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Genetic variability in peas (*Pisum sativum* L.) from Turkey asssessed with molecular and morphological markers

Fatih Hanci

Erciyes University
Kayseri, Turkey

ABSTRACT

The aim of this study was to identify the molecular and morphological characteristics of Turkish pea accessions (*Pisum sativum* L.). The genetic diversity among 130 Turkish landraces and 2 commercial varieties in a total of 132 pea accessions was assessed with 14 simple sequence repeat (SSR) markers. Forty-eight (48) polymorphic alleles were identified using 14 SSR markers. The pairwise Dice coefficients of similarity between accessions ranged from 0.091 to 0.960. The polymorphism information content (PIC) value ranged from 0.585 to 0.861. Overall, 50 morphological traits were evaluated. Cluster analysis was carried out on a matrix of Euclidean distances. The accessions were divided into three main groups. Principal component analysis (PCA) was used to identify the weight of each morphological characteristic. According to the results, the highest eigenvalue was observed in PC-I (13.88) followed by PC-II (11.42), and PC-III (7.32). The first fifteen PCs with eigenvalues > 1 explained 74.08% of the variability. The results showed that the molecular markers were useful and polymorphic, sufficient to allocate all the evaluated accessions. This research has provided significant insights into the genetic variability of Turkish pea accessions.

Key words: breeding, cluster analysis, diversity, pea, polymorphism

INTRODUCTION

Having more than 650 genera and 18,000 species, the legumes are the third uppermost family of flowering plants (Lewis et al., 2005). Globally, the pea (*Pisum sativum* L.) is the second most important pulse crop after the common bean (*Phaseolus vulgaris* L.) in terms of grain yield and sixth in terms of cultivation area (Kumari et al., 2013).

It is assumed that the "Fertile Crescent" through Turkey, Iraq, Lebanon, Israel, and Syria is the centre of pea genetic diversification (Smýkal et al., 2013). Morphological characteristics and agronomical traits have been used by several studies conducted on the genetic diversity in the genus *Pisum* (Yirga et al., 2013; Gixhari et al., 2014; Ouafi et al., 2016). A few techniques are effective for investigating morphological variation in a genetic resource. The principal component analysis (PCA), as a multivariate statistical technique, can convert numerous contingent correlated factors into a few factors that are termed principal components (Ziegel, 2006).

Morphological markers are influenced by environmental factors to a greater extent in comparison with biochemical and molecular markers. Molecular markers serve as a tool to overcome the deficiencies of morphological markers (Rao, 2004), because molecular markers are not influenced by environmental factors (Tatikonda et al., 2009). For high polymorphisms, co-dominance,

Corresponding author.
e-mail: tanerfatih@gmail.com (F. Hanci).

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and locus specificity widely distributed throughout the genome, simple sequence repeat (SSR) markers have increasingly become the favourite marker set for genetic work (Cuevas and Prom, 2013; Izzah et al., 2013). As with many other species, these markers have previously been preferred to define genetic variation of pea accessions (Tar'an et al., 2005; Nasiri et al., 2009; Nisar et al., 2017).

Despite their high economic value, an important part of Turkish pea accessions has not been described genetically. The aim of this research was to characterize the genetic variation of this germplasm by means of morphological and molecular markers, and to identify/examine the extensive implications of the research for prospective breeding and gene-bank conservation programmes.

MATERIAL AND METHODS

Plant materials

The experiments were carried out at the Atatürk Central Horticultural Research Institute, Yalova, Turkey. The 130 pea accessions, which originated from different regions of Turkey, had been kindly obtained from Plant Gene Banks [Western Regional Plant Introduction Station, USDA, Pullman, (USA); John Innes Centre (UK); Gene Bank of the Aegean Agricultural Research Institute (TR)]. The cultivars Kaysee and Serge were used as the control. The seeds were planted in the field at the end of November 2015 and November 2016. The morphological data were collected from two one-year experiments. Seeds of each accession were sowed in 1.0 × 1.6 m plots. At least sixty plants were grown in each plot in a Randomized Complete Block Design with three replications. Routine maintenance procedures such as irrigation, weeding, disease and pest control were performed throughout the growing season.

Molecular characterization

Total genomic DNA was isolated according to Hanci and Gökçe (2016a). For the DNA isolation studies, parts of fresh young leaves were collected from 20-day-old pea (*P. sativum* L.) seedlings. For the extraction, a bulk sample was prepared from six plants for each accession. A Macherey-Nagel NucleoSpin® Plant II kit (Macherey-Nagel GmbH and Co. KG., Düren, Germany) was used for isolation. The steps of the work were carried out according to the manufacturer's instructions.

The SSR assay was carried out using fourteen primers (Tab. 1). The high-quality SSR markers with a relatively high polymorphic information content were selected based on the data provided by previous studies (Loridon et al., 2005). The PCR reaction volume was 25 µL, consisting of 0.6 mM reverse and forward primers, 200 µM deoxyribonucleotide triphosphates, 20-25 ng genomic DNA, 1X Taq buffer, 2 mM $MgCl_2$, 1 U Taq-DNA polymerase (Fermentas, Pittsburgh, PA, USA) (Kumari et al., 2013). A typical PCR procedure was as follows: initial denaturation for 3 min. at 94°C, followed by 40 cycles of 94°C, 51°C or 61°C for 30 s, 1 min. at 72°C, and the final extension for 10 min. at 72°C before cooling at 4°C. The amplified fragments were separated by electrophoresis on a 3% agarose gel containing ethidium bromide.

For data analysis, the amplified bands generated by SSR-PCR amplification were scored based on the presence (1) or absence (0) of bands for each primer (Nisar et al., 2017). Cluster analysis was performed on the molecular data using the un-weighted pair group method based on arithmetic means (UPGMA) algorithm. The information of each pair primer was deduced using the polymorphic information content (PIC) as described by Hildebrand et al. (1992):

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2\, p_j^2$$

where p_i and p_j are the population frequency of the ith and jth allele. The similarity matrix was formed using Dice's coefficient. XLSTAT software (Garcia-Vallve et al., 1999) was used for generating the similarity matrices and UPGMA clustering.

Morphological characterization

Data on different agronomic characteristics from fifteen individuals randomly chosen from each plot were analysed according to the guide of the International Union for the Protection of New Varieties of Plants (UPOV, 2009) (Tab. 2). The cluster analysis was applied using UPGMA. The Ward method was used to establish a dendrogram from the Euclidean distances of each accession (Gixhari et al., 2014). PCA was used to identify the weight of each characteristic. Numerical scores of 50 traits for the accessions were transformed to standardize the units for PCA. The number of principal components was determined using the minimum eigenvalue (Hanci and Gökçe, 2016b). All the statistical procedures for morphological traits were performed using the SAS Institute Inc. JMP® and IBM SPSS® Statistics Ver. 221.

