

RESEARCH ARTICLE

Phenotypic Characterization and Diversity Analysis of a Collection of *Gladiolus* Cultivars in India using Morpho-physiological Traits

Varun M. Hiremath*, Kanwar P. Singh, Kishan Swaroop and Pooja Anand

Abstract

Gladiolus is a beautiful ornamental geophyte valued for varieties with attractive florets and various colors. The genetic diversity of *Gladiolus* is tremendous due to species richness, outcrossing nature and heterozygosity. In the current study, phenotypic characterization and diversity assessment of 84 Indian and exotic-bred genotypes were investigated. Among different quantitative and qualitative traits, the coefficient of variation varied from 7.90% (androecium length) to 48.23% (chlorophyll 'b'). All qualitative characteristics were polymorphic. In correlation analysis, 136 out of 253 pairwise inter-trait combinations revealed significant and positive correlations, although a significant and negative connection was found between the duration of flowering and the time of the beginning of flowering. Indirect selection for plant height, rachis length, and corm fresh weight may aid in the development of the linked traits. Principal component analysis revealed that six out of the 17 principal component axis had Eigen-values greater than one, accounting for 79.31% of the overall variability. The stable expression and better performance of *Gladiolus* genotypes for quantitative traits, such as plant height, length of the leaf blade, corm fresh weight, floret length, tepal length, and floret width would be useful in selection of superior offspring from hybridization. Cluster analysis divided whole germplasm into two major clusters: cluster I (29 genotypes) and cluster II (55 genotypes). Genotypes observed in subcluster Ia (08 genotypes) and subcluster IIc (13 genotypes) are relatively dissimilar from one another. Cultivar 'Victor' is found to be relatively distinct among all genotypes because of taller plants, bigger corms, short flowering duration, and maximum corm fresh weight. Therefore, these set of genotypes can serve as a reference collection for DUS testing of novel varieties and genotype-specific information generated from this study can be useful for breeders to claim protection for their varieties

Keywords: Characterization, Corm, Descriptors, Diversity, *Gladiolus*.

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Received: 29/08/2022 **Revised:** 23/01/2023

Accepted: 03/04/2023

How to cite this article: Hiremath, V.M., Singh, K.P., Swaroop, K., Anand, P. (2023). Phenotypic Characterization and Diversity Analysis of a Collection of *Gladiolus* Cultivars in India using Morpho-physiological Traits. *Indian J. Plant Genetic Resources*. 36(2), 265-279.
DOI: 10.61949/0976-1926.2023.v36i02.09

Introduction

Gladiolus (*Gladiolus* × *grandiflorus* L.) is a significant member of the Iridaceae family. It is a high-value bulbous flower crop grown all over the world for its eye-catching multi-colored spikes with long vase life. *Gladiolus* has 270 species (Goldblatt *et al.*, 2014), with around 263 species found in Sub-Saharan Africa and Madagascar, seven species endemic to Eurasia (Goldblatt & Manning, 2008), about 170 species native to Southern Africa, and 93 species unique to tropical Africa. According to *Statista*, an international organization located in Germany, *Gladiolus* ranks fifth in terms of area and production among bulbous flowers grown worldwide (Pas, 2018). *Gladiolus* is grown on an estimated 11.66 thousand hectares in India, with a production of 106 crore cut flowers (agricoop.nic.in). *Gladiolus* is mostly a winter flower crop, but it may be produced throughout the year in mild climates. Its cut flowers are used to decorate vases, prepare bouquets, and for flower arrangements.

Gladiolus is a highly heterozygous, cross-pollinated crop. Its cultivars are said to have evolved naturally by

hybridization between the wild species *Gladiolus cruentus*, *G. natalensis*, *G. oppositiflorus*, *G. papilio* and *G. saundersii* (Imanishi, 1989). Because *Gladiolus* species are very cross-compatible, interspecific hybridization has become an important breeding strategy. Most species of *Gladiolus* have an identical basic chromosome number of $x = 15$ (Sinha and Roy, 2002) and a similar chromosomal configuration. Therefore, it became a popular plant for hybridization and the development of new colorful and vigorous hybrids; these new hybrids are estimated to number over 10,000 (Sinha and Roy, 2002). Flower patterns, floral colors, and flowering behavior of cultivated species vary greatly due to decades of interspecific hybridization and selection. New fascinating cultivars with novel floret color, vase life, pest and disease resistance are released each year to meet consumer demand.

Gladiolus is an exotic commercial crop in India with no native *Gladiolus* species found yet (Cantor and Tolety, 2011). *Gladiolus* hybrids and varieties have been routinely brought to India from South Africa and Europe since the 18th century for breeding and cultivation. Many *Gladiolus* hybrids and variants were subsequently developed utilizing these exotic varieties and released for cultivation in India. Systematic characterization involves distinguishing plant genetic resources by virtue of any difference in their phenotype or genetic make-up (De Vicente *et al.*, 2006). Genetically diverse germplasm broadens the genetic base by selecting distinct cross combinations in the hybridization programme. Accurately identifying and documenting cultivars is also important to protect the plant breeder's rights. Characterization data is used to monitor germplasm behavior, identify and distinguish between different accessions conserved in field or *in-vitro* gene banks. In this direction, different workers have characterized selected germplasm and delineated genetic diversity in previous studies in *Gladiolus* (Kadam *et al.*, 2014; Singh *et al.*, 2018). Szczepaniak *et al.* (2016) have even clearly differentiated *G. palustris* and *G. imbricatus* using phenotypic traits (plant height, flower number, leaf width, leaf number and corm morphology) to find out the possibility of natural hybridization. Despite the importance of genus *Gladiolus*, research in India is still limited and comprehensive genetic diversity studies covering a wide range of accessions are inadequate. The objective of the study was to phenotypically characterize and assess genetic diversity of 84 popular cultivars of *Gladiolus* grown in India by using morpho-physiological descriptors.

Materials and Methods

Plant Material and Data Collection

A total of 84 diverse *Gladiolus* genotypes collected from various sources were used in the present study (Table 1). Commercially cultivated varieties by farmers in India include

Table 1: Indian and exotic *Gladiolus* cultivars used in the study.

