International Journal of Agricultural and Natural Sciences
E-ISSN: 2651-3617 16(3): 322-330, 2023
doi: 10.5281/zenodo.10434225
Research Article

EVALUATION OF GENETIC DIVERSITY IN DAISY SPECIES BASED ON MORPHOLOGICAL AND MOLECULAR MARKERS

Hüseyin Özsoy, Akife Dalda Şekerci*, Özhan Şimşek

Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri, Türkiye

**Corresponding Author:*
E-mail: akifedalda@erciyes.edu.tr

(Received 14th November 2023; accepted 06th December 2023)

ABSTRACT. The daisy, a member of the *Asteraceae* family, represents the most diverse family in both species and genus. Beyond its role as a prized ornamental plant, the daisy holds significance as a medicinal, aromatic herb owing to its active constituents. This study aims to assess the genetic diversity and levels of similarity among 21 daisy genotypes, encompassing 11 distinct species, utilizing morphological and molecular markers. The results of this investigation reveal extensive variability in daisies concerning morphological traits. Moreover, the findings underscore the high variability among daisy species regarding plant growth patterns, leaf characteristics, flower shapes, and flower colors. Genetic diversity was evaluated utilizing Inter-primer binding site (iPBS) markers, a molecular marker system based on retrotransposons. A total of 9 iPBS primers were identified, resulting in a polymorphism rate of 98%, as evidenced by the detection of 99 polymorphic bands. The dimensions of genetic diversity were quantified, revealing similarity indexes among genotypes ranging from 0.41 to 0.83. Through the construction of two and three-dimensional principal component analysis (PCA) graphics aligning with genetic parameters, it was observed that genotypes clustered based on species. This study contributed to the characterization of 11 distinct daisy species. Although this study was conducted in a limited species, it is remarkable that there is a high variation in morphological and genetic characteristics. The sizes of genetic differences and similarities among daisy genotypes of different species were highlighted. The results indicate a broad variation in both morphological and genetic features of daisy species, providing valuable guidance for future research endeavors.

Keywords: *Asteraceae, iPBS, ornamental plant, daisy*

INTRODUCTION

The *Compositae* (*Asteraceae*) is the largest and most thriving flowering plant family globally, comprising 1700 genera and 25,000 species. Found in diverse ecosystems worldwide, excluding Antarctica, these plants thrive in open areas and include familiar garden varieties such as sunflowers, daisies, artichokes, thistles, and lettuce, collectively constituting approximately 10% of all flowering plants [1-4]. The Asteraceae family holds significant taxonomic richness among flowering plants [5]. With three subfamilies, 1535 genera, and nearly 23,000 species, the *Asteraceae* family is a pivotal contributor to the diversity of flowering plants. According to the Turkish Plant List, it represents the most abundant family in our flora, with 136 genera and 1345 species [6, 7]. Daisy, a valuable plant belonging to the *Asteraceae* family, serves both as an ornamental species and a significant medicinal herb due to its bioactive compounds. Embracing annual and perennial species, daisy exhibits diverse leaf forms and draws attention with its delightful white and purple flowers. Despite its importance, studies on daisies' ornamental use and genetic diversity are limited. Investigations into the genetic diversity of daisies are crucial for their effective conservation and adaptation to various applications.

The *Asteraceae* Family, characterized by many species, showcases considerable morphological variations. However, comprehensive studies elucidating these species' genetic similarities and differences remain limited. Molecular markers are pivotal in validating recorded morphological variations, particularly when subjected to environmental influences [8]. These markers, defined as DNA sequences on the genome, aid in identifying genetic diversity and similarity levels within populations [9].

Molecular techniques, integral applications within biotechnology, accelerate the process of plant breeding by harnessing genetic traits. DNA-based molecular markers, particularly those reliant on polymerase chain reaction (PCR), provide autonomous assessments of genetic diversity. Notably, the iPBS method, a primer binding approach based on retrotransposons, emerges as a powerful tool for discerning genetic variations [10]. Retrotransposons, distributed throughout the genome, possess common structural features, and contribute to genome-wide regulations, making them suitable as molecular markers [11]. This study aims to characterize 21 distinct daisy genotypes from diverse species, employing a combination of morphological and molecular traits.

