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Assessment of Genetic Diversity and Relationships among Gypsophila and Silene Species from Türkiye based on SRAP Markers

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ABSTRACT

Gypsophila is a member of the Caryophyllaceae family and its genus consists of approximately 150 species. Several species are grown commercially, including herbal medicine and food. Its most common use is as a cut flower worldwide. Gypsophila species are native and widely distributed in Türkiye, the main genetic resource center. In this study, *Gypsophila* L. genotypes were first collected from native areas in Türkiye. Secondly, genetic diversity using molecular markers provided valuable information for breeding programs and strategies of germplasm conservation. Sequence-related amplified polymorphism (SRAP) as a molecular marker was used to determine diversity and relationships among 41 Gypsophila (Caryophyllaceae) genotypes including 13 species (*G. viscose*, *G. simonii*, *G. venusta*, *G. bicolor*, *G. simulator*, *G. bitlisensis*, *G. germanicopolitana*, *G. perfoliata*, *G. acrostic*, *G. eleganas*, *G. paniculata* and *G. aucheri*) and two *Silene* types (*S. vulgaris* L. and *Silene* spp.) as outgroups. Results revealed that twenty primer combinations produced 153 scorable fragments, and all markers showed 100% polymorphism for 43 genotypes. The cophenetic correlation (r = 0.80) between the Dice similarity matrix and the corresponding dendrogram obtained by the SRAP marker revealed good compliance. The *Gypsophila* and *Silene* species were grouped according to subspecies and by region. Results indicated that SRAP markers were useful for investigating diversity and relationships among *Gypsophila* L. germplasm. Additionally, this data could be used to develop new *Gypsophila* L. varieties in the breeding program.

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Türkiye'deki Gypsophila ve Silene Türleri Arasındaki Genetik Çeşitlilik ve İlişkilerin SRAP Belirteçleri Kullanılarak Değerlendirilmesi

ÖZET

Cipsofilya, Caryophyllaceae familyasının bir üyesi olup cinsi yaklaşık 150 türden oluşur. Bazı türleri, bitkisel ilaç ve gıda gibi çeşitli kullanımlar için ticari olarak yetiştirilmektedir. Dünya çapında en yaygın kesme çiçek olarak kullanılmaktadır. Gypsophila türleri ana genetik kaynak merkezi olan Türkiye'de yaygın olarak bulunmaktadır. Bu çalışmada, ilk olarak Türkiye'deki yerel bölgelerden *Gypsophila* L. genotipleri toplandı. İkinci olarak, moleküler belirteçler kullanarak genetik çeşitlilik, ıslah programları ve germplazmayı koruma stratejileri için değerli bilgiler elde edildi. Moleküler belirteç olarak dizi ilişkili çoğaltılmış polimorfizm (SRAP), 13 tür içeren (*G. viscosa*, *G. simonii*, *G. venusta*, *G. bicolor*, *G. simulatrix*, *G. bitlisensis*, *G. germanicopolitana*, *G. perfoliata*, *G. arrostii*, *G. eleganas*, *G. paniculata* ve *G. aucheri*) 41 Gypsophila (Caryophyllaceae) genotip ve iki *Silene* tipin (*S. vulgaris* L. ve *Silene* spp.) genetik farklılık ve akrabalık durumunu belirlemek amacıyla kullanılmıştır. Sonuç olarak, 20 primer kombinasyonundan 153 skorlanabilir fragment üretilmiş ve ayrıca tüm belirteçlerr 43 genotip için %100 polimorfizm göstermiştir. Kofenetik korelasyon r değerleri (r ≥ 0,80) hesaplanarak, SRAP belirteçlerinin oluşturduğu dendrogramların

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önemi bilgiler sunmuştur. *Gypsophila* ve *Silene* türleri alt türlere ve bölgelere göre gruplandırılmıştır. Sonuçlar, SRAP belirteçlerinin *Gypsophila* L. genotipleri arasındaki çeşitliliği ve ilişkileri araştırmak için yararlı olduğunu göstermiştir. Ayrıca, bu veriler ıslah programında yeni *Gypsophila* L. çeşitlerinin geliştirilmesinde de kullanılabilir.

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INTRODUCTION

The genus Gypsophila is a member of the Caryophillaceae and includes about 150 species. The main diversification centers of the Gypsophila genus are in the Caucasus, the Transcaucasian region (northern Iraq and northern Iran), and particularly in the Eastern part of Türkiye (Barkoudah, 1962; Madhani et al., 2023). The feature of these regions is temperate or warm temperate regions in the Northern Hemisphere, especially the Mediterranean region and Near East (Ataslar et al., 2009; Intrieri et al., 2010). Gypsophila L. is the third largest genus of the Caryophyllaceae family in Türkiye and 60 taxa belonging to 56 species grow naturally (Korkmaz and Özçelik, 2011a). Gypsophila species are seen as one of the important alternatives in the cut flower industry and product diversification (Karagüzel and Ortaçeşme, 2000; Korkmaz and Özçelik, 2011b).

The genus Gypsophila contains several ornamental species, of which *G. paniculata* L. is the most important species used in cut flower production worldwide (Zvi et al., 2008; Madhani et al., 2023). *G. paniculata* L. is one of the indispensable elements of bouquets and arrangements in fresh and dry-cut flowers in the domestic market (Karagüzel, 2003). Gypsophila has male sterility, so in the classical breeding program, new varieties are obtained artificially from wild species through *in vitro* vegetative propagation and the selection of clonal variants. Another method is based on open pollination of wild plants (Bogani et al., 2012).

Knowing the genetic structure and germplasm diversity found in Gypsophila's germplasm can provide valuable information for Gypsophila breeding programs to tackle a variety of traits and select new cultivars for conservation purposes (Calistri et al., 2014). For successful breeding, it is crucial to have prior knowledge of the genotypes, their origin, genetic variability, and relationships. Molecular markers may prove precious in supporting Gypsophila germplasm development through characterization of the genetic diversity.

DNA molecular markers are used to evaluate plant diversity, plant breeding, phylogenetic and systematic analyses (Kanayama et al., 2007; Martínez-Nieto et al., 2013; Bolger et al., 2014, Jin et al., 2022). DNA-based markers can be detected at all stages of plant development, contain the entire genome, and can provide large amounts of information; they are highly polymorphic and evaluation to easy and simple (van Zonneveld et al., 2014; Serrote et al., 2020). In ornamental plants, molecular markers are an extremely effective tool for genetic characterization and variety conservation (Mahmood et al., 2013). Studies on the genetic variation of the genus Gypsophila have focused on Gypsophila varieties and their wild ancestors. Random amplification of polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSRs) analysis have been used to identify genetic variations between Gypsophila species and their genetic structure (Rady, 2006; Barakat and El-Sammak, 2011; Lachmayer, 2009). Calistri et al. (2014) investigated the genetic relationship of five Gypsophila spp., sixty-two *G. paniculata* L. within their native range and thirteen commercial hybrid strains using a combination of 63 AFLPs, ISSRs, 64 TRAP, and 65 cpSSRs. As a result, they determined that the markers used were dominant. Initially, Korkmaz and Doğan (2015) were found to be correlated with geographic and phytogeographic regions of genetic diversity of fourteen Gypsophila species from Türkiye using RAPD and ISSR markers. Jin et al. (2022) constructed a genome-wide InDel marker system of *G. paniculata* L. following genome resequencing of another white-flowered wild-type accession.