Origin	Institute/organization	Cultivars
Indian	BHU, Varanasi	Malaviya Shatabdi, Malaviya Kiran, Malaviya Kundan
	NBRI, Lucknow	Kalima, Neelima, Roshni, Mohini
	IARI, New Delhi	Pusa Gunjan, GS-2, Anjali, Dhanvantari, Pusa Shagun, Pusa Shabnam, Pusa Lohit, Pusa Manmohak, Pusa Sindhuri, Pusa Sringarika, Pusa Red Valentine, Neel Rekha, Pusa Suhagin, Pusa Urmi, Pusa Chirag, Pusa Archana, Pusa Sarang, Urmil, Delhi Callianthus, Pusa Urvashi, Pusa Chandni, Suchitra, Surya Kiran, Pusa Shweta, Pusa Srijana, Pusa Gulal, Pusa Kiran, Pusa Bindiya
	IIHR, Bengaluru	Arka Tilak, Arka Nazrana, Arka Aayush, Arka Sapna, Arka Kesar, Arka Amar, Arka Poonam, Arka Gold, Arka Aarti, Arka Naveen, Arka Darshan
	PAU, Ludhiana	Punjab Glad 1, Punjab Morning, Punjab Glance, Punjab Glad 2, Punjab Dawn, Punjab Lemon Delight, Punjab Pink Elegance, C.P.G
Exotic	-	Yellow Star, Amethyst, Aldebaran, Tiger Flame, Nova Lux, Euro Vision, Nicole, Fidelio, True Love, Vink's Glory, Anglia, Sancerre, Victor, Creamy Green, Friendship, Pink Lady, Plumtart, Lucky Shamrock, Peter Pear's, Vicki Lin, African Star, Apple Blossom, Summer Sunshine, Algarve, Fire Flame, Lady Jane, Yellow Stone, Rose Supreme, Jacksonville Gold, Praha

mostly exotic varieties. Characterization of these genotypes based on 35 morpho-physiological descriptors was carried out at the research farm of Division of Floriculture and Landscaping, IARI, New Delhi. It is situated at 77° 12' E longitude, 28°40' latitude and at an altitude of 228.16 m above mean sea level. *Gladiolus* corms (4–5 cm diameter) of respective genotypes were planted in last week of October at a spacing of 30 cm × 30 cm in randomized block design (RBD) with three replications under open field conditions during 2017-18 and 2018-19. Standardized uniform cultivation practices were followed throughout the cropping season to raise a good crop. DUS descriptors for all varieties, hybrids and parental lines of *Gladiolus* spp. and its cultivars developed by the Protection of Plant Varieties and Farmers' Rights Authority, New Delhi were used as reference guide for data collection (Kalloo *et al.*, 2013). As mentioned in Tables 2 and 3, observations on 23 quantitative and 12 qualitative characteristics were recorded when the plants were at full flowering or expression stage. Data were recorded on ten randomly selected plants in each replication, avoiding border plants from each genotype.

Table 2: Descriptive statistics for morpho-physiological characteristics of 84 *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

Trait	Min.	Max.	Mean	SE	CV	Skewness	Kurtosis
Plant height (cm)	40.42	78.00	57.73	0.84	13.32	0.42	2.86
Length of the leaf blade (cm)	30.33	62.25	43.70	0.64	13.38	0.66	3.75
Width of the leaf blade (cm)	1.81	4.30	3.01	0.06	17.17	0.33	2.72
Rachis length (cm)	18.50	52.25	35.35	0.72	18.79	0.34	2.90
Number of florets per spike (No.)	7.33	18.17	13.10	0.23	16.25	0.03	2.83
Bract length (cm)	3.47	7.03	4.90	0.09	16.77	0.43	2.64
Floret length (cm)	7.07	11.17	9.40	0.08	8.09	-0.16	3.14
Floret width (cm)	6.77	11.13	8.76	0.09	9.54	-0.05	3.06
Tepal length (cm)	4.53	8.47	5.96	0.07	11.34	1.05	5.61
Tepal width (cm)	2.50	5.70	3.97	0.07	15.62	0.55	3.18
Androecium length (cm)	3.78	5.65	4.63	0.04	7.90	0.25	2.98
Anther length (cm)	0.53	1.78	1.27	0.02	16.12	-0.68	4.77
Stigma lobe length (cm)	0.47	1.37	0.71	0.02	24.93	1.38	5.56
Style length (cm)	3.88	7.47	5.96	0.06	9.09	-0.26	5.06
Time of beginning of flowering (days)	72.33	122.50	104.39	1.15	10.12	-0.62	2.91
Duration of flowering (days)	6.83	36.50	14.65	0.60	37.28	1.23	5.12
Corm diameter (days)	3.92	6.88	5.36	0.07	12.30	-0.12	2.56
Corm fresh weight (g)	23.08	90.83	44.64	1.37	28.04	0.63	4.06
Chlorophyll 'a' (mg/g)	1.28	6.06	4.04	0.10	22.73	-0.18	3.07
Chlorophyll 'b' (mg/g)	0.03	1.77	0.62	0.03	48.23	1.14	4.90
Total chlorophyll (mg/g)	1.27	7.33	4.65	0.12	23.44	-0.09	3.24
Total carotenoids (mg/g)	0.69	2.11	1.51	0.03	18.27	-0.18	3.03
Normal difference vegetation index	0.43	0.66	0.53	0.01	9.50	0.44	2.94

Qualitative data were collected per DUS guidelines and described with characteristics and their states. Floret color and corm skin color were recorded during day time (11.00 AM to 1.00 PM) using Royal Horticultural Society (RHS) color chart (6th edition, 2015) as per the guidelines recommended by the International Union for the Protection of New Varieties of Plants (UPOV), Geneva, Switzerland.

Physiological characteristics like leaf chlorophyll content (mg/g FW) was estimated by a non-maceration method using dimethyl sulphoxide (DMSO) as described by Hiscox and Israelstam (1979). A total of 50 mg of finely chopped leaf samples were placed in test tubes to which 10 mL DMSO was added. The test tubes were covered with aluminum foil and kept in an oven at 65°C for 4 hours. After 4 hours, the absorbance of chlorophyll solution was recorded at 470, 645 and 663 nm against DMSO as blank. Chlorophyll 'a', 'b' and total chlorophyll were calculated by using the formulae chlorophyll 'a' = $12.7 A_{663} - 2.69 A_{645}$; chlorophyll 'b' = $22.9 A_{645} - 4.68 A_{663}$, where 'A' is OD value (Arnon, 1949). The total carotenoid content (mg/g FW) was calculated as per the formulae total carotenoids = $[1000 A_{470} - (3.27 \text{ Chlorophyll 'a' } + 104 \text{ Chlorophyll 'b'})]/229$, where 'A' is OD value (Lichtenthaler and Wellburn, 1983). Normalized Difference Vegetation Index was recorded on random group of plants

in each plot using 'GreenSeeker' NDVI meter to estimate the relative chlorophyll content in each *Gladiolus* genotype.

Statistical Analysis

Morpho-physiological data recorded during two consecutive years of 2017-18 and 2018-19 was subjected to pooled analysis and mean values of each character for all *Gladiolus* genotypes were calculated. The recorded data for quantitative characters were analyzed using the software package R of version 4.2.0 developed by the R Core Team (2022). Descriptive statistics, including the mean, median, range, variance, skewness and kurtosis were calculated for all quantitative variables. Coefficients of variation [CV = (Std. Dev./Mean) *100] were estimated as the indicator of variability. Box plots for quantitative characters were created using the "ggplot2" package. The Pearson correlation coefficients (r) among the 23 different qualitative traits were estimated using the package "Corrplot". Principal component analysis (PCA) was done to determine the extent of the variation and percentage similarity within the *Gladiolus* genotypes. The Euclidean distance was used to calculate the distance coefficients matrix for cluster analysis using UPGMA method. The dendrogram was constructed using the "factoextra" R package.

