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Research Article

Comprehensive assessment of genetic variation in native heterostylous primrose genotypes of Türkiye

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Abstract

Primula vulgaris L. is an essential species of ornamental plant value with attractive flowers distributed in humid and cold regions. It spreads around Giresun, Gümüşhane, Trabzon, and Rize in Türkiye's Eastern Black Sea region. Through molecular and morphological markers, this study determined the genetic diversity and similarity levels of *Primula* genotypes sampled from different areas (Samsun, Trabzon, Ordu, and Giresun). Variation was determined regarding morphological characteristics, and that plant shape, flower, and leaf properties were highly variable. Molecular data support morphological features. Thirteen ISSR primers determined the dimensions of genetic diversity, and the genotypes' similarity indexes were between 0.70 and 0.92. It is seen that there is no clustering in the two- and three-dimensional graphics created in line with the genetic parameters, and the genotypes are distributed. Also, plant characteristics were correlated with the ISSR data, and many DNA profiles were detected. This study has characterized *Primula* genotypes that naturally spread in the Black Sea region of Türkiye. It is a guiding study for transferring some observed properties to culture varieties.

Keywords: ISSR; ornamental plant; *Primula*

Introduction

Primula, the largest genus of the *Primula*ceae family (Kovtonyuk and Gontcharov, 2009; Baasanmunkh *et al.*, 2020), is distributed throughout the humid and cold regions of the Northern Hemisphere, including East Asia and Europe. So far, it has been divided into 37 sections, and about 500 species have been characterized according to morphological features (Richard, 2003). Most *Primula* species have attractive flowers and are critical ornamental plants with high value and easy reproduction (Kato *et al.*, 2018). It is represented by nine species, one of which is a natural hybrid species in Türkiye (Güner *et al.*, 2012). Some species belonging to the *Primula* genus are used medicinally and primarily in treating conditions like cramps, rheumatic pain, insomnia, and paralysis (Majid *et al.*, 2014; Khan *et al.*, 2022).

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Primula vulgaris L. is a perennial herbaceous plant with rosette leaves and white, yellow, and purple flowers (Baytop, 1994). It spreads around Giresun, Gümüşhane, Trabzon, and Rize in the Eastern Black Sea region in Türkiye (Anşin *et al.,* 1994). There are many studies on the genus *Primula*, and morphological (Smith and Fletcher, 1948; Zhang and Kadereit, 2005), cytological (Bruum, 1932), biochemical (Yayli, 2001; Morozowska, 2004; Fico *et al.*, 2007.), and molecular (Cservenka *et al.*, 2002; Zhang and Kadereit, 2004; Zhang and Kadereit, 2005; Manfield *et al.*, 2005; Gültepe *et al.*, 2010) studies have been carried out.

Genetic diversity and population similarity can be measured using molecular and morphological markers. One of the significant applications of biotechnology is known as molecular markers. Adapting molecular techniques to plant breeding can shorten the plant breeding process. DNA-based molecular markers help provide a relatively independent estimate of genetic diversity. Molecular markers are DNA sequences on the genome used to identify individual similarities and differences (Yang *et al.*, 2015). PCR-based marker methods are based on amplifying the DNA region between the binding sites of the primers, using primers suitable for the species and varieties that are wanted to detect the characteristics. Among these molecular techniques, ISSR markers with high usage rates are important. Molecular markers overcoming the limitations of morphological and biochemical markers avoid the influence of the environment on the performance of genotypes (Simsek *et al.*, 2020). The ISSR method is highly reproducible based on the random distribution of repetitive nucleotide units such as 2, 3, 4, and 5 in eukaryotic genomes, independent of the locus (Heidari *et al.*, 2016; Parveen *et al.*, 2016). Therefore, the ISSR technique assessed genetic diversity among *Primula* genotypes. This study aimed to characterize different *Primula* genotypes sampled from some Black Sea regions of Türkiye regarding morphological and molecular properties.

Materials and Methods

Plants material

Primula vulgaris L. genotypes were gathered from the natural flora of the Black Sea region in Türkiye in February 2020 (Table 1 and Figure 1). Each genotype was potted in 18 cm diameter pots containing a substrate mixture (1:1:1, v/v, turf, sand, perlite). Plants were maintained in pots in an unheated plastic greenhouse under natural daylight. During winter, plants were irrigated once a week (250 ml/pot) and twice a week during the hot season. Additionally, the greenhouse was covered with a shade net (80% shade factor) during summer. Morphological parameters were monitored in cultivated plants in the next flowering period, 2021.

Table 1. Data of the locations where *Primula* vulgaris L. genotypes were sampled

Genotype	Genotype code	Location	Altitude (m)	Habitat
G1	KER-4	Kerpiçli/Samsun	138	Roadside, scattered forest trees
G2	KER-1	Kerpiçli/Samsun	132	Open field, meadow
G3	KER-19	Kerpiçli/Samsun	157	Roadside, scattered forest trees
G4	KER-18	Kerpiçli/Samsun	157	Roadside, scattered forest trees
G5	KER-2	Kerpiçli/Samsun	140	Open field, meadow
G6	KER-13	Kerpiçli/Samsun	144	Roadside, scattered forest trees
G7	KER-15	Kerpiçli/Samsun	152	Roadside, scattered forest trees
G8	KER-3	Kerpiçli/Samsun	133	Open field, meadow
G9	KER-10	Kerpiçli/Samsun	130	Roadside, scattered forest trees
G10	KER-6	Kerpiçli/Samsun	153	Roadside, scattered forest trees
G11	NEB-17	Nebiyan/Samsun	1245	Forest boundary

G12	NEB-2	Nebiyan/Samsun	1041	Forest boundary
G13	NEB-13	Nebiyan/Samsun	1124	Forest boundary
G14	NEB-10	Nebiyan/Samsun	1178	Forest boundary
G15	NEB-5	Nebiyan/Samsun	1020	Forest boundary
G16	NEB-1	Nebiyan/Samsun	1041	Forest boundary
G17	NEB-19	Nebiyan/Samsun	1140	Forest boundary
G18	NEB-16	Nebiyan/Samsun	1207	Forest boundary
G19	NEB-20	Nebiyan/Samsun	940	Forest boundary
G20	NEB-3	Nebiyan/Samsun	938	Forest boundary
G21	NEB-4	Nebiyan/Samsun	1015	Forest boundary
G22	NEB-18	Nebiyan/Samsun	1245	Forest boundary
G23	OMU-7	Atakum/Samsun	145	Scattered *Quercus* sp. trees and wild low bushes
G24	OMU-5	Atakum/Samsun	198	Scattered *Quercus* sp. trees and wild low bushes
G25	OMU-9	Atakum/Samsun	113	Scattered *Quercus* sp. trees and wild low bushes
G26	DEM-8	Demirli/Trabzon	911	Dominated by a meadow and a few forest trees
G27	DEM-9	Demirli/Trabzon	923	Foothill dominated by forest trees
G28	ÇAM-8	Çambaşı/Ordu	579	Open field, meadow
G29	SIS-18	Görele/Giresun	2172	Located on a plateau, an open field dominated by a meadow