Table 1. Details of the SSR primers (Loridon et al., 2005) and observeded results

Marker	Sequence (5′–3′)	Tm* (°C)	Linkage group	Band size (bp)	Number of bands	PIC
AA122	F:GGGTCTGCATAAGTAGAAGCCA R:AAGGTGTTTCCCCTAGACATCA	61	IV	175-225	4	0.820
AA205	F:TACGCAATCATAGAGTTTGGAA R:AATCAAGTCAATGAAACAAGCA	51	II	175-225	2	0.585
AA446	F:TTAGCTTGCAGCCCACTC R:ATCCGACCCATGGATTTA	51	VII	650-900	5	0.859
AA5	F:TGCCAATCCTGAGGTATTAACACC R:CATTTTTGCAGTTGCAATTTCGT	61	III	225-250	3	0.755
AB141	F:ATCCCAATACTCCCACCAATGTT R:AGACTTAGGCTTCCCTTCTACGACTT	61	III	175-225	3	0.752
AB23	F:TCAGCCTTTATCCTCCGAACTA R:GAACCCTTGTGCAGAAGCATTA	61	V	200-225	3	0.777
AC58	F:TCCGCAATTTGGTAACACTG R:CGTCCATTTCTTTTATGCTGAG	61	V	200-225	3	0.781
AD146	F:TGCTCAAGTCAATATATGAAGA R:CAAGCAAATAGTTGTTTTGTTA	51	VII	375-425	6	0.861
AD147	F:AGCCCAAGTTTCTTCTGAATCC R:AAATTCGCAGAGCGTTTGTTAC	61	I	300-325	3	0.786
AA67	F:CCCATGTGAAATTCTCTTGAAGA R:GCATTTCACTTGATGAAATTTCG	51	I	330-390	4	0.779
AB72	F:ATCTCATGTTCAACTTGCAACCTTTA R:TTCAAAACACGCAAGTTTTCTGA	55	II	450-500	4	0.799
AA175	F:TTGAAGGAACACAATCAGCGAC R:TGCGCACCAAACTACCATAATC	61	III	225-250	3	0.740
AA285	F:TCGCCTAATCTAGATGAGAATA R:CTTAACATTTTAGGTCTTGGAG	51	IV	250-275	3	0.609
AB64	F:GCATTCATTGCGGTTGCATTAT R:GAGTGACAGGTGCCACATTGA	61	III	350-400	2	0.609

*Tm: melting temperature

RESULTS AND DISCUSSION

Molecular characterization

The 14 SSR markers used in this study yielded reproducible polymorphic bands in all of the 132 pea accessions. The primers employed revealed a total of 48 polymorphic alleles. The size of the alleles ranged from 175 bp to 900 bp. The number of polymorphic alleles ranged from two to six. The highest number of polymorphic alleles was obtained with primer AD146. The primers AA205 and AB64 generated only two polymorphic alleles. The mean number of alleles per locus was 3.43. In general, the number of alleles revealed by the SSR markers was similar to those in previous reports. Loridon et al. (2005) had reported an average of 3.8 alleles per locus using 309 SSR markers in pea accessions. The PIC values ranged from 0.585 to 0.861 (Tab. 1). The improvements in molecular techniques have enabled us to observe genetic narrowing at the allelic grade. The abundance of allelic variation is of significance with regard to both evolutionary and breeding aspects (van de Wouw et al., 2010). Nisar et al. (2017) reported that the newly developed Pakistani pea lines showed an average of 4.69 alleles per SSR locus. Similarly, an average number of 4.5 alleles per locus was reported in European pea accessions (Cupic et al., 2009). Ahmad et al. (2012), evaluating 35 pea accessions from various sources with 15 SSR loci, found 41 alleles (bands) with an average of 2.73 alleles, i.e. less than the value reported in this study. Similarly, the number of alleles per locus averaged 2.1 in the study by Kumari et al. (2013).

Higher PIC values were calculated in the present study compared with the results of other researchers.

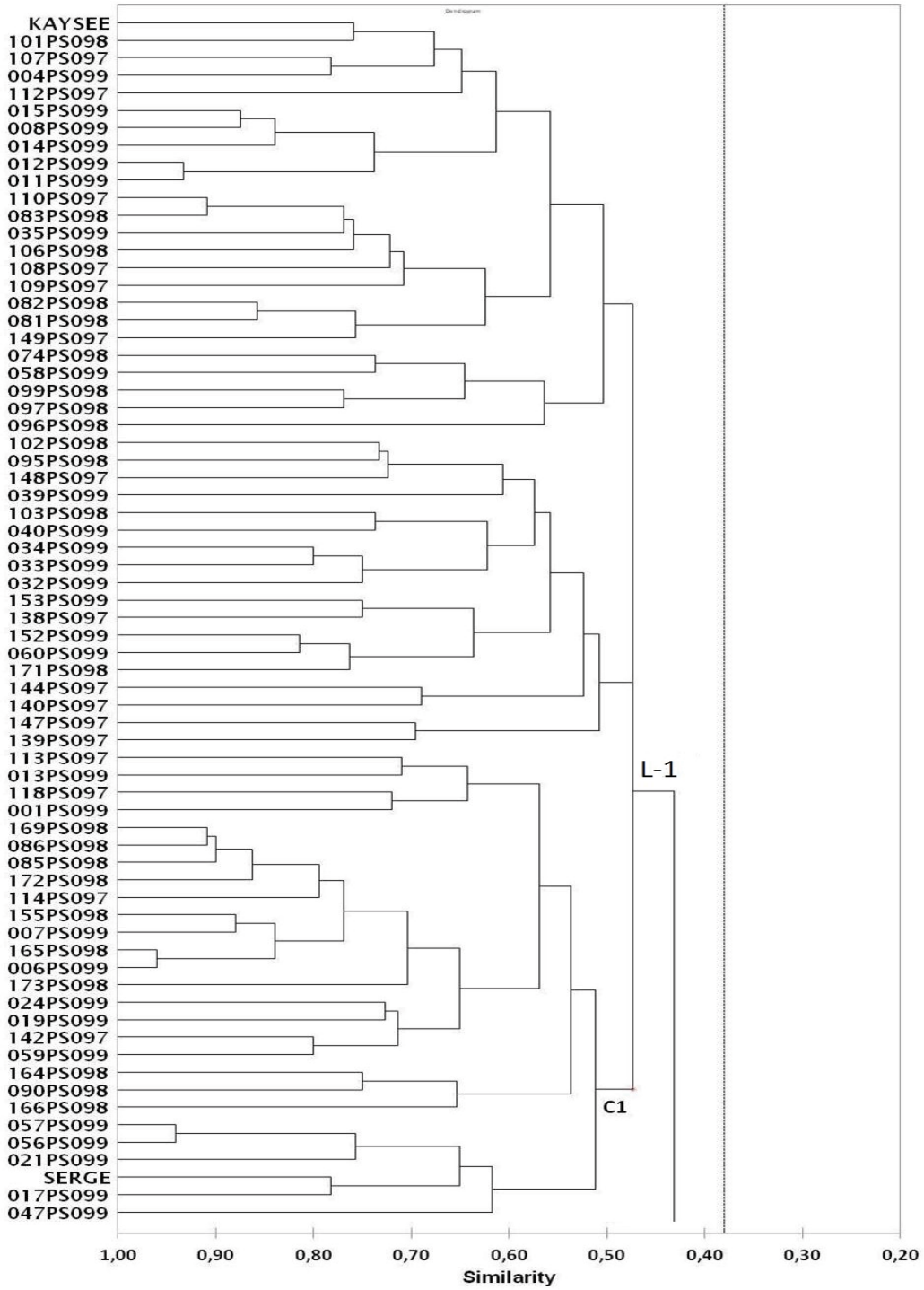

Figure 1A. Dendrogram of pea accessions based on SSR primers. Scale at bottom is Dice's coefficient of similarity (Part I, Linkage-1, Cluster-1)