Table 3: Twelve qualitative characteristics and their states in 84 *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

Cv. No.	Cultivar name	Leaf blade color	Anthocyanin coloration of bracts	Shape of apex of bracts	Floret color*	Flower secondary color	Outer skin color of corolla	Outer whorl shape	Filament main color	Stigma lobe color	Floret shape	Flower attitude	Arrangement of florets on spike®
1	Anglia	DG	W	O	Fan1, Yellow group 8(B)	Yellow	Yellowish -Orange	O	Light yellow	Yellow	T	U	T
2	Amethyst	YG	W	A	Fan 2, Purple - Violet group N80 (C)	White (pure)	Light yellow	O	Light pink	Pink purple	T	SU	Z
3	Pusa Gunjan	DG	W	O	Fan1, Orange group 27(A)	Red	Dark yellow	E	Light pink	White	S	SU	Z
4	Punjab Glad 1	LG	W	BO	Fan1, Orange-Red Group 33(D)	Yellow	Dark yellow	O	Light yellow	Pink purple	T	SU	Z
5	Aldebaran	DG	W	A	Fan1, Yellow group 9(D)	Red	Dark yellow	O	Light yellow	Yellow	S	SU	Z
6	Fire Flame	LG	A	O	Fan 1, Red group (45B)	Yellow	Yellowish -orange	E	White	Pink purple	T	SU	Z
7	Tiger Flame	DG	W	O	Fan 1, Red Group 38(A)	Yellow	Dark yellow	E	Light yellow	Yellow	T	SU	T
8	Kalima	LG	S	O	Fan 1, Red group (45B)	None	Purple	O	Light yellow	Pink purple	T	SU	Z
9	Neelima	YG	W	A	Fan 2, Purple group 77(B)	White (pure)	Light yellow	O	White	Blue purple	S	U	T
10	GS-2	DG	W	BO	Fan 1, Yellow group 4(D)	Red	Light yellow	E	Light yellow	Yellow	R	SU	O
11	Praha	DG	M	A	Fan 1, Orange Red Group N30(A)	Yellow	Purple	O	Light yellow	Red	T	SU	T
12	Anjali	LG	W	O	Fan 4, White group NN155(B)	Pink	Dark yellow	O	White	White	R	SU	Z
13	Malaviya Shatabdi	LG	W	O	Fan 2, Red purple group 69 (C)	Pink	Yellowish -orange	O	White	White	T	U	T
14	Roshni	DG	M	A	Fan 1, Red group 43(B)	Yellowish-green	Orange	E	Light pink	Pink purple	T	U	Z
15	Yellow Stone	YG	W	O	Fan 1, Yellow group 8(C)	None	Orange	E	Light yellow	Yellow	T	SU	T
16	Dhanvantari	LG	W	A	Fan 1, Yellow group 4(D)	Red	Yellowish -orange	O	Light yellow	Yellow	S	SU	Z

17	Punjab Morning	DG	W	O	Fan 2, Red purple group 65(C)	Red	Yellowish -orange	White	White	T	U	T
18	Rose Supreme	DG	W	A	Fan 1, Red Group 48(D)	Yellowish-green	Light yellow	Light yellow	White	T	U	T
19	Punjab Glance	DG	W	A	Fan 1, Red Group 38(A)	Yellow	Light yellow	White	White	T	U	Z
20	Sancerre	DG	W	A	Fan 4, White group NN155(B)	Pink	Light yellow	White	White	S	SU	Z
21	Pusa Shagun	LG	M	O	Fan 1, Orange Red Group N30(B)	Purple red	Purple	Light pink	Red	T	SU	T
22	Punjab Glad 2	LG	A	A	Fan 1, Yellow group 8(D)	Pink	Light yellow	Light yellow	Pink purple	S	SU	T
23	Pusa Shabnam	DG	M	O	Fan 1, Yellow group 8(D)	Pink	Yellowish -orange	White	White	T	U	T
24	Punjab Dawn	DG	W	O	Fan 1, Red Group 38(A)	Red	Orange	White	White	T	SU	T
25	Arka Tlak	DG	M	O	Fan 1, Orange red group N30A	None	Red	Light red	Red	T	U	Z
26	Victor	DG	W	O	Fan 3, Yellow Green 154(D)	None	Yellowish -orange	Light yellow	Yellow	S	SU	T
27	Pusa Lohit	LG	S	O	Fan 1, Red group 44(D)	Pink	Red	Light pink	Pink purple	S	U	Z
28	Malaviya Kiran	LG	M	A	Fan 4, White group NN155(A)	Purple red	Yellowish -orange	White	White	T	U	T
29	Pusa Manmohak	DG	M	O	Fan 1, Red group (40B)	Red	Red	Light pink	Red	S	SU	T
30	Pusa Sindhuri	DG	M	BO	Fan 1, Red group 51(A)	Yellowish-green	Light yellow	Light yellow	Pink purple	S	SU	T
31	Arka Nazrana	DG	VS	A	Fan 2, Red purple group 61(B)	None	Purple	White	Pink purple	S	U	T
32	Nova Lux	DG	W	A	Fan 1, Yellow group 11(B)	Purple red	Light yellow	Light yellow	Yellow	T	U	T
33	Euro Vision	DG	S	A	Fan 1, Red group 40(A)	None	Purple	Light yellow	Pink purple	T	SU	Z
34	Lady Jane	LG	M	BO	Fan 1, Red group 41(B)	Yellow	Orange	Light yellow	Yellow	T	SU	Z

35	Jacksonville Gold	YG	W	A	Fan 1, Yellow group 6(D)	Red	Light yellow	E	Light yellow	Yellow	T	U	T
36	Punjab Lemon Delight	YG	M	BO	Fan 1, Yellow group 8(D)	Pink	Light yellow	O	Light yellow	Yellow	S	U	Z
37	Arka Aayush	LG	S	BO	Fan 1, Red group 41(B)	Yellow	Dark yellow	E	Light yellow	Yellow	T	SU	T
38	Pusa Sringerika	DG	W	O	Fan 2, Pink group NN 74(B)	Yellow	Light yellow	E	White	Yellow	T	SU	T
39	Pusa Red Valentine	DG	M	BO	Fan 1, Red group 45(C)	Pink	Red	Ob	Medium pink	Pink purple	T	SU	Z
40	Neel Rekha	DG	M	O	Fan 2, Red-purple group 64(B)	None	Orange	O	Light pink	Blue purple	S	SU	Z
41	Yellow Star	DG	W	O	Fan 1, Yellow group 10(B)	None	Light yellow	E	Light yellow	Yellow	R	U	Z
42	Creamy Green	LG	W	A	Fan 4, White group NN155(D)	None	Light yellow	O	White	White	T	U	Z
43	Friendship	YG	W	A	Fan 4, White group NN155(D)	None	Dark yellow	Ob	White	White	R	SU	T
44	Pink Lady	DG	W	A	Fan 1, Red group 49(C)	Orange	Dark yellow	E	Light pink	White	R	SU	Z
45	Arka Sapna	DG	W	O	Fan 4, White group 155(B)	Yellowish-green	Dark yellow	E	White	Pink purple	S	SU	T
46	Arka Kesar	LG	W	BO	Fan 1, Orange group 29(B)	Orange	Light yellow	E	Light yellow	Yellow	T	U	Z
47	Pusa Suhagin	DG	S	A	Fan 1, Red group 46(A)	None	Red	E	Light red	Red	T	SU	Z
48	Pusa Urmi	DG	W	O	Fan 2, Pink group NN74(A)	Violet blue	Orange	E	Light pink	Blue purple	T	SU	Z
49	Pusa Chirag	DG	W	BO	Fan 1, Red group 41(C)	Yellow	Light yellow	O	Light pink	Yellow	T	U	Z
50	Nicole	YG	M	O	Fan 1, Orange red group 30(A)	Yellow	Purple	E	Light yellow	Pink purple	T	U	Z
51	Pusa Archana	YG	W	BO	Fan 2, Red purple 68(B)	Yellowish-green	Purple	O	White	Pink purple	S	SU	Z
52	Arka Darshan	DG	M	BO	Fan 2, Red purple 63(B)	White (pure)	Orange red	E	Light pink	Pink purple	T	U	T

