

Analysis of diversity and relationships among *Gladiolus* cultivars using morphological and RAPD markers

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ABSTRACT

A study was conducted during 2003–05 to identify and characterize 54 *Gladiolus* cultivars using morphological traits and RAPDs and to estimate the genetic relationships between the selected genotypes. Nine morphological traits and 225 random amplified polymorphic DNA (RAPD) markers amplified with 25 arbitrary primers were employed to discriminate between the cultivars and to evaluate the relatedness between them. A total of 225 RAPD bands (250–3000 bp) were obtained of which 211 (93.78%) were polymorphic. Number of bands generated per primer ranged from 4 to 14 with an average of 9 bands. For morphological data, Euclidean distances were calculated and an unweighed pair-group method using an arithmetic average was used to construct a dendrogram. In addition, principal component analysis was carried out based on RAPD data. In both the cluster analyses, cultivar 'Pusa Lohit' branched out from the dendrograms, confirming that it is quite different from all other genotypes. Furthermore, cultivar pairs 'Ripples' and 'Anglia', 'Friendship' and 'Yellow Jame', 'Creamy Green' and 'Morello', 'Pusa Suhagin' and 'Pusa Archana', 'The Barton' and 'Christian Jane', 'Plumtart' and 'Chantiler' and 'Peter Pears' and 'Picardy' were very close in both the dendrograms indicating that they are quite similar to each other and this result was also supported by PCA analysis. However, incongruence existed between these cluster analyses, and the relationships between some cultivars require further investigation.

Key words: Diversity analysis, *Gladiolus*, Molecular marker, RAPD

Gladiolus (*Gladiolus* spp), the queen of the bulbous ornamentals, is the leading geophyte grown worldwide for cut flower trade and garden displays. It occupies a pristine place in the garden for its magnificent inflorescence, wide array of colours, and fascinating varieties of shapes and sizes. It 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. As the number of gladiolus cultivars is continuously increasing, the newly constituted cultivars need to be more intensively monitored for novelty, distinctness, uniformity, stability and molecular markers useful for the protection of Plant Breeder's Rights. This would make

protection of new gladiolus cultivars more specific and effective. Moreover, characterization of accessions is vital for their conservation and management as well as in understanding the genetic relationships between them, which could further be very useful for breeding in supporting the selection of cross combinations from large sets of parental genotypes, thus broadening the genetic base of breeding programmes.

Morphological traits have long been used to estimate systematic relationships in crops (Chen *et al.* 2004) and ornamentals (Wen and Hsiao 2001). Though simple and irreplaceable, these descriptors suffer from many drawbacks, such as influence of environment on trait expression, epistatic interaction and pleiotropic effects. Furthermore, paucity of sufficient number of stable morphological markers for unequivocal identification of increasing number of reference collection of varieties enforces to look for alternatives. Molecular approaches collectively represent a potential goldmine of important informations that can be applied as an efficient tool for effective characterization of germplasm. Characterization by methods that directly utilize DNA can potentially address the limitations associated with

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morphological and biochemical methods since all genetic differences between individuals are laid down in the primary sequence of their genomic DNA. Among the available molecular marker techniques, RAPD is most widely exploited by horticulturists largely due to the fact that results are obtained quickly and are fairly inexpensive to generate (Meerow 2005). Although RAPD analyses have been performed on some species of the genus *Gladiolus* (Takatsu *et al.* 2001), this technique has not been applied to characterize different cultivars of gladiolus and to estimate the genetic relationships among them. Further, other more robust marker systems such as simple sequence repeats (SSR) are not yet available in *Gladiolus*.

The objectives of this study were to identify and characterize 54 gladiolus cultivars using morphological traits and RAPDs and to estimate the genetic relationships between the selected genotypes.

MATERIALS AND METHODS

A total of 54 *Gladiolus* cultivars representing majority of varieties under cultivation in India and a duplicate sample of 'Pusa Shweta' (Table 1) were analyzed using morphological traits and selected RAPD markers. The

materials were planted in randomized block design with 3 replications at Floriculture Research Farm, Indian Agricultural Research Institute, New Delhi during winter season of 2003–2005 for morphological characterization.

Nine morphological traits, viz flower colour, plant height, spike length, rachis length, floret diameter, number of florets/spike, number of corms/plant, number of cormels/plant and weight of corm were recorded. All the morphological characters were scored as quantitative traits except flower colour. Data for each trait were collected from 10 to 15 plants on a single day to minimize experimental error.

Young expanding leaves were collected from field grown plants and stored at -80°C prior to DNA extraction. Total genomic DNA was extracted as per the protocol of Saghai-Maroo *et al.* (1984) using 4–5 g finely ground leaf tissue in 20 ml of DNA extraction buffer. The random amplified polymorphic DNA analysis (RAPD) was performed in 25 μl reaction mixture containing 1X PCR assay buffer, 2.5 mM MgCl_2 , 20 ng template DNA, 0.2 mM dNTPs, 0.06 μM of the decamer primer and 1 U Taq DNA polymerase (all chemicals were from M/S Bangalore Genei Ltd, Bangalore). Twenty-five random decamer primers were selected from 100 primers after pre-screening the commercially available

Table 1 *Gladiolus* cultivars analyzed for morphological traits and RAPDs along with their parentages

