

Original Research Paper

Genetic divergence in Chrysanthemum (*Dendranthema x grandiflora* Tzvelev) based on morphological traits

Gurung A., Kumar R. *, Aswath C., Tejaswini P. and Bennurmth P.

Division of Flower and Medicinal Crops,
ICAR-Indian Institute of Horticultural Research, Bengaluru - 560089, Karnataka
*Corresponding author Email : Rajiv.Kumar11@icar.gov.in

ABSTRACT

Genetic diversity of thirty-one genotypes of Chrysanthemum were analysed for various growth and flowering related traits. Analysis of variance revealed significant differences among the genotypes for all the morphological traits studied. The clustering pattern based on Mahalanobis D^2 statistics categorised genotypes into six distinct clusters. The largest cluster *i.e.* cluster III composed of eleven genotypes followed by cluster II with nine genotypes, cluster I having eight genotypes and cluster IV, V, and VI with one genotype each. The maximum inter-cluster distance was recorded between clusters IV and cluster V (376.87) followed by clusters IV and cluster VI (344.96) and, cluster II and cluster IV (196.81). The maximum intra-cluster distance was observed for cluster III (56.57), followed by cluster II (46.87) and cluster I (29.52). Among all the clusters, genotypes in cluster II recorded highest cluster mean values for number of branches per plant (7.15), number of leaves (119.72) and flowers (91.69) per plant. Among nine characters, number of flowers per plant contributed maximum to divergence (32.26%). Therefore, for chrysanthemum improvement, highly diverse genotypes can be used as parents for crossing to generate high variability.

Keywords: Chrysanthemum, genetic diversity, Mahalanobis D^2 statistics, morphological traits

INTRODUCTION

Chrysanthemum is a high-profit floricultural crop belonging to the family Asteraceae and ranked second to rose in market trade (Nguyen et al., 2020), mainly grown for cut flower, loose, pot plant and landscaping. There are approximately 200 species in the genus Chrysanthemum, but most of them are sub-divided into 38 satellite genera of the chrysanthemum complex (Dalda-Sekerci, 2023). More recently, only 41 species have been attributed to the genus Chrysanthemum (Hadizadeh et al., 2022). The cultivated chrysanthemum is originally native of Asia (China and Japan) and northeastern Europe (Baliyan et al., 2014).

Morphological trait measurement is a commonly used index, since it provides a simple technique of quantifying genetic variation, while, simultaneously assessing genotype performance under normal growing environments (Fu et al., 2008). The hybridization of florist chrysanthemum began after 1850 when the practice of culturing chrysanthemum in greenhouses began. Several hundred cultivars have been commercialized, indicating that there are substantial

genetic variations that can be manipulated under cultivations to produce a wide array of phenotypic variation (Jo et al., 2015).

Germplasm collection and evaluation have received a lot of attention in India. However, the information for higher flower yield and yield contributing characters is limited. In order to choose diverse parents to complement various breeding programmes, it is necessary to estimate the genetic variation and method of inheritance of various plant traits in chrysanthemum.

In chrysanthemum, the investigation, classification, identification, and diversity analysis of the cultivars became highly challenging due to the large number of cultivars, abundant morphological variation, wide distribution and complex genetic background. Therefore, the present study was carried out to select suitable genotypes for breeding programmes based on the genetic diversity estimation conducted on a group of chrysanthemum genotypes obtained from various locations in India.



