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Genetic divergence (D^2) analysis in gladiolus genotypes (*Gladiolus hybrid* Hort.) under Southern Rajasthan

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Abstract
Gladiolus, the queen of flowers has revolutionized the world floriculture trade, occupying the important place in the floriculture trade next to rose. The main emphasis is on the development of varieties, having attractive colours, increasing number of well-placed florets, long spike, efficient corm production and longevity of florets. Therefore, for developing elite varieties, it is essential to explore the range of variability present in the crop. For making further improvement in number of florets, there had been consistent efforts on the part of breeders and floriculturists to bring about variation in the cultivated Gladiolus for the characters attributed to number of florets per spike. Greater variability ensures better chances of selecting new improved forms. To evaluate Gladiolus germplasm the present investigation was carried out to analyse the genetic divergence among 22 Gladiolus genotypes. The present experiment was carried out from October 2019 to May 2020 in the Horticulture Farm, department of horticulture, Rajasthan College of Agriculture, MPUAT, Udaipur. The field experiment was carried out in randomised block design (RBD) with twenty- two variety of Gladiolus. The findings indicate that though there is strong inherent association between various characters, the phenotypic expression is lessened under the influence of environment and that genotypes have substantial diversity and variability for most of the characters.

Keywords: Gladiolus, D^2, genetic divergence, Intra and Inter cluster distance, genetic contribution

Introduction
Gladiolus (*Gladiolus hybrid* L.) also known as the "sword lily," is an important cut flower in both Indian and international markets. It originates in South Africa and is a member of the Iridaceae family. Its fascinating spikes show various florets with tepals that are smooth, ruffled, deeply crinkled, or laciniated and that are blotched or have distinct patches or markings of different colours and colour combinations. One of the most significant bulbous crops cultivated for commercial purposes, it is used to make cut flowers, bouquets, floral arrangements, home decoration, and garden exhibits.

Basic chromosome number is n=15. Ploidy in the genus ranges from diploid (2n=30) to dodecaploid (2n=180). The modern garden gladiolus is a complex hybrid from at least 12 species and most of the cultivars are tetraploids (2n=60) and highly heterozygous, they will not breed true to the type if grown from seeds due to cross pollination by honey bees. The genus gladiolus has 255 species over the world. (Goldblatt and Manning 1998) [25]. In the cut flower trade, gladiolus rank fourth in the International market after the rose, carnation and chrysanthemum (Rathod *et al*. 2011) [10]. Major gladiolus-producing states in India are Uttar Pradesh, West Bengal, Odisha, Chhattisgarh, Haryana, and Maharashtra. Although gladiolus is primarily a winter-season flower crop, it may be grown all year round in locations with a moderate environment. There are numerous varieties of gladiolus with magnificent inflorescence in a range of colours, sizes and shapes. Number of florets on each spike. For the classification and use of germplasm resources in breeding programmes, genetic variation and the genetic link among genotypes are crucial factors (Kumar *et al.*, 2013) [26]. The requirement of the breeding programme is the level and magnitude of genetic heterogeneity in the gene pool (Bhujbal *et al.*, 2013) [1].

Genetic divergence analysis
Genetic diversity is the basic requirement for successful breeding programme. The more diverse the parents, greater are the chances of increased spectrum of variability. However, major difficulties are encountered in the measurement of such variability.

Due to lack of precise statistical method to estimate genetic divergence, ecological divergence was considered as an index of genetic diversity in the past and varieties from different localities have usually been included in hybridization programme. However, this being an inferential criterion, it cannot always be used in quantifying the degree of divergence between biological population at the genotypic level. Mahalanobis outlined a statistical procedure D^2 statistics, to measure the genetic divergence in a given population. This concept is based on the technique of utilizing measurement in respect of an aggregate of characters. Being a numerical estimate, it permits precise comparison among all possible pair of populations. Therefore several workers have used this technique for inter and intra-specific levels. De and Misra reported high range of genetic diversity in Gladiolus and reported three clusters. Cluster I consisted maximum number of genotypes. In 1999, Arya and co-workers evaluated 21 Gladiolus varieties in relation to 16 quantitative traits and varieties were grouped in seven clusters. Cluster I and II comprised of seven genotypes while the remaining clusters had only one or two genotypes in each. Deshraj and Misra (2000) [4] measured multivariate analysis by Mahalanobis's D^2 statistics for 20 quantitative characters in 25 cultivars. The cultivars differed significantly for all the 20 characters of Gladiolus considered collectively and were grouped into five clusters on the basis of relative magnitude of D^2 value at individual environment and pooled analysis. Based on cluster means character like days to 50 per cent heading, first floret showing colour, first floret opening, last floret opening, number of florets per spike, average weight of a corm and propagation coefficient were the major factors for differentiating among 25 cultivars. However, no close correspondence is evident between geographical distributions to genetic divergence. Using D^2 statistics, Nimbaker *et al*., indicated the existence of genetic diversity in a set of 101 genotypes of Gladiolus. No parallelism was observed between geographic diversity and genetic diversity. The maximum intra-cluster distance was exhibited by genotypes of the cluster III, while lowest by cluster VII. The intra-cluster distance was highest between cluster VIII and cluster XI. The traits, number and weight of corms and cormels per plant, number of florets per spike and plant height contributed considerably to divergence. Based on the D^2 value and per se performance, divergent pair for hybridization programme and other genotype in possible combination are suggested to obtain superior types to secure yield improvement and removing yield constraints in Gladiolus.