53	Plumtart	LG	S	A	Fan 2, Red purple 71(B)	None	Red	E	Medium pink	Violet	S	SU	Z
54	Pusa Sarang	DG	S	O	Fan 2, Purple group 76(A)	White (pure)	Purple red	E	Medium pink	Blue purple	T	SU	T
55	Fidelio	DG	W	O	Fan 2, Purple-violet group N80(A)	None	Orange	O	White	Blue purple	T	SU	T
56	Urmil	DG	W	A	Fan 2, Purple-violet group N80(B)	White (pure)	Orange	E	Light yellow	Blue purple	T	SU	Z
57	Lucky Shamrock	DG	W	O	Fan 1, Orange red group 33(D)	Orange	Orange	E	Light pink	Orange	T	SU	Z
58	Delhi Callianthus	LG	W	O	Fan 1, Red group 43(C)	Pink	Dark yellow	E	White	Orange	T	SU	Z
59	Pusa Urvashi	DG	M	O	Fan 2, Red purple 70(A)	White (pure)	Yellowish -orange	E	White	Blue purple	T	SU	T
60	Pusa Chandni	DG	M	A	Fan 1, Yellow group 11(D)	Yellow	Yellowish -orange	E	White	White	S	U	T
61	Suchitra	DG	W	A	Fan 1, Red group 56(B)	Red	Light yellow	O	White	White	S	U	T
62	Surya Kiran	YG	W	A	Fan 1, Red group 37(B)	Red	Yellowish -orange	E	White	White	T	SU	T
63	Arka Amar	LG	VS	O	Fan 1, Red group 48(B)	Yellow	Red	E	Light pink	Red	T	U	T
64	True Love	DG	M	O	Fan 2, Red purple N66(C)	White (pure)	Orange	E	Light pink	Pink purple	S	SU	O
65	Peter Pear's	LG	W	A	Fan 1, Orange-Red group 32(C)	White (pure)	Orange	E	White	White	T	SU	T
66	Pusa Shweta	DG	W	O	Fan 4, White group 155(B)	None	Dark yellow	O	Light yellow	White	T	U	T
67	Arka Poonam	YG	W	O	Fan 4, White group 155(A)	None	Others	E	White	White	T	U	Z
68	Pusa Srijana	DG	VS	A	Fan 2, Red purple group 71(C)	Yellow	Yellowish -orange	O	Light pink	Blue purple	S	U	Z
69	Pusa Gulal	DG	M	O	Fan 2, Red purple 61(B)	None	Red	E	Medium pink	Pink purple	S	SU	T
70	Vicki Lin	DG	W	A	Fan 1, Orange red group 33(D)	Orange	Orange	O	Light pink	Orange	T	H	Z

71	African Star	DG	W	O	Fan 1, Red group 48(C)	Red	Light yellow	O	White	White	T	U	Z
72	Arka Gold	DG	W	BO	Fan 1, Yellow group 8(D)	Red	Others	O	Light yellow	Yellow	T	SU	Z
73	Pusa Kiran	YG	M	O	Fan 1, Yellow group 11(D)	Pink	Yellowish-orange	E	White	White	R	SU	Z
74	Arka Aarti	DG	VS	A	Fan 1, Red group 44(B)	Red	Yellowish-orange	E	Light pink	Red	T	SU	Z
75	Arka Naveen	YG	A	O	Fan 2, Violet group 84 (C)	Yellow	Light yellow	O	White	White	T	U	Z
76	Apple Blossom	DG	A	O	Fan 1, Yellow group 11(D)	Purple Red	Light yellow	O	White	Blue purple	T	U	Z
77	Punjab Pink Elegance	LG	W	A	Fan 1, Red group 56 (D)	Red	Light yellow	E	White	White	T	U	T
78	Vink's Glory	DG	W	O	Fan 1, Yellow group 8(C)	None	Light yellow	E	Light yellow	Yellow	T	U	Z
79	Pusa Bindiya	YG	W	O	Fan 1, Red group 36(D)	Red	Light yellow	O	White	White	T	U	Z
80	Pusa Mohini	DG	W	O	Fan 2, Pink group NN74(C)	White (Pure)	Light yellow	E	White	White	S	U	Z
81	Summer Sunshine	LG	A	A	Fan 1, Yellow group 6(C)	None	Dark yellow	E	Light yellow	Yellow	T	SU	Z
82	Algarve	DG	M	O	Fan 1, Orange group 25(B)	None	Light yellow orange	E	Light yellow	Pink purple	S	SU	T
83	Malaviya Kundan	YG	W	BO	Fan 1, Yellow orange 18(B)	Yellowish-Green	Light yellow	O	Light yellow	Yellow	T	SU	Z
84	C.P.G	YG	S	A	Fan 1, Red group 46(A)	None	Red	O	Light red	Red	T	SU	Z

T= Upright
 Triangular
 S= Star
 R= Round
 H= Horizontal

E= Elliptic
 O= Ovate
 Ob= Obovate

*Refer Figure 3
 #Refer Figure 4

A= Absent, W=Weak, M= Medium, VS=Very Strong

LG=Light green, YG=Yellowish green, DG= Dark green

A=Acute, O=Obtuse, BO=Broadly Obtuse

Table 4: Eigen values, proportion of variability and factor loadings (Eigen vectors) of correlation matrix for 17 morpho-physiological traits of 84 *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

