African Journal of Basic & Applied Sciences 7 (3): 153-159, 2015
ISSN 2079-2034

DOI: 10.5829/idosi.ajbas.2015.7.3.22296

The Genetic Divergence among 22 *Gladiolus* Genotypes Using D^2 Analysis

[1,2]*Kanika Malik and* [3]*Krishan Pal*

[1]CSIR-NISCAIR, Dr. K S Krishnan Marg, Pusa Campus, 110 012, New Delhi, India
[2]Biotech Department, Mewar University, Chittorgarh, 312 001, Rajasthan, India
[3]Department of Bioscience, Shri Ram College, Muzzafarnagar, 251 001, Uttar Pradesh, India

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. The basic information which a breeder usually needs for improvement of a particular crop is the extent of genetic variability present in the available germplasm. Greater variability ensures better chances of selecting new improved forms. Variation at phenotypic level is a combination of genotypic as well as environmental variability. It is the genotypic variability which is of primary interest because it is the deciding factor for character selection. Statistics like range, mean, coefficient of variability, heritability and genetic advance provides basic information for successful breeding programme. To evaluate *Gladiolus* germplasm the present investigation was carried out to analyse the genetic divergence among 22 *Gladiolus* genotypes. 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.

Key words: Genotype · Genetic variability · Hybridization programme · Qualitative · Quantitative characters

INTRODUCTION

Gladiolus ranks fifth next to tulip (*Tulipa* spp.), lily (*Lilium* spp.), freesia (*Freesia* spp.) and hippeastrum (*Hippeastrum* spp.) among the geophytes in international florist trade (Flower Council of Holland, 2008) and first in domestic bulbous flower trade. In India, its cultivation dates back to nineteenth century (Apte, 1958)[1] and valued for its magnificent flower spike with massive florets of brilliant colours, attractive shapes, varying size and excellent shelf life. Fascinating spikes of *Gladiolus* bear a large number of florets which exhibit varying size, forms, with smooth, ruffled, deeply crinkled or lacerated petals in different shades of brilliant colours. These characters make it attractive for use as cut flower for vase and bouquet, herbaceous borders, bedding and pot cultivation. The cultivated *Gladiolus* developed from six different species. *Gladiolus* is rich in its varietal wealth and every year there is an addition of about 200 new varieties (Deshraj and Misra, 1998)[2], hence varietal trials become necessary to find out suitable varieties for specific region. In *Gladiolus*, the range of variability exists for various qualitative and quantitative characters like number of leaves per plant, number of flowers per spike, length of spike, duration of flowering, number of corms per plant. Crop improvement ultimately depends upon the availability of variability to be utilized in breeding programme.

The occurrence of genotype and environmental interaction has provided a major challenge for obtaining complete understanding of the genetic control of variability. For planning a selection strategy, understanding the inter relationship among yield and components/characters is of vital importance. The choice

Corresponding Author: Kanika Malik, CSIR-NISCAIR, Dr. K S Krishnan Marg, Pusa Campus, 110 012, New Delhi, India.

of stable parents is of paramount importance for planned hybridization to improve productivity; therefore, efforts are to be intensified to identify the best parent with wide genetic base from the large germplasm pool for characters of importance to their efficiency in hybridization programme. The information on heritability with the genetic advance as percentage of mean for different parameters is of paramount importance for breeder to identify the characters to be relied upon and to decide the selection strategies to get good quality bloom and maximum corm production.

For developing improved varieties it is essential to explore the range of inability present in the crop. If the relationship among cultivars is not known, variability assessment and validation of genetic relatedness among different varieties becomes important to proceed for crop improvement programme. The present study estimates the relative extent of genetic variability through genetic divergence in twenty two diverse genotypes of *Gladiolus*.

Genetic Divergence Studies: 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 [3] 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*.

MATERIALS AND METHODS

The experimental material consisting of 22 genotypes of *Gladiolus* with the name, origin and collection of place of the cultivars is given in Table 1. The experiment consisted of estimations of genetic variability for qualitative and quantitative characters in *Gladiolus* germplasm during two successive years 2011-2012 and 2012-2013. The experiment was laid out in Randomized Block Design (RBD) with two replications and each genotype had 10 plants or corms per replication by keeping row to row distance of 30 cm and plant to plant distance of 20 cm. Each replication had two rows of 10 corms out of which six corms were selected randomly and observations were recorded.

Observations belonging to number of characters were recorded on the basis of six plants per replication in each treatment and the characters studied in the present investigations are listed below [8].