Table 1 : List of thirty-one chrysanthemum genotypes used for diversity study

Genotype	Source	Flower form	Growth habit
A1 Collection	Dooblabylekere, Bengaluru	Semi-double	Semi-erect
Appu	CSIR-NBRI, Lucknow	Semi-double	Erect
Arka Chandrakant	ICAR-IIHR, Bengaluru	Decorative	Spreading
Arka Chankdrika	ICAR-IIHR, Bengaluru	Decorative	Erect
Arka Kirti	ICAR-IIHR, Bengaluru	Double Korean	Erect
Arka Pink Star	ICAR-IIHR, Bengaluru	Semi-double	Spreading
Arka Usha Kiran	ICAR-IIHR, Bengaluru	Semi- double	Erect
Arka Yellow Gold	ICAR-IIHR, Bengaluru	Decorative	Erect
Autumn Joy	PAU, Ludhiana	Decorative	Spreading
Coffee	CSIR-NBRI, Lucknow	Semi- double	Spreading
Fitonia	CSIR-NBRI, Lucknow	Single	Semi-erect
Flirt	CSIR-NBRI, Lucknow	Decorative	Erect
Garden Beauty	PAU, Ludhiana	Spoon	Spreading
Gulmohar	CSIR-NBRI, Lucknow	Double Korean	Erect
Heritage	CSIR-NBRI, Lucknow	Semi-double	Semi-erect
Jubilee	CSIR-NBRI, Lucknow	Semi-double	Erect
Marigold	Local Collection	Pompon	Erect
Mayur	CSIR-NBRI, Lucknow	Semi-double	Erect
NBRI Little Kusum	CRIS-NBRI, Lucknow	Pompon	Spreading
Pachai Local	Local Collection	Semi-double	Spreading
Pink Cloud	CSIR-NBRI, Lucknow	Semi- double	Erect
Ratlam Selection	CSIR-NBRI, Lucknow	Decorative	Erect
Rekha	CSIR-NBRI, Lucknow	Single	Spreading
Shukla	CSIR-NBRI, Lucknow	Semi-double	Semi-erect
Statesman	PAU, Ludhiana	Semi-double	Erect
Sunil	CSIR-NBRI, Lucknow	Spoon	Erect
Vasanthika	CSIR-NBRI, Lucknow	Single	Semi-erect
White Dolley	CSIR-NBRI, Lucknow	Pompon	Spreading
White Local	Local Collection	Semi-double	Erect
White Prolific	CSIR-NBRI, Lucknow	Semi-double	Erect
Winter Queen	PAU, Ludhiana	Spoon	Spreading

MATERIALS AND METHODS

Plant material

The present study was carried out in the Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, during 2019-20 and 2020-21. The experimental site was geographically located at 13°58' N Latitude, 78°E Longitude and at an elevation of 890 meter above mean sea level. In total, thirty-one chrysanthemum genotypes obtained from different locations of India

(Table 1) were evaluated for various growth and flowering traits under naturally ventilated polyhouse in completely randomized design with three replications.

The plants of all genotypes were raised through terminal cuttings taken from healthy stock plants. After transplanting, plants were imposed with photoperiod of 15/9 hours for 30 days and 'black in' (dark conditions) until flower bud initiation. Uniform package of practices was followed throughout the experiment to ensure good growth. Five uniformly

Table 2 : Combined analysis of variance of nine characters for 31 genotypes of chrysanthemum

Source of variation	df	Plant height (cm)	No. of branches plant ⁻¹	No. of leaves plant ⁻¹	Days to bud initiation	Days to first flower opening	Days to optimum flowering	Flower diameter (cm)	No. of flowers plant ⁻¹	Flowering duration (days)
Treatment	30	8,230.95 **	58.67 **	13,872.46 **	471.52 **	3,867.80 **	3,852.28 **	22.18 **	9,507.31 **	902.86 **
Error	62	0.25	12.81	10.19	1.00	4.74	6.02	4.22	0.08	5.29

grown plants per replication were tagged for recording observations of various growth and flowering traits, viz., plant height (cm), number of branches and leaves per plant, days to bud initiation and first flower opening, number of flowers per plant, optimum flowering (the number of days taken to 50% flowering from the date of 'black in' treatment), flower diameter (cm) and flowering duration (days) to check superiority of genotypes. The data recorded for both the years were pooled and analyzed statistically.

Quantitative traits data analysis

The mean values of the genotypes in each replication for quantitative characters were used for statistical analysis. The data were processed with the help of the software programme SPAR-1 (Doshi & Gupta, 1991) utilizing various standard statistical procedures. The data recorded on nine different quantitative traits were subjected to the D^2 statistic of Mahalanobis, and grouping of the genotypes into different clusters was done by using Ward's minimum variance (Rao, 1952) and average intra-and-inter cluster distances were calculated following Tocher's method.