MATERIAL AND METHODS

Plant Materials

The genotypes of 21 different daisy genotypes belonging to commercial outdoor ornamental plants, including *Leucanthemum maximum, Argyranthemum frutescens, Euryops pectinatus, Dimorphotheca ecklonis, Coreopsis grandiflora, Leucanthemum vulgare, Felicia amelloides*, also commonly used for outdoor landscaping, as well as naturally occurring varieties such as *Anthemis arvensis, Anthemis cotula, Matricaria recutita,* and *Senecio vulgaris* have been collected. Various Daisy species exhibiting distinct morphological characteristics and serving as ornamental plants were sampled from commercial varieties and naturally occurring plants in Türkiye (Table 1).

Morphological Characterization

The 21 daisy genotypes, representing 11 diverse species, were characterized based on morphological traits, including plant height, leaf type, leaf length, leaf width, petal color, and disc color. The experiments followed the parameters of *Asteraceae* UPOV (International Union for the Protection of New Varieties of Plants, UK).

DNA Isolation and PCR Amplification

Genomic DNA extraction from young leaves of the daisy genotypes was performed using the modified CTAB method [12], followed by purification through [13] methodology. Subsequently, the genomic DNA underwent PCR amplification using 9 iPBS primers, as outlined in Table 2. The PCR reaction was conducted in a 15 µl volume, comprising 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) for each iPBS primer. The cycling conditions for PCR were as follows: an initial denaturation at 95 °C for 4 minutes, followed by 35 cycles at 95 °C for 1 minute, 48-63 °C for 1 minute, 72 °C for 1 minute, and a final extension step at 72 °C for 7 minutes. The amplified products were separated on a 1.5% agarose gel at 110 V for 3 hours, employing TBE (Tris-Boric acid-EDTA) buffer. Subsequently, the gel was visualized under UV light following staining with ethidium bromide, and images were captured using a gel documentation system. DNA

fragments were scored as a binary data matrix (1 for the presence and 0 for the absence of a band). Cluster analysis among the 21 Daisy genotypes was conducted utilizing Dice's similarity coefficient [14] with the unweighted pair-group with arithmetic average method (UPGMA) SAHN clustering algorithm. The analyses were performed using NTSYS-pc (Numerical Taxonomy Multivariate Analysis System, NTSYS, 2.11, USA). Various parameters were determined, including the total number of fragments, number of polymorphic fragments, mean polymorphism, allele frequency, number of effective alleles, Shannon's information index, expected heterozygosity, and unbiased expected heterozygosity ratio. Mantel test was executed to assess the correlation between the *Dice* and *Jaccard* similarity matrices. Principal Component Analysis (PCA) based on the variance-covariance matrix was conducted. In the initial stage of PCA, a correlation matrix was generated using the SİMINT module. Subsequently, eigen vectors were calculated using this matrix through the EIGEN module. Two-dimensional and three-dimensional graphics were then generated using the eigen vectors in the PROJ module.

RESULTS AND DISCUSSION

Morphological Results

The 21 daisy genotypes, representing 11 distinct species, were characterized based on morphological traits. Notable differences in growth patterns were observed among the species after scrutinizing their plant growth tendencies. Leaf characteristics displayed considerable variation across all species, with certain species like *Leucanthemum maximum* showcasing long and narrow leaves. In contrast, naturally occurring species such as *Anthemis cotula, Matricaria recutita*, and *Senecio vulgaris* were identified by shorter and more delicate leaf structures (Figure 1). In naturally occurring species, the predominant colors of the ray florets are yellow and white, whereas in commercial varieties, a spectrum of ray floret colors has been observed, including white, pink, dark red, and purple. Among the species, *Leucanthemum maximum* stands out with the largest flower diameter, while *Felicia amelloides* is identified as having the smallest. Additionally, it has been observed that in most species, the color of the flower disc is yellow. Furthermore, genotypes of the *Dimorphotheca ecklonis* species were found to exhibit a purple disc color (Table 1).