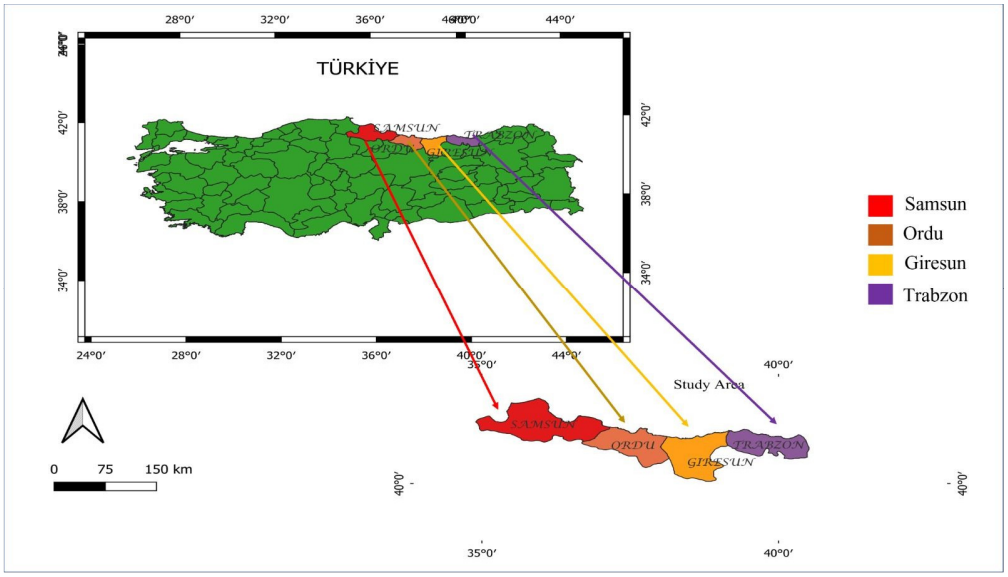

Figure 1. Map of provinces where *Primula* genotypes were sampled

Morphological characterization method

Five quantitative and nine qualitative parameters were monitored for morphological characterization (Table 2). The three longest leaves and randomly selected three flowers at the full bloom stage for each genotype were collected and photocopied (Figure 2). Leaf length (LL), leaf width (LW), and flower diameter (FD) were measured using a digital calliper, and leaf area and flower area were estimated using a hand planimeter. Flower type (FT), flower colour (FC), colour variety of the flowers (CT), homogeneity of primary flower colour (CH), and flower number per plant (FP) (low = flower numbers <3, medium = 3 to 10 flowers, high = flower numbers > 10), plant shape (PS), leaf colour (LF), anthocyanin content of pedicel (PA) and pedicel hairiness were visually determined.

Table 2. Morphological characterization parameters in *Primula vulgaris* L. genotypes

Criteria	1	2	3	4
Flower type	Pin	Thrum	-	-
Colour variety	Monocolor	Bicolor	-	-
Main flower color	White	Cream	Lilac	Yellow
Homogeneity of the color	Even	Uneven		-
Flower number per plant	Low (<3)	Medium (3-9)	High (=10, >10)	-
Plant shape	Compact	Equal	Large	-
Leaf color	Light green	Green	Dark green	-
Anthocyanin content of pedicel (PA)	None (green)	Low (scattered light red color)	Medium (scattered red color)	High (red color covers the whole pedicel)
Pedicel hairiness	Low	Moderate	High	-

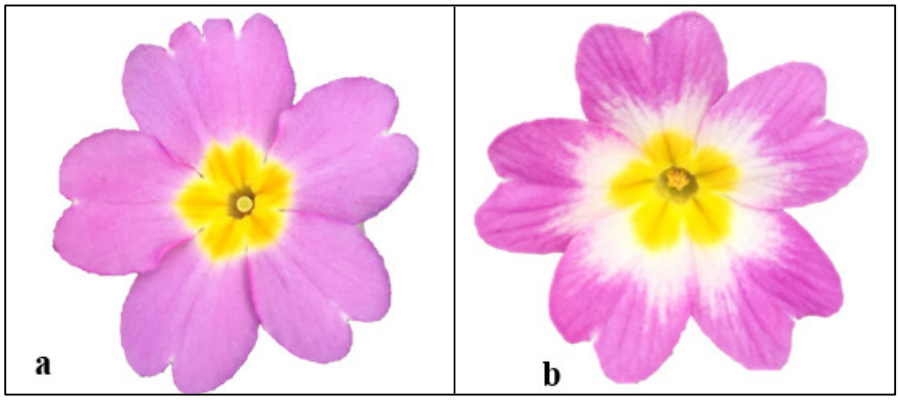

Figure 2. A visual of flower morphological properties in *Primula vulgaris* L. genotypes

Molecular characterization method

Genomic DNA was extracted from young leaves of the *Primula* genotypes according to the modified CTAB method (Doyle & Doyle 1987) and purified. The genomic DNA was subjected to PCR amplification with 13 ISSR primers (Table 5). PCR reaction carried out 15 µl volume containing 1.5 µl Taq buffer, 0.33 µl of 2.5mM dNTPs, 0.2 µl of Taq DNA polymerase, 2.5 µl (20 ng) of template genomic DNA and 1 µl (5 pM) each of ISSR primers. PCR reactions were performed on a thermocycler (Bio-Rad, C1000, USA). Cycling conditions were as follows: initial denaturation at 95 °C for 4 min followed by 35 cycles at 95 °C for 1 min, 52-56 °C for 1min, 72 °C for 1min, and a final extension step at 72 °C for 7min. The amplified products were resolved on 1.5% agarose gel at 110 V for 3 h, using TBE (Tris-Boric acid-EDTA) buffer, visualized under UV light after staining with ethidium bromide and photographed using gel documentation system (Kodak EL Logic 200, USA). Then, clear and reproducible DNA fragments were scored as a 1–0 binary data matrix for the presence and absence of a band, respectively. Cluster analysis among the 29 genotypes of *P. vulgaris* L. species was based on Dice's similarity coefficient (Dice, 1945) using the unweighted pair-group with average arithmetic method (UPGMA) SAHN clustering algorithm. Analyses were conducted using NTSYS-pc (Numerical Taxonomy Multivariate Analysis System, NTSYS, 2.11, USA). The total number of fragments (TNF), number of polymorphic fragments (NPF), and mean polymorphism (MP) for each primer combination were determined.