RESULTS AND DISCUSSION

Analysis of variance indicated highly significant differences among the genotypes for all the characters studied indicating the presence of sufficient amount of variability (Table 2).

Cluster constellation

Thirty-one genotypes were classified into six clusters on the basis of D^2 values calculated from the analysis of nine morphological traits (Fig. 1). Among all the clusters, cluster III being the largest consisted of eleven genotypes, followed by cluster II with nine genotypes, cluster I having eight genotypes and cluster IV, V, and VI with one genotype each (Table 4). The variable number of accessions in different clusters pointed toward the presence of wide range of genetic diversity within as well as between the clusters. Similarly,

Bhargav et al. (2023) recorded two clusters in China aster, while, Baliyan et al. (2014) reported four clusters in chrysanthemum, and Kavitha & Anburani (2009) recorded eight clusters in African marigold.

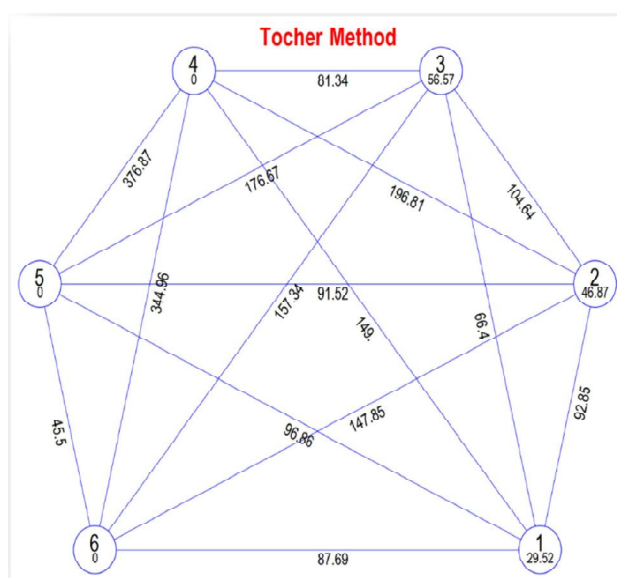


Fig. 1 : Inter and intra cluster distances for thirty-one genotypes in chrysanthemum

The group constellation of thirty-one chrysanthemum genotypes was also plotted in the form of a dendrogram (Fig. 2), where, Euclidian distances in dendrogram highlighted the genetic divergence between and within the clusters. The pattern of distribution of genotypes from various eco-geographical regions into various clusters with different divergence values was random, supporting that genetic diversity and geographical diversity are unrelated. Natural or artificial selection, breeding material exchange, genetic drift, and environmental variation may be the primary factors influencing this genetic diversity in addition to geographic origin. The results of present study are substantiated with the similar conclusions reported on diversity (Kumar et al., 2011; Baliyan et al., 2014) indicated that geographic diversity cannot always be used as an index of genetic diversity. It is quite

possible that many of the genotypes obtained from different geographical regions could share a common ancestor. Moreover, progenies obtained through breeding incorporate genes from varied sources, thus losing the basic geographical identity of the genotype (Bharathi & Jawaharlal, 2014).

Average inter as well as intra-cluster distances in all the genotypes were computed through Mahalanobis (D^2) analysis (Table 3). On the basis of D^2 values between two genotypes or two clusters, choice of divergent parents can be made for hybridization purpose. The inter-cluster distance ranged from