Table 1: List of genotypes of *Gladiolus* with their origin and place of collection

Name	Origin	Place of collection
American Beauty	USA	NBRI, Lucknow
Candyman	USA	PAU, Ludhiana
Eurovision	USA	NBRI, Lucknow
Happy End	-	NBRI, Lucknow
Her Majesty	India	NBRI, Lucknow
Invitation	USA	PAU, Ludhiana
Jester	USA	NBRI, Lucknow
Melody	Netherlands	PAU, Ludhiana
Novalux	-	PAU, Ludhiana
Oscar	Netherlands	NBRI, Lucknow
Pink Friendship	Dutch	IARI, New Delhi
Poppy Tear	-	PAU, Ludhiana
Red Beauty	UK	PAU, Ludhiana
Rose Supreme	USA	PAU, Ludhiana
Shobha	India	IARI, New Delhi
Snowprincess	Holland	NBRI, Lucknow
Spic and Span	-	PAU, Ludhiana
Subhangini	India	GBPAUT, Pantnagar
Sylvia	Netherlands	NBRI, Lucknow
Trader Horn	-	PAU, Ludhiana
White Friendship	-	NBRI, Lucknow
White Prosperity	USA	NBRI, Lucknow

Vegetative Characters

Days Taken to Sprouting: Day to sprouting was noted down as number of days from planting to initiation of vegetative bud.

Days to 50 per Cent Sprouting: Number of days required from planting to 50 per cent sprouting of corms were recorded.

Sprouting Percentage: It was calculated as number of corms sprouted over the total number of corms planted multiplied by 100.

Number of Shoots per Planted Corm: Number of shoots/surviving buds per corm were counted.

Number of Leaves per Plant: Number of leaves per shoot per plant counted at full maturity.

Plant Height at 45 Days of Planting: Plant height was measured in centimeters from the ground level to the tip of the spike.

Area of 3rd Leaf at 1st Flower Colour Show: Length and width of each 3rd leaf was measured. Area of leaf was computed using the following formula:

$$\text{Leaf area} = L \times B \times 0.635 + 12.9$$

where, L = length, B = width.

Floral Characters

Days Taken to 50 per Cent Heading: The number of days from planting to the appearance of the 50 per cent spike tip was recorded.

Days Taken to First Floret Opening: The number of days taken from the appearance of spike tip to the opening of the first lower most floret was recorded.

Spike Length: The length of spike was measured from the base to the tip of the spike in meters.

Rachis Length: Length of rachis was measured in centimeters from the basal floret to the tip of the spike in centimeters.

Number of Florets per Spike: The total number of florets per spike were counted and recorded.

Size of Second Standard Floret: The size of floret (maximum diameter) was measured in centimeters at full bloom stage.

Flower Colour: Flower colour was measured according to Munshel's plant colour chart.

Shape of Flower: Flower shape was recorded as open faced or close faced (hooded) floret.

Total Blooming Life: Total blooming life was recorded as days taken to first flower opening to last floret wilting.

Cormel/corm Characters

Number of Cormels per Plant: Cormels collected from each plant were counted and recorded.

Number of Corms per Plant: Number of corms per plant collected and data recorded.

Weight of Corm: The corms were weighed in grams at the time of harvesting.

Size of the Corms: Diameter of corms was measured in centimeter with help of Vernier Calliper at harvest.

Propagation Coefficient: It was expressed in percentage dividing the collective weight of corm and cormels produced per plant by weight of a mother corm planted.

$$\text{Propagation coefficient (\%)} = \frac{\text{Total weight of corms and control/plants}}{\text{Weight of the planted corm}}$$

Environmental

Per Cent Disease Severity (*Fusarium* Wilt): Data was recorded as occurrence of *Fusarium* wilt disease during plant life.

Temperature Tolerance: Corms were planted in the month of June and the survival of plant was recorded.

Genetic Divergence: The genetic divergence in 22 genotypes was estimated by Mahalanobis D^2 statistics (generalized distance) as suggested by Rao (1952). The twenty characters viz., days taken to sprouting, days taken to 50 per cent sprouting, sprouting percentage, number of shoots per planted corms, number of leaves per plant, plant height, leaf area of 3rd leaf at flower colour shows, days taken to 50 per cent heading, days taken to first floret opening, spike length, rachis length, number of florets per spike, size of second standard floret, total blooming life, number of cormels per plant, number of corms per plant, weight of corm, size of corm, per cent disease severity of *Fusarium* wilt and propagation coefficient 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)[5].

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)[6]. 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, V_1 and V_2 are close together and V_3 and V_2 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 combination of 231 (combination between 22 genotypes).