Statistical analysis

The findings obtained in the morphological characterization studies were evaluated by performing a variance analysis in the SAS (version 9.00) statistical program. Means were compared with the DUNCAN test at the 0.05 and 0.001 significance levels.

As a result of the statistical analysis of molecular studies, data files were prepared by recording '1' in the presence of bands and '0' in the absence of bands. The obtained data were analysed using NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1, Exeter Software, Setauket, N.Y., USA) package program (Rohlf, 2000). UPGMA dendrogram based on the Dice similarity matrix was created from the similarity index. A mantel test was performed between the Dice and SM similarity matrix. Principal Component Analysis (PCA) based on the variance-covariance matrix was performed. The correlation matrix is created using the SIMINT module for Principal Component Analysis. Using this matrix EIGEN module, eigenvectors are calculated. Two-dimensional and three-dimensional graphics were obtained using Eigenvectors in the PROJ module. In addition, a correlation among all the obtained morphological parameters and molecular marker characterization results was conducted through Spearman's Correlation Test using the statistical program PAST at $p \leq 0.01$ and $p \leq 0.05$ significance levels.

Results and Discussion

The *P. vulgaris* L. genotypes naturally distributed in the Black Sea region of Türkiye were collected and characterized by morphological and molecular methods. Studies were carried out with 29 *P. vulgaris* L. genotypes. Observation results of the morphological properties of *P. vulgaris* L. genotypes are presented in Table 3. While 12 genotypes are pin flower types, 17 are thrum flower types. Similarly, while bicolor flower color was observed in 17 genotypes, it was determined that flower color was mono colour in 12. Some genotypes tend to form many flowers, and more than 10 were counted in 5 genotypes (G3, G18, G3, G24, G25). The number of flowers is relatively high, especially in the genotypes sampled from Samsun/Atakum. Genotype 29, sampled from Giresun, has a different yellow flower color from other genotypes. While some genotypes have a large plant habitus, some have a more compact structure (Table 3). Relatively dark green leaves were observed in genotypes collected from areas shaded by trees. Different pedicel hairiness conditions and different rates of anthocyanin accumulation were observed in genotypes collected from the same regions. There was observed a high variation in morphological characteristics between genotypes.

Table 3. Morphological observation results of *P. vulgaris* L. genotypes

Genotype no	FT	CT	FC	CH	PS	LC	FPP	PA	PH
G1	Pin	Bicolor	Light lilac-white	Uneven	Equal	Dark Green	Moderate	Low	Low
G2	Pin	Bicolor	Light lilac-white	Uneven	Compact	Dark Green	Moderate	None	High
G3	Thrum	Bicolor	Lilac-white	Uneven	Compact	Dark Green	High	Low	High
G4	Thrum	Bicolor	Light lilac-white	Uneven	Compact	Dark Green	Moderate	Low	Low
G5	Thrum	Bicolor	Light lilac-white	Uneven	Compact	Dark Green	Moderate	Low	Moderate
G6	Thrum	Monocolor	Light lilac	Uneven	Compact	Dark Green	Moderate	None	High
G7	Thrum	Monocolor	Light lilac	Uneven	Large	Green	Moderate	High	High
G8	Pin	Bicolor	Lilac-white	Even	Equal	Dark Green	Few	High	High
G9	Thrum	Bicolor	Lilac-white	Uneven	Compact	Green	Moderate	High	Low

G10	Thrum	Monocolor	Light lilac	Uneven	Equal	Dark Green	Moderate	Low	High
G11	Pin	Bicolor	Light lilac-white	Uneven	Equal	Light Green	Moderate	None	High
G12	Thrum	Bicolor	Light lilac-white	Uneven	Large	Green	Few	None	Low
G13	Thrum	Monocolor	Light Yellow	Even	Large	Green	Few	Medium	Low
G14	Thrum	Monocolor	Light Lilac	Even	Large	Green	Moderate	Low	Low
G15	Pin	Bicolor	Lilac-white	Even	Large	Green	Few	None	Low
G16	Thrum	Bicolor	Lilac-white	Uneven	Large	Green	Few	None	Moderate
G17	Pin	Bicolor	Light lilac-white	Uneven	Large	Light Green	Few	None	High
G18	Thrum	Bicolor	Light lilac-white	Uneven	Large	Green	High	High	Low
G19	Pin	Bicolor	Light lilac-white	Uneven	Large	Green	Few	None	Low
G20	Thrum	Monocolor	Cream	Even	Compact	Green	Few	None	Low
G21	Thrum	Bicolor	Light lilac-white	Uneven	Large	Green	Few	None	Low
G22	Pin	Monocolor	White	Even	Equal	Green	Moderate	None	Moderate
G23	Thrum	Monocolor	Lilac	Even	Equal	Green	High	Low	Moderate
G24	Pin	Bicolor	Lilac-white	Even	Equal	Green	High	Low	Moderate
G25	Pin	Bicolor	Lilac-white	Even	Equal	Green	High	Low	Moderate
G26	Pin	Monocolor	Lilac	Even	Compact	Light Green	Few	Low	Low
G27	Thrum	Monocolor	Lilac	Even	Compact	Dark Green	Few	Medium	Low
G28	Pin	Bicolor	Light lilac-white	Uneven	Compact	Green	Few	High	Moderate
G29	Thrum	Monocolor	Yellow	Even	Compact	Dark Green	Few	None	Low

* Flower type (FT), color variety of the flowers (CT), flower color (FC), homogeneity of main flower color (CH), and flower number per plant (FPP) (few = flower numbers <3, moderate = 3 to10 flowers, high = flower numbers > 10), plant shape (PS), leaf color (LC), anthocyanin content of pedicel (PA) and pedicel hairiness (PH)

The measurement results of the morphological features of the *P. vulgaris* genotypes are presented in Table 4. When the leaf characteristics were examined, it was determined that genotype 29 and genotype 28 had small leaves. Genotypes collected from Samsun/Nebiyan region were found to have relatively large leaves. Genotypes collected from the Trabzon/Demirli region forest had rather large flowers. When we look at the flower characteristics, genotypes obtained from the same place show high variation in terms of flower size and flower color (Table 4 and Figure 3). Although the flowers seem quite uniform in appearance, it has been reported that they are quite different morphologically (pedicel, corolla, and calyx properties), and flower diameters have been reported to vary between 23-45 mm (Cservenka *et al.*, 2002).