SRAP markers have been recognized as important molecular marker systems for gene tagging and mapping in Brassica (Li and Quiros, 2001). These PCR-based markers target open reading frames in genomic sequences, generating a set of codominant markers per amplification using forward and reverse primers. SRAP markers are more consistent and reproducible than RAPDs and require less labor and time than AFLP markers (Lia and Quiros, 2001; Budak et al., 2004). For this reason, it has widely been used to evaluate the genetic diversity and population structure of species (Bargish and Rahmani 2016; Kelemen et al., 2018; Akçali Giachino, 2023).

Türkiye is a major diversity center for Gypsophila, as are many other important plant species. Gypsophila is among one of the most important varieties of cut flowers in Türkiye, where it is grown in more than 250 decare greenhouses. Currently, its cultivation is spreading (Anonymous, 2018). The cut flower sector has many problems; one of them is production material, which is imported. Improvement of a new variety that is suitable for Türkiye's

ecological condition. Therefore, the project named "Cut Flower Breeding Program; conducted to generate gene pools of Carnation and *Gypsophila* L." was carried out for the exploration of Gypsophila germplasm. Gypsophila genetic materials were examined for their potential for use as ornamental plants, and seeds and herbarium samples were collected from different locations that may hold rich genetic diversity (Kaya et al., 2012).

This study aimed to evaluate SRAP to determine the genetic variability and relationships within and among Gypsophila and Silene species from different regions (Eastern Anatolia, Middle Anatolia, and the Mediterranean) of Türkiye to provide further insight and develop useful strategies for its conservation and evaluation in a breeding program.

MATERIALS and METHODS

Plant Materials

Fourty-one genotypes of Gypsophila accessions (Caryophyllaceae) including 13 species (*G. Simonis, G. viscose, G. venusta Fenzl., G. bicolor, G. simulatrix, G. bitlisensis, G. germanicopolitana, G. perfoliata, G. acrostic, G. eleganas, G. paniculate* and *G. aucheri*) and two *Silene* types (Caryophyllaceae) including *Silene vulgaris* L. and *Silene* spp. as outgroups were evaluated in this study (Table 1). Numbers of species were given in Figure 1 as *G. simonii* 14, *G. viscose* 1, *G. venusta* 3, *G. bicolor* 4, *G. simulatrix* 2, *G. bitlisensis* 4, *G. germanicopolitana* 1, *G. perfoliata* 3, *G. acrostic* 4, *G. eleganas* 1, *G. paniculata* 1, *G. aucheri* 1, and *Silene vulgaris* 2. details of original locations of collected genotypes.

DNA Extraction and SRAP Experimental Design

Seeds of the collected Gypsophila genotypes were germinated under greenhouse conditions in March 2008 at Batı Akdeniz Agricultural Research Institute, Antalya, Türkiye (36º55'46,30" N and 30º58'47,96" E, altitude 10 m). After 4–6 weeks, the young leaf parts were collected and genomic DNA was isolated using the modified CTAB method developed by Doyle and Doyle (1990). The resulting DNA concentration was measured in a 1% agarose gel stained with ethidium bromide, compared with the known concentration of Lambda DNA (0.5µg/µl, Fermantas).

SRAP analysis was performed as described by Li and Quiros (2001) with some modifications. A total of 36 different SRAP primer combinations were employed using six forward and six reverse primers (Table 2), of which 20 pairs produced clear and reproducible bands. The PCR amplifications were carried out using a thermal cycler (Eppendorf Mastercycler Gradient) in reaction volumes of 15 µl containing 15 ng of genomic DNA and 0.2 µM each of forward and reverse primers, 100 mM of dNTPs, 2 mM of $MgCl_2$, 10 x Taq buffer and 1 unit Taq DNA Polymerase (Biorun), and ddH_2O. PCR reactions were performed under the following conditions: 5 min of denaturing at 94 ºC and 5 cycles of three steps: 1 min of denaturing at 94 ºC, 1 min of annealing at 35 ºC and 2 min of elongation at 72 ºC. In the subsequent 34 cycles; 1 min of denaturing at 94 ºC, 1 min of annealing at 47 ºC, 1 min of elongation at 72 ºC, 1 cycle of 5 min at 72°C. PCR products were separated on 2.5% agarose gel 1X in TAE buffer at 100 V for 3 h. A 100 bp DNA ladder was used as a molecular standard. The gels were stained in ethidium bromide solution (0.5 µg/ml) and then photographed under UV light using the Kodak GelLogic200 Image Analysis System.

Figure 1 Location of sampling site of genotypes
Şekil 1. Genotiplerin elde edildiği bölgeler

Table 1. Gypsophila and Silene species and location of 43 genotypes evaluated in this study
Tablo 1. Çalışmada kullanılan 43 adet Gypsophila ve Silene türleri ve lokasyonları

Genotype	Taxon name	Location
1	*Gypsophila viscose* Muray	Ankara-Şereflikoçhisar
2	*Gypsophila simonii* Hub. Mor	Sivas – Gürün
3	*Gypsophila simonii* Hub. Mor	Sivas – Gürün
4	*Gypsophila venusta* Fenzl.	Erzurum -Aşkale - Tercan
5	*Gypsophila bicolor* (Freyn & Sint.) Grossh	Erzurum - Aşkale
6	*Silene* spp.	Nevşehir-Ürgüp
7	*Gypsophila simulatrix* Bornm. & Woron	Konya – Ereğli
8	*Silene vulgaris* (Moench) Garcke	Sivas-Refahiye location
9	*Gypsophila simonii* Hub. Mor	Erzincan - Refahiye
10	*Gypsophila bitlisensis* Bark.	Erzurum – Aşkale
11	*Gypsophila simonii* Hub. Mor	Erzincan - Refahiye
12	*Gypsophila simonii* Hub. Mor	between Yozgat-Boğazlayan and Sarıkaya
13	*Gypsophila germanicopolitana* Hub.-Mor	between Kayseri and Kırşehir
14	*Gypsophila* spp.	Artvin-Hopa
15	*Gypsophila bitlisensis* Bark.	Kars- Digor
16	*Gypsophila arrostii* Guss.	Konya-Beyşehir
17	*Gypsophila bitlisensis* Bark.	Erzurum – Aşkale
18	*Gypsophila bicolor* (Freyn & Sint.) Grossh	Van- Gürpınar -Başkale
19	*Gypsophila simonii* Hub. Mor	Erzincan-Tercan
20	*Gypsophila simonii* Hub. Mor	between Kayseri and Kırşehir
21	*Gypsophila simonii* Hub. Mor	between Kayseri and Kırşehir
22	*Gypsophila simonii* Hub. Mor	between Kayseri and Kırşehir
23	*Gypsophila simonii* Hub. Mor	Yozgat-Boğazlayan
24	*Gypsophila simonii* Hub. Mor	Sivas- Zara
25	*Gypsophila perfoliata* L. var. *perfoliate*	Konya-Ereğli
26	*Gypsophila perfoliata* L. var. *perfoliate*	Konya-Ereğli
27	*Gypsophila perfoliata* L. var. *perfoliata*	Konya-Ereğli
28	*Gypsophila arrostii* Guss.	Konya- Beyşehir
29	*Gypsophila arrostii* Guss.	Konya- Beyşehir
30	*Gypsophila arrostii* Guss.	Antalya- Elmalı
31	*Gypsophila simulatrix* Bornm. & Woron	Konya-Ereğli
32	*Gypsophila elegans* Bieb.	Konya-Karapınar
33	*Gypsophila venusta* Fenzl.	between Konya- Seydişehir and Bozkır
34	*Gypsophila venusta* Fenzl.	between Konya- Seydişehir and Bozkır
35	*Gypsophila paniculata* L.	Isparta-University
36	*Gypsophila arrostii* Guss.	between Isparta-Eğirdir and Senirkent
37	*Gypsophila simonii* Hub. Mor	Yozgat-Boğazlayan
38	*Gypsophila bicolor* (Freyn & Sint.) Grossh	Ağrı- Doğu Beyazıt
39	*Gypsophila bitlisensis* Bark.	Erzurum – Aşkale
40	*Gypsophila simonii* Hub. Mor	Kırşehir
41	*Gypsophila simonii* Hub. Mor	between Van- Gürpınar and Gevaş
42	*Gypsophila bicolor* (Freyn & Sint.) Grossh	between Van- Gevaş and Tatvan
43	*Gypsophila aucheri* Boiss.	Erzincan-Tercan