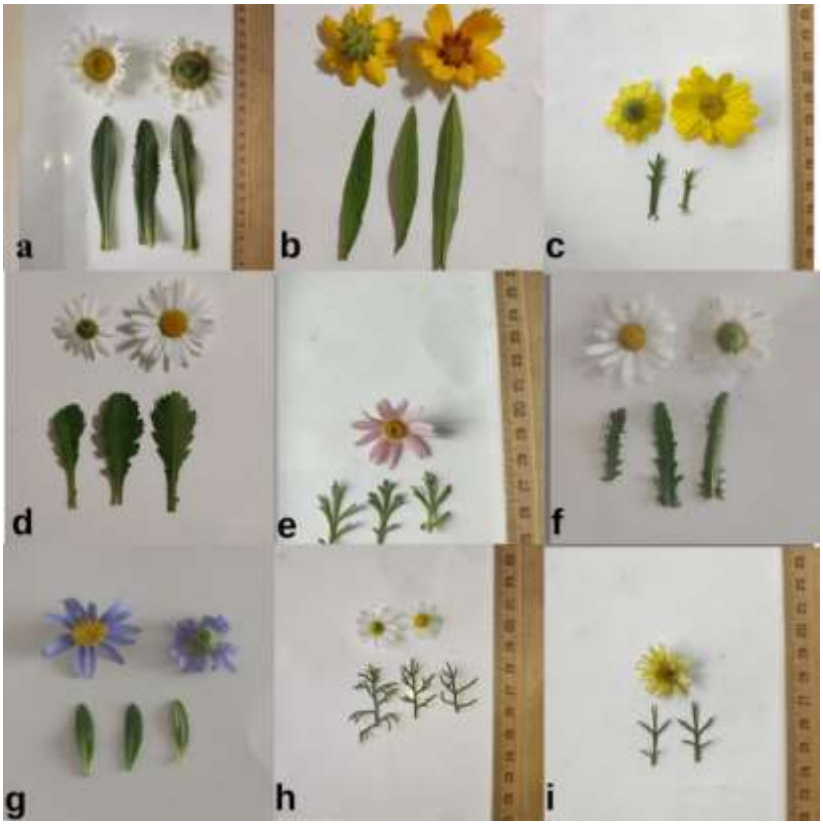

Figure 1. *Visual of flower morphological properties in daisy genotypes (**a:** Leucanthemum maximum; **b:** Coreopsis grandiflora; **c, e, i:** Argyranthemum frutescens; **d,f:** Leucanthemum vulgare **g:** Felicia amelloides; **h:** Anthemis cotula)*

Table 1. *Morphological observation results of daisy genotypes*

Genotype	Species	Plant Height	Leaf Type	Leaf Length	Leaf Width	Petal Color	Disc Color
1	*Leucanthemum maximum*	tall	simple	long	narrow	white	yellow
2	*Argyranthemum frutescens*	medium	pinnate	medium	medium	pink	yellow
3	*Argyranthemum frutescens*	medium	pinnate	medium	medium	purple	yellow
4	*Argyranthemum frutescens*	medium	pinnate	medium	medium	multicolor	yellow
5	*Argyranthemum frutescens*	medium	pinnate	medium	medium	yellow	yellow
6	*Argyranthemum frutescens*	medium	pinnate	medium	medium	white	yellow
7	*Argyranthemum frutescens*	medium	pinnate	medium	medium	red	yellow
8	*Euryops pectinatus*	tall	pinnate	medium	broad	yellow	yellow
10	*Argyranthemum frutescens*	medium	pinnate	medium	medium	yellow	yellow
12	*Dimorphotheca ecklonis*	medium	simple	long	narrow	purple	purple
13	*Dimorphotheca ecklonis*	medium	simple	long	narrow	pink	purple
14	*Dimorphotheca ecklonis*	medium	simple	long	narrow	white	purple
15	*Argyranthemum frutescens*	medium	pinnate	medium	medium	yellow	yellow
16	*Coreopsis grandiflora*	tall	simple	long	narrow	orange	yellow
18	*Coreopsis grandiflora*	tall	simple	long	narrow	yellow, red	yellow
20	*Leucanthemum vulgare*	medium	simple	long	narrow	white	yellow
21	*Felicia amelloides*	short	simple	short	narrow	purple	yellow
23	*Matricaria recutita*	medium	pinnate	medium	narrow	white	yellow
24	*Senecio vulgaris*	medium	simple	medium	narrow	yellow	yellow
27	*Anthemis cotula*	tall	pinnate	short	medium	white	yellow
28	*Anthemis arvensis*	tall	pinnate	short	medium	white	yellow