Table 4. Morphological measurement results of *Primula* vulgaris L. genotypes

Genotype	LW	LL	LA	FD	FA
G1	42.00f	86.80j	2678.40ı	33.23fed	642.93g
G2	36.83ı	79.10k	2080.33m	39.06a	893.27b
G3	44.16e	86.26j	2832.10h	31.90fhg	551.27ı
G4	41.20gf	85.23j	2392.37k	36.46b	732.63d
G5	49.70c	96.43ı	3169.23g	32.67feg	587.03h
G6	35.70ı	78.50k	1897.70n	33.60fed	696.23ef
G7	39.66h	94.03ı	2512.90j	30.46ıh	575.13ıh
G8	40.10gh	119.00f	3679.57d	31.90fhg	656.90g
G9	32.86kj	84.56j	1983,267n	34.63d	729.73d
G10	105.46a	148.06b	3522,733e	35.50cb	770.50c
G11	51.73b	162.63a	5683,40a	32.56feg	641.30g
G12	45.60ed	110.70g	3379.87f	21.20l	291.60n
G13	45.93d	122.93e	3103.83g	23.30k	356.80m
G14	39.16h	133.10c	3311.33f	34.90cbd	786.63c
G15	33.83j	99.93h	1929.97n	25.66j	421.10kj
G16	48.36c	129.00d	3664.03d	36.06cb	779.27c
G17	42.40f	128.00d	3789.07c	33.66ed	715.20ed
G18	49.03c	159.26b	4519,93b	26.83j	449.93j
G19	43.92e	129.03d	3754.91c	34.61d	818.26b
G20	32.53kjl	100.10h	2218.43l	22.10lk	203.13o
G21	38.83h	111.16h	2565.20j	25.93j	373.43ml
G22	52.43b	125.43e	4463,93b	31.40ıhg	606.73h
G23	38.63h	80.56k	2087.57m	26.70j	440.87j
G24	31.20l	59.96n	1240.80p	26.33j	402.57kl
G25	34.00j	85.96j	1953.10n	29.80ı	580.23h
G26	31.93kl	64.26m	1409.27o	39.01a	1002.97a
G27	35.56ı	73.20l	1933.27n	33.20fed	673.77gf
G28	21.33m	65.06m	919.60q	33.45fed	671.43gf
G29	19.23n	48.70o	648.50r	25.73j	374.43lm
LSD	**1.38**	**2.56**	**93.49**	**1.76**	**29.43**
CV	**2.04**	**1.61**	**2.11**	**3.46**	**3.03**

*LW-leaf width; LL-leaf length; LA-leaf area; FD- flower diameter; FF-flower area

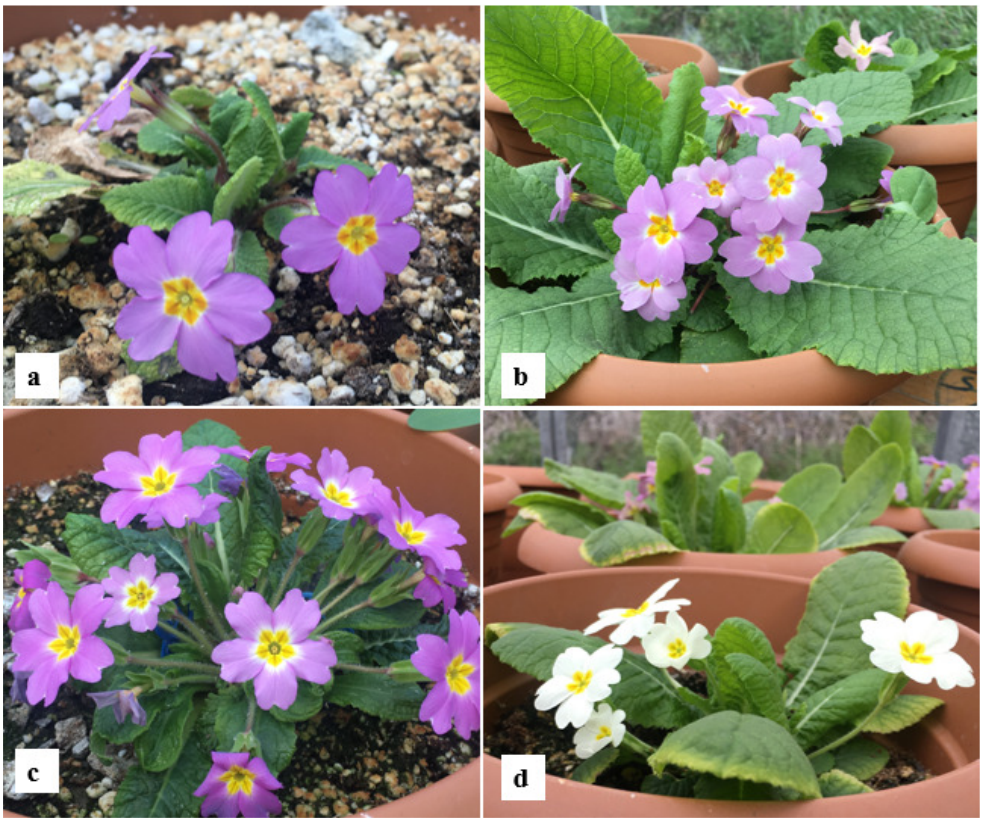

Figure 3. Visual of *P. vulgaris* L. genotypes collected from different regions

Molecular methods determined the dimensions of genetic diversity of *Primula* genotypes. Studies were carried out with 13 primers showing amplification.

In the study, a total of 81 bands were obtained by using 13 ISSR primers. Seventy-one bands were polymorphic, 10 were monomorphic, and the average polymorphism value was calculated as 90%. While band sizes of ISSR primers were detected in the range of 100-1100 Bp, primers (AGC)6G and (GACA)4 had the highest amplification, and the primer (TCC)5RY showed the lowest amplification with one band (Table 5). The expected and observed allelic frequency values (p, q) depending on the ISSR primers ranged from 0.309 to 0.696 and from 0,304 to 0.641, respectively. The number of effective alleles (Ne) ranged from 1.367 (AGCAGC)3G to 1,899 (TCC)5RY (average 1,582), Shannon's information index (I) values ranged from 0,395 to 0,669 (average 0,495), expected heterozygosity (He) values from 0.247 to 0.476 and unbiased expected heterozygosity (uHe) values from 0.252 to 0.487 (Table 5).