Table 2. Sequence of SRAP primers used in this study
Tablo 2. Çalışmada kullanılan SRAP primerlerin baz dizilimi

Primer	Forward primer Sequence (5'–3')		Reverse primer Sequence (5'–3')
ME3	TGAGTCCAAACCGGAAT	EM3	GACTGCGTACGAATTCGA
ME4	TGAGTCCAAACCGG CC	EM6	GACTGCGTACGAATTCCA
ME7	TGAGTCCTTTCCGGTCC	EM8	GACTGCGTACGAATTCAC
ME8	TGAGTCCTTTCCGGTGC	EM11	GACTGCGTACGAATTCTA
ME11	TGAGTCCTTTCCGGAAC	EM12	GACTGCGTACGAATTCTC
ME13	TGAGTCCTTTCCGGAAG	EM15	GACTGCGTACGAATTGAT

Data Scoring and Analysis

All clear and reproducible PCR products were scored as the presence (1) and absence (0) of a band for Gypsophila genotypes. The total number of bands, polymorphic bands, *Gypsophila* species-specific bands, and the average number of bands per primer were calculated. The statistical assessments, which are the evaluation method and variability formula, PIC (Polymorphic Information Content), MI (Marker Index), PI (Primer Index), and EMR (Effective Multiplex Ratio) were calculated to determine the polymorphism information and discriminating ability of each primer combinations (Table 3). The discriminatory ability of each SRAP marker was determined by calculating the PIC. PIC values were estimated according to the formula described by Smith et al. (1997). PIC= 1-Σ (fi^2), where fi^2 is the frequency of the i th allele. EMR is calculated as EMR = np × ß, where np is the total number of polymorphic loci per primer and ß is the rate of polymorphic loci from their total number (Powell et al.,1996; Nagaraju et al., 2001). MI is a statistical parameter used to estimate the total utility of the maker system. MI is the product of the polymorphism information content value and effective multiplex ratio. MI was calculated using the formula MI = PIC × EMR (Zitouna et al., 2015). The SRAP primer index was calculated by summing the PIC values of all loci amplified with the same SRAP primer combination, PI = PIC × total bands (Anderson et al., 1993; Ghislain et al., 1999; Rajwade et al., 2010).

The genetic similarity coefficient was calculated using the procedures in the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc version 2.02) (Rohlf, 2000). The similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average), using the SAHN function of the NTSYS to illustrate the genetic relationships among the germplasm studied. The representativeness of the dendrogram was determined by estimating the cophenetic correlation for the dendrogram and comparing it with the similarity matrix using Mantel's matrix correspondence test (Mantel, 1967). Principal Coordinate Analysis (PCoA) was obtained with the use of Dice's coefficient (Dice, 1945) to confirm associations among 43 species, and a two-dimensional plot (2D) was constructed.

RESULTS

The 41 Gypsophila (Caryophyllaceae) germplasm lines, including 13 species and 2 *Silene* (Caryophyllaceae) types, were analyzed using 20 different combinations of SRAP primers. For all that, 16 SRAP primer combinations produced only monomorphic DNA bands. The 20 primer combinations generated a total of 153 DNA fragments with distinct scoreable polymorphic bands and showed 100% polymorphism. The number of bands scored per primer ranged from 4 bands (Me4Em12 and Me4Em15) to 13 bands (Me3Em6) and 12 bands (Me4Em3), with 7.65 fragments for primers average (Table 3). The size of the amplified products generated using different primer combinations ranged from 200 bp to 1800 bp. The gel view obtained from the Me3Em12 primer combination is given in Figure 2.

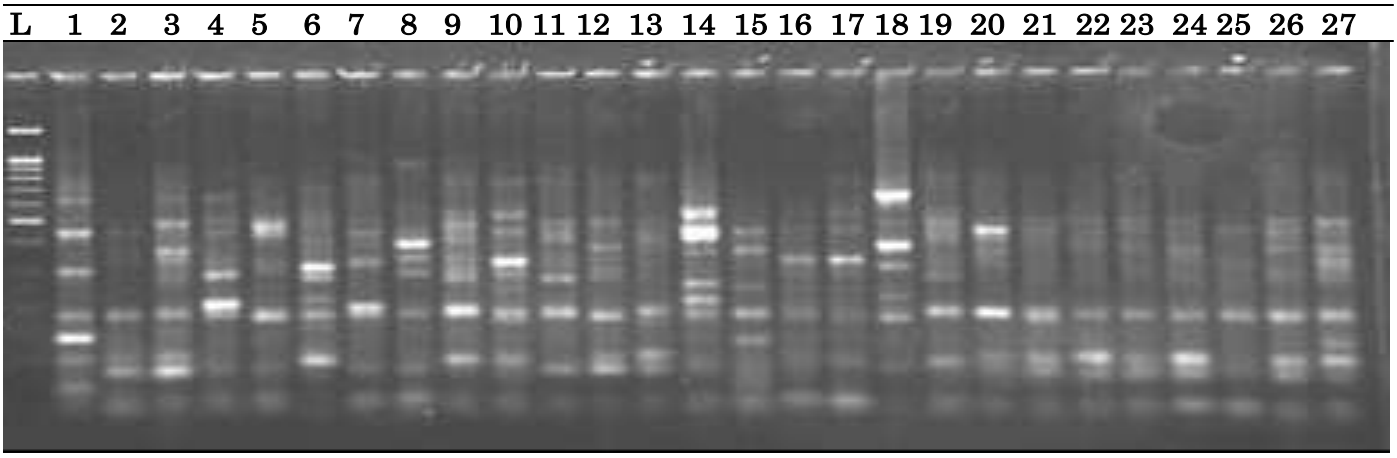

Figure 2. Gel view obtained from Me3Em12 primer combination. L: ladder DNA, 1-27: genotypes
Şekil 2. Me3Em12 primer kombinasyonundan elde edilen jel görünümü. L: DNA ladder, 1-27: genotipler

The informative and discriminating power of the PIC, EMR, MI, and PI values of markers at individual SRAP primer combinations were calculated (Table 3). The PIC values for 20 primer combinations ranged from 0.67 (Me7Em3) to 0.98 (Me8Em8 and Me7Em123), with a mean of 0.88 (Table 3). The EMR value ranged from 4.00 to 13.00 and the MI and PI values ranged from 3.35 to 9.96. The maximum EMR (13.00), MI, and PI (9.96) values were observed for the primers Me3Em16 and Me4Em3, respectively, whereas minimum scores for EMR (4.00) at

Me4Em12 and Me4Em13, MI and PI (3.35) at Me7Em3 SRAP primers combinations were recorded. Overall, the 15 SRAP primers used in this study showed an average EMR value of 7.64 and a mean of 6.72 scores for MI and PI (Table 3).