Ahmad et al. (2012) had obtained much lower PIC values, ranging from 0.055 to 0.660, when assessing pea accessions with 15 SSR loci. Nisar et al. (2017) calculated the maximum PIC value of 0.630 in 23 pea accessions, while Kumari et al. (2013) obtained the maximum PIC value of 0.657 in 28 accessions. In this study, the high polymorphism rate (average PIC, 0.751; maximum PIC, 0.861) stemmed from the efficiency of the selected SSR primers. The 132 accessions were classified into three linkage groups at genetic distances of 37% in the cluster analysis (Figs 1A and 1B). Eight clusters were additionally classified into these main groups.

Morphological characterization

Descriptive statistics for traits demonstrated a substantial variability in the accessions under investigation (Tab. 2). Standard deviations were

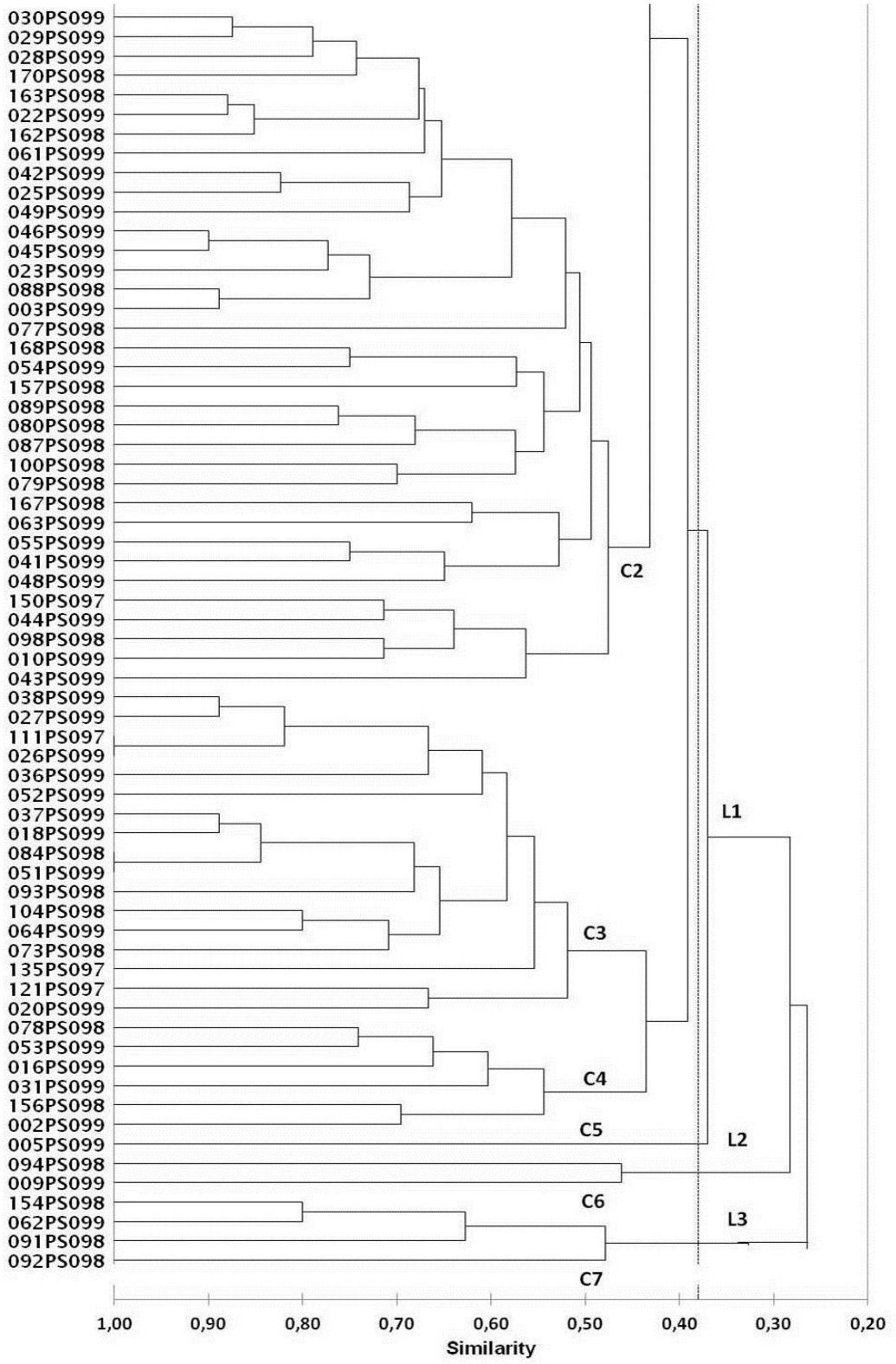

Figure 1B. Dendrogram of pea accessions based on SSR primers. Scale at bottom is Dice's coefficient of similarity (Part II, Linkage-1Cluster 2; Linkage-2 and Linkage-3)

observed at the levels of 19.52 and 7.03, which were relatively high, for the length of the plant (LS17) and the time of flowering (F1), respectively. Cluster analysis performed on the matrix of Euclidean distances generated a dendrogram using the Ward method based on the variations associated with fifty quantitative and qualitative characteristics (Figs 2A and 2B). The average dissimilarity index for all the investigated accessions was 9.74. Two main groups were obtained (L1-2) in the cluster analysis.

Table 2. Morphological traits related to leaf-stem (LS), flower (F), and seed-pod (SP) characteristics, with standard errors and standard deviations (UPOV, 2009)