Molecular Results

The genetic diversity dimensions of 21 daisy genotypes were assessed through molecular methods, employing 9 primers for amplification. In the study, 99 bands were generated using the 9 iPBS primers, of which 95 were identified as polymorphic and 4 as monomorphic. The calculated average polymorphism value stood at 96%. The band sizes observed for iPBS primers ranged from 200 to 1500 base pairs (bp), with iPBS 2226 exhibiting the highest amplification with 18 bands, while iPBS-2252 and iPBS-2231 displayed the lowest amplification, each with 7 bands (Table 5). The expected and observed allelic frequency values (p, q) depending on the iPBS primers ranged from 0,189 to 0,351 and from 0,811 to 0,649, respectively. The number of effective alleles (Ne) ranged from 1,424 iPBS-2226 to 1,703 iPBS-2232, Shannon's information index (I) values ranged from 0,493 to 0,590, expected heterozygosity (He) values from 0.283 to 0.401 and unbiased expected heterozygosity (uHe) values from 0.291 to 0.413 (Table 2). Cluster analysis was conducted through the UPGMA method utilizing the Dice similarity index derived from 9 iPBS primers across 21 daisy genotypes, forming a dendrogram (Figure 2). Based on this dendrogram and the associated Dice similarity matrix, the genetic similarity levels among the daisy genotypes ranged from 0.41 to 0.88. The dendrogram analysis revealed distinct clusters formed within each species. Genotypes of *Argyranthemum frutescens* belonging to individuals numbered 2, 3, 4, 5, and 6 are observed to be grouped in the same branch, with a similarity index exceeding 80%. Similarly, genotypes 12, 13, and 14 are identified to belong to the species *Dimorphotheca ecklonis*, as they cluster together on the same branch with a genetic similarity of 82% (Figure 2). While genotypes belonging to the same species are closely positioned, the noticeable occurrence of genetic variation within the species is noteworthy. Additionally, identifying high genetic variation among different species of daisies, popular ornamental plants, through this study is promising for breeding efforts. The diverse range of species within the same ornamental category and the high genetic variation among these species offer hopeful prospects for future breeding endeavors.

Table 2. *Polymorphism values of iPBS primers*

Primary	**Sequence 5'-3'**	TNF	NPF	Band size (bp)	**p**	**q**	**Ne**	**I**	**He**	**uHe**
iPBS 2389	ACATCCTTCCCA	10	10	250-1150	0,239	0,761	1,535	0,493	0,323	0,331
iPBS 2393	TACGGTACGCCA	10	9	300-1100	0,250	0,742	1,581	0,535	0,344	0,363
iPBS 2226	CGGTGACCTTTGATACCA	18	17	200-1500	0,189	0,811	1,424	0,446	0,283	0,291
iPBS 2379	TCCAGAGATCCA	9	9	200-1100	0,303	0,697	1,640	0,517	0,354	0,363
iPBS 2380	CAGACGGCGCCA	12	11	250-1200	0,246	0,754	1,556	0,517	0,341	0,349
iPBS 2381	GTCCATCTTCCA	8	8	400-1200	0,207	0,793	1,482	0,468	0,301	0,328
iPBS 2383	GCATGGCCTCCA	15	14	225-1300	0,304	0,696	1,635	0,500	0,370	0,379
iPBS 2232	AGAGAGGCTCGGATACCA	10	9	300-1100	0,300	0,700	1,703	0,590	0,401	0,413
iPBS 2231	ACTTGGATGCTGATACCA	7	7	500-1350	0,351	0,649	1,662	0,548	0,374	0,386
Mean	-	**11**	**10.4**	**150-1200**	**0,265**	**0,733**	**1.579**	**0.512**	**0.343**	**0.355**