Table 3. Details of data produced by screening Gypsophila and Silene genotypes using SRAP markers
Tablo 3. Gypsophila ve Silene genotiplerinin ayrımında kullanılan SRAP belirteçlerin verileri

Primer Combinations	Total DNA fragments	Number of Polymorphic fragments	Percentage of polymorphism	PIC	EMR	MI	PI
ME3EM15	5	5	100.00	0.95	5.00	4.75	4.75
ME3EM3	7	7	100.00	0.94	7.00	6.58	6.58
ME3EM6	13	13	100.00	0.73	13.00	9.49	9.49
ME4EM12	4	4	100.00	0.89	4.00	3.56	3.56
ME4EM15	4	4	100.00	0.91	4.00	3.64	3.64
ME4EM3	12	12	100.00	0.83	12.00	9.96	9.96
ME4EM6	11	11	100.00	0.87	11.00	9.57	9.57
ME7EM12	7	7	100.00	0.98	7.00	6.86	6.86
ME7EM3	5	5	100.00	0.67	5.00	3.35	3.35
ME7EM6	8	8	100.00	0.95	8.00	7.60	7.60
ME8EM11	7	7	100.00	0.82	7.00	5.74	5.74
ME8EM12	10	10	100.00	0.96	10.00	9.60	9.60
ME8EM3	8	8	100.00	0.85	8.00	6.80	6.80
ME8EM8	7	7	100.00	0.98	7.00	6.86	6.86
ME11EM3	10	10	100.00	0.85	10.00	8.50	8.50
ME11EM6	9	9	100.00	0.93	9.00	8.37	8.37
ME13EM15	7	7	100.00	0.82	7.00	5.74	5.74
ME13EM3	6	6	100.00	0.88	6.00	5.28	5.28
ME13EM6	8	8	100.00	0.83	8.00	6.64	6.64
ME13EM8	5	5	100.00	0.96	5.00	4.80	4.80
Total	153	153	-	-			
Average	7.64	7.64	100.00	0.88	7.64	6.72	6.72

PIC: Polymorphic Information Content, EMR: Effective Multiplex Ratio, MI: Marker Index, PI: Primer Index

According to the genetic relationship analysis, Dice's genetic similarity coefficient of 41 Gypsophila genotypes and two Silene species indicated a high genetic variation among the genotypes, ranging between 0.31 and 0.85. However, the genetic closeness of the two varieties was observed between genotypes 25 and 27, including *G. perfoliata* L. both of which belonged to the same location of origin (Konya). The dendrogram created using UPGMA cluster analysis showing the general genetic relationship between genotypes is given in Figure 3.

UPGMA clustering was used to determine the rare germplasms of Gypsophila species found in different locations of Türkiye and the relationships between genotypes. UPGMA cluster analysis showed the presence of two major clusters (I and II) (Figure 3). Forty-one Gypsophila and two Silene genotypes were clustered separately. Group I is divided into two branches and the second branch is divided into four subgroups (1A, 1B, 1C, and 1D). There were no identical genotypes on the dendrogram (Figure 3). Sub-group 1A consisted of two genotypes (1 and 14), of which genotype 1 was identified as *G.viscosa* Muray and 14 number genotype were selected to Artvin, was determined as *Gypsophila* spp, two types were located on the border of the dendrogram and their genetic distance was 0.415 from other Gypsophila species (Figure 3). Sub-group 1B included in *G. simonii*, *G. perfoliata* var. *perfoliata* L. and *G. germanicopolitan* species. All *G. simonii* types were collected from different regions in Türkiye: *G. perfoliata* var. *perfoliata* L. and *G. germanicopolitan* species, collected from nearer regions, Konya and Kayseri, respectively. Sub-group 1C was formed by seven *Gypsophila* species, which were *G. venusta, G. bitlisensis, G. bicolor, G. simulatrix* from Ulukışla, *G. arrostii* L. and *G. paniculata* L. species from Isparta. Sub-group 1D included four genotypes of *G. arrostii* L. which were selected from nearby locations in Konya, Isparta, and Antalya, and the other *G. perfoliata* L. var. *perfoliata*, *G. simulatrix,* and *G. elegans* species that were collected from Konya formed a joint group of clusters. Additionally, sub-group 1D comprised *G. bitlisensis* and *G bicolor* species that were collected to Erzurum and Van, respectively. The genetic distance among Gypsophila species ranged from 0.58 to 0.85, and genetic diversity among the individuals of sub-cluster 1B is lower than that of the other sub-groups individuals. The greatest similarity was observed among two types (26 and 27) of *G. perfoliata* var. *perfoliata* L. collected from the same location in Konya.

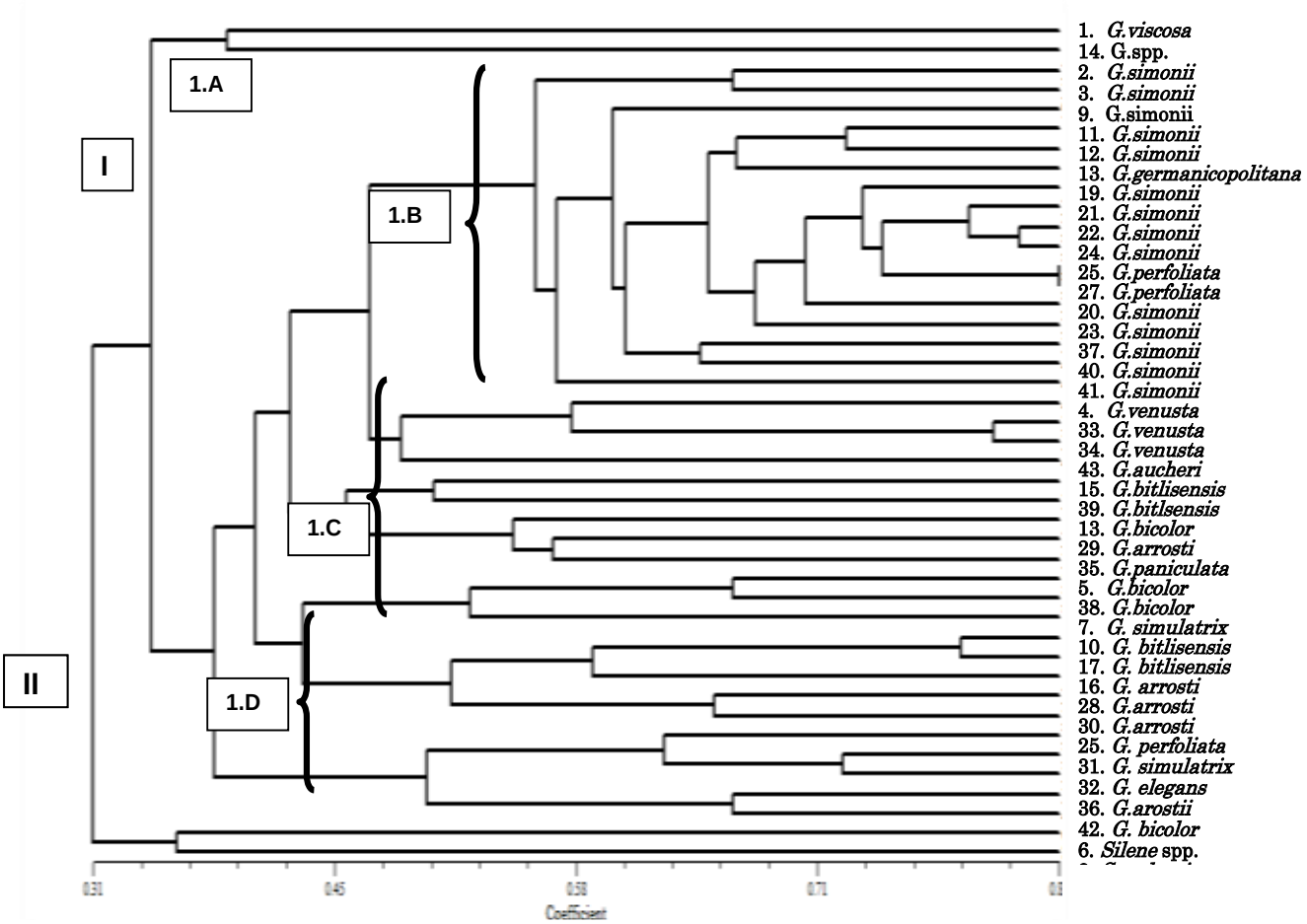

Figure 3. UPGMA dendrogram based on similarity matrix constructed from the 153 SRAP markers amplified for the 41 Gypsophila (Caryophyllaceae) and the two Silene accessions