Characters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17
Plant height	0.36	0.13	0.21	0.12	0.34	-0.22	0.08	-0.19	0.26	0.14	0.02	0.06	-0.16	-0.23	-0.44	0.48	0.02
Length of the leaf blade	0.36	0.14	0.17	0.13	0.37	-0.18	0.06	-0.16	0.35	-0.13	0.08	0.03	0.16	0.35	0.36	-0.43	-0.01
Rachis length	-0.01	0.08	-0.34	0.49	0.14	0.24	0.19	0.09	0.01	-0.04	0.36	-0.12	0.46	-0.36	-0.14	-0.12	-0.01
Number of florets per spike	-0.10	0.00	-0.28	0.49	0.18	0.39	0.12	0.00	0.06	-0.04	-0.27	-0.02	-0.45	0.42	0.01	0.16	0.01
Bract length	0.20	0.24	-0.16	-0.11	0.09	0.23	-0.61	-0.42	-0.21	-0.02	0.03	-0.46	0.02	0.04	-0.05	0.02	-0.02
Floret length	0.22	0.42	-0.17	-0.06	-0.15	0.06	-0.01	-0.04	-0.23	-0.54	-0.06	0.58	0.06	0.04	-0.14	0.05	0.01
Floret width	0.24	0.37	-0.14	-0.03	-0.26	-0.08	0.26	0.18	0.16	0.02	-0.59	-0.39	0.12	-0.20	0.15	0.03	0.02
Tepal length	0.23	0.31	-0.27	0.00	-0.20	-0.29	0.16	0.18	-0.22	0.30	0.50	-0.10	-0.42	0.13	0.07	-0.09	-0.02
Time of beginning of flowering	0.05	0.17	-0.25	-0.38	0.38	0.30	-0.05	0.23	0.05	0.56	-0.12	0.34	0.18	0.05	0.03	-0.01	0.00
Duration of flowering	-0.08	0.09	0.00	0.49	-0.33	-0.25	-0.30	-0.29	-0.03	0.46	-0.21	0.33	0.17	0.01	0.08	-0.05	-0.01
Corm diameter	0.30	-0.07	0.39	0.13	-0.09	0.13	0.14	0.23	-0.44	0.13	-0.02	-0.14	0.37	0.46	-0.23	0.13	-0.02
Corm fresh weight	0.27	-0.01	0.41	0.16	0.07	0.36	0.00	0.05	-0.31	0.07	-0.06	0.12	-0.33	-0.48	0.31	-0.21	0.01
Chlorophyll 'a'	0.32	-0.38	-0.24	0.01	-0.02	-0.10	-0.11	0.10	0.01	-0.02	-0.19	0.04	-0.10	-0.05	-0.29	-0.30	-0.66
Chlorophyll 'b'	0.18	-0.26	-0.18	-0.17	-0.15	0.16	0.46	-0.59	-0.10	0.10	0.08	0.06	0.11	0.01	0.31	0.26	-0.18
Total chlorophyll	0.32	-0.39	-0.25	-0.04	-0.06	-0.03	0.03	-0.08	-0.03	0.03	-0.11	0.04	-0.05	-0.01	-0.23	-0.28	0.72
Total carotenoids	0.30	-0.30	-0.20	0.12	0.02	-0.13	-0.36	0.37	0.04	-0.13	0.09	0.05	0.10	-0.04	0.46	0.48	0.06
NDVI	0.19	0.02	0.16	-0.04	-0.52	0.46	-0.13	0.07	0.58	0.06	0.25	0.06	-0.04	0.08	-0.11	0.00	-0.01
Eigen value	3.92	2.84	2.35	2.06	1.20	1.12	0.87	0.81	0.55	0.37	0.31	0.18	0.16	0.13	0.09	0.06	0.00
%Variance	23.06	16.72	13.79	12.12	7.04	6.58	5.10	4.78	3.22	2.16	1.79	1.05	0.96	0.76	0.53	0.33	0.01
%Cumulative variance	23.06	39.77	53.57	65.69	72.73	79.32	84.41	89.19	92.41	94.56	96.36	97.40	98.36	99.12	99.65	99.99	100.00

Table 5: Cluster wise means for different quantitative traits of 84 *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19.

Characters	PH	LLB	RL	NFS	BL	FL	FW	TL	TBF	DF	CD	CFW	Chl 'a'	Chl 'b'	Total Chlorophyll	Total carotenoids	NDVI
Sub-cluster I	Mean	52.36	30.02	11.19	5.95	10.03	9.04	6.11	109.54	13.19	6.11	63.85	3.90	0.46	4.36	1.52	0.57
la	±SE	2.44	1.87	1.46	0.27	0.27	0.25	0.17	2.51	1.85	0.21	5.10	0.19	0.03	0.19	0.05	0.02
Sub-cluster II	Mean	60.97	46.44	39.24	5.09	9.63	9.08	6.34	104.56	14.55	5.57	46.84	4.93	0.85	5.78	1.74	0.54
lb	±SE	1.06	0.75	1.60	0.18	0.11	0.14	0.16	2.23	1.01	0.12	1.77	0.12	0.08	0.14	0.05	0.01
Sub-cluster III	Mean	53.57	41.89	38.07	5.03	9.85	9.26	6.31	114.32	14.86	4.80	34.31	2.91	0.47	3.39	1.23	0.52
IIa	±SE	2.52	2.19	1.42	0.39	0.22	0.31	0.16	1.68	0.98	0.12	2.57	0.22	0.07	0.26	0.07	0.01
Sub-cluster IV	Mean	54.48	40.44	30.17	11.89	8.92	8.29	5.48	107.01	11.07	4.91	36.09	4.41	0.71	5.08	1.54	0.51
IIb	±SE	1.31	0.93	1.12	0.42	0.17	0.18	0.09	1.61	0.59	0.15	2.17	0.18	0.07	0.20	0.06	0.01
Sub-cluster V	Mean	55.74	41.78	35.90	13.48	9.12	8.46	5.74	95.67	17.52	5.53	47.82	3.68	0.50	4.16	1.44	0.53
IIc	±SE	1.22	0.76	1.09	0.39	0.13	0.14	0.12	1.89	1.39	0.09	1.82	0.10	0.04	0.13	0.04	0.01

Results and Discussion

Descriptive Characteristics

The data pertaining to the variation among 84 *Gladiolus* genotypes for morpho-physiological characters are given in Table 2. The coefficient of variation varied from 7.90% (androecium length) to 48.23% (chlorophyll 'b'). Chlorophyll 'b' content (Chl' b) and duration of flowering (DF) showed high CV values of greater than 30%. Corm fresh weight (CFW), stigma lobe length (SLL), total chlorophyll (TCH), chlorophyll 'a' (Chl' a), rachis length (RL), total carotenoids (TC), width of leaf blade (WLB), bract length (BL), number of florets per spike (NFS), anther length (ATL), tepal width (TW) showed moderate CV values ranging from 15 to 30%. Length of leaf blade (LLB), plant height (PH), corm diameter (CD), tepal length (TL), time of beginning of flowering (TBF), floret width (FW), normal difference vegetation index (NDVI), style length (SL), floret length (FL) and androecium length (AL) showed low CV values of less than 15%. In a similar line of study, Pattanaik *et al.* (2015) also reported the highest CV (>20%) for corm weight, rachis length and number of spikes per plant. CV is very important in selecting elite parents in the breeding of *Gladiolus* × *hybridus* (Hort *et al.*, 2014). Skewness and kurtosis values indicated that morpho-physiological data distributed among all cultivars is symmetrical and follows a normal distribution. This variability in *Gladiolus* germplasm could be related to the high heterozygosity achieved during outcrossing.