Trait	SE	SD	Trait	SE	SD	Trait	SE	SD
Length of leaflet (LS1)	0.10	1.17	Intensity of colour of foliage (LS19)	0.08	0.95	Shape of seed (SP1)	0.09	1.00
Width of leaflet (LS2)	0.09	1.07	Stem length (LS20)	0.12	1.36	Colour of cotyledon of seed (SP2)	0.07	0.82
Size of leaflet (LS3)	0.08	0.89	Number of nodes up to first fertile node (LS21)	0.10	1.18	Marbling of testa (SP3)	0.08	0.93
Length of stipule (LS4)	0.10	1.16	Length from axil to first leaflet of tender (LS22)	0.10	1.18	Violet or pink spots on testa (SP4)	0.06	0.65
Width of stipule (LS5)	0.10	1.11	Time of flowering (F1)	0.61	7.03	Hilum colour on seed (SP5)	0.18	2.11
Colour of leaflet (LS6)	0.06	0.71	Maximum number of flowers per node (F2)	0.14	1.63	Colour of testa (SP6)	0.05	0.55
Intensity of colour of leaflet (LS7)	0.08	0.89	Colour of wing (F3)	0.13	1.48	Wrinkling of seed cotyledon (SP7)	0.23	2.64
Leaflets (absent or present) (LS8)	0.04	0.43	Intensity of colour of wings (F4)	0.27	3.14	Type of starch grains (SP8)	0.03	0.33
Waxiness of upper leaflet (LS9)	0.05	0.56	Intensity of colour of standard (F5)	0.22	2.47	Width of seed (SP9)	0.13	1.43
Dentation of leaflet (LS10)	0.04	0.50	Colour of standard (F6)	0.09	0.98	Curvature on pod (SP10)	0.04	0.47
Degree of dentation of leaflet (LS11)	0.12	1.35	Width of standard (F7)	0.12	1.43	Type of curvature of pod (SP11)	0.08	0.88
Size of stipule (LS12)	0.02	0.19	Shape of base of standard (F8)	0.08	0.95	Shape of distal part of pod (SP12)	0.03	0.39
Shape of stipule (LS13)	0.11	1.21	Undulation of standard (F9)	0.08	0.94	Colour of pod (SP13)	0.03	0.36
Flecking of stipule (LS14)	0.10	1.10	Width of upper sepal (F10)	0.08	0.88	Intensity of green colour of pod (SP14)	0.11	1.22
Density of flecking of stipule (LS15)	0.09	0.99	Shape of apex of upper sepal (F11)	0.04	0.47	Anthocyanin coloration of parchment (SP15)	0.06	0.71
Anthocyanin coloration of stem (LS16)	0.09	1.01	Length of peduncle (from first flower) (F12)	0.12	1.40	Anthocyanin coloration of pod (SP16)	0.03	0.31
Length of plant (LS17)	1.70	19.52						
Fasciation of stem (LS18)	0.06	0.69						

SE: Standard error, SD: Standard deviation

Group-I (L1) consisted of two clusters (C1-2). A relationship was observed between the accessions in these clusters based on days to flower initiation. The first cluster (C1) consisted of 46 accessions and, in general, late-flowering accessions clustered in this group (avg. 63 days). The second cluster consisted of 31 accessions, and the most prominent feature of this cluster was that it included the earliest flowering accessions (avg. 45 days). The commercial cultivars Serge and Kaysee were in cluster C2. The second group (L2) consisted of two clusters. The third cluster (C-3) consisted of only seven accessions. These accessions showed radically different morphological features compared to the others. Finally, the fourth cluster (C4) had 48 accessions.

The first fifteen principal components (PCs) with eigenvalues greater than one accounted for 74.08% of total variability amongst the 132 pea accessions. The proportion of total variation explained by these principal components of more than 2/3 was used for the next step (Hanci and Gökçe, 2016b). The percentages of cumulative variation explained by each of the seven PCs were 13.88%, 25.29%, 32.61%, 38.05%, 43.21%, 47.65% and 51.545, respectively (Tab. 3). In each principal component, a coefficient equal to or greater than 0.3 was determined as the threshold to define the cut-off limit for the coefficients of the accurate vectors (Raji, 2002). The first principal component (PC1) had a high positive value for the colour of the wings (F3), the intensity of the colour of the wings (F4), and the intensity of the colour of the standard banner (F5). PC1 had a negative value for the colour of the standard (F6). The second principal component had a high positive value for the width

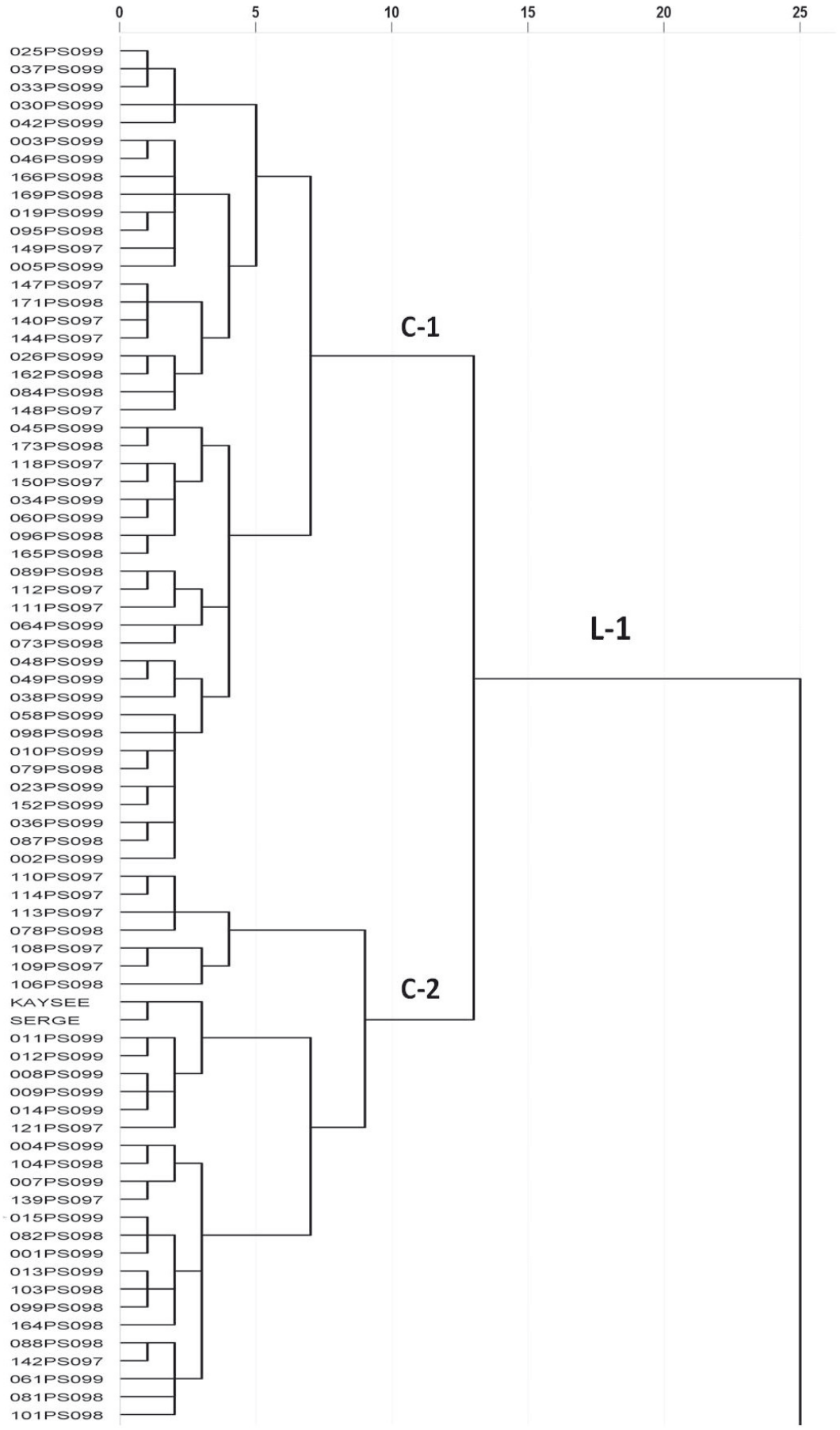

Figure 2A. Dendrogram of pea accessions constructed using UPGMA based on morphological data (Part-I, Linkage-1)