Şekil 3. 153 adet SRAP belirteciyle 41 Gypsophila (Caryophyllaceae) ve 2 Silene türleri arasındaki benzerlik indeksini gösteren UPGMA yöntemiyle elde edilmiş dendrogram

Interestingly, the other *G. perfoliata* type (25) was shown to be dissimilar and take place in the sub-group 1D. The genetic distance of the same genotype was nearer *G. simulatrix* and *G. elegans* than the other two *G. perfoliata* types. *Gypsophila simonii* (22 and 24 number) were placed close to each other, with a genetic distance of 0.81. The reason is that these two genotypes were collected in Kayseri and Sivas, which are near provinces. However, it was noted that the genetic dissimilation of genotype 41 selected from Van was quite far from the other *G. simonii* types. The genetic distance of all genotypes selected from different localities representing *G. simonii* species ranged from 0.58 to 0.81. High similarity was observed between 33 and 34 number genotypes (*G. venusta* Fenzl), which were selected for the same location. Similarly, the genetic distance between 10 and 17 numbers (*G. bitlisensis* Bark.) was 0.78. Both genotypes were collected from Erzurum, the near location. *G. paniculata* L. is widely used in commercial cut flower production and is the primary source of commercial varieties. Clustering analysis showed the nearest genetic similarity of *G. paniculata* was determined to be *G. arrostii* L. and *G. bicolor* species from Konya and Van placed in the same sub-group, 1C. In this study, Silene spp. (6 numbers, from Nevsehir) and Silene vulgaris (8 numbers, from Sivas) were used as outgroups, and the genetic similarity of both was determined to be very low. Gypsophila and Silene taxa were distinguished and the genetic similarity (0.31) was very low, as expected.

Cluster analysis was supported by high bootstrap values and confirmed by PCoA analysis (Figure 3). The cophenetic correlation was determined as $r \geq 0.80$. Figure 3 shows the distribution of the different species of Gypsophila and Silene according to the two principal axes of variation using principal coordinates analysis (PCoA). PCoA analysis showed that the first and second principal components accounted for 12.01% and 36.18% of the total variation, respectively (Figure 4). The classification of all species derived from PCoA was similar to the result of the UPGMA analysis. Substantial dispersion of Gypsophila species in the PCoA plot and the result of the UPGMA analysis indicate high genetic diversity among different species of Gypsophila. The genetic diversity among two Silene types (*Silene vulgaris* L. and Silene spp.) was very high (0.35).

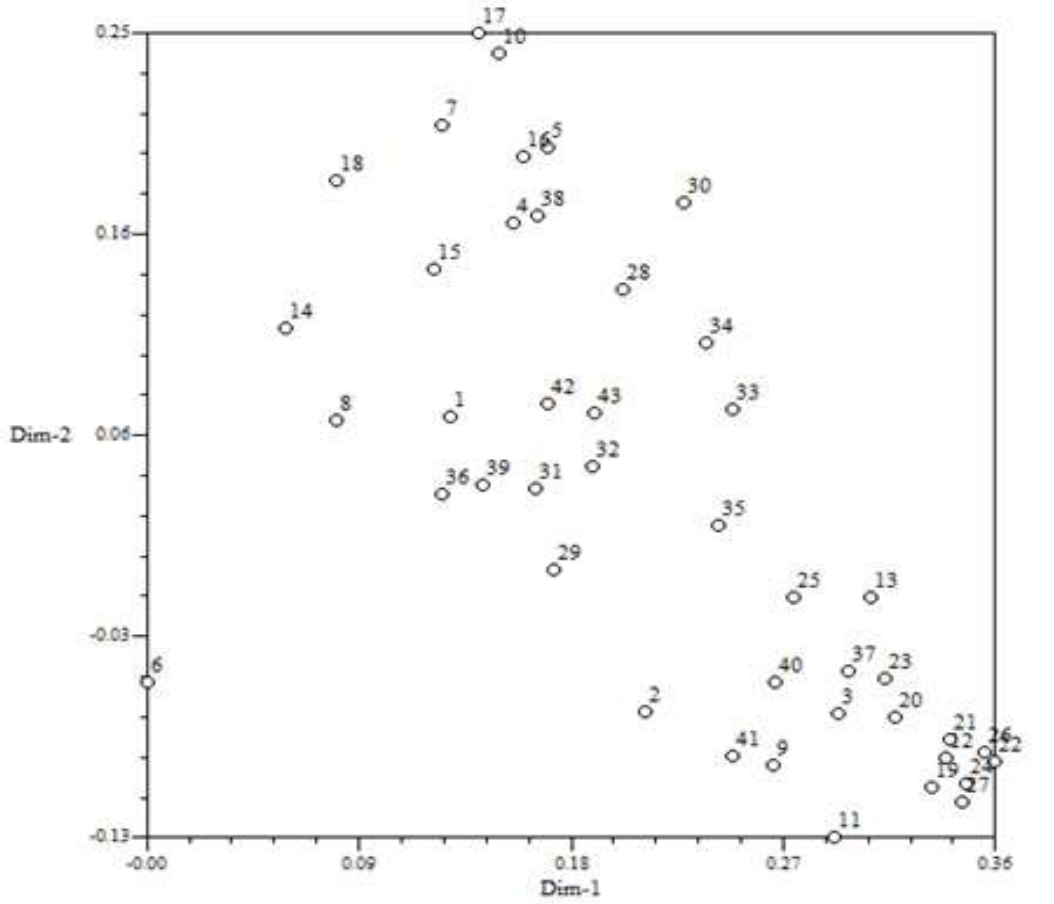

Figure 4. Diagram showing the relationships among 41 Gypsophila and two Silene accessions based on principal coordinates analysis using SRAP

Şekil 4.SRAP analizi sonucunda 41 Gypsophila ve 2 Silene türlerinin principle koordinat analizi (PCoA) sonucu göstermiş olduğu akrabalık dağılım deseni

DISCUSSION and CONCLUSIONS

In our study, genetic diversity within and between species and genotypes of the genus *Gypsophila* L. was evaluated using SRAP molecular markers. The findings of our study revealed that SRAP was found to be effective in assessing the genetic variation in 41 *Gypsophila* species and 2 *Silene* species from different locations in Türkiye. The results show that SRAP markers can be used in developing varieties, understanding relationships, and creating germplasm collections. It also shows that SRAP markers can be used in developing varieties, understanding relationships, and creating germplasm collections.