Phenotypic characterization is the primary criteria for classifying and identifying crop species and varieties. It helps to obtain robust genetic information that would guide us in germplasm conservation. Vegetative and reproductive characteristics generally allow for quick genotype determination and genetic trait association (Datta and Chakrabarty, 2016). The expression of phenotypic features at the gene level allows for genetic differentiation of crops, and these traits become transmissible in subsequent generations (De Vicente *et al.*, 2006). In the case of *Gladiolus*, it also aids in the identification of more reliable features other than commonly used criteria such as floret color, spike length, number of florets per spike, floret diameter, and vase life for the purpose of selecting superior genotypes in hybridization.

Box plots showing genetic variability among 84 *Gladiolus* genotypes with respect to various morpho-physiological characters and outliers have been depicted in Figure 1. In the present study, box plots displayed highly informative means suggesting considerable variation in selected *Gladiolus* germplasm. *Gladiolus* cultivars showing extreme variability were identified and plotted as outliers for morphological characters viz., length of leaf blade (Praha, Pusa Shagun), corm fresh weight (Victor), duration of flowering (Malaviya Shatabdi, Yellow Stone), floret

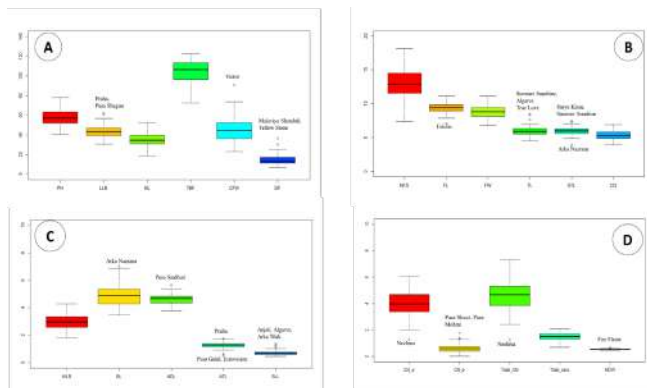


Figure 1: Box plots indicating genetic variability in quantitative characteristics in *Gladiolus* germplasm. Graph A - boxplots of plant height (PH), length of the leaf blade (LLB), rachis length (RL), time of beginning of flowering (TBF), corm fresh weight (CFW), duration of flowering (DF); Graph B – boxplots of number of florets per spike (NFS), flower length (FL), flower width (FW), tepal length (TL), stigma length (STL), corm diameter (CD); Graph C – boxplots of width of leaf blade (WLB), bract length (BL), androecium length (ADL), anther length (ATL), stigma lobe length (SLL); Graph D – boxplots of chlorophyll ‘a’ (Chl’a), chlorophyll ‘b’ (Chl’b), total chlorophyll (Total Chl), total carotenoids (Total caro), Normal Difference Vegetation Index (NDVI). For every box plot, the horizontal line represents the median of the trait, the box represents the interquartile range, the bars outside the box represent the extremes and the dots indicate the outliers

length (Fidelio), tepal length (True Love, Algarve, Summer Sunshine), stigma length (Surya Kiran, Summer Sunshine, Arka Nazrana), bract length (Arka Nazrana), androecium length (Pusa Sindhuri), anther length (Praha, Pusa Gulal, Euro Vision) and stigma lobe length (Anjali, Algarve, Arka Tilak). Kumar and Kulkarni (2009) assessed seven parents and their 21 hybrids using vegetative and flowering characteristics for phenotypic variation. It has been reported that days to sprouting, plant height, stem girth, number of leaves, leaf length, days required for bud initiation, days required for first floret opening and days required for first to last floret opening can be useful criteria for selection of better progenies subsequent to hybridization. For physiological characteristics such as chlorophyll ‘a’ (Neelima), chlorophyll ‘b’ (Pusa Shwet, Pusa Mohini), total chlorophyll (Neelima) and for NDVI (Fire Flame), varieties with extreme values were also detected. All qualitative characters are found to be polymorphic among all *Gladiolus* genotypes. States of each qualitative characteristic for all *Gladiolus* genotypes used in the study are given in Table 3. Spikes of selected *Gladiolus* genotypes are presented in Figure 2. Floret color and corm skin color are the most reliable morphological descriptors for differentiating *Gladiolus* varieties. It is feasible to employ them for the certification of genetic purity in *Gladiolus* cultivars. Outer corm skin color of selected *Gladiolus* genotypes is depicted in Figure 3.

Correlation Analysis

Information obtained by analyzing the coefficients of correlation between the plant traits helps us understand



Figure 2: Spike characteristics of representative *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

each character’s relative influence on flower quality and yield in *Gladiolus* production. Correlation coefficients between the morpho-physiological traits could determine whether selection for one trait may affect the other ones. In the present study, altogether positive and negative correlations were observed among twenty three different traits (Figure 4). The figure represents that the correlation coefficients with the absolute measures more than 0.22 and 0.28 were statistically significant at 5 and 1% probability, respectively. Meanwhile, significant correlations of ‘r’ value more than ±0.50 between the traits indicated relative influence of each character. Significant and positive correlations were obtained for plant height with length of leaf blade ($r = 0.90$) and corm fresh weight ($r = 0.51$); bract length and floret length ($r = 0.54$); floret width with floret length ($r = 0.67$) and tepal length ($r = 0.69$); rachis length with number of florets per spike ($r = 0.78$); corm diameter with corm fresh weight ($r = 0.80$). The relationship between plant height, length of leaf blade and corm fresh weight was apparent because length of leaf blade serves as a source of photosynthesis, directing photosynthates towards plant growth and eventually carbohydrate accumulation in corms. Correlations for floret characteristics *viz.*, bract length, floret

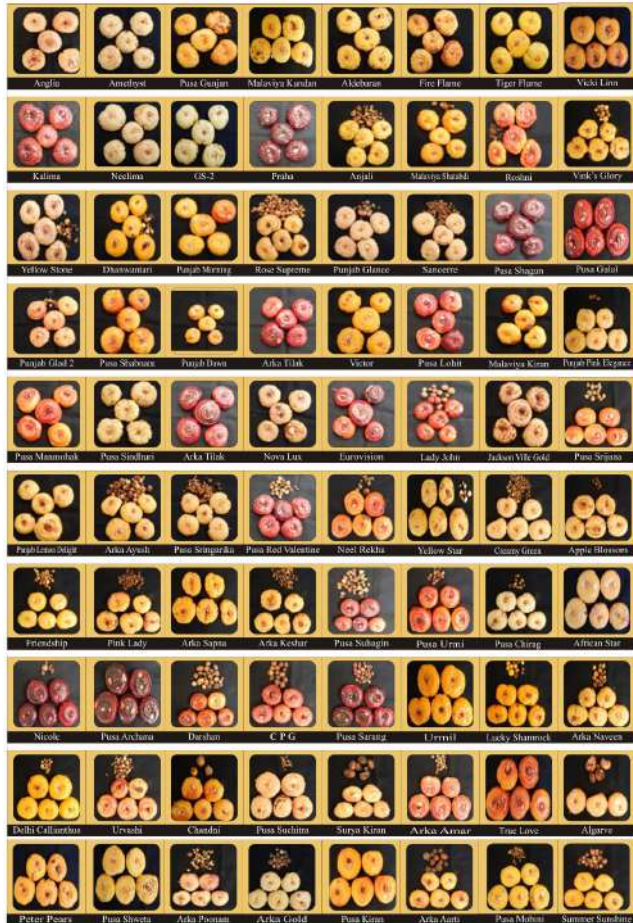


Figure 3: Corm skin color of representative *Gladiolus* genotypes evaluated at Delhi during 2017-18 and 2018-19