*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

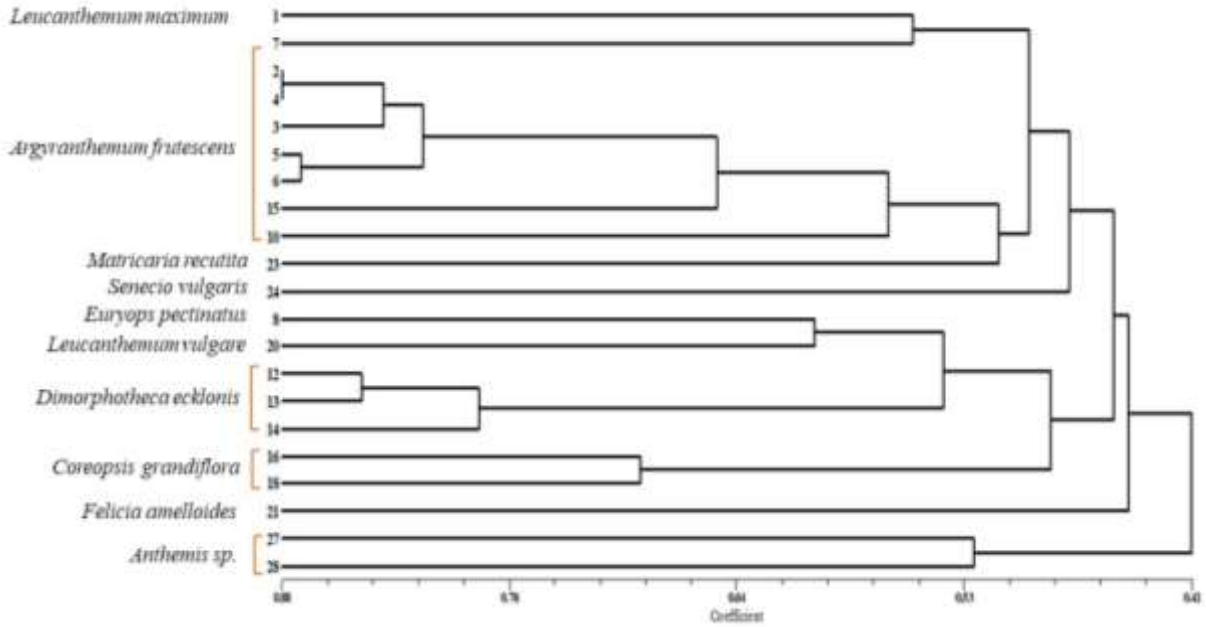

Figure 2. *The UPGMA analysis based on Dice coefficients of iPBS markers from the daisy genotypes.*

DNA matrix data generated from different similarity matrices using primers underwent correlation analysis through the mantel test in the NTSYS program. The resulting Mantel correlation value (r = 0.99543) was high. Two and three-dimensional graphics were created using principal component analysis in the NTSYS program. In the principal components analysis, the cumulative sum of the first three eigenvalues was 43.91, explaining 44% of the total variation. According to the two-dimensional principal component analysis plot, most genotypes were dispersed rather than clustered. However, genotypes belonging to the same species and those with similar morphological properties were observed to be genetically close to each other (Figure 3).