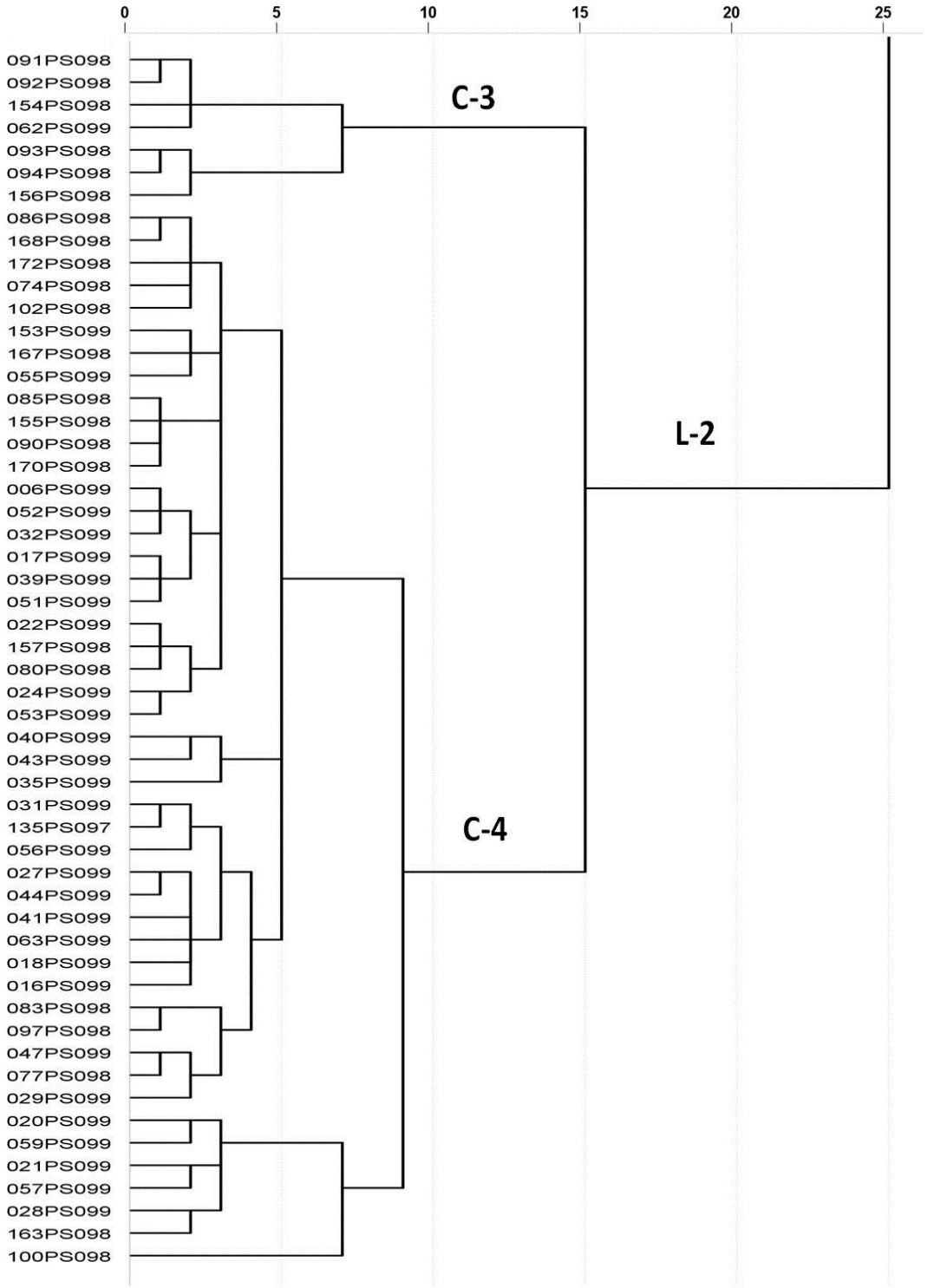

Figure 2B. Dendrogram of pea accessions constructed using UPGMA based on morphological data (Part-II, Linkage-2)

of the leaflet (LS2), the size of the leaflet (LS3), and the width of the stipule (LS5). Having such high positive or negative component values, these traits reveal high genetic diversity. In the study by Smýkal et al. (2008), the PCA of the morphological traits disclosed that 82% of the total variation was explained by 3 principal components comprising 48.8%, 27.0%, and 6.0%, respectively. In the Albanian pea germplasm, 86.91% of the variation was explained by the first three PCs (Gixhari et al., 2014). In the same study, the total contribution of quantitative traits included in PC1 accounted for 58.1% of PC1 variance.

Dice's similarity coefficient varied in the range from 0.091 to 0.960, with an average of 0.439, showing the genetic distance between Turkish