length, floret width and tepal length were found significant with each other. Rachis length exhibited significant and positive correlation with number of florets per spike. For corm characters, correlation was obvious between corm diameter and corm fresh weight. The correlations for these characters observed in our study were consistent with those reported earlier in *Gladiolus* by Chaudhary *et al.* (2011) and Zaharia *et al.* (2018). Furthermore, it is evident that a heavier corm and/or a larger corm size result in a higher plant height and rachis length. Among physiological characteristics, significant and positive correlations were found for total chlorophyll with chlorophyll 'a' ($r = 0.97$), chlorophyll 'b' (0.68) and total carotenoids ($r = 0.75$). This revealed a close relationship between the various chlorophyll components in leaves. Flowering duration had a significant but negative correlation with time taken to flowering ($r = -0.50$). Duration of flowering may be longer as there is an increase in rachis length and the number of florets per spike. Since *Gladiolus* flower 3 to 4 months after the vegetative phase, they have a lengthy juvenile period. In light of this finding, our work hypothesizes that indirect selection for plant height, rachis length, and corm fresh weight may aid in the development of the linked traits. In the same line of study, Ramzan *et al.* (2016)

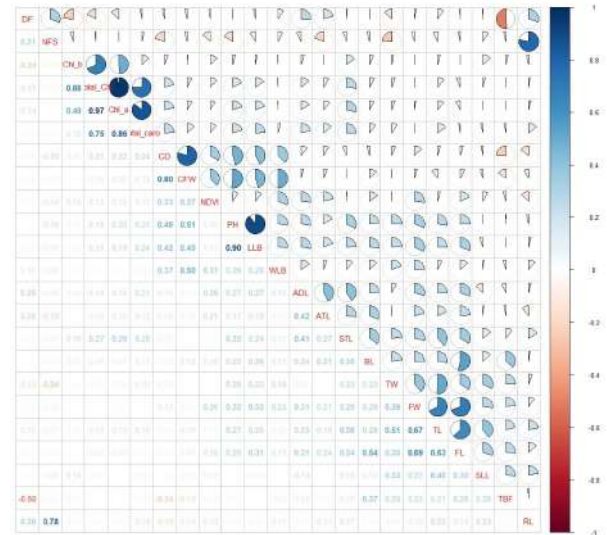


Figure 4: Pearson correlation coefficients among 23 quantitative traits of 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19. The correlation coefficients with the absolute values more than 0.22 and 0.28 were significant at the statistical probability level of 5 and 1%, respectively

stated that indirect selection for floret breadth in hybrid progenies simultaneously improve number of florets per spike and rachis length due to direct and positive correlation between them. Among the 253 potential trait combinations, 136 pairwise inter-trait combinations revealed significant and positive correlations, although a significant and negative connection was found between flowering duration and time of beginning of flowering. Positive correlations between two attributes typically indicate that improving one trait will improve the other.

Principal Component Analysis

Principal component analysis (PCA) allows us to determine the traits that contribute the most to variability as well as recognize trait-specific germplasm for breeding. It is also a successful method of evaluating germplasm for desirable characteristics. In the current study, 17 morpho-physiological characters with significant correlations were used to create principal components that can explain most of the variance. Eigen-values, proportion of variability and factor scores obtained from PCA were used to determine the axis's relative discriminative power and associated characters (Table 4). Scree plot showing the contribution of each principal component towards variability in *Gladiolus* germplasm is presented in Figure 5. Table 4 reveals that six out of the 17 principal component axis had Eigen-values greater than one, and all together account for 79.31% of the total variability. The bold-marked values indicate the highest correlations between variables and the corresponding factor or principal component (PC). The first factor which accounted for 23.06% of the variation was strongly associated with plant height, length of leaf blade,

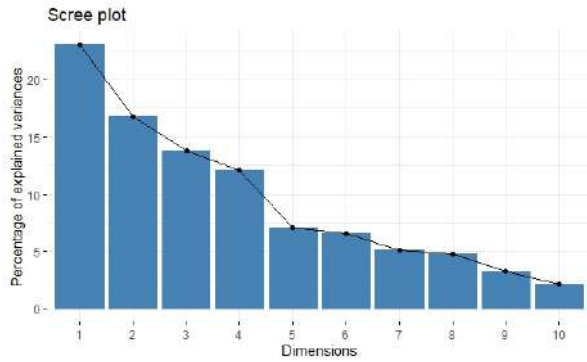


Figure 5: Scree plot showing contribution of each principal component towards variability of 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19

bract length, floret length, floret width, tepal length, corm diameter, corm fresh weight, chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll, total carotenoids and normal difference vegetation index. PC 1 can thus be considered a component of productivity because it contains multiple characteristics that are components of production. A similar study on *Gladiolus* found that corm diameter, days to first floret opening and colour bud display, spike and stem diameter accounted for the greater part of variation in genotypes (Zahoor *et al.*, 2019). The second factor which accounts for 16.72% of the variation, has affected bract length, floret length, floret width and tepal length. Positive loadings were found for all variables in the first and second factors. The third factor with significant positive loadings, accounting for 13.79% of total variation contributed by plant height, corm diameter and corm fresh weight. The variation accounted in the fourth factor (12.12%) was found to be significantly related to rachis length, number of florets per spike and flowering duration. The fifth factor (7.04%) consisted of variation contributed from traits plant height, length of leaf blade and time of beginning of flowering whereas the sixth factor was characterized by rachis length, number of florets per spike, bract length, time of beginning of flowering, corm fresh weight and normal difference vegetative index. Among all quantitative traits, plant height, length of the leaf blade, corm fresh weight, floret length, tepal length and floret width contributed more to the total variation in *Gladiolus* germplasm in the present study. Hybridization among *Gladiolus* genotypes with stable expression and better performance for these traits would be useful in selection of desirable progenies. Variable correlation plot within the circle on bifactorial plane represents contributions of quantitative traits (Figure 6).