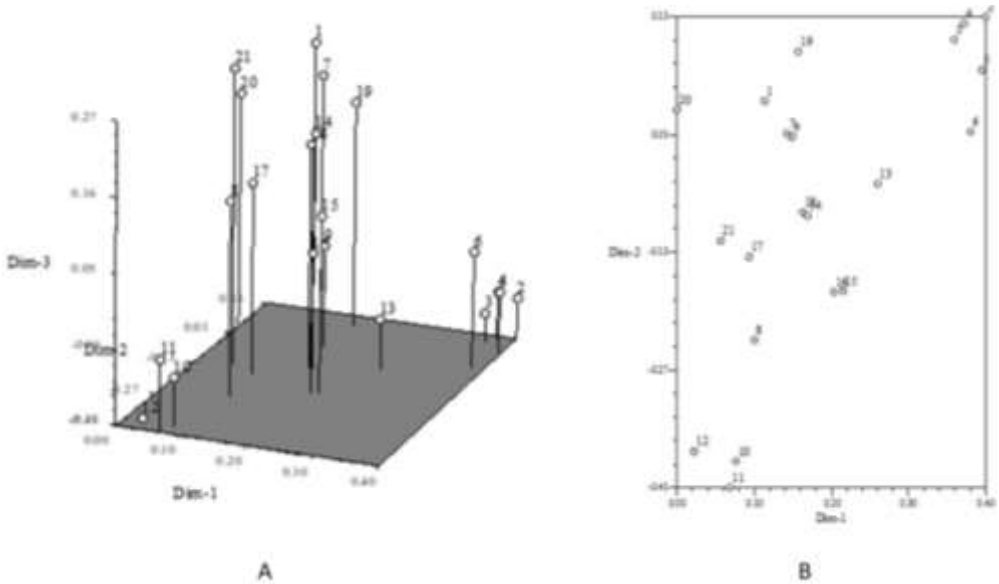

Figure 3. *Two-dimensional (B) and three-dimensional (A) graphics based on principal component analysis with iPBS data in 21 daisy genotypes.*

The results obtained in this study revealed a broad spectrum of daisy genotypes concerning flower shape, color, and size. Consistent with these findings, prior studies, such as the work conducted by [15], have indicated considerable variations in flower characteristics among members of the Asteraceae family. Moreover, these studies highlighted that the observed morphological differences in flowers could be subject to variation based on climatic conditions [3, 15].

In this study, notable distinctions were identified in both leaf sizes and shapes. The significance of leaf characteristics in breeding studies was underscored, as they are crucial in selecting parental sources for breeding programs. Moreover, these characteristics offered valuable insights into the extent of variation within the studied daisy genotypes. Similarly, the study highlighted the importance of leaf length and petiole length as crucial parameters in evaluating hybrid varieties within breeding studies. It was reported that these lengths were anticipated to reflect an average value derived from the parent plants in the developed hybrid cultivars [16].

Many researchers state that molecular markers minimize the effect of environmental factors in breeding studies and distinguish varieties and genotypes. The present study attempted to probe genetic diversity through iPBS molecular markers. The genetic similarity level of daisy genotypes was between 0.41 and 0.88. The findings obtained in this study were consistent with previous studies. In the study conducted with 43 genotypes of *A. frutescens* species, the variation was reported to be relatively high, with an average of 38 polymorphic markers produced per primer combination by the AFLP molecular marker method [17]. Similar results were reported by Doveri et al. [18] with a high level of polymorphism in *A. frutescens*. It has been reported that the high degree of polymorphism may result from the outcrossing nature of the species, and the varieties may be chimeras, or the primary explants may be heterozygous. It has been reported that a significant degree of variation has been identified using Random Amplified Polymorphic DNA (RAPD) markers in chrysanthemum, a member of the *Asteraceae* family [19]. Genetic diversity in chrysanthemum has been explored using various molecular markers. Huang et al. [20] investigated three hybrid combinations of chrysanthemum employing 24 RAPD primers to assess the genetic similarity between parents and new plants. Martin et al. [19] also characterized fifteen commercial chrysanthemum cultivars with RAPD molecular markers, reporting genetic similarity levels between cultivars ranging from 0.50 to 0.80. In another study using molecular markers ISSR and SRAP, the genetic similarity coefficients ranged from 0.6154 to 0.9835, from 0.6214 to 0.9959, or from 0.5350 to 0.9794, depending on the respective analysis techniques of ISSR, SRAP, and combined ISSR and SRAP [21].