Table 5. Polymorphism values of studied ISSR primers

Primary	Sequence 3'-5'	TNF	NPF	Band size (Bp)	p	q	Ne	I	He	uHe
(GACA)4	GACAGACAGACAGACA	11	9	250-900	0,628	0,372	1,608	0,508	0,346	0,352
(CA)8R	CACACACACACACACAR	5	4	100-400	0,547	0,453	1,617	0,498	0,345	0,352
(AGCAGC)3G	AGCAGCAGCAGCAGCAGCG	9	8	100-550	0,367	0,633	1,367	0,395	0,247	0,252
(CAC)3GC	CACCACCACGC	5	4	350-800	0,573	0,427	1,654	0,510	0,357	0,365
(AG)7YC	AGAGAGAGAGAGAGYC	6	5	450-900	0,413	0,587	1,587	0,498	0,341	0,348
(GT)8YA	GTGTGTGTGTGTGTGTYA	6	6	300-1000	0,415	0,585	1,678	0,569	0,388	0,395
(AGC)6G	AGCAGCAGCAGCAGCAGCG	12	11	300-1100	0,637	0,363	1,618	0,513	0,350	0,357
HVH(TCC)7	HVHTCCTCCTCCTCCTCCTCCTCC	7	7	300-800	0,309	0,691	1,719	0,589	0,404	0,412
BDB(CA)7C	BDBCACACACACACACAC	3	3	400-800	0,401	0,599	1,912	0,669	0,476	0,487
(CT)8TG	CTCTCTCTCTCTCTCTTG	4	3	300-700	0,479	0,521	1,440	0,400	0,256	0,270
(TCC)5RY	TCCTCCTCCTCCTCCRY	1	1	500-500	0,615	0,385	1,899	0,666	0,474	0,482
(CA)8 YG	CACACACACACACACAYG	8	7	300-1100	0,359	0,641	1,485	0,443	0,291	0,296
(CAC) 6	CACCACCACCACCACCAC	4	3	400-850	0,696	0,304	1,552	0,453	0,312	0,317
	Total	81	71	-	-	-	-	-	-	-
	Mean	6,23	5,46	100-1100	0,484	0,516	1,582	0,495	0,335	0,342

* Abbreviations for Single Letter Bases: Y (C, T); R (A, G); D (A, G, T); B (C, G, T); H (A, C, T); V (A, C, G)

**TNF: Total Number of Fragments, NPF; Number of Polymorphic Fragments, MP: Mean Polymorphism, p and q: Allele Frequency, Ne: Number of Effective Alleles, I: Shannon's Information Index, He: Expected Heterozygosity and uHe: Unbiased Expected Heterozygosity

Clustering analysis was performed by the UPGMA method using the Dice similarity index of 13 ISSR primers in 29 *Primula* genotypes, and a dendrogram was formed (Figure 4). According to the dendrogram based on the Dice similarity matrix, it is seen that the genetic similarity levels of *Primula* genotypes vary between 0.70 and 0.92. In the dendrogram obtained, genotype 17 and genotype 18 showed low similarity with other genotypes and were collected in a separate arm. Similarly, genotypes 1 and 3 were separated from other genotypes. Genotypes 5 and 16 have more than 90% similar genetic structures. Also, genotypes 11 and 12 were determined as close to each other with a similarity level of 0.88.

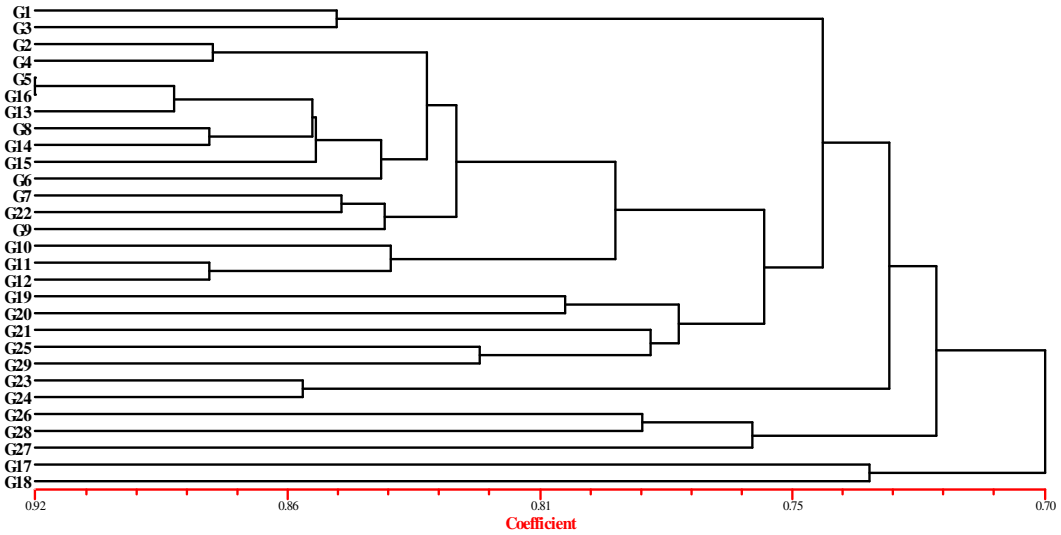

Figure 4. The UPGMA analysis based on Dice coefficients of ISSR markers from the *Primula* genotypes

DNA matrix data at different similarity matrices generated with primers. It was subjected to correlation analysis with the mantel test in the NTSYS program. Mantel correlation value (r = 0.9722) was found to be high.

Two and three-dimensional graphics were created with principal component analysis in the NTSYS program. In the main components analysis, the cumulative sum of the first three eigenvalues was 44.58, which explains 45% of the total variation. According to the two-dimensional principal component analysis graph, most genotypes were distributed rather than clustered. However, it is observed that genotype 1, genotype 3, and genotype 17 are located separately from other genotypes (Figure 5). In this study, *Primula* genotypes collected from different provinces in the Black Sea region showed differences in terms of some morphological features but were found to be genetically similar. This can be explained by all the genotypes collected were plants of the genus *P. vulgaris* L.

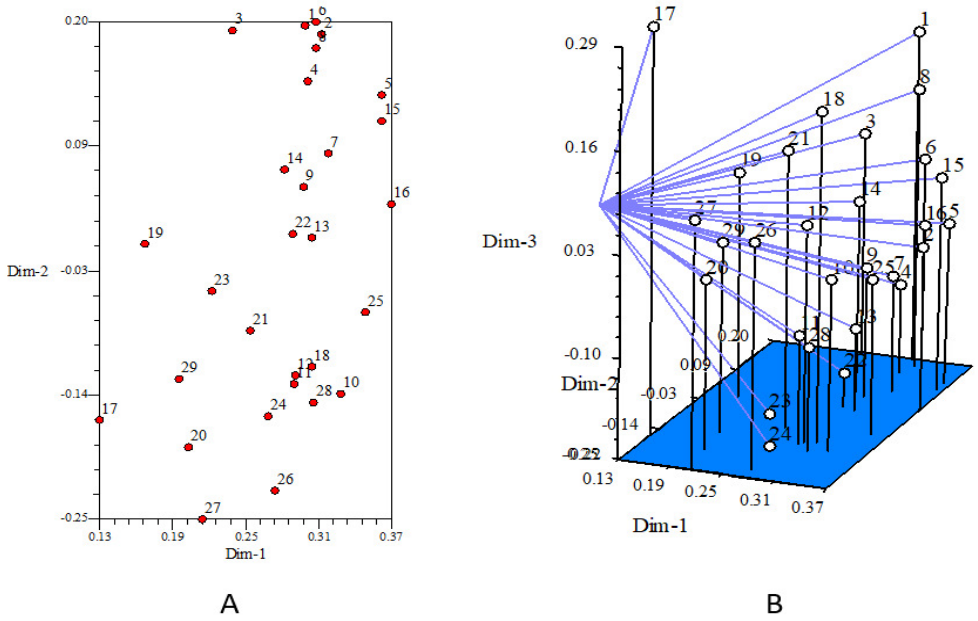

Figure 5. Two-dimensional (A) and three-dimensional (B) graphics obtained because of Principal Component Analysis with ISSR Data in 29 *Primula* genotypes