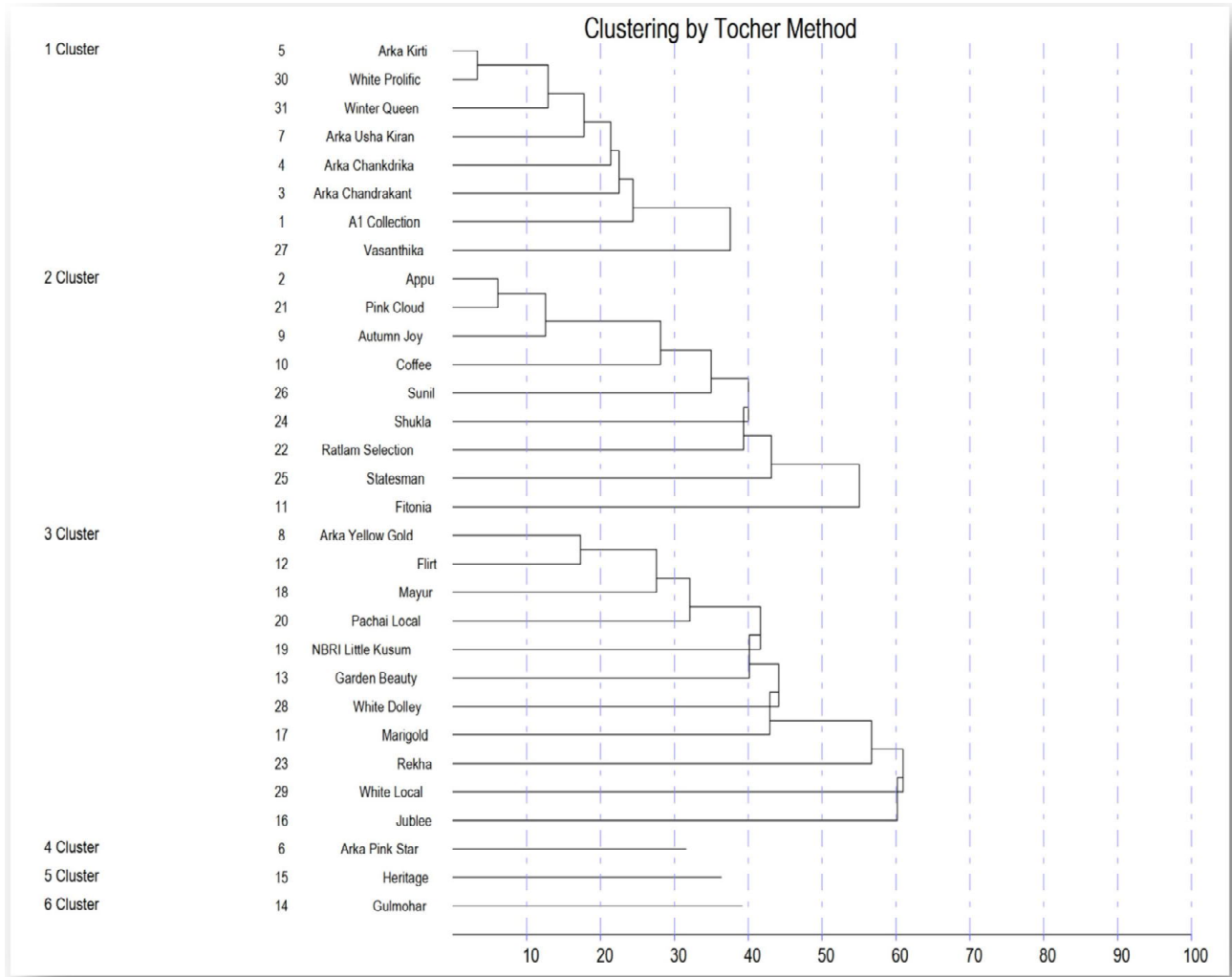


Fig. 2 : Dendrogram showing Euclidean distances based on nine quantitative traits of thirty-one chrysanthemum genotypes

Table 3 : Average intra and inter-cluster distances computed through D^2 analysis

Clusters	Cluster distances					
	I	II	III	IV	V	VI
I	29.52	-	-	-	-	-
II	92.85	46.87	-	-	-	-
III	66.40	104.64	56.57	-	-	-
IV	149.00	196.81	81.34	0.00	-	-
V	96.86	91.52	176.67	376.87	0.00	-
VI	87.69	147.85	157.34	344.96	45.50	0.00