In a study on *Chrysanthemum morifolium*, Chang et al. [22] utilized 10 simple sequence repeat (SSR) markers to identify 88 chrysanthemum accessions. The observed similarity coefficient varied between 0.53 and 0.88. Another investigation characterized the genetic variation of a population of 38 Indian chrysanthemum cultivars using RAPD molecular markers, revealing a genetic similarity ranging from 0.41 to 0.90 [23]. Olejnik et al. [24] emphasized the difficulty of distinguishing chrysanthemum genotypes based solely on morphological features, highlighting the importance of employing molecular methods. Their study used 14 SSR markers to evaluate the genetic diversity of 97 chrysanthemum cultivars, determining that these cultivars were genetically classified into four groups based on their morphological characteristics. In another study on species within the *Asteraceae* family, Dalda-Şekerci [13] reported high morphologically and genetically variations in this family.

CONCLUSION

In this study, an evaluation was conducted on 21 daisy genotypes belonging to commercially preferred and naturally occurring species. The results demonstrate that these daisy genotypes, characterized by significant variations in morphological features such as plant, leaf, and flower characteristics, also exhibit extensive genetic diversity. Despite the dual significance of daisies as both ornamental and medicinal aromatic plants, there is limited prior research on various daisy species. Furthermore, the identification of genetic variability in ornamental plant breeding is deemed crucial based on the study's findings. This study aimed to assess the genetic similarities and differences, alongside ornamental characteristics, of 21 daisy genotypes. Despite the limited number of genotypes examined, the combined analyses of both morphological and molecular aspects unveiled a noteworthy level of variation. These findings offer essential insights for including these daisy species in breeding programs. The results of this study underscore the high variation in genetic and morphological characteristics among daisy genotypes. Therefore, it is recommended that future breeding programs undertake more comprehensive studies based on a broad genetic pool.

Further research on the genetic diversity and morphological features of different chamomile species could be beneficial, particularly in better understanding the potential for their use in ornamental plant breeding. The findings of this study have contributed to the understanding of genetic similarities and differences among daisy genotypes. Consequently, future similar studies could conduct more extensive genetic analyses with larger genotype populations. In conclusion, the findings establish an important foundation for enhancing ornamental plant breeding and understanding genetic diversity. Larger-scale and more comprehensive studies may shed further light on the genetic richness of daisy genotypes, contributing to developing more effective and resilient plant varieties in horticulture.

Acknowledgement. This study has been supported by TÜBİTAK (The Scientific and Technological Research Council of Turkey) under Project Number 2209-A. Also, we would like to thank the Office of the Dean for Research at Erciyes University for providing the necessary infrastructure and laboratory facilities at the ArGePark research building.

REFERENCES

[1] Jeffrey C. (2007): Compositae: Introduction with key to tribes. In: Kubitzki K. ed. The families and genera of vascular plants. Berlin: Springer Verlag, 61–87.

[2] Barreda, V. D., Palazzesi, L., Katinas, L., Crisci, J. V., Telleria, M. C., Bremer, K., Corsolini, R. (2012): An extinct Eocene taxon of the daisy family (*Asteraceae*): evolutionary, ecological, and biogeographical implications. Annals of botany, *109*(1), 127-134.

[3] McDonald-Spicer, C., Knerr, N. J., Encinas-Viso, F., & Schmidt-Lebuhn, A. N. (2019): Big data for a large clade: bioregionalization and ancestral range estimation in the daisy family (*Asteraceae*). Journal of Biogeography, *46*(2), 255-267.

[4] Rolnik, A., & Olas, B. (2021): The plants of the Asteraceae family as agents in the protection of human health. International journal of molecular sciences, *22*(6), 3009.

[5] Seçmen, Ö., Gemici, Y., Görk, G., Bekat, L. ve Leblebici E., (2000): Tohumlu Bitkiler Sistematiği, Ege Üniversitesi, Fen Fakültesi, Bornova, İzmir.

[6] Bremer, K., (1994): *Compositae*. Cladistics and classification. Portland, Oregon. Timber Press. U. S. A.