A correlation between all the obtained morphological parameters and ISSR data results was conducted through Spearman's Correlation Test. (GT)8YA-625, (AG)7YC-600, (AG)7YC -700 DNA profiles showed a positive correlation with leaf colour. However, HVH(TCC)7-350, (CA)8YG -300, (CA)8YG-900 showed a negative correlation with leaf colour. Similarly, DNA profiles of (GT)8YA-900, (CT)8TG-450, (GACA)4-650, HVH(TCC)7-500 also showed a negative correlation with leaf width. Furthermore, (CA)8R -200 DNA profile was positively correlated with flower type, while (GACA)4-650 and (CAC)6-850 DNA profiles were negatively correlated with flower colour. Similarly, the (CA)8R-100 DNA profile showed a positive correlation with the flower number, while the (CA)8YG -600 and CAC)3GC -350 DNA profiles showed a negative correlation with the flower number (Table 6).

Table 6. Correlations between all the obtained morphological properties and ISSR data

Primers	FT	CT	FC	CH	PS	LC	FPP	PA	PH	LW	LL	LA	FD	FA
(GT)8YA-300	-0,154	0,156	-0,001	0,168	-0,343	0,348	0,096	0,09	-0,018	-0,087	-0,36	-0,329	-0,045	0,199
(GT)8YA -600	-0,02	0,02	-0,003	0,06	-0,187	0,342	0,098	0,069	0,02	-0,247	-0,039	-0,164	-0,126	0,122
(GT)8YA -625	0,01	-0,148	-0,049	-0,233	-0,265	,376*	0,057	-0,029	-0,01	-0,179	-0,059	-0,103	-0,165	0,075
(GT)8YA -900	0,042	-0,241	-0,164	-0,224	0,336	0,189	0,346	-0,253	0,081	-,404*	0,313	,368*	-,535**	-0,141
(GT)8YA -950	0,149	-0,015	-0,007	-0,211	,426*	0,078	-0,19	-0,142	0,104	0,055	0,273	,399*	-,373*	0,003
(GT)8YA-1000	-0,185	-0,175	-0,221	-,382*	-0,183	0,316	0,327	-0,101	0,345	0,117	-0,153	0,201	-,446*	0,205
(CA)8R -100	0,337	-0,014	0,02	0,042	-0,099	0,117	,438*	0,15	-0,199	-0,236	-0,049	-0,11	0,108	-,380*
(CA)8R -200	,403*	0,034	0,069	-0,101	0,056	-0,18	0,189	0,134	-0,27	0,213	0,059	0,081	0,155	-0,165
(CA)8R -250	0,267	-0,103	-0,069	-0,267	0,186	-0,243	0,174	0,09	-0,298	0,248	0,155	0,218	0,006	-0,115
(CA)8R -300	0,229	-0,213	-0,221	-0,229	0,329	-0,108	0,071	0,061	-0,279	0,008	0,277	0,211	0,163	-0,293
(CA)8R -400	0,084	0,114	0,125	-0,084	-0,023	-0,143	0,115	0,138	-0,135	0,214	-0,257	-0,103	0,35	-0,037
BDB(CA)7C-400	0,127	-0,038	-0,004	-0,216	0,029	-0,218	-0,017	-0,001	-0,025	0,01	0,011	-0,098	-0,075	-0,012
BDB(CA)7C-500	0,057	0,165	0,205	0,095	0,18	-0,247	-0,331	-0,108	-0,163	0,123	0,264	0,123	0,159	0,04
BDB(CA)7C-800	0,169	0,071	0,107	-0,15	0,125	0,004	-0,109	-0,241	0,165	0,157	0,267	0,331	-0,119	0,009
(TC)8TG -450	0,181	-0,223	-0,268	-0,181	-0,02	0,293	0,251	-0,097	0,186	-,487**	-0,034	-0,005	-0,352	-0,13