Material and Methods

The present investigation was carried out in Randomized Block Design with three replications in the Experimental block of Department of Horticulture, Horticulture Farm, Rajasthan College of Agriculture, MPUAT, Udaipur during the year of 2019-20. Twenty two different varieties *viz*., Angalia, Arka Amar, Arka Kesar, Arka Pratham, Astralian Fair, Chandani, Friendship, Green Spire, GS-2, Mohini, Nathan Red, Punjab Beauty, Punjab Dawn, Punjab Glad-2, Praha, Priscella, Pusa Dhanavantri, Pusa Gunjan, Pusa Kiran, Pusa Sinduri, Pusa Srijan, Pusa Subham were used for the study. The entire experimental land was divided into subplots measuring 1.2 m x 1.8 m and there were totally 66 plots. Before planting Bavistin (3g/litre) treated corms were planted on the ridges to a depth of 6-8 cm by adopting a spacing of 30 × 20 cm.

Genetic Divergence

The observations were recorded on 13 quantitative characters selected for genetic divergence analysis. Observations belonging to number of characters were recorded on the basis of five plants per replication in each treatment and the characters studied in the present investigations such as days to spike emergence, days to first floret show colour, days to last floret show colour, spike length (cm), rachis length (cm), spike per plant, Florets per spike, vase life (days), spike durability in field, corm per plant, weight of corm per plant (g), cormlets per plant and weight of cormlets per plant. as outlined in beginning of this chapter were included for this analysis. The calculation of D^2 values involved following steps (Murthy and Arunachalam, 1966) [16].

Group Constellations

Treating D^2 as the square of generalized distance, all genotypes were grouped into a number of clusters according to the method described by Tocher (Rao, 1952) [17]. The criterion used in clustering by this method in any two groups of genotypes belonging to same cluster should at least on an average show a smaller D^2 value than those belonging two different clusters. In other words, if genotypes, V1 and V2 are close together and V3 and V2 form a cluster, the average D^2 value of all possible combinations of genotypes in one cluster with those in the other cluster was computed and its square root was used to represent the statistical distance between two clusters.

Contribution of different characters towards divergence

The relative combinations of different characters to the total D^2 between each pair of genotypes was given a score of 1-20 (20 number of characters) based on the magnitude of D^2 value due to each character. A rank of 1 represented highest contribution and 20th the lowest.

$$\text{Percentage contribution of character 'X'} = \frac{N(x) \times 100}{N\,(n-1)/2}$$

Where,

N(X) = number of genotypic combinations which were ranked first for character "X" out of total genotypic combinations of 231(Combinations between 22 genotypes).

Table 1: List of Gladiolus genotypes with their place of collection used for genetic divergence ($D^{2)}$ analysis

Sr. No.	Name of Genotype	Place of Collection
1	Anglia	AICRP, Udaipur
2	Arka Amar	IIHR, Bangalore
3	Arka Kesar	IIHR, Bangalore
4	Arka Pratham	IIHR, Bangalore
5	Australian Fair	AICRP, Udaipur
6	Chandani	AICRP, Udaipur

7	Friendship	AICRP, Udaipur
8	Green Spire	AICRP, Udaipur
9	GS-2	AICRP, Udaipur
10	Mohini	AICRP, Udaipur
11	Nathan Red	AICRP, Udaipur
12	Punjab Beauty	PAU, Ludhiana
13	Punjab Dawn	PAU, Ludhiana
14	Punjab Glad-2	PAU, Ludhiana
15	Praha	AICRP, Udaipur
16	Priscella	AICRP, Udaipur
17	Pusa Dhanavantri	IARI, New Delhi
18	Pusa Gunjan	IARI, New Delhi
19	Pusa Kiran	IARI, New Delhi
20	Pusa Sinduri	IARI, New Delhi
21	Pusa Srijan	IARI, New Delhi
22	Pusa Subham	IARI, New Delhi