The 153 markers were found using 20 SRAP primers analyzed in this study and presented valuable information about genetic variations in Gypsophila species/germplasms originating from diverse geographical locations. SRAP markers allowed the obtaining of highly polymorphic fragments. This result demonstrated a good choice of method for the analysis of Gypsophila species with 20 SRAP markers revealed an average of 100% polymorphism, which was too high compared to earlier studies using a different marker system. Percentage of polymorphism (100 %) was found to be higher than in other SRAP-based studies, e.g. 95.76 % for Dianthus accessions (Xiao et al., 2008), 93 % for coffee species (Mishra et al., 2011) and 71.90 % for *Silene* species (Bargish and Rahmani, 2016). In a previous study of Gypsophila species in Türkiye, Korkmaz and Doğan (2015) and Kołodziej et al. (2018) studied the genetic diversity and relationships among the accessions were determined using RAPD and ISSR markers. Similar results were presented by Korkmaz and Doğan (2015) who found 92.7, 93.8, and 92.9% polymorphism for the 14 species based on RAPD, ISSR, and RAPD + ISSR data, respectively. The other study based on the RAPD and ISSR markers showed 80.31% and 95.86% polymorphic products, respectively (Kołodziej et al., 2018). Calistri et al. (2014) used AFLP, ISSR, cpSSR, and TRAP for the analysis of the genetic distance of 5 Gypsophila wild species from Europe and Asia and 13 commercial hybrids with similar phenotypes and reported that the higher number of polymorphic products was (96.3%) for ISSR markers.

The percentage of polymorphic fragments, as well as gene diversity, showed a high range of variability in the analyzed Gypsophila and Silene accessions. The high polymorphism of selected SRAP markers provides a unique

opportunity to study genetic variation and relatedness of Gypsophila germplasm. Thus, the assessment of genetic variance among Gypsophila species in Türkiye has a greater importance in Gypsophila breeding programs and in situ conservation. Another important point is to cross genotypes in highly diverse and distinct clusters to increase the opportunity for the over-segregation of alleles at various loci (Souza and Sorrells, 1991).

The number of polymorphic markers analyzed is important to detect true relationships between taxa. Dudley (1994) suggested that when numbers reach 50 to 100 markers, results are consistent with pedigree information. One hundred fifty-three SRAP bands have been obtained to determine the relationship between and within the Gypsophila species. For this reason, the numbers of markers and informative markers number were over the suggested range by Dudley (1994).

In this study, the average values of PIC, EMR, MI, and PI, were 0.88, 7.64, 6.72, and 6.72, respectively. PIC value shows the discrimination ability of the marker depending on the number of known alleles and their frequency distribution. PIC values were higher due to markers with equal distribution in the population (Botstein et al., 1980). High, medium, or low polymorphism is expressed by PIC >0.5, 0.5 > PIC > 0.25, and PIC < 0.25, respectively (Xie et al., 2010).

PIC results were within a relatively narrow range, indicating a uniform distribution of SRAP polymorphisms among the genotypes collected; this is a desirable trait for their use in genetic diversity analyses (Al-Faifi et al., 2013). All PIC values in this analysis were found to be higher than 0.5, thus indicating that the observed polymorphism was high. On the other hand, except for Me7Em3 primer combinations, the other 19 primer combinations could be considered highly informative in determining genetic diversity. Overall, the 20 SRAP primer combinations used in this study showed that EMR, MI, and PI values are higher in Me3Em6, Me4Em3, Me4Em6, and Me8Em12 primers. The average values of PIC, EMR, MI, and PI, were higher than >0.5 for PIC and >5.0 for others. The higher value of EMR and MI explains that the selected marker system is a more efficient primer-marker and suitable method (Chesnokov and Artemyeva, 2015). Diversity parameters such as the polymorphism rate, PIC, EMR, MI, and PI had high values, indicating high variability of the tested population. High levels of diversity may increase the adaptation of Gypsophila genotypes to a wide range of environments (Nagl et al., 2011). Additionally, a high genetic variation of Gypsophila germplasm should be considered as a background for breeding programs

Based on the SRAP marker system, the genetic distance between Gypsophila and Silene accessions/species of distinct geographical regions (Southeast Anatolia, the Middle and the Mediterranean regions of Türkiye) was revealed. The dendrogram delineated the genetic distance of two taxa which formed two major clusters. This sub-cluster formed in the dendrogram was mainly displaying the genetic structure and grouped in the different climatic zones. The emergence of high polymorphism can be explained by accessions/species in different climatic zones by changing selection pressure throughout the evolution process (Mishra et al., 2011). The genetic structure and geographic distribution of species appear to greatly influence levels of genetic diversity (Hamrick and Godt, 1989). *G. simonii* types were collected from different regions in Türkiye and grouped into the same cluster (subgroup 1B). However, it is noteworthy that the genetic variation within *G.simonii* species is quite high and the germplasm pool may help improve new varieties.

The dendrogram derived from SRAP data showed that the species *G. viscosa* Muray from Ankara and Gypsophila spp. from Artvin differed significantly from the 13 Gypsophila species. The distinctiveness of these species was associated with its geographic distribution. Ankara and Artvin have diverse topography (particularly altitude) and the wide range of climatic and ecological conditions during the growing period correlate with altitude. Higher variation of both germplasms may be created due to higher mutation rates and/or selection pressure in those regions. Mhiret and Heslop-Harrison (2018) noted that molecular markers can group *Linum usitatissimum* L. accessions by both altitude and region, indicating a lack of gene flow across the country and/or selection of specific genotypes in each environment. Similarly, Korkmaz and Dogan (2015) used RAPD and ISSR markers and reported that the genetic distance of *G. glomerata* and *G. muralis* with 14 *Gypsophila* species correlated with their different phytogeographic regions.

The dendrogram delineated that the clusters of *Gypsophila* species were closely related to their geographic origins and surrounding geographic environments. *G. simonii* included 14 genotypes, *G. venusta* (3 genotypes), *G. germanicopolitana* and *G. perfoliata* (2 types) collected along the nearer cities (Konya, Kayseri, Kırşehir, Sivas, Yozgat, Ercincan, and Erzurum) in the Middle Anatolia region formed one major cluster except one genotype (41) from Van, which is located in the South-eastern Anatolia. The seven locations in the Middle Anatolia part of Türkiye have relatively similar climates and altitudes; therefore, it can be said that geographic distance and environmental factors including local climates may impact the genetic differentiation of the native Gypsophila populations.

Characterization of the genetic structure of *Gypsophila* L. can be very useful in establishing breeding strategies

that enable selection. Kaya et al. 2019, developed the named GA8, which is a new Gypsophila type as cut flowers using a selection of clones from *G. arrosti* in Türkiye and the cultivation performance of this type has been investigated. Clustering analysis showed that the genetic distance of *G. paniculata* L. among *G. arrostii* L. is nearer than other Gypsophila species.

Clustering analysis was supported by high bootstrap values and confirmed by PCoA analysis (Figure 4). Also, the significance of the resulting dendrograms was confirmed by calculating the cophenetic correlation ($r \geq 0.80$). In statistics, the cophenetic correlation coefficient is a measure of how faithfully a dendrogram preserves the pairwise distances between the original unmodeled data points. Thus, it is a measure of how faithfully the tree represents the dissimilarities among observations (Rohlf and Fisher, 1968). PoCA helps in analyzing genetic variation among plant species and determining the most important variables contributing to variation (Chesnokovand Artemyeva, 2015). The classification of all species derived from PCoA was similar to the result of the UPGMA analysis. Substantial dispersion of Gypsophila species in the PCoA plot and the result of the UPGMA analysis indicate high genetic diversity among different species of Gypsophila. Similar results have been reported by Korkmaz and Doğan (2015). The Gypsophila and Silene species were grouped according to subspecies and by region. Silene spp. separated from Gypsophila species, while *Silene vulgaris* was closer to *G. viscosa* Murav. This result may reveal a high genetic relationship between *Gypsophila* and *S. vulgaris* studied. The genetic diversity among two Silene types (*Silene vulgaris* and Silene spp.) was very high (0.35). Similar results were reported for 13 different Silene species in Iran. Their results revealed sufficient level of genetic distance (0.10 to 0.52) (Bargish and Rahmani, 2016). The genetic diversity among two Silene types (*S. vulgaris* and Silene spp.) was very high (0.35). Similarly, Bargish and Rahmani (2016) reported that the genetic distance of 13 different Silene species from Iran was from 0.10 to 0.52.