(TC)8TG -800	-0,193	0,09	0,136	0,344	0,028	-0,3	-0,193	-0,194	0,019	-0,036	0,08	-0,062	-0,027	-0,134
(TC)8TG-1000	-0,209	0,121	0,135	0,353	0,032	-0,343	-0,221	-0,054	-0,036	0,025	0,085	-0,127	0,042	-0,017
(TCC)5RY-500	-0,133	0,107	-0,115	-0,07	-0,248	0,158	0,155	0,172	,411*	0,197	-0,275	-0,096	-0,143	,514**
(AGCAGC)3G-100	-0,087	0,088	0,092	0,095	0	0,356	0,111	-0,015	-0,136	0,163	-0,11	-0,046	0,186	-0,138
(AGCAGC)3G -110	-0,006	-0,356	-0,221	-,382*	0,018	0,281	0,054	-0,224	-0,061	-0,077	-0,069	0,044	-0,215	-0,152
(AGCAGC)3G -125	0,097	0,055	0,073	-0,064	0,159	0,22	0,26	-0,071	-0,06	-0,099	0,147	0,255	-0,113	-0,245
(AGCAGC)3G -175	-0,154	-0,241	-0,164	-0,224	0,336	0,189	0,196	-0,253	0,205	-0,151	0,244	0,287	-0,241	-0,21
(AGCAGC)3G -275	0,135	0,088	0,092	0,095	0	0,356	0,281	-0,015	-0,276	-0,124	-0,031	0,045	-0,146	-0,06
(AGCAGC)3G -300	-0,154	-0,241	-0,327	-0,224	0,336	-0,091	0,196	0,341	-0,018	0,143	0,187	0,264	-0,086	-0,106
(AGCAGC)3G -350	-0,054	-0,251	-0,193	-0,215	0,239	0,034	-0,162	-0,278	-0,079	-0,108	0,307	0,184	-0,335	-0,015
(AGCAGC)3G -400	-0,225	-0,148	-0,154	-0,159	0,012	0,237	0,098	0,06	-0,194	0,023	-0,068	0,023	-0,181	0,068
(AGCAGC)3G -450	-0,095	0,096	0,082	0,119	0,088	0,218	0,121	-0,175	0,054	-0,143	0,038	0,101	-0,067	-0,195
(GACA)4-250	-0,225	-0,148	-0,154	0,225	0,012	-0,075	0,294	0,06	0,049	-0,305	-0,271	-0,271	0,09	-0,226
(GACA)4-275	-0,302	-0,123	-0,073	0,161	-0,103	0,058	0,077	0,069	-0,076	-0,105	-0,146	-0,312	0,098	0,157
(GACA)4-300	-0,349	-0,241	-0,164	-0,042	-0,007	-0,08	-0,061	-0,253	0,196	-0,283	-0,105	-0,151	-0,234	0,066
(GACA)4-400	-0,152	-0,325	-0,277	0,152	-0,104	0,225	0,139	0,212	-0,322	0,231	-0,193	-0,119	0,151	0,017
(GACA)4-350	-0,279	0,085	0,002	0,279	-0,207	-0,279	0,121	0,243	-0,021	0,007	-,469*	-,480**	,421*	-0,051
(GACA)4-500	0	0	0	0,267	-0,15	0,165	0,34	0,184	-0,135	-0,204	-0,353	-0,353	0,212	-0,243
(GACA)4-600	-0,21	-0,33	-0,225	0,032	0,301	-0,182	0,122	-0,137	-0,048	-0,211	0,234	0,245	-0,204	-0,073
(GACA)4-650	-0,087	-0,362	-,448*	-0,135	0,125	-0,135	0,22	0,269	0,111	-,405*	-0,073	-0,125	-0,093	-0,282
(GACA)4-800	0	0	0	0,267	-0,15	0,165	0,34	0,184	-0,135	-0,204	-0,353	-0,353	0,212	-0,243
(GACA)4-900	-0,087	-0,137	-0,093	0,309	0	0,002	0,22	0,269	-0,253	-0,046	-0,197	-0,158	0,266	-0,25
(GACA)4-950	0,036	-0,184	-0,063	0,254	0,067	0,031	0,343	-0,092	0,014	-0,228	0,141	0,184	-0,192	-0,179
HVH(TCC)7-300	0,064	-0,065	-0,176	-0,296	0,19	0,139	0,304	0,075	0,338	-0,052	0,338	,484**	-,446*	0,181
HVH(TCC)7-350	-0,107	0,277	0,296	0,238	0,136	-,429*	-0,273	-0,321	-0,188	-0,035	0,291	0,154	0,093	0,036
HVH(TCC)7-450	0,067	0,068	-0,018	0	0,152	-0,041	-0,102	-0,098	0,093	-0,367	0,147	-0,026	-0,179	0,167
HVH(TCC)7-500	-0,262	-0,01	-0,049	0,175	-0,054	-0,164	0,163	0,056	0,261	-,442*	0,146	-0,07	-0,037	-0,112
HVH(TCC)7-600	-0,043	-0,092	-0,059	-,441*	0,17	-0,023	-0,007	-0,136	0,306	0,108	0,189	0,283	-,484**	0,157
HVH(TCC)7-700	0,097	0,039	-0,005	-0,155	0,069	0,103	0,282	-0,018	0,26	-0,03	0,048	0,128	-0,256	-0,105
HVH(TCC)7-800	-0,126	-0,148	-0,23	-0,097	0,022	0,141	0,163	0,149	0,261	-0,246	0,034	0,002	-0,197	0,088
(AG)7YC -450	0,264	0,099	0,03	-0,023	0,023	0,111	0	0,367	-0,041	0,141	0,154	0,21	-0,079	0,02
(AG)7YC -500	0,249	0,119	0,035	-0,127	-0,197	0,182	-0,281	0,195	-0,008	0,289	0,07	0,144	0,036	0,349
(AG)7YC -600	0,196	0,17	0,077	0,168	-0,259	,368*	-0,093	,404*	-0,086	0,223	-0,186	-0,106	0,144	0,013
(AG)7YC -650	0,183	0,062	0,045	0,183	-0,036	0,225	-0,031	0,084	0	-0,191	0,108	-0,007	0,038	-0,054
(AG)7YC -700	0,055	-0,266	-0,276	-0,055	-0,252	,426*	0	0,245	0,058	0,122	0,068	0,149	-0,074	0,027
(AG)7YC -900	0,042	0,08	0,129	0,079	-0,11	0,215	-0,215	0,081	0,094	0,257	0,052	0,041	0,112	-0,123
(CA)8 YG -300	0,089	0,224	0,271	0,256	,379*	-,641**	-0,221	-0,027	-0,262	0,193	0,183	0,051	0,214	-0,193
(CA)8 YG -450	0,053	-0,133	-0,216	-0,053	0,115	0,243	-0,108	0,159	-0,107	-0,037	0,236	0,145	-0,203	0,17
(CA)8 YG -550	0,049	0,313	0,35	,421*	0,118	-0,341	0,12	0,025	-0,158	-0,346	0,161	0,051	-0,074	-0,198
(CA)8 YG -600	0,049	-0,005	0,099	-0,205	0,113	-0,056	-,400*	-,392*	0	-0,014	0,175	0,148	-0,249	0,009
(CA)8 YG -900	0,017	0,109	0,131	0,147	,451*	-,708**	-0,125	0,061	-0,166	0,087	0,308	0,183	0,183	-0,318
(CA)8 YG -1100	0,013	-0,169	-0,062	-0,198	0,232	-0,259	-0,095	-0,058	-0,047	0,311	0,366	,398*	-0,06	-0,147
(CA)8 YG -1200	0,083	-0,048	0,104	-0,083	-0,081	-0,275	-0,265	-0,146	-0,131	-0,02	0,188	0,148	-0,254	-0,127
(CAC)3GC -300	-0,048	-0,213	-0,221	0,048	0,329	-0,108	-0,282	-0,294	-0,105	-0,114	0,179	0	0,033	0,016
(CAC)3GC -350	0	-0,191	-0,157	-0,189	0,212	0	-,481**	-0,26	-0,119	0,15	0,322	0,178	-0,078	0,211
(CAC)3GC -500	-0,144	-0,136	-0,049	0,144	0,117	-0,221	-0,045	-0,038	-0,025	-0,063	0,119	-0,07	0,099	-0,268
(CAC)3GC -700	0,096	-0,229	-0,16	-0,096	0,23	0,061	-0,126	-0,325	0,08	-0,171	0,346	0,141	-0,23	0,055
(CAC)3GC -800	-0,112	-0,271	-0,297	-0,014	0,341	-0,04	-0,136	-0,162	0,053	-0,118	0,314	0,179	-0,163	0,123
(AGC)6G -300	0,048	0,213	0,221	0,229	-0,009	0,054	-0,071	0,104	-0,349	-0,024	-0,106	-0,203	0,334	0,008