Table 3. Eigenvectors of the first seven principal components

Trait*	Eigenvectors							Trait	Eigenvectors						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7		PC1	PC2	PC3	PC4	PC5	PC6	PC7
LS1	-0.01	0.28	0.14	0.03	0.03	0.12	0.07	SP4	-0.03	0.07	-0.13	0.05	-0.08	0.27	0.10
LS2	-0.05	0.33	0.12	0.13	0.12	0.08	0.07	SP5	0.22	0.01	0.02	0.05	0.10	-0.08	0.13
LS3	-0.09	0.31	0.12	0.08	0.11	0.08	0.06	SP6	-0.22	-0.02	-0.10	0.08	0.03	0.06	-0.01
LS4	-0.05	0.29	0.10	0.00	0.06	0.13	0.14	SP7	-0.13	-0.04	0.14	-0.39	0.29	-0.05	0.07
LS5	-0.08	0.31	0.10	0.08	0.13	0.08	0.08	SP8	-0.16	-0.02	0.11	-0.36	0.29	-0.05	0.05
LS6	0.02	-0.19	-0.12	0.01	0.20	0.06	0.00	SP9	-0.12	0.20	0.16	0.03	0.09	0.03	0.00
LS7	0.01	-0.23	-0.06	0.05	0.14	0.19	0.05	SP10	0.10	-0.04	-0.13	0.21	0.26	-0.29	0.11
LS8	-0.04	-0.02	-0.08	-0.07	0.28	0.11	-0.19	SP11	0.11	-0.09	-0.10	0.26	0.27	-0.27	0.11
LS9	-0.02	0.03	-0.09	0.10	-0.03	0.12	0.11	SP12	-0.03	-0.11	0.09	0.00	0.09	-0.02	0.31
LS10	0.10	-0.11	-0.03	0.07	-0.09	0.20	0.39	SP13	-0.09	0.00	0.17	0.05	0.01	0.24	-0.12
LS11	0.13	-0.20	-0.04	0.08	-0.05	0.17	0.30	SP14	-0.07	-0.02	0.11	0.08	-0.05	0.25	0.03
LS12	-0.09	0.26	-0.10	-0.17	-0.11	-0.03	0.02	SP15	0.02	-0.01	0.25	0.22	0.12	0.11	-0.24
LS13	0.02	0.04	0.08	-0.13	-0.24	-0.24	0.16	SP16	0.06	-0.17	0.23	0.26	0.17	0.04	-0.13
LS14	0.10	0.08	0.20	0.03	-0.20	-0.28	0.26	F1	0.13	0.07	-0.25	-0.12	-0.01	0.09	0.06
LS15	0.06	0.11	0.17	0.01	-0.05	-0.24	0.22	F2	-0.06	0.10	-0.02	-0.11	-0.02	0.04	0.14
LS16	0.26	-0.06	0.17	-0.02	0.03	0.07	-0.06	F3	0.33	-0.01	0.15	-0.10	-0.04	0.12	-0.08
LS17	0.17	0.13	-0.10	0.16	0.02	-0.10	-0.04	F4	0.33	-0.01	0.16	-0.09	-0.02	0.12	-0.09
LS18	0.04	0.03	-0.04	-0.16	-0.22	-0.03	-0.23	F5	0.32	0.05	0.15	-0.11	-0.02	0.08	-0.05
LS19	0.24	0.05	0.17	-0.10	0.07	-0.11	-0.03	F6	-0.30	-0.04	-0.08	0.01	-0.06	-0.18	0.02
LS20	0.00	0.21	-0.12	0.13	0.11	-0.09	-0.22	F7	0.15	0.12	-0.16	-0.05	0.25	0.09	0.12
LS21	0.10	0.14	-0.17	-0.02	-0.11	-0.05	-0.20	F8	-0.07	-0.06	0.11	0.18	0.13	0.03	0.12
LS22	0.03	0.10	-0.14	0.10	0.04	-0.10	-0.14	F9	0.11	0.08	-0.28	-0.13	0.16	0.01	-0.03
SP1	-0.02	-0.10	0.11	-0.36	0.22	-0.02	0.07	F10	-0.03	0.10	0.02	0.10	0.14	-0.13	0.01
SP2	0.22	0.12	0.04	-0.01	0.02	-0.12	-0.03	F11	-0.12	-0.10	0.26	0.11	-0.16	-0.08	-0.07
SP3	0.01	0.07	-0.18	0.05	-0.12	0.24	0.17	F12	0.22	0.15	-0.19	-0.03	0.07	0.04	0.06

*Explanations: see Table 2

pea accessions. Kumari et al. (2013) had reported narrow diversity (0.11-0.73) among 28 pea cultivars. In the study by Cupic et al. (2009), the estimated genetic distance among pea accessions based on SSR markers ranged from 0.24 to 0.84. In another study, the RAPD and AFLP markers were compared to determine effectiveness in pea germplasm. At the end of the study, similar ranges of genetic distance coefficients were obtained with RAPD and AFLP markers, 0.80-0.94 and 0.85-0.94, respectively (Simioniuc et al., 2002). However, a much wider range of similarity (0.0-1.0) was determined in 148 Pisum germplasm using protein and PCR-based markers (Baranger et al., 2004).

No relationship was observed between molecular data and morphology according to the genetic similarity results. All the accessions were classified into two linkage groups in accordance with the morphological data. This result was similar to the results of previous studies where 35 pea accessions had been classified into two major clusters and seven sub-clusters (Nisar et al., 2017), and 28 pea lines into three groups (Gixhari et al., 2014).

In the literature, the pea genotypes which flowered after more than 60 days from sowing were grouped within the class of "late varieties" (Solberg et al., 2015). In our study, the flowering time ranged from 43 to 80 days, with an average of 62 days. Eighty seven accessions, in total, were late flowering (60 days or later). Nisar (2008) had reported that days to flower initiation ranged from 45 to 141 days in Pakistani conditions. The number of flowers per node ranged from one to seven. The majority of accessions had fewer than five flowers per node (77%). The accessions differed significantly in plant height, averaging 60.3 cm, and it varied between 25 and 120 cm among all the accessions. Researchers have obtained similar results for maximum plant height, varying between 65.67 and 132 cm (Ceyhan and Avci, 2015), 51.20 and 111.30 cm (Georgieva et al., 2016), and 65.67 and 126 cm (Khan et al., 2013). The largest fresh

seed diameter was measured in the commercial cultivar Serge and 020PS099 (13 mm), while 41PS099 showed the smallest (4 mm). The results obtained revealed the presence of a great genetic diversity for all characteristics studied, which is in concordance with the findings of Ouafi et al. (2016), Gixhari et al. (2014), and Khan et al. (2013), who analyzed genetic variation in pea germplasm.

CONCLUSIONS

This study was designed to look into the genetic richness in Turkish pea accessions by analysing morphological characteristics and molecular markers (SSRs). The application of modern molecular markers in pea, such as marker-assisted selection, determination of regions influencing quantitative trait loci (Tar'an et al., 2005), and assessment of variation (Baranger et al., 2004) provide great benefit for breeding programs. The determination of genetic diversity could benefit genetic and genomic analyses and the exploitation of genetic variation in pea breeding (Nisar et al., 2017). Kwon et al. (2012) reported that the range of the genetic distance values (0.0280 to 0.5147) proved that redundancy had, for the most part, been successfully eliminated from the core collection. The morphological traits examined in this research revealed substantial differences between accessions. Clustering of accessions by multivariate techniques may provide breeders with advantages.

The pea is a crop plant of significant importance for Turkey due to its contribution to the advancement of the agricultural sector. The results indicate that the Turkish pea collection preserves a relatively high variability. According to our results, the richness of the Turkish pea genetic resources can be of benefit in cultivar improvement programmes and breeding studies. Members representing certain groups may be recommended for particular breeding programmes.

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AUTHOR CONTRIBUTIONS

All stages of the study were carried out by Fatih Hanci.

CONFLICT OF INTEREST

Author declare no conflict of interest.