Genetic Divergence Analysis: The genetic divergence was estimated by Mahalanobis D^2 statistics as described by Rao (1952). 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)[6]. All the 22 genotypes were grouped into four clusters. The cluster I comprised of the highest number of genotypes (15) followed by cluster II (5), cluster III and cluster IV (1) each have one genotype.

In cluster I, the constituently genotypes were American Beauty, Spic and Span, Syl*via*, White Prosperity, White Friendship, Red Beauty, Pink Friendship, Trader Horn, Candyman, Her Majesty, Rose Supreme, Poppy Tear, Eurovision, Happy End and Jester. In cluster II, Melody, Snow Princess, Subhangini, Oscar and Novalux were found. Cluster III and IV consisted of Invitation and Shobha, respectively (Table 2)[7-15].

Intra and Inter-Cluster Divergence: Intra and inter-cluster average D^2 values are given in Table 3 to 5. Intra-cluster average D^2 value ranged from 0.00 to 23.61. It was maximum in cluster I with 15 genotypes followed by cluster II (25.15) which was indicative of wide genetic divergence. In cluster III and IV intra-cluster distance was zero because it consists of only one genotype.

The inter-cluster average D^2 value was maximum (54.19) between cluster II and IV followed by (50.37) between cluster III and cluster IV. The minimum inter cluster distance was obtained between cluster I and cluster II. It indicates that genotypes of cluster I and cluster II are very close to each other.

Table 2: Composition of different clusters of genotypes in *Gladiolus*

Cluster	Name of genotypes	No. of genotypes
I	American Beauty, Spic and Span, Sylvia, White Friendship, Red Beauty, Pink Friendship, White Prosperity, Trader Horn, Candyman, Her Majesty, Rose Supreme, Poppy Tear, Eurovision, Happy End and Jester	15
II	Melody, Snow princess, Subhangini, Oscar, Novalux	5
III	Invitation	1
IV	Shobha	1

Table 3: Average inter-cluster D^2 value

	I	II	III	IV
I	557.41	740.25	1593.75	1954.07
II		632.11	2081.15	2936.97
III			0.00	2537.64
				0.00

Table 4: Average intra and inter-cluster D value.

	I	II	III	IV
I	23.61	27.21	39.42	44.20
II		25.14	45.62	54.19
III			0.00	50.37
				0.00

sTable 5: Cluster group mean for various characters in *Gladiolus*

Characters	I	II	III	IV
Days taken to sprouting	10.97	10.56	10.33	11.83
Days taken to 50% sprouting	10.33	10.07	10.33	11.33
Spouting percentage	96.31	98.00	67.33	100.00
Number of shoots/planted corm	1.57	1.78	1.53	1.60
Number of leaves/plant	8.36	8.58	7.33	8.00
Plant height	38.12	41.28	43.55	32.66
Leaf area	52.63	52.96	81.50	43.67
Days taken to 50% heading	77.88	78.11	106.60	104.66
Days taken to first floret opening	94.67	96.56	77.33	95.40
Spike length	87.58	95.14	83.88	83.33
Rachis length	58.48	62.88	48.11	53.00
Number of florets per spike	13.90	14.21	11.83	16.55
Size of second floret	9.74	10.08	8.87	8.22
Total blooming life	24.86	29.74	22.44	25.66
Number of cormels per plant	32.10	37.93	26.00	90.67
Weight of corm	47.54	68.00	65.33	47.86
Number of corms per plant	1.57	1.67	1.76	1.44
Size of corm	5.41	5.76	6.07	5.41
Per cent disease severity of *Fusarium* wilt	16.49	6.42	22.40	50.67
Propagation coefficient	148.19	170.05	187.90	143.50

A considerable range of variation was found in cluster mean value in respect of all 20 characters given in Table 5. A close perusal of these cluster mean for different characters indicated that cluster III had highest cluster mean for propagation coefficient (187.9), leaf area (81.5 cm^2). Cluster II showed the highest clusters mean for weight of corm (68.0 g) and minimum cluster mean for disease severity (6.42). Highest mean for number of florets per spike was found in cluster IV (16.55). Highest cluster mean for number of cormels per plant was found 90.67 in cluster IV followed by 37.93 in cluster II. In cluster III, plant height (43.55 cm) cluster mean was maximum while highest cluster mean for spike length and rachis length were found (95.14) and (62.88), respectively in cluster II.