[7] Uncu, H. (2019): Kırıkkale'de Doğal Yayılış Gösteren *Asteraceae* (Papatyagiller) Familyasına Ait Bazı Taksonlarda Yaprak Epidermisi Üzerine Bir Çalışma (master's thesis, Kırıkkale University).
[8] Li, F., Zhang, H., Zhao, H., Gao, T., Song, A., Jiang, J., Chen, S. (2018): Chrysanthemum Cm HSFA 4 gene positively regulate salt stress tolerance in transgenic chrysanthemum. Plant Biotechnology Journal, 16(7), 1311-1321.
[9] Yang HB, Kang WH, Nahm SH, Kang, BC (2015): Methods for developing molecular markers. In Current technologies in plant molecular breeding (pp. 15-50). Springer, Dordrecht.
[10] Kalendar, R., Flavell, A. J., Ellis, T. H. N., Sjakste, T., Moisy, C., Schulman, A. H. (2011): Analysis of plant diversity with retrotransposon-based molecular markers. Heredity, 106(4), 520-530.
[11] Kalendar, R., Schulman, A. H. (2014): Transposon-based tagging: IRAP, REMAP, and iPBS. Molecular plant taxonomy: methods and protocols, 233-255.
[12] Doyle JJ, Doyle JL (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15
[13] Dalda-Sekerci, A. (2023): Comprehensive assessment of genetic diversity in chrysanthemum germplasm using morphological, biochemical, and retrotransposon-based molecular markers. Genetic Resources and Crop Evolution, 1-16.
[14] Dice LR (1945): Measures of the amount of ecologic association between species. Ecology 26:297–302
[15] Wang, Y., Jung, J. A., Kim, W. H., Lim, K. B., Hwang, Y. J. (2021): Morphological and rDNA fluorescence in situ hybridization analyses of Chrysanthemum cultivars from Korea. Horticulture, Environment, and Biotechnology, 62(6), 917-925.
[16] Tang, F., Chen, S., Deng, Y., Chen, F. (2010): Intergeneric hybridization between Dendranthema crassum and Ajania myriantha. Acta horticulturae, 855, 267-272.
[17] Kjos, M., Fjellheim, S., Rognli, O. A., Hvoslef-Eide, A. K. (2010): Amplified Fragment Length Polymorphism (AFLP) markers for fingerprinting of Argyranthemum frutescens cultivars. Scientia Horticulturae, 124(4), 506-510.
[18] Doveri, S., Donini, P., Lee, D., Morreale, G., Berio, T., Allavena, A., Venturo, R., (2002): AFLP Fingerprinting in Marguerite Daisy (*Argyranthemum frutescens*). In: Proceedings of the XLVI Italian Society of Agricultural Genetics—SIGA Annual Congress Giardini Naxos, Italy, September 18–21.
[19] Martin, C., Uberhuaga, E., Pérez, C. (2002): Application of RAPD markers in the characterisation of Chrysanthemum varieties and the assessment of somaclonal variation. Euphytica, *127*, 247-253.
[20] Huang, S. C., Tsai, C. C., Sheu, C. S. (2000): Genetic analysis of *Chrysanthemum* hybrids based on RAPD molecular markers. Botanical Bulletin of Academia Sinica, *41*.
[21] Shao, Q. S., Guo, Q. S., Deng, Y. M., Guo, H. P. (2010): A comparative analysis of genetic diversity in medicinal chrysanthemum morifolium based on morphology, ISSR and SRAP markers. Biochemical Systematics and Ecology, 38(6), 1160-1169.
[22] Chang, L. U. O., Dongliang, C. H. E. N., Cheng, X., Hua, L. I. U., Yahui, L. I., Huang, C. (2018): SSR Analysis of genetic relationship and classification in chrysanthemum germplasm collection. Horticultural Plant Journal, *4*(2), 73-82.
[23] Kumar, S., Kumar, M., Yadav, H. K., Sharma, S. (2017): Genetic diversity and population structure analysis of chrysanthemum (Dendranthema grandiflora Tzvelev) germplasm based on RAPD markers. Journal of Environmental Biology, *38*(3), 457.
[24] Olejnik, A., Parkitna, K., Kozak, B., Florczak, S., Matkowski, J., Nowosad, K. (2021): Assessment of the Genetic diversity of chrysanthemum cultivars using SSR markers. Agronomy, *11*(11), 2318.