To our knowledge, this is the first study to use SRAP markers to analyze genetic variation between and within Gypsophila and Silene species. The results provide a more detailed understanding of the genetic diversity and evolutionary relationships of the above-mentioned Gypsophila and Silene species and may be a useful tool for plant breeding and the conservation of genetic resources. The most important step for the breeding program to be successful is to work with the right genotypes. Additionally, genetic resources are national treasures of countries. UPGMA cluster analysis showed that most conspecific accessions tend to have high genetic similarity and cluster into the same group or subgroups. While *G. simonii* and *G. perfoliata* were found to be the most closely related species, *G. viscosa* appeared to be a separate species. Finally, using more genotypes in different species may provide more understandable results. The germplasm in this study should be a valuable source in Gypsophila breeding.

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Conflict of interest

The author declares that they have no conflict of interest.

REFERENCES

Akçali Giachino, R.R. (2023). Evaluation of some local and registered safflower (*Carthamus tinctorius* L.) Varieties based on SRAP markers. *KSU Journal of Agriculture and Nature 26* (6), 1325-1336. https://doi.org/10.18016/ksutarimdoga.vi.1168608

Al-Faifi, S.A., Migdadi, H.M., Al-doss, A., Ammar, M.H., El-Harty, E.H., Khan, M.A., Javed, M.M., & Alghamdi, S.S. (2013). Morphological and molecular genetic variability analyses of Saudi lucerne (*Medicago sativa* L.) landraces. *Crop and Pasture Science 64*,137-46. https://doi.org/10.1071/CP12271

Anderson, J.A., Churchill, G.A., Sutrique, J.E., Tanksley, S.D., & Sorrells, M.E. (1993). Optimizing parental selection for genetic linkage maps. *Genome 36*,181-186. https://doi.org/10.1139/g93-024

Anonymous (2018). Cut flower sector report. Republic of Turkey, Prime Ministry Foreign Trade Undersecretariat, Directorate General for Export, Antalya Exporters Union General Secretary, 32p. www.aib.org.tr, www.dtm.gov.tr. (Accessed: 20.10.2022).

Ataslar, E., Potoğlu, E., & Tokur, S. (2009). Pollen morphology of some *Gypsophila* L. (Caryophyllaceae) species and its taxonomic value. *Turkish Journal of Botany 3*, 335-351. https://doi.org/10.3906/bot-0810-19

Barakat, M.N., & El-Sammak, H. (2011) . In vitro culture and plant regeneration from shoot tip and lateral bud explants of *Gypsophila paniculata* L.. *Journal of Medicinal Plants Research 5*, 3351-3358. http://www.academicjournals.org/JMPR

Bargish, T.A., & Rahmani, F. (2016). SRAP Markers based genetic analysis of *Silene* species. *Journal of Tropical Biology and Conservation 13*, 57-70.

Barkoudah, Y.I. (1962). A revision of Gypsophila, Bolanthus, Ankyropetalum and Phryna. *Wentia 9,* 1-203.

Bogani, P., Calistri, E., Intrieri, M.C., Buiatti, M., Vettori, L., Schiff, S., et al. (2012). Novel tools for the genetic breeding of Gypsophila. *Acta Italus Hortus 4*, 58-64.

Bolger, M.E., Weisshaar, B., Scholz, U., Stein, N., Usadel, B., & Mayer, K.F.X. (2014). Plant genome sequencing - applications for crop improvement. *Current Opinion in Biotechnology 26,* 31-37. https://doi.org/10.1016/j.copbio.2013.08.019

Botstein, D., White, R.L., Skalnick, M.H., & Davies, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. *American Journal of Human Genetics 32*, 314-331.

Budak, H., Shearman, R.C., Parmaksiz, I., Gaussoin, R.E., Riordan, T.P., & Dweikat, I. (2004). Molecular characterization of buffalograss germplasm using sequence-related amplified polymorphism markers. *Theoretical and Applied Genetics 108*, 328-334. https://doi.org/10.1007/s00122-003-1428-4

Calistri, E., Buiatti, M., & Bogani, P. (2014). Characterization of *Gypsophila* species and commercial hybrids with nuclear whole-genome and cytoplasmic molecular markers. *Plant Biosystems 1*-11. https://doi.org/10.1080/11263504.2014.944609

Chesnokov, Yu.V.,& Artemyeva, A.M. (2015). Evaluation of the measure of polymorphism information of genetic diversity. *Agricultural Biology 50*, 571-578.

Dice, L.R. (1945). Measures of the amount of ecologic association between species. *Ecology 26*, 297-302.

Doyle, J.J., & Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus, 12*, 13-15.

Dudley, J.W. (1994). Comparison of genetic distance estimators using molecular marker data. Joint Plant Breeding Symposia 3-7.

Ghislain, M., Zhang, D., Fajardo, D., Huaman, Z., & Hijmans, R. (1999). Marker-assisted samplingof the cultivated andean potato *Solanum phureja* collection using RAPD markers. *Genetic Resources and Crop Evolution 46*, 547-555. https://doi.org/10.1023/A:1008724007888

Hamrick, J.L., & Godt, M.J.W. (1989). Allozyme diversity in plant species. In: Brown A.H.D., Clegg M.T., Kahler A.L. and Weir B.S. Eds. Plant Population Genetics, Breeding, and Germplasm Resources, 43-63, Sinauer Associates, Sunderland.

Intrieri, M,C., Calistri, E., Nieddu, F., Pasqualetto, P., Marcello, B., & Patrizia, B. (2010). Molecular characterization of gypsophila cultivars with ISSR and TRAP markers. Proceedings of the 54th Italian Society of Agricultural Genetics Annual Congress.

Jin, C., Liu, B., Ruan, J., Yang, C.,& Li, F. (2022). Development of InDel Markers for *Gypsophila paniculata* based on genome resequencing. *Horticulturae 8*, 921. https://doi.org/10.3390/horticulturae8100921

Kanayama, Y., Kato, K., & Moriguchi, R. (2007). Genetic and molecular aspects of *Gypsophila*. *Genes, Genomes and Genomics 1*(1), 63-65.

Karagüzel, O. (2003). Influence of different greenhouse conditions on growth and flowering of *Gypsophila paniculata* 'Perfecta'. *Mediterranean Agricultural Sciences 16*, 51-60. https://dergipark.org.tr/tr/pub/akdenizfderg/issue/1585/19690

Karagüzel, O., & Ortaçeşme, V. (2000). The effect of planting frequency on yield, quality and efficient use of lighting energy in Gypsophila cultivation. *Turkish Journal of Agriculture and Forestry 24* (6), 691-697. https://journals.tubitak.gov.tr/agriculture/vol24/iss6/8

Kaya A.S., Karagüzel Ö., Aydınşakir K., Kazaz S., Özçelik A. 2012. Usage possibilities of some Gypsophila (*Gypsophila* sp.) species naturally grown in Turkey as ornamental plants. *Derim 29,* 37-47.

Kaya, A.S., Aydinşakir, K., Erdal, Ş., & Kazaz, S. (2019). The effects of different applications on the cut flower performance of the promising *Gypsophila* genotype (GA 8). *Derim 36*, 13-23.