45.5 (cluster V and VI) to 376.87 (cluster IV and V). The maximum (376.87) inter-cluster distance was recorded between clusters IV (Arka Pink Star) and cluster V (Heritage) followed by clusters IV (Arka Pink Star) and cluster VI (Gulmohar) (344.96) and, cluster II (Appu, Pink Cloud, Autumn Joy, Coffee, Sunil, Shukla, Ratlam Selection, Statesman and Fitonia) and cluster IV (Arka Pink Star) (196.81). This clearly indicated that the genotypes present in these clusters contain a wide range of genetic diversity which could be exploited through hybridization program to get better recombinants in the segregating generations. The results suggested that cross combination of genotypes from most divergent clusters IV and V, and IV and IV, respectively would be more vigorous when used as parent in hybridization programme for obtaining wide spectrum of variation among the segregates.

The least intercluster distance was observed between clusters V (Heritage) and cluster VI (Gulmohar) followed by cluster I (Arka Kirti, White Prolific, Winter Queen, Arka Usha Kiran, Arka Chankdrika, Arka Chandrakant, A1 Collection and Vasanthika) and cluster II (Appu, Pink Cloud, Autumn Joy, Coffee, Sunil, Shukla, Ratlam Selection, Statesman and Fitonia). The less inter-cluster distances indicated lower degree of divergence and close genetic makeup of these genotypes (Kaur et al., 2021), making them less suited for use in a hybridization programme between the genotypes in the cluster.

In the present investigation, cluster III (56.57) showed the highest intra-cluster distance followed by cluster II (46.87) and cluster I (29.52). Genotypes belonging

to cluster III had the maximum heterogeneity among the assembled genotypes that is also clear from the assembling pattern of the genotypes in the dendrogram (Fig. 2 and Table 4). It means that the genotypes in this cluster were highly diverse for the traits under study. However, null intra cluster distances were recorded in cluster IV, V and VI because of the inclusion of single genotype in each. The lower intra-cluster distances indicated homogenous nature of genotypes within the clusters, while, higher values displayed heterogeneous nature of clusters (Kaur et al., 2021). Lower level of intra- cluster distances explained narrow genetic variation within the cluster. Therefore, the members of same cluster are not expected to yield desirable recombinants. These results for genetic variation within and between the clusters are substantiated with the previous reports (Kavitha & Anburani, 2009; Baliyan et al., 2014). The genetic diversity observed within and between the clusters can be utilized in selection of genetically diverse genotypes which is important for exploitation of heterosis and development of desirable recombinants (Panwar et al., 2014).

Cluster-wise performance

The considerable morphological diversity of different clusters could also be explained from the average performance of the genotypes for nine morphological traits (Table 5).

Among all the clusters, genotypes in cluster II had highest cluster mean values for number of branches per plant (7.15), number of leaves per plant (119.72), and number of flowers per plant (91.69). The high yield potential in chrysanthemum is associated with

Table 4 : Distribution of chrysanthemum genotypes in different clusters through D² analysis based on quantitative traits

Clusters	Number of Genotype(s)	Genotype(s)
I	8	Arka Kirti, White Prolific, Winter Queen, Arka Usha Kiran, Arka Chankdrika, Arka Chandrakant, A1 Collection and Vasanthika
II	9	Appu, Pink Cloud, Autumn Joy, Coffee, Sunil, Shukla, Ratlam Selection, Statesman and Fitonia
III	11	Arka Yellow Gold, Flirt, Mayur, Pachai Local, NBRI Little Kusum, Garden Beauty, White Dolley, Marigold, Rekha, White Local and Jublee
IV	1	Arka Pink Star
V	1	Heritage
VI	1	Gulmohar

Table 5 : Cluster means for nine quantitative traits in thirty-one chrysanthemum genotypes

