin 2023).

Screening for disease resistance

TMV and TSWV viral infections cause substantial damage to yield and quality in *Solanaceae* species. Disease screening was performed using the dominant 189D23M SCAR marker determined by Tomita et al. (2008) for the *L3* allele, which is linked with resistance to TMV, the dominant 060I2END marker determined by Yang et al. (2009) for the *L4* allele, the co-dominant marker 087H3T7CAPS used for analysis. 087H3T7HRM analysis and 087H3T7 CAPS markers same resistance was observed and results were compared with the dominant 060I2END marker for the *L4* locus. When the capillary electrophoresis electropherogram was examined, it was observed that it was possible to distinguish homozygote-resistant alleles from heterozygote alleles through the size of the fields of allele integrals with a high amplification power. In samples with similar initial DNA concentrations, genotypes that display a high amplification effective in the dominant marker for the *L4* locus highly overlap (91.6%) with homozygote-resistant individuals for the 087H3T7 CAPS marker analyses. In addition, the 087H3T7caps marker can be used effectively for codomaninat selection in screening for this disease. TMV resistance was obtained by evaluating and scoring the dominant/co-dominant markers of *L3* and *L4* loci. According to the test results of pepper plants for resistance to this virus, 43 lines were resistant (AA/Aa), and 77 lines were susceptible (aa) for the *L3* allele. In comparison, 14 lines were homozygote-resistant (AA), 28 lines were heterozygote-resistant (Aa), and 78 lines were susceptible (aa) for the *L4* allele (Table 5). These findings supports the idea that *L3* and *L4* may be different genes closely linked within the region instead of different alleles at the same locus. Also, the markers we used in our study to determine TMV-resistant lines were successfully used in numerous investigations on resistance to viral diseases in Turkey (Şimşek et al. 2015; Göksu 2016; Bozkuş, 2018). These markers also worked effectively in our study and showed parallels with the previous research above.

The screening of TSWV was performed with SCAC568, a co-dominant marker for the *Tsw* allele. According to the results, 30 lines were detected to be resistant (13 homozygote-resistant lines and 17 heterozygote-resistant lines), and 90 lines were not resistant (Table 5). The use of mechanical inoculation of plants, one of the early selection tests to screen resistance among stages in pepper breeding, has provided significant benefits (Boiteux 1995). However, the selection of TSWV resistance through mechanical inoculation is prevented due to various disadvantages of this method. Inoculation tests require storing and multiplying a TSWV inoculation and controlling inoculated plants in restricted areas to prevent a viral spread (Moury et al. 1997). At the same time, high temperatures may destabilize the resistance provided by *Tsw* (Moury et al. 1998). Therefore, using molecular indicators related to the *Tsw* locus is beneficial in obtaining more reliable results (Moury et al. 2000). In addition, obtaining results in a shorter time compared to inoculation tests is another advantage of this method. The co-dominant CAPS marker (SCAC 568+XbaI) used in this study worked successfully in 120 lines. As a result of our study, 13 lines that developed homozygote resistance to the *Tsw* gene will be included in breeding programs in the future to develop hybrids. The marker used in our study to determine TSWV-resistant lines was successfully used in many other investigations about resistance to viral diseases in Turkey (Özkaynak et al. 2014; Çelik et al. 2018; Okay 2019). Moreover, this CAPS marker has been frequently used in our country in various pepper breeding programs, and TSWV-resistant long green pepper and sweet pepper types have been bred (Polat et al. 2012; Özkaynak et al. 2014). Şimşek et al. (2015) produced TSWV-resistant bell pepper types using the SCAC568 marker, and Çelik et al. (2018), on the other hand, developed TSWV-resistant long green pepper types. This marker worked

Table 5 Disease testing results

Lines name	TSWV (*Tsw*)	TMV (*L4*)	TMV (*L3*)	Lines name	TSWV (*Tsw*)	TMV (*L4*)	TMV (*L3*)	Lines name	TSWV (*Tsw*)	TMV (*L4*)	TMV (*L3*)
1	rr	RR	+	36	RR	RR	+	73	rr	Rr	−
2	rr	Rr	−	37	rr	rr	−	74	rr	rr	−
3	rr	RR	+	38	rr	rr	−	75	rr	rr	−
4	rr	rr	−	39	rr	rr	−	76	rr	rr	−
5	rr	Rr	+	40	rr	rr	−	77	rr	rr	−
6	rr	rr	−	41	rr	rr	−	78	rr	rr	−
7	rr	Rr	+	42	rr	rr	−	79	rr	Rr	−
8	rr	Rr	+	43	rr	rr	−	80	rr	rr	−
9	rr	rr	−	44	rr	rr	−	81	rr	rr	−
10	rr	rr	−	45	rr	RR	+	82	rr	rr	−
11	Rr	Rr	+	46	RR	RR	+	83	Rr	rr	−
12	Rr	Rr	+	47	RR	RR	+	84	RR	RR	+
13	Rr	Rr	+	48	RR	RR	+	85	RR	RR	+
14	rr	rr	−	49	rr	RR	+	86	Rr	Rr	+
15	rr	rr	−	50	rr	rr	−	87	rr	rr	−
16	rr	rr	−	51	rr	Rr	+	88	rr	rr	−
17	rr	rr	−	52	rr	rr	−	89	rr	rr	−
18	rr	rr	−	53	rr	rr	−	90	rr	rr	−
19	rr	rr	−	54	rr	rr	−	91	rr	rr	−
20	rr	rr	−	55	rr	rr	−	92	rr	rr	−
21	rr	Rr	+	56	rr	rr	−	93	RR	Rr	+
22	rr	rr	−	57	rr	rr	−	94	Rr	Rr	+
23	Rr	Rr	+	58	RR	rr	−	95	Rr	Rr	+
24	Rr	Rr	+	59	RR	Rr	+	96	rr	Rr	+
25	Rr	rr	+	60	rr	Rr	+	97	rr	Rr	+
26	rr	rr	−	61	rr	rr	−	98	rr	rr	−
27	rr	rr	−	62	rr	rr	−	99	rr	rr	−
28	rr	rr	−	63	Rr	Rr	+	100	rr	rr	−
29	rr	rr	−	66	Rr	Rr	+	101	rr	rr	−
30	rr	rr	−	67	Rr	Rr	+	102	rr	rr	−
31	rr	rr	−	68	Rr	Rr	+	103	rr	rr	−
32	rr	rr	−	69	Rr	Rr	+	104	rr	rr	−
33	RR	Rr	+	70	rr	rr	−	105	rr	rr	−