Experimental results

Genetic divergence analysis

The genetic divergence was estimated by Mahalanobis D^2 statistics as described by Rao (1952) [17]. It is obvious from the Table 2 to 6 that there was wide range of variability among genotypes for all the characters. Based on the D^2 value, the constellation of genotypes into cluster was done following Tocher's optimization procedure (Rao, 1952) [17]. All the 22 genotypes were grouped into 10 clusters. The cluster I comprised of the highest number of genotypes (5) followed by cluster II (4), cluster III (3), cluster IV (3) cluster V,VI and Vll each have one genotype cluster VIII consists 2 genotypes and cluster IX and X have one genotype In cluster - I the constituently genotypes were Australian Fair, Pusa Sinduri, Nanthan Red, Parha, Pusa Srijan. In cluster II- Chandani, Punjab Beauty, Arka Pratham, Pusa Kiran. Cluster III -Punjab Glad-2, Mohini, Arka Kesar. Cluster IV- GS-2, Punjab Dawn, Pusa Dhanavantri. Cluster V-Arka Amer. Cluster VI- Pusa Gunjan. Cluster VII- Green Spire. Cluster VIII- Friendship and Priscella. Cluster IX- Angalia and Cluster X- Pusa Subham respectively (Table 2).

Table 2: Distribution of 22 gladiolus genotypes into different clusters

Cluster	No. of Genotypes	Members
I	5	Australian Fair, Pusa Sinduri, Nathan Red, Parha, Pusa Srijan
II	4	Chandani, Punjab Beauty, Arka Pratham, Pusa Kiran
III	3	Punjab Glad-2, Mohini, Arka Kesar
IV	3	GS-2, Punjab Dawn, Pusa Dhanavantri
V	1	Arka Amer
VI	1	Pusa Gunjan
VII	1	Green Spire
VIII	2	Friendship, Priscella
IX	1	Anglia
X	1	Pusa Subham

Intra and inter-cluster divergence

Intra and inter- cluster average D^2 values are given in Table 3 Intra- cluster average D^2 value ranged from 0.00 to 114.57. It was maximum in cluster III (114.57) followed by cluster IV (105.84) which was indicative of wide genetic divergence. In cluster V, VI, VII, IX and X intra-cluster distance was zero because it consists of only one genotype. The inter cluster average D^2 value was maximum between cluster VII and IX (1288.92) followed by between cluster VII and cluster X. (994.23). The minimum inter cluster distance was obtained between cluster II and cluster VI (105.06). It indicates that genotypes of cluster II and cluster VI are very close to each other.

Table 3: Average Intra and inter cluster Distance D^2 values

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X
I	67.21	507.69	142.49	280.00	130.09	534.78	153.55	221.31	892.52	571.48
II		74.05	332.78	406.37	408.66	105.06	713.25	396.29	196.04	262.67
III			114.57	378.74	177.09	403.28	358.62	202.03	508.18	285.87
IV				105.84	267.40	282.82	192.36	319.75	907.86	861.81
V					0.00	529.13	291.48	420.22	726.13	590.03
VI						0.00	583.32	305.84	348.83	416.78
VII							0.00	293.80	1288.92	994.23
VIII								99.14	692.41	452.52
IX									0.00	176.48
X										0.00

Cluster group means

Wide range of variation was found in cluster mean value in respect of all 13 characters given in Table 4. A close perusal of these cluster mean for different characters indicated that cluster I had highest cluster mean for Days to last floret show colour (121.40), Days to first floret show colour (110.36). Cluster II showed the highest clusters mean for Days to last floret show colour (104.66), and minimum cluster mean for corm per plant (2.01). Highest mean for florets per spike was found in cluster VII (19.80). Highest cluster mean for number of cormels per plant was found 40.20 in cluster II followed by 38.33 in cluster IV respectively.