Clusters	Plant height (cm)	Branches per plant (Nos.)	Leaves per plant (Nos.)	Days to flower initiation	Days to first flower opening	Days to optimum flowering	Flowers per plant (Nos.)	Flower diameter (cm)	Flowering duration (days)
I	58.02	4.35	85.54	23.61	60.55	78.29	50.11	5.65	43.75
II	65.74	7.15	119.72	20.61	63.82	79.74	91.69	5.87	47.17
III	50.31	7.14	93.47	19.15	52.93	69.59	54.56	4.73	41.91
IV	26.92	7.00	110.33	11.16	31.00	51.33	56.67	3.93	37.33
V	92.50	5.83	101.67	28.84	88.33	104.50	67.83	5.70	47.50
VI	103.27	6.33	69.50	27.83	80.83	97.83	31.67	6.03	57.00

the number of branches per plant, number of leaves and flowers per plant. However, cluster IV recorded least plant height (26.92), days to flower initiation (11.16), days to first flower opening (31.00), days to optimum flowering (51.33), flower diameter (3.93) and flowering duration (37.33). The clustering pattern could be used to select parents for cross combinations that are expected to generate the highest possible variability for various economic characters. Similar type of observations for morphological variability in chrysanthemum has been reported (Kameswari et al., 2014; Kumar et al., 2016).

In the present study, higher mean for plant height was recorded in cluster VI followed by cluster V, while, number of branches per plant was recorded maximum in cluster II, followed by cluster III. The maximum number of leaves per plant was recorded in cluster II, followed by IV.

Minimum days to flower initiation, first flower opening and optimum flowering was in cluster IV, followed by cluster III. Maximum flower diameter and flowering duration was recorded in cluster VI, followed by cluster II and cluster V, respectively, while, maximum number of flowers per plant was recorded in cluster II, followed by cluster V.

Highly varied genotypes from these clusters may be exploited in a hybridization programme to develop highly desired recombinants for the improvement of yield. Among various groups, the genotype belonging to cluster VI (Gulmohar) and cluster V (Heritage) can be used to develop variety suitable for cut flower purpose. The genotypes were more distinct between cluster IV and III, therefore, hybridization of Arka Pink Star from cluster IV and Arka Yellow Gold, Flirt, Mayur, Pachai Local, NBRI Little Kusum, Garden Beauty, White Dolley, Marigold, Rekha, White Local and Jublee from cluster III may result in earliness to flowering. Similarly, the high yielding genotypes of cluster II can also be utilized in breeding programmes. Additionally, the precise selections for specific characteristics may help in the development of trait-specific high-yielding inbreds. To generate a wide range of variability or segregation for the target traits, the parents for hybridization should be selected from clusters expressing moderate to high D^2 values (Kaur et al., 2021).

Trait-wise contribution to diversity

The relative contribution of individual characters towards genetic divergence has been computed (Table 6) and revealed that among all the characters,

Table 6 : Contribution (%) of the traits towards genetic divergence in chrysanthemum genotypes

Source	Contribution (%)	Times ranked 1 st
Plant height (cm)	11.40	53
Number of branches per plant	15.91	74
Number of leaves per plant	10.32	48
Days to flower initiation	2.80	13
Days to first flower opening	13.98	65
Days to optimum flowering	0.43	2
Number of flowers per plant	32.26	150
Flower diameter (cm)	10.11	47
Flower duration (days)	2.80	13

number of flowers per plant contributed maximum to divergence (32.26%), followed by number of branches per plant (15.91%), days to first flower opening (13.98%) and plant height (11.40%). These characters can be given more emphasis during selection of at least one parent for hybridization program.

Number of leaves per plant, flower diameter, flower duration and days to optimum flowering showed contribution of 10.32%, 10.11%, 2.80% and 0.43%, respectively. The importance of morphological traits in genetic diversity has been substantiated with the findings of Kameswari et al. (2014) and Kumar et al. (2016).

CONCLUSION

Genetic divergence has been considered as an important factor in selecting genetically diverse parents for efficient and successful hybridization programme. Clustering pattern based on Mahalanobis D^2 statistic revealed that diverse geographic origins of the genotypes could not necessarily be an index of variation and the factors such as genetic drift, selection pressure and environment may be responsible for discrepancy of genotypes. Among nine characters, number of flowers per plant contributed maximum to divergence. Therefore, for chrysanthemum improvement, the clustering pattern could be used to select parents for cross combinations that are expected to generate the highest possible variability.