Eigen-values demonstrated the PCA’s relative discriminating capacity. PCA 1 had the greatest discriminating capacity, as evidenced by its Eigen-value of 3.92, followed by PCA 2 with an Eigen-value of 2.84. Principal component analysis was successful in identifying linear combinations of the 17 different quantitative features that distinguished

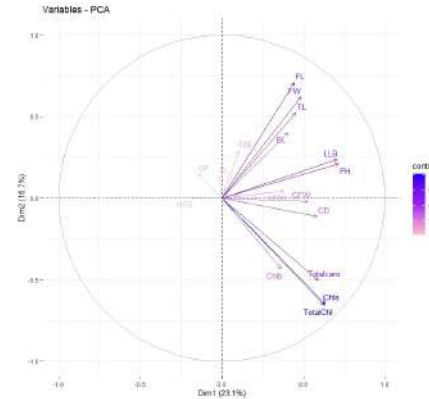


Figure 6: Variable correlation plot for seventeen quantitative traits of 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19

various *Gladiolus* genotype clusters. A two-dimensional scatter plot of PCA visualized the relationships between *Gladiolus* genotypes (Figure 7). Therefore, PCA revealed that the cumulative variation explained by the first six components accounted over 79.31% variation indicating great variability in quantitative traits, a high genetic diversity between *Gladiolus* genotypes. This suggested that these traits had significant contribution in *Gladiolus* diversity.

Cluster Analysis

Hierarchical cluster analysis based on squared Euclidian distance (distance coefficient=13) classified all 84 *Gladiolus* genotypes in to two major clusters I and II in a dendrogram (Figure 8). Cluster wise mean data of 17 quantitative characteristics is given in Table 5. Cluster I consists of 29 genotypes, which was further classified into two sub clusters Ia and Ib. Clusterwise average data indicated that sub-cluster Ia comprised of 08 genotypes characterized by taller plants (60.08–78.00 cm), longer leaves (47.42–56.67 cm), longer bracts (4.53–6.83 cm), longer florets (9.03–11.17 cm), bigger (5.19–6.88 cm) and heavier corms (44.92–90.83 g) in comparison with other clusters. Sub cluster Ib included 21 genotypes characterized by longest spikes (27–52.25 cm)

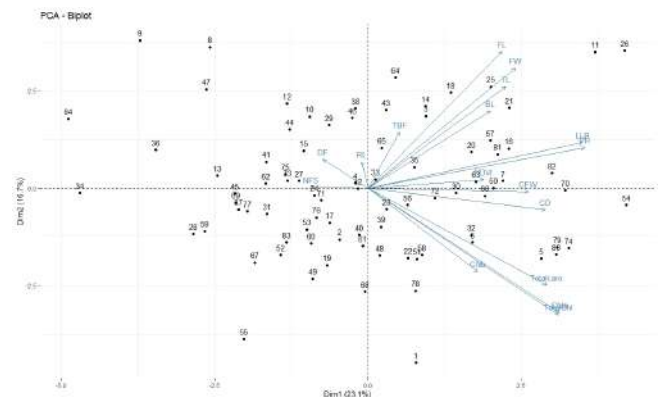


Figure 7: A two-dimensional scatter plot of PCA visualizing the relationships between 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19

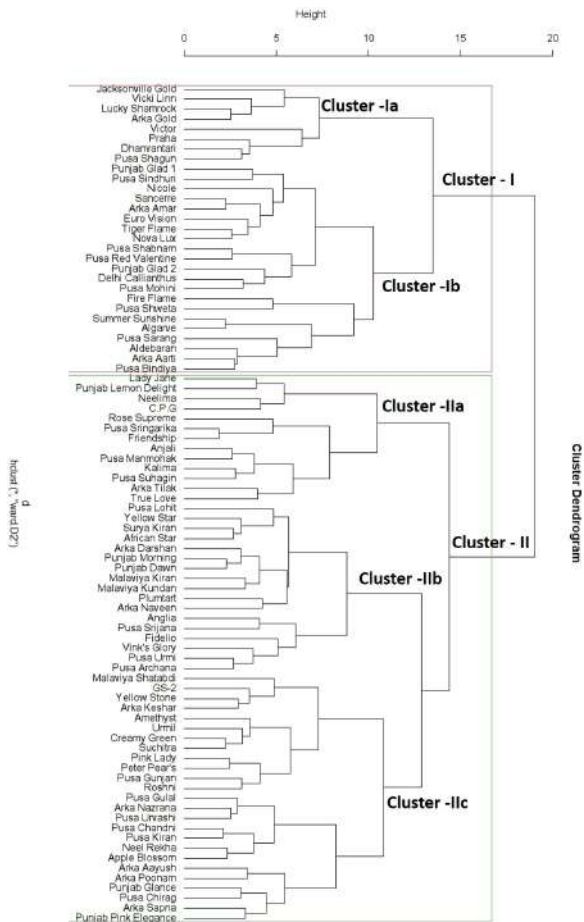


Figure 8: Cluster diagram based on squared Euclidian distance using phenotypic data of 84 *Gladiolus* genotypes evaluated during 2017-18 and 2018-19

with higher floret counts (9.67–17.50), longer tepals (5.72–8.47 cm), rich in chlorophyll and carotenoid contents. The major cluster II consists of 55 genotypes, which was further divided into three sub clusters IIa, IIb and IIc. Sub-cluster IIa consist of 13 genotypes that had broader florets (6.80cm - 11.30 cm) and were late flowering types (102–120 days). Sub-cluster IIb had 17 genotypes characterized by moderate leaf length (35.42–49.83 cm), shorter florets (7.07–9.90 cm) and short flowering duration (7.33–15.83 days). Sub-cluster IIc comprised of 25 genotypes which usually had early flowering genotypes (72.33–114.50 days) with longer flowering duration (7.67–36.50 days). In a similar line of study, Singh *et al.* (2017) morphologically assessed 50 *Gladiolus* cultivars for plant height (80.30–134.70 cm), spike length (40.30–81.70 cm), and number of florets per spike (9.30–18.0). Based on these phenotypic traits, fifty genotypes were grouped into seven primary clusters separated by Euclidean distance coefficients of 14.75. Cluster analysis in our study indicated that genotypes found in sub-cluster Ia are characterized by comparatively taller plants bearing longer florets and heavier corms whereas sub-cluster IIc genotypes possessed early flowering plants with

medium spikes with more floret count and longer blooming period. In this way, both clusters are relatively distinct from each other. Hence, genotypes from these clusters can be used in hybridization to obtain desirable progenies. Among all genotypes, cv. Victor is found to be relatively distinct owing to its tallest plants, heavier corms, short flowering duration and maximum corm fresh weight.

Conclusions

This study on 84 genotypes of *Gladiolus* revealed that huge variability exists for 35 morpho-physiological traits in the germplasm sourced from India and abroad. Genotypes for specific traits such as Victor for corm fresh weight and green florets, Malaviya Shatabdi and yellow stone for the longest flowering duration, Praha and Pusa Shagun for plant height and longest leaves, Arka Poonam and Pusa Shabnam for highest count of florets, Vicki Linn, Pusa Sarang and Victor for bigger corms were identified as important material for breeders and for all stake holders. Among all 84 studied genotypes, cv. Victor was found to be relatively distinct owing to its tallest plants, heavier corms, short flowering duration and maximum corm fresh weight. Distinct genotypes identified in the study would help breeders in selecting desirable parents to obtain better segregants. Characterization data generated during the work will assist the breeders in understanding varietal behavior and selection of reliable traits in breeding. It could serve as a reference collection for identifying and cataloguing *Gladiolus* varieties.

Acknowledgments

Authors acknowledge ICAR-IARI, New Delhi for providing Senior Research Fellowship to the first author.

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