Table 4: Cluster mean for different characters in gladiolus genotypes

Cluster	Days to spike emergence	Days to first floret show colour	Days to last floret show colour	Spike length (cm	Rachis length (cm)	Spike per plant	Florets per spike	Vase life (days)	Spike durability in field	Corm per plant	Weight of corm per plant (g)	Cormlets per plant	Weight of cormlets per plant
I	103.0133	110.3600	121.4047	89.6400	58.6000	1.3600	17.9587	12.1000	17.3007	1.2400	45.4400	23.3007	5.7000
II	86.6042	93.2492	104.6659	75.4500	48.2508	2.2000	15.2508	10.6008	15.7742	2.0175	40.7333	40.2008	7.3008
III	96.6778	103.6000	117.5578	80.3333	51.6667	1.5333	16.8667	11.5678	16.3333	1.0645	49.4444	21.9333	5.4900
IV	91.6667	98.4656	112.0000	99.9989	66.6667	2.3744	19.3333	12.6433	17.8567	2.0667	49.2800	38.3344	9.8656
V	93.0067	99.0000	111.0000	95.0033	63.3333	1.2000	17.9967	12.0000	17.0000	1.0000	42.0000	27.2000	5.1000
VI	86.9967	92.9967	102.9967	79.0000	53.0033	2.8000	17.3967	11.6000	16.7000	2.5000	47.9333	41.0000	10.0000
VII	102.9967	110.0000	121.0000	100.6000	68.0000	2.0033	19.8000	13.0033	18.0000	2.0033	52.9300	30.0000	6.1000
VIII	103.7600	110.5000	122.0017	78.4167	48.8333	1.7983	16.2000	11.5017	16.1334	1.5983	58.6000	32.3016	8.9500
IX	79.1500	86.0000	97.0000	63.0033	41.6000	2.0000	13.2000	9.0000	13.6667	1.4000	50.9300	35.5000	5.8000
X	93.0000	100.0000	110.0000	59.0000	39.3333	1.6000	13.8000	10.0000	15.0000	1.4000	41.0700	18.8033	4.8200

Contribution of different characters towards genetic divergence

The per cent (%) contribution of different quantitative characters under evaluation towards the expression of genetic divergence is given in Table 5. vase life (days) contributed maximum (38.96%) towards genetic divergence followed by cormlets per plant (20.77), weight of corm per plant (14.71), Corm per plant (10.82), Weight of cormlets per plant (7.35), Days to first floret show colour (2.16), Spike length (2.16) and Days to spike emergence (1.29) respectively.

Table 5: Contribution of different characters towards genetic divergence

Sr. No.	Characters	Per cent (%) contribution
1	Days to spike emergence	1.29
2	Days to first floret show colour	2.16
3	Days to last floret show colour	0.00
4	Spike length (cm)	0.00
5	Rachis length (cm)	2.16
6	Spike per plant	0.86
7	Florets per spike	0.00
8	Vase life (days)	38.96
9	Spike durability in field	0.86
10	Corm per plant	10.82
11	Weight of corm per plant (g),	14.71
12	Cormlets per plant	20.77
13	Weight of cormlets per plant	7.35

Discussion

In the present investigation, the findings indicate that though there is high genetic divergence between various characters, the phenotypic expression is lessened under the influence of environment. It is obvious from the foregoing discussion on correlations that for the improvement of Gladiolus both for market value and maintaining quality, the characters like vase life (days), cormlets per plant, weight of corm per plant, corm per plant, weight of cormlets per plant, Days to first floret show colour and Spike length are of primary significance. The selection of cultivars on the basis of these traits can help to find better recombinants through a suitable breeding programme. Several measures of distance have been proposed over the past two decades to suit various objectives of which Mahalanobis's generalized distance has occupied a unique place in plant breeding. Using the Mahalanobis technique in the present investigation 22 Gladiolus genotypes were classified into 10 clusters with inter-cluster average D^2 ranging from 105.06 (cluster II and cluster VI) to 1288.92 (cluster VII and IX). Five genotypes fell in cluster I indicating overall genetic similarity among them, cluster II consisted of four genotypes followed by III and IV each having three genotypes. The genotypes from cluster II having maximum genetic divergence from Angalia (cluster IX) (Table 3 and 4). The characters contributing most towards genetic divergence were vase life, high cormlets per plant, weight of corm per plant, corm per plant, rachis length (cm) and days to first floret show colour The results are in agreement with those of Arya *et al.,* (1999) [18]. Based on inter-cluster distant crosses and selection from more diverse parent expected to get better genotype, these clusters constituent genotype could be used in yield improvement. The maximum inter cluster distance between cluster VII and IX could be expected to exert high heterosis effect in the hybrids when crossed and consequently might generate desirable segregants. The characters which contribute maximum in genetic divergence *viz*., vase life, high cormlets per plant, weight of corm per plant, corm per plant, rachis length (cm), days to first floret show colour and Days to spike emergence can be used in selecting diverse parent for hybridization programme.

Conclusion

Based on Mahalanobis D^2 analysis, 10 clusters were formed and clustering pattern indicated substantial genetic diversity among 22 genotypes of gladiolus. The inter cluster average D^2 value was maximum between cluster VII and IX (1288.92) It can be concluded that genotypes were having substantial diversity and variability for most of the characters. A promising gladiolus genotype could be obtained by selection on the basis with an excellent vase life, high cormlets per plant, weight of corm per plant, corm per plant, rachis length (cm) and days to first floret show colour. Further studies on correlation among the other characters and its relation with spike length, plant height, number of florets per spike and durability of spike are recommended for better information and understanding the improvement process.

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