Kelemen, C.D., Hârta, M., Borsai, O., Szabo, K., Clapa, D., Kokoska, L., & Pamfil, D. (2018). Genetic diversity and relatedness among six Ranunculus species unraveled by SRAP markers. *Bulletin UASVM Horticulture 75*,169-176. http://dx.doi.org/10.15835/buasvmcn-hort:2018.0032

Kołodziej, B., Okoń, S., Nucia, A., Ociepa, T., Luchowska, K., Sugier, D., Gevrenova, R., & Henry, M. (2018). Morphological, chemical, and genetic diversity of *Gypsophila* L. (Caryophyllaceae) species and their potential use in the pharmaceutical industry. *Turkish Journal of Botany 42,* 257-270. Doi:10.3906/bot-1707-13

Korkmaz M., Özçelik H. 2011a. Systematical and morphological characteristics of annual Gypsophila L. (Caryophyllaceae) taxa of Turkey. *Biological Diversity and Conservation (Biodicon) 4*:79-98.

Korkmaz, M., & Özçelik, H. (2011b). Economic importance and using purposes of *Gypsophila* L. and *Ankyropetalum* Fenzl and *Saponaria* L. (Caryophyllaceae) taxa of Turkey. *African Journal of Biotechnology 10,* 9533-9541. http://dx.doi.org/10.5897/AJB10.2500

Korkmaz, M., & Doğan, N.Y. (2015). Biogeographic pattern of genetic diversity detected by RAPD and ISSR analysis in *Gypsophila* (Caryophyllaceae) species from Turkey. *Genetics and Molecular Research 14*,8829-8838.

https://doi.org/10.4238/2015.august.3.6

Lachmayer, M. (2009). Genetic patterns within and among highly isolated populations of *Gypsophila fastigiata* subsp. *arenaria* (Caryophyllaceae) at its distribution margins and evaluation of restoration measures [dissertation]. Department of Systematic and Evolutionary Botany, University of Vienna.

Li, G., & Quiros, C.F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. *Theoretical and Applied Genetics 103,* 455-461.

Madhani, H., Rabeler, R.K., Heubl, G., Madhani5, N., & Zarre, S. (2023). Dynamics of evolution in Irano-Anatolian and Caucasus biodiversity hotspots: Evolutionary radiation and its drivers in *Gypsophila* (Caryophyllaceae). bioRxiv 2023.11.24.568494 https://doi.org/10.1101/2023.11.24.568494

Mahmood, M.A., Hafiz, I.A., Abbasi, N.A., Faheem, M. (2013). Detection of genetic diversity in Jasminum species through RAPD techniques. *International Journal of Agriculture and Biology* 15, 505-510. http://www.fspublishers.org

Martínez-Nieto, M.I., Segarra-Moragues, J.G., Merlo, E., Martínez-Hernández, F., & Mota, J.F. (2013). Genetic diversity, genetic structure and phylogeography of the Iberian endemic *Gypsophila struthium* (Caryophyllaceae) as revealed by AFLP and plastid DNA sequences: connecting habitat fragmentation and diversification. Botanical Journal of the Linnean Society, 173(4), 654-675. https://doi.org/10.1111/boj.12105

Mantel, N.A. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research 27*, 209-220.

Mhiret, W.N., & Heslop-Harrison, J.S. (2018). Biodiversity in Ethiopian linseed (*Linum usitatissimum* L.):molecular characterization of landraces and some wild species. *Genetic Resources and Crop Evolution 65*, 1603-1614. https://doi.org/10.1007/s10722-018-0636-3

Mishra, M.K., & Jayarama, S.N. (2011). Genetic relationship among Indigenous Coffee species from India using RAPD, ISSR, and SRAP marker analysis. *Biharean Biologists 5*, 17-24. http://biologie-oradea.xhost.ro/BihBiol/index.html

Nagaraju, J., Damodar, R.K., Nagaraja, G.M.,& Sethuraman, B.N. (2001). Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silkworm, *Bombyx mori*. *Heredity 86*, 588-597. doi: 10.1046/j.1365-2540.2001.00861.x.

Nagl, N., Taški-Ajduković, K., Popovic, A., Curcic, Z., Danojevic, D., & Kovacev, L. (2011). Estimation of genetic variation among related sugar beet genotypes by using RAPD. *Genetika 3*, 575-582. http://dx.doi.org/10.2298/GENSR1103575N

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., & Rafal-Ski, A. (1996). The utility of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding 2*, 225-238. https://doi.org/10.1007/BF00564200

Rady, M.R. (2006). In vitro culture of *Gypsophila paniculata* L. and random amplified polymorphic DNA analysis of the propagated plants. *Biologia Plantarum 50*, 507-513. https://doi.org/10.1007/s10535-006-0080-7

Rajwade, A.E., Arora, R.S., Kadoo, N.Y., Harsulkar, A.M., Ghorpade, P.B.,& Gupta, V.S. (2010). Relatedness of Indian flax genotypes (*Linum usitatissimum* L.): An Inter-Simple Sequence Repeat (ISSR) primer assay. *Molecular Biotechnology 45*, 161-170. https://doi.org/10.1007/s12033-010-9256-7

Rohlf, F. J., & Fisher, D.L. (1968). Test for hierarchical structure in random data sets. *Systematic Biology, 17*, 407-412. https://doi.org/10.1093/sysbio/17.4.407

Rohlf, F.J. (2000). NTSYS-pc, numerical taxonomy and multivarite analysis system, version 2.02. New York, Exeter, Setauket.

Serrote, C.M.L., Reiniger, L.R.S., Silva, K.B., dos Santos Rabaiolli, S.M., & Stefanel, C.M. (2020). Determining the polymorphism information content of a molecular marker. *Gene 726*, 144175. https://doi.org/10.1016/j.gene.2019.144175

Smith, J.S.C., Chin, E.C.L., Shu, H., Smith, O.S., Wall, S.J., Senior, M.L., Mitchell, S.E., Kresovich, S., & Ziehl,e L. (1997). An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPS and pedigree. *Theoretical and Applied Genetics 95*, 163-173. https://doi.org/10.1007/s001220050544

Souza, E., & Sorrells, M.E. (1991). Relationships among 70 North American Oat germplasms:I. Cluster analysis using quantitative characters. *Crop Sciences 31*, 599-605. http://dx.doi.org/10.2135/cropsci1991.0011183X003100030010x

Xiao Peng, F., Guo Gui, N., Li Ping, G., & Man Zhu, B. (2008). Genetic diversity of Dianthus accessions as assessed using two molecular markers system (SRAPs and ISSRs) and morphological traits. *Scientia Horticulturae 117*, 263-270. http://dx.doi.org/10.1016/j.scienta.2008.04.001

Xie, W., Zhang, X., Cai, H., Liu, W., & Peng, Y. (2010). Genetic diversity analysis and transferability of cereal EST-SSR markers to orchardgrass (*Dactylis glomerata* L.). *Biochemical Systematics and Ecology 38*, 740-749.

https://doi.org/10.1016/j.bse.2010.06.009

van Zonneveld, M., Dawson, I., Thomas, E., Scheldeman, X., van Etten, J., Loo, J., et al. (2014). Application of molecular markers in spatial analysis to optimize in situ conservation of plant genetic resources. In: Tuberosa R, Graner A, Frison E, editors. Genomics of plant genetic resources. The Netherlands: Springer 67-91.

Zvi, M.M.B., Zuker, A., Ovadis, M., Shklarman, E., Ben-Meir, H., Zenvirt, S., & Vainstein, A. (2008). Agrobacterium- mediated transformation of Gypsophila (*Gypsophila paniculata* L.). *Molecular Breeding 22*, 543-553. https://doi.org/10.1007/s11032-008-9197-z