(AGC)6G -350	0,055	-0,032	0,031	-0,055	-0,122	-0,09	-0,176	-0,209	-0,087	-0,217	0,156	0,074	-0,169	-0,02
(AGC)6G -375	0,013	,396*	0,328	0,358	-0,197	0,006	-0,047	0,087	-0,352	0,295	-0,011	-0,033	0,295	0,316
(AGC)6G -400	0,205	-0,164	-0,188	-0,049	0,025	0,041	0,08	-0,235	0,02	-0,106	-0,134	-0,106	-0,125	0,014
(AGC)6G -450	0,048	0,213	0,221	0,229	0,147	-0,341	-0,282	-0,087	-0,035	-0,049	0,114	-0,049	0,309	0,049
(AGC)6G -500	0,055	0,201	0,184	-0,055	0,014	0,097	-0,176	-0,209	0,174	0,25	-0,223	0,047	-0,183	,386*
(AGC)6G -600	-0,209	0,268	0,197	0,209	-0,045	-0,108	0,184	0,023	,456*	0,157	-0,174	0,106	0,098	0,106
(AGC)6G -800	-0,159	0,148	0,154	0,159	0,216	-0,237	-0,098	-0,06	0,194	0,045	0,045	0,045	0,068	-0,158
(AGC)6G -900	-0,029	0,098	0,033	0,169	0,202	-0,187	0,036	0,075	-0,018	-0,182	0,082	0,049	0,082	-0,066
(AGC)6G -1000	-0,209	-0,172	-0,154	0,065	0,113	-0,108	-0,331	-0,176	-0,146	-0,034	0,153	0,085	0,034	-0,059
(AGC)6G -1100	0,147	-0,275	-0,268	-0,147	0,02	0,208	-0,042	0,036	0,083	0,222	0,159	0,236	-0,106	-0,092
(CAC) 6-500	-0,133	-0,099	-0,115	-0,273	-0,019	0,158	-0,104	0,153	0,154	,472**	0,131	0,227	-0,143	,514**
(CAC) 6-550	0,225	0,148	0,154	0,159	-0,228	0,312	0,196	0,204	-0,243	0,011	-0,237	-0,237	0,215	-0,124
(CAC) 6-850	-0,053	-0,296	-,432*	-0,228	-0,04	0,302	0,252	0,256	0,16	-0,245	0,133	0,058	-0,216	0,174

Flower type (FT), colour variety of the flowers (CT), flower colour (FC), homogeneity of main flower colour (CH) and flower number per plant (FPP), plant shape (PS), leaf colour (LC), anthocyanin content of pedicel (PA), pedicel hairiness (PH), leaf width (LW), leaf length (LL), leaf area (LA), flower diameter (FD), flower area (FF)

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

In a study conducted in the same region where *Primula* genotypes were previously sampled, Gültepe *et al*. (2010) examined 23 populations belonging to 10 native *Primula* L. taxa. They analyzed the nrDN ITS regions and performed base sequence analysis to determine the phylogenetic relationship between *Primula* taxa. The dendrograms generated in the study were compatible with traditional taxonomic data at the subgenus level. Notably, *P. vulgaris* L. genotypes were included in the same clade, and the taxa of *P. vulgaris* L. were closely linked to each other with high bootstrap values of 92%. In another study by Cservenka *et al.* (2002), phenotypic and genetic properties were evaluated together in *Primula* populations. Using RAPD primers, they obtained 54% polymorphism. The study reported that 44% of *P. vulgaris* genotypes differed from hybrids, while *P. veris* genotypes showed a higher clustering frequency with hybrids (87.5%). Furthermore, Van Geert *et al.* (2006) developed microsatellite loci for *P. vulgaris*. They tested eighteen microsatellite primer pairs on 267 *Primula* individuals and found that only three loci were polymorphic. These loci exhibited a range of 4 to 11 alleles per locus, with expected heterozygosity values ranging from 0.496 to 0.620. These studies highlight using molecular techniques, such as base sequence analysis, RAPD markers, and microsatellite loci, to investigate the genetic diversity and relationships among *Primula* taxa. The findings provide insights into the phylogenetic relationships and genetic variation between *Primula* species, including *P. vulgaris*.

Conclusions

In conclusion, this study delved into the analysis of 29 diverse genotypes of *Primula vulgaris*, meticulously collected from four distinct provinces of Türkiye's Black Sea region. These primrose genotypes, indigenous to this ecologically rich area, revealed intriguing variations in their morphological traits, underscoring the remarkable adaptability and diversity present in this population. To comprehensively gauge the genetic diversity within these genotypes, the study employed ISSR markers, uncovering both expected and unexpected genetic differentiations among genotypes originating from the same region while also unveiling surprising genetic similarities between genotypes hailing from different provinces. This genetic tapestry, characterized by diversity and convergence, positions these genotypes as an exceptionally promising initial population for future breeding endeavours.

Within the realm of breeding studies, the paramount significance of establishing a strong foundational population cannot be overstated. In this pursuit, the incorporation of genotypes boasting both high genetic variation and a natural distribution is of utmost importance. The rationale behind this approach is rooted in the ecological compatibility of naturally occurring species, finely tuned to the specific conditions of their native regions. This research further served as a platform for a comprehensive examination of *Primula* genotypes in the Black Sea region, encompassing their morphological and molecular attributes and the subsequent evaluation of genetic diversity. This multifaceted investigation not only deepened our understanding of these intriguing primroses but also illuminated the path forward for future research.

Moving forward, this well-considered initial population, rich in genetic diversity and adaptive potential, will be instrumental in advancing breeding studies aimed at enhancing agronomic characteristics. These characteristics may encompass heightened flowering tendencies and a spectrum of flower colours, ultimately contributing to the cultivation of more resilient, beautiful, and productive *Primula* cultivars. In essence, this study has provided valuable insights and a robust foundation for the cultivation of superior *Primula* varieties. By harnessing the innate genetic treasures of the Black Sea's primrose population, we set the stage for a flourishing future in the world of plant breeding. This study promises discoveries and innovations in the realm of primula research and breeding.

Authors' Contributions

Data curation: MT, Formal analysis: MT and ADS, Investigation: MT, ADS and FB, Methodology: MT, Project administration: MT and OS; Resources and Software: ADS, Supervision: OS, Validation; Visualization, Writing and editing: MT, ADS, FB, and OS. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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