REFERENCES

- Baliyan, D., Sirohi, A., Kumar, M., Kumar, V., Malik, S., Sharma, S., & Sharma, S. (2014). Comparative genetic diversity analysis in chrysanthemum: A pilot study based on morpho-agronomic traits and ISSR markers. *Scientia Horticulturae*, *167*, 164-168.
- Bharathi, T.U., & Jawaharlal, M. (2014). Genetic divergence of African marigold (*Tagetes erecta* L.). *Biosciences*, p. 2233.
- Bhargav, V., Kumar, R., Bharathi, T.U., Dhananjaya, M.V., & Rao, T.M. (2023). Assessment of genetic diversity in China aster [*Callistephus chinensis* (L.) Nees. *Journal of Horticultural Sciences*, *18*(1), 84-89. doi: <https://doi.org/10.24154/jhs.v18i1.2138>
- 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*, *70*(08), 1-16. doi: 10.1007/s10722-023-01634-4
- Doshi, S.P., & Gupta, K.C. (1991). SPAR-1 software. Indian Agricultural Statistical Research, New Delhi, India.
- Fu, X., Ning, G., Gao, L., & Bao, M. (2008). Genetic diversity of *Dianthus* accessions as assessed using two molecular marker systems (SRAPs and ISSRs) and morphological traits. *Scientia Horticulturae*, *117*(3), 263-270. doi: 10.1016/j.scienta.2008.04.001
- Hadizadeh, H., Samiei, L., & Shakeri, A. (2022). Chrysanthemum, an ornamental genus with considerable medicinal value: A comprehensive review. *South African Journal of Botany*, *144*, 23-43. <https://doi.org/10.1016/j.sajb.2021.09.007>
- Jo, K.M., Jo, Y., Chu, H., Lian, S., & Cho, W.K. (2015). Development of EST-derived SSR markers using next-generation sequencing to reveal the genetic diversity of 50 chrysanthemum cultivars. *Biochemical Systematics and Ecology*, *60*, 37-45. <https://doi.org/10.1016/j.bse.2015.03.002>
- Kameswari, P. L., Pratap, M., Anuradha, G., & Begum, H. (2014). Genetic divergence studies in chrysanthemum (*Dendranthema grandiflora* Tzvelev). *Indian Journal of Scientific Research and Technology*, *2*, 4-10.
- Kaur, S., Sidhu, M.K., & Dhatt, A.S. (2021). Genetic diversity analysis through cluster constellation in brinjal (*Solanum melongena* L.). *Genetika*, *53*(2), 629-640. doi: 10.2298/GENSR2102629K
- Kavitha, R., & Anburani, A. (2009). Genetic diversity in African marigold (*Tagetes erecta* L.) genotypes. *Journal of Ornamental Horticulture*, *12*(3), 198-201.
- Kumar, R., Kumar, S., Kumar, P., & Mer, R. (2011). Genetic variability and divergence analysis in snapdragon (*Antirrhinum majus* L.) under Tarai conditions of Uttarakhand. *Progressive Horticulture*, *43*(2), 332-336.



- Kumar, S., Kumar, M., Kumar, R., Malik, S., Singh, M.K., & Kumar, S. (2016). Analysis of genetic divergence in chrysanthemum (*Dendranthema grandiflora* Tzvelev) germplasm using morphological markers. *International Journal of Agricultural and Statistical Sciences*, 12(2), 255-260.
- Nguyen, T.K., Ha, S.T.T., & Lim, J.H. (2020). Analysis of chrysanthemum genetic diversity by genotyping-by-sequencing. *Horticulture, Environment, and Biotechnology*, 61, 903-913.
- Panwar, S., Singh, K.P., & Janakiram, T. (2014). Assessment of genetic diversity of marigold (*Tagetes erecta* L.) genotypes based on morphological traits. *Journal of Ornamental Horticulture*, 17(3&4), 77-81.
- Rao, C.R. (1952). *Advanced statistical methods in biometric research*. John Wiley and Sons, New York, pp. 390. <https://doi.org/10.1002/ajpa.1330120224>

(Received : 09.10.2023; Revised : 12.12.2023; Accepted : 15.12.2023)