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Table S1. List of pea accessions used in this study and some morphological traits (UPOV, 2009)

Accession	Province	Leaflet: length	Leaflet: width	Stipule: length	Stipule: width	Leaflet: dentation	Plant: length	Stem: length	Grain surface appearance	Width of seed	Initiation of flowering	Maximum number of flowers per node	Width of upper sepal
		3:short, 5:medium, 7:tall	*3:narrow, 5:medium, 7:broad*	*3:short, 5:medium, 7:tall*	*3:narrow, 5:medium, 7:broad*	*0:absent 1:present*	*cm*	*1:too short, 3:short, 5:medium, 7:tall, 9:too tall*	*1:smooth 2:wrinkled*	*mm*	*days*	*numeral*	*3:narrow, 5:medium, 7:broad*
001PS099	Anatolia*	5	5	5	5	0	50	5	1	10	52	4	5
002PS099	Anatolia	7	7	7	7	1	65	5	1	8	71	7	5
003PS099	Anatolia	5	5	7	7	0	35	5	1	8	78	3	5
004PS099	Anatolia	7	7	7	7	0	25	3	1	8	48	1	5
005PS099	Anatolia	5	5	7	7	0	45	5	1	10	53	3	5
006PS099	Anatolia	7	5	5	5	0	60	5	1	7	64	2	3
007PS099	Anatolia	7	7	7	7	0	60	5	1	10	53	2	5
008PS099	Anatolia	5	5	5	5	0	30	5	2	9	56	6	5
009PS099	Anatolia	5	4	7	5	1	25	3	2	8	57	6	5
010PS099	Anatolia	5	5	5	5	1	30	3	1	8	67	7	5
011PS099	Anatolia	5	3	5	5	0	35	3	2	7	55	3	5
012PS099	Anatolia	5	5	5	5	0	50	3	2	6	52	3	5
013PS099	Anatolia	5	5	5	5	1	25	3	1	8	51	5	5
014PS099	Anatolia	5	5	7	5	1	35	3	2	7	62	6	3
015PS099	Anatolia	7	5	7	5	1	35	5	1	8	51	7	5
016PS099	Anatolia	5	3	5	5	1	40	3	2	7	69	4	5
017PS099	Konya	5	5	7	5	0	55	5	1	8	63	6	3
018PS099	Mugla	7	5	7	5	1	65	5	1	10	60	2	5
019PS099	Kastamonu	5	7	7	7	1	75	7	1	7	66	4	5
020PS099	Sakarya	7	7	7	7	0	65	5	1	13	64	3	5
021PS099	Istanbul	7	7	7	7	0	45	5	1	8	64	4	5
022PS099	Izmir	5	5	5	5	1	85	7	1	8	64	4	3
023PS099	Izmir	7	5	7	5	1	55	5	1	9	69	3	3
024PS099	Manisa	5	5	5	5	1	25	5	1	8	70	2	5
025PS099	Manisa	7	7	7	7	1	60	7	1	9	58	4	5
026PS099	Izmir	5	5	7	7	1	55	5	1	8	62	6	3

Accession	Province	Leaflet: length	Leaflet: width	Stipule: length	Stipule: width	Leaflet: dentation	Plant: length	Stem: length	Grain surface appearance	Width of seed	Initiation of flowering	Maximum number of flowers per node	Width of upper sepal
		3:short, 5:medium, 7:tall	*3:narrow, 5:medium, 7:broad*	*3:short, 5:medium, 7:tall*	*3:narrow, 5:medium, 7:broad*	*0:absent 1:present*	*cm*	*1:too short, 3:short, 5:medium, 7:tall, 9:too tall*	*1:smooth 2:wrinkled*	*mm*	*days*	*numeral*	*3:narrow, 5:medium, 7:broad*
027PS099	Izmir	7	5	7	5	1	65	5	1	8	69	1	5
028PS099	Sakarya	7	7	7	7	1	80	5	2	10	63	6	5
029PS099	Tokat	5	5	7	5	1	60	5	1	8	67	6	5
030PS099	Ordu	7	7	7	7	1	65	5	1	10	57	4	5
031PS099	Gumushane	5	3	5	5	1	55	5	1	5	71	2	3
032PS099	Agrı	7	5	5	5	0	75	7	1	6	66	2	5
033PS099	Artvin	7	7	7	7	0	65	5	1	8	64	2	5
034PS099	Mardin	5	5	5	5	0	40	5	1	8	64	3	5
035PS099	Mus	5	5	5	5	1	50	7	1	11	62	2	3
036PS099	Van	5	5	5	5	1	75	5	1	10	55	5	5
037PS099	Elazig	7	7	7	7	1	85	7	1	9	58	3	5
038PS099	Izmit	5	5	7	7	1	60	3	1	9	67	4	5
039PS099	Bilecik	7	5	7	5	0	55	5	1	8	68	7	5
040PS099	Istanbul	5	5	7	7	0	65	5	1	8	62	4	3
041PS099	Kars	5	5	5	5	1	45	5	1	4	61	4	5
042PS099	Yalova	7	7	7	7	1	65	7	1	8	68	4	3
043PS099	Konya	5	5	5	5	0	75	5	1	6	64	3	5
044PS099	Hakkari	5	5	5	5	1	55	5	1	8	68	3	5
045PS099	Erzurum	5	3	5	3	1	55	5	1	7	70	3	5
046PS099	Erzurum	5	5	5	5	0	70	7	1	8	70	4	5
047PS099	Tekirdag	3	3	5	5	1	65	3	1	6	49	4	5
048PS099	Malatya	3	5	7	7	0	55	5	1	6	67	2	5
049PS099	Izmir	3	5	7	7	0	80	5	1	7	62	3	3
051PS099	Eskisehir	7	5	5	5	0	55	3	1	6	71	4	3
052PS099	Eskisehir	5	5	5	5	0	65	5	1	8	62	4	5
053PS099	Eskisehir	5	5	5	5	1	40	5	1	6	70	3	3
054PS099	Eskisehir	3	3	3	3	1	35	5			78	3	5

Accession	Province	Leaflet: length	Leaflet: width	Stipule: length	Stipule: width	Leaflet: dentation	Plant: length	Stem: length	Grain surface appearance	Width of seed	Initiation of flowering	Maximum number of flowers per node	Width of upper sepal
		3:short, 5:medium, 7:tall	*3:narrow, 5:medium, 7:broad*	*3:short, 5:medium, 7:tall*	*3:narrow, 5:medium, 7:broad*	*0:absent 1:present*	*cm*	*1:too short, 3:short, 5:medium, 7:tall, 9:too tall*	*1:smooth 2:wrinkled*	*mm*	*days*	*numeral*	*3:narrow, 5:medium, 7:broad*
055PS099	Eskisehir	5	5	5	5	0	55	7	1	6	68	4	5
056PS099	Mugla	7	5	7	5	0	60	3	1	6	63	3	3
057PS099	Mugla	7	7	7	7	0	90	5	2	8	62	4	5
058PS099	Anatolia	5	5	5	5	0	80	5	1	8	68	4	5
059PS099	Antalya	7	7	7	7	1	75	7	1	11	57	4	5
060PS099	Mersin	5	3	5	3	0	75	5	1	8	65	4	5
061PS099	Antalya	7	5	5	5	1	50	5	1	8	60	2	5
062PS099	Antalya	3	3	3	3	1	65	3	1	8	60	1	3
063PS099	Mersin	5	5	5	5	1	55	5	1	7	66	2	5
064PS099	Ankara	5	3	5	3	1	25	3	1	7	68	2	3
073PS098	Anatolia	5	3	5	3	1	65	5	1	5	71	6	5
074PS098	Anatolia	5	5	5	5	1	110	5	1	8	64	4	5
077PS098	Anatolia	5	5	5	5	1	90	3	1	7	53	2	5
078PS098	Anatolia	7	5	7	5	0	60	7	2	12	48	4	5
079PS098	Anatolia	5	5	5	5	1	45	3	1	8	68	6	5
080PS098	Anatolia	5	5	5	5	1	70	7	1	7	68	7	5
081PS098	Anatolia	5	5	5	5	0	40	5	1	9	55	5	5
082PS098	Anatolia	7	5	7	5	1	50	5	1	8	56	5	5
083PS098	Anatolia	7	5	7	5	1	65	5	1	7	64	5	5
084PS098	Anatolia	7	5	7	5	0	80	5	1	7	57	3	5
085PS098	Anatolia	7	5	7	5	1	70	5	1	8	70	4	5
086PS098	Anatolia	7	5	7	5	0	70	5	1	6	64	6	5
087PS098	Anatolia	5	5	5	5	1	55	5	1	7	67	7	5
088PS098	Denizli	5	5	5	5	0	50	3	1	8	55	7	5
089PS098	Anatolia	5	5	5	5	0	45	3	1	8	78	4	3
090PS098	Anatolia	7	5	7	5	1	55	5	1	8	66	4	5
091PS098	Anatolia	3	3	3	3	1	60	3	1	5	61	1	3