Contribution of Different Characters Towards Genetic Divergence: The relative contribution of different quantitative characters under evaluation towards the expression of genetic divergence is given in Table 6. Leaf area contributed maximum (24.67%) towards genetic divergence followed by disease severity (24.24), number of cormels per plant (17.75), spike length (9.09), number of florets per spike (8.65) and weight of corm (5.20).

Table 6: Relative contribution of different characters to genetic divergence among *Gladiolus*

Characters	Per cent contribution
Days taken to sprouting	0.00
Days taken to 50% sprouting	0.00
Spouting percentage	1.72
Number of shoots/planted corm	0.00
Number of leaves/plant	0.00
Plant height	0.00
Leaf area	24.67
Days taken to 50% heading	5.19
Days taken to first floret opening	0.00
Spike length	9.09
Rachis length	0.00
Number of florets per spike	8.65
Size of second floret	1.73
Total blooming life	1.29
Number of cormels per plant	17.75
Weight of corm per plant	0.00
Size of corm	0.00
Per cent disease severity of *Fusarium* wilt	24.24
Propagation coefficient	0.43

DISCUSSION

In the present investigation, the findings indicate that though there is strong inherent association 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 number of florets per spike, plant height, rachis length, leaf area, weight of daughter corms and propagation coefficient are of primary significance. The selection of cultivars on the basis of these characters can help to find good 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 3 clusters with inter-cluster average D^2 ranging from 27.21 (cluster I and cluster II) to 54.19 (cluster II and IV). Fifteen genotypes fell in cluster I indicating overall genetic similarity among them, cluster II consisted of five genotypes followed by III and IV each having one genotype. The genotype from cluster II having maximum genetic divergence from Shobha (cluster IV) (Table 3 and 4). The characters contributing most towards genetic divergence were leaf area, number of cormels per plant, number of florets per plant and weight of corm (Table 6). The results are in agreement with those of Arya *et al.,* (1999).

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 highest inter-cluster distance between cluster II and IV 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., leaf area, number of cormels, number of florets per spike and weight of corm can be used in selecting diverse parent for hybridization programme.

CONCLUSION

Based on Mahalanobis D^2 analysis, four clusters were formed and clustering pattern indicated substantial genetic diversity among twenty two genotypes of *Gladiolus*. Inter-cluster distance was found maximum between cluster II and cluster IV. It can be concluded that genotypes were having substantial diversity and variability for most of the characters. A promising *Gladiolus* genotype with a good number of florets per spike could be obtained by selection on the basis of plant height, rachis length, number of cormels per plant, weight of corm and size of corm. Therefore, selection should be based on leaf area, number of florets per spike and number of cormels per plant for better genotypes. Further studies on correlation among the 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.

REFERENCES

1. Apte, S.S., 1958. Glad Culture in Puna, India. *Gladiolus,* 33: 31-36.
2. Deshraj and R.L. Misra, 1998. Genetic variability in gladiolus, J. Orn. Hort., 4(1-2): 1-8.
3. Deshraj and R.L. Misra, 2000. Genetic divergence for economic characters in gladiolus under different environment, J. Orn. Hort. New Series, 3(1): 37-42.
4. Murthy, B.R. and V. Arunachalam, 1966. The nature of genetic divergence in relation to breeding system in crop plants, Indian J. Genet., 26: 188-189.
5. Rao, C.R., 1952. Advanced Statistical Methods in Biometrics Research. John Wiley and Sons, New York.

6. Arya, S., R. Kumar and K. Gupta, 1999. Genetic divergence in gladiolus cultivars. Proc. National Symposium on Emerging Scenario in Ornamental Horticulture in 2000 AD and Beyond, New Delhi.
7. De, L.C. and R.L. Misra, 1993. Genetic diversity in gladiolus, Indian J. Plant Genet. Resources, 6(1): 9-14.
8. Nimbalkar, C.A., S.M. Katwate, M.T. Patil, V.T. Desai and R.L. Misra, 2002. Genetic divergence and selection of genotypes in gladiolus. *In:* Floriculture Research Trends in India. Proc.: National Symposium on Indian Floriculture in the New Millennium, Lalbagh, Bangalore, pp: 100-104.
9. Mahlanobis, P.C., 1930. On the generalized distance in statistics. Proc. Nat. Inst. Sci., 12: 49-55.
10. http://www.aboutgardens.com/Plants/Bulbs/Gladiolus/
11. http://www.clustal.org/clustal2/
12. http://www.ebi.ac.uk/Tools/msa/clustalw2/
13. http://www.genome.jp/kegg/genes.html
14. http://www.ncbi.nlm.nih.gov/genev
15. http://muextension.missouri.edu/xplor/agguides/-hort/g06620.htm