Table 5 (continued)

Lines name	TSWV (*Tsw*)	TMV (*L4*)	TMV (*L3*)	Lines name	TSWV (*Tsw*)	TMV (*L4*)	TMV (*L3*)	Lines name	TSWV (*Tsw*)	TMV (*L4*)	TMV (*L3*)
34	RR	RR	+	71	rr	rr	–	106	rr	rr	–
35	RR	RR	+	72	rr	rr	–	107	rr	rr	–
108	rr	rr	–	113	rr	Rr	+	118	rr	rr	–
109	rr	rr	–	114	rr	rr	–	119	rr	rr	–
110	RR	Rr	+	115	rr	rr	–	120	rr	RR	+
111	Rr	Rr	+	116	rr	rr	–	121	rr	RR	+
112	Rr	Rr	+	117	rr	rr	–				

RR homozygous resistant, *Rr* heterozygous resistant, *rr* susceptible to disease, + resistant (homozygous and heterozygous), – susceptible to disease

effectively in our study and helped determine resistant lines in line with the above mentioned research.

Conclusion

Turkey is one of the significant pepper-growing countries of the world, and pepper cultivation is possible in all its regions. A lot of different populations emerged in places where pepper is cultivated due to both natural selection and selections performed by breeders. These populations have significant genetic potential in terms of breeding. Identifying these populations is vital to reveal genetic diversity and provide materials with well-known characteristics for future breeding programs. TMV and TSWV viruses are an important problem limiting production in pepper growing areas. These virus diseases are one of the most difficult pathogen groups to control and one of the most effective strategies to control them is the use of pepper cultivars resistant to these two diseases. The aim of this study was to collect genetic material with different traits from capia peppers for fresh and industrial use, to study their characteristics, to identify promising genotypes and to include them in breeding programs by testing resistance to two viral diseases (TMV, TSWV) that severely damage the yield and quality of *Solanaceae* species. Furthermore, this study provided an overview of the status and extent of the existing morphological variation of local capia pepper populations. By providing detailed information on the morphological variability of the local pepper genotypes studied, it constitutes a starting point for future research on capia pepper breeding. In the study, PCA was applied to determine the relationship between genotypes. In principal component analysis (PCA), the qualitative (anthocyanin colouration in the internode, fruit set, fruit cross-sectional shape, fruit apex shape) and quantitative (plant height, plant width, plant index, fruit length, fruit width, fruit flesh thickness, seed cavity length, stem diameter) characters which were effective in the first three components explained 32.5% of the variation. According to PCA, 10 PC axes explained 69.9% of the total variation. In addition, 3 main groups were determined in the cluster analysis. In order to be used in various breeding research, if the important criteria are yield, long fruit, high soluble solid content value and high fruit flesh thickness of capia type peppers, in

the 2nd and 3rd groups in the dendogram showing the similarity coefficients between pepper samples can be selected. Again, if the criterion is plant habitat, these groups should be evaluated. In addition, if the populations that show great affinity with the cultivars we use as standard are taken into breeding programmes and evaluated, new cultivars suitable for the market can be provided. In this direction, morphological identification research will bring important advantages in determining the characteristics of half-way materials to be used in gene accessions. This is because agronomic traits are necessary for classical breeding programmes. In addition, 48 plant lines belonging to lines 34, 35, 36, 46, 47, 48, 84 and 85, which were screened for resistance to TSWV and TMV with the help of molecular markers in 120 pepper lines of the S2 stage, were determined to be homozygous resistant to *L4* allele and *Tsw* gene. In addition, in order to be used in various breeding investigations, if the important criterion is disease resistance, the populations in the 1st and 3rd groups in the dendogram where the lines resistant to these two diseases are found can be selected. In addition, the half-way materials whose resistance levels to these two diseases are determined are very important for future breeding research to develop disease resistant cultivars.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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