Accession	Province	Leaflet: length	Leaflet: width	Stipule: length	Stipule: width	Leaflet: dentation	Plant: length	Stem: length	Grain surface appearance	Width of seed	Initiation of flowering	Maximum number of flowers per node	Width of upper sepal
		3:short, 5:medium, 7:tall	*3:narrow, 5:medium, 7:broad*	*3:short, 5:medium, 7:tall*	*3:narrow, 5:medium, 7:broad*	*0:absent 1:present*	*cm*	*1:too short, 3:short, 5:medium, 7:tall, 9:too tall*	*1:smooth 2:wrinkled*	*mm*	*days*	*numeral*	*3:narrow, 5:medium, 7:broad*
092PS098	Anatolia	3	3	3	3	1	65	3	1	6	60	1	3
093PS098	Anatolia	3	3	3	3	1	50	3	1	5	53	1	5
094PS098	Anatolia	5	3	5	3	1	40	3	1	5	55	1	5
095PS098	Anatolia	5	5	5	5	1	65	7	1	8	80	1	5
096PS098	Anatolia	5	5	5	5	0	75	7	1	7	64	4	5
097PS098	Anatolia	5	5	7	5	1	55	3	1	9	66	4	3
098PS098	Anatolia	7	5	7	5	0	60	5	1	5	67	6	5
099PS098	Anatolia	5	5	5	5	1	85	5	1	8	54	2	5
100PS098	Anatolia	7	7	7	7	0	65	7	1	11	53	3	5
101PS098	Anatolia	5	5	5	5	0	25	7	1	8	54	3	5
102PS098	Anatolia	7	5	7	5	0	75	7	1	7	58	3	5
103PS098	Anatolia	5	5	5	5	1	40	5	1	8	55	2	5
104PS098	Anatolia	7	7	7	7	0	30	5	1	10	55	5	5
106PS098	Anatolia	5	5	5	5	0	50	5	1	8	64	4	5
108PS097	Anatolia	5	5	7	5	0	40	7	2	7	58	4	3
109PS097	Anatolia	5	5	7	7	0	25	5	2	10	57	3	5
110PS097	Anatolia	5	5	5	5	0	40	7	2	9	65	4	5
111PS097	Anatolia	5	5	5	5	0	30	5	1	8	75	3	5
112PS097	Anatolia	5	5	5	5	0	35	5	1	8	74	3	5
113PS097	Anatolia	7	7	7	7	0	40	5	2	10	57	3	5
114PS097	Anatolia	5	5	5	5	0	80	7	2	9	57	3	5
118PS097	Mersin	5	5	5	5	0	95	5	1	6	46	2	5
121PS097	Manisa	7	5	7	5	1	50	3	2	8	52	7	5
135PS097	Mugla	7	5	7	5	1	65	3	1	6	66	2	3
139PS097	Antalya	7	5	7	7	0	55	3	1	8	54	2	5
140PS097	Izmir	5	5	5	5	0	95	5	1	6	64	4	5
142PS097	Aydın	7	5	7	5	0	45	5	1	10	54	7	5

Accession	Province	Leaflet: length	Leaflet: width	Stipule: length	Stipule: width	Leaflet: dentation	Plant: length	Stem: length	Grain surface appearance	Width of seed	Initiation of flowering	Maximum number of flowers per node	Width of upper sepal
		3:short, 5:medium, 7:tall	*3:narrow, 5:medium, 7:broad*	*3:short, 5:medium, 7:tall*	*3:narrow, 5:medium, 7:broad*	*0:absent 1:present*	*cm*	*1:too short, 3:short, 5:medium, 7:tall, 9:too tall*	*1:smooth 2:wrinkled*	*mm*	*days*	*numeral*	*3:narrow, 5:medium, 7:broad*
144PS097	Kutahya	7	5	7	5	1	105	5	1	7	55	5	5
147PS097	Tekirdag	5	5	5	5	1	85	7	1	7	69	4	5
148PS097	Tekirdag	5	5	5	5	0	95	7	1	9	63	4	5
149PS097	Kirklareli	5	5	7	5	1	45	3	1	7	62	6	3
150PS097	Tekirdag	5	5	5	5	0	55	5	1	8	56	1	5
152PS099	Adana	7	7	7	7	1	70	5	1	8	70	6	5
153PS099	Erzurum	5	5	5	5	0	80	7	1	6	65	2	5
154PS098	Anatolia	5	3	5	3	1	85	3	1	6	59	2	5
155PS098	Anatolia	7	5	7	5	1	85	5	1	6	65	2	5
156PS098	Anatolia	3	3	3	3	1	60	3	1	6	57	4	5
157PS098	Anatolia	5	5	5	5	1	90	7	1	8	68	6	5
KAYSEE	Commercial variety	5	5	5	5	0	60	5	2	8	47	1	5
SERGE	Commercial variety	5	5	5	5	0	45	5	2	8	60	4	5
162PS098	Anatolia	5	5	7	5	1	55	3	1	9	67	3	5
163PS098	Anatolia	7	7	7	7	0	120	3	1	10	61	4	7
164PS098	Anatolia	5	5	5	5	1	80	7	1	8	43	1	5
165PS098	Anatolia	7	5	7	5	0	90	7	1	8	59	3	5
166PS098	Anatolia	5	5	5	5	1	60	5	1	5	66	2	5
167PS098	Anatolia	7	5	7	5	1	100	7	1	6	60	4	7
168PS098	Anatolia	5	5	5	5	0	75	7	1	6	60	4	5
169PS098	Anatolia	5	5	5	5	1	65	7	1	8	67	4	5
170PS098	Anatolia	7	7	7	7	1	85	5	1	7	60	3	5
171PS098	Anatolia	5	5	5	5	1	65	5	1	7	66	6	5
172PS098	Anatolia	7	5	7	5	0	70	5	1	7	64	4	5
173PS098	Anatolia	5	3	5	3	0	60	3	1	7	62	4	5
MEAN		5.6	5.0	5.8	5.2	0.5	60.3	5.0	1.1	7.7	62.0	3.7	4.7

*There is no exact information about on collected province