Cultivar	Parentage	Cultivar	Parentage
'Friendship'	(Maid of Orleans \times Picardy) \times (Gloaming \times Seedling)	'Viola'	Pastorale \times unknown
'Yellow Jame'	Unknown	'Bonaire'	Unknown
'Creamy Green'	Unknown	'Regency'	Ruffled Ebony \times Ace of spades
'Light Purple'	Unknown	'Australian Fair'	Unknown
'Morello'	Unknown	'Little Fawn'	Old South \times Statuette
'Ripples'	Crusador \times Statuette	'Bis- Bis'	Unknown
'Priscilla'	Diamond \times Leona	'Melody'	Comm Koehl \times Moorish King
'Apricot Queen'	Unknown	'Apple Blossom'	Beauty's Blush \times Rose Charm
'Anglia'	Eloise \times Rosemarie Pfitzer	'Rose Delight'	Picardy \times Early Rose
'Pusa Archana'	Creamy Green \times American Beauty	'Pusa Chandani'	Unknown
'Gold Dust'	Parfait \times Domino	'George Mazure'	Unknown
'Punjab Dawn'	Unknown	'Pusa Bindiya'	Open pollinated seedling of Ratna's Butterfly
'Pusa Suhagin'	Sylvia seedling	'Pusa Sarang'	Open pollinated seedling of White Oak
'Smoky Lady'	Big Time \times Plum Pudding	'Neelam'	Sylvia \times Partica
'The Barton'	Marjorie Nadean \times R S Mayne	'Pusa Mohini'	Ave \times Christian Jane
'Christian Jane'	Unknown	'Pusa Lohit'	Creamy Green \times American Beauty
'Errato'	Unknown	'Pusa Chirag'	Cygnets \times Little Fawn
'Royal Supreme'	Unknown	'Pusa Rangmahal'	Red Bantam \times Laura Belle
'Ave'	Unknown	'Pusa Shweta 1'	Seedling of (Wind Song \times Pink Frost)
'Picardy'	Apricot Glow \times Emile Aubrun	'Pusa Shweta 2'	Seedling of \times (Wind Song \times Pink Frost)
'Summer Rose'	Unknown	'Pusa Swarnima'	Spontaneous mutant of Dhanvantari
'Jester Gold'	Jester sport	'Pusa Urvashi'	Red soft glow open
'Peter Pears'	Salome \times Maolete	'Pusa Shringarika'	Mayur \times Heady Wine
'Pusa Dhanvantari'	Jr Prom \times Lucky Star	'Pusa Urmi'	Berlew \times Heady Wine
'Snow Princess'	Unknown	'Pusa Swapnil'	Viola open
'Pusa Shagun'	White Oak \times Oscar	'Pusa Gulaal'	Snow Princess \times Viola
'Plumtart'	Unknown	'Pusa Gunjan'	White Oak \times Oscar
'Chantiler'	Unknown		

kits (Operon A, B, C, D, M, O and P series) for DNA amplification. Amplifications were performed in a PTC 200 Thermal Cycler (MJ, USA) programmed for an initial denaturation step at 94°C for 4 min., followed by 40 cycles at 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min, with a final extension step at 72°C for 5 min. The amplification products were separated on 1.8% agarose gel, stained with 1µg/ml ethidium bromide, and was photographed under UV light of wave length 325nm. DNA molecular weight marker, 1kb DNA ladder from M/S Fermentas, Germany was loaded in each gel to estimate the sizes of bands. Each RAPD band was visually scored as '1' for presence, and '0' for absence in a lane, and the binary data were used for statistical analysis.

Morphological data were standardized before Euclidean morphological distances were calculated (Wen *et al.* 2004), and the distance matrix was subjected to unweighed pair-group method using arithmetic average (UPGMA) analysis to construct a dendrogram. RAPD data were also used to calculate pair-wise Jaccard's similarity coefficients (Jaccard 1908) and the resulting coefficients were used to construct a UPGMA dendrogram. The robustness of the nodes of dendrogram was tested by bootstrap analyses using 1000 re-samplings with Free Tree Software (Pavlicek *et al.* 1999). The correlations among variables was subjected to 'Eigen vectors' analyses and the resulting Eigen values were projected on 3-dimensional plane to obtain a 3-dimensional plot of first 3 principal components. All statistical analysis were conducted using the programme NTSYS-pc (numerical taxonomy and multivariate analysis system) Version 1.80 software (Rohlf 1993).

RESULTS AND DISCUSSION

A wide variation was recorded for all the 9 morphological traits (Table 2). Although ornamentals could be identified by their morphological traits, the easiest practical method of identification so far has been by comparing colour photographs of the inflorescence as in case of *Heliconia* (Berry and Kress 1991). On the basis of spike colour, all cultivars could be distinguished. Based on pair-wise comparisons among the selected cultivars, a matrix of Euclidean distances was obtained and a dendrogram was constructed (Fig 1). The genotypes with superior commercial characters, such as spike length, rachis length, floret diameter etc. were clustered together. At a distance of 2.65, 4 clusters could be obtained. The lowest distance value was observed between cultivar 'Punjab Dawn' and 'Pusa Dhanvantari' (0.84). 'Regency' and 'Pusa Gunjan' branched out from the dendrogram showing that they are quite different from other genotypes. Cluster I was dominated with exotic cultivars and was divided into 2 sub-clusters in which the first one was formed by pinkish-yellow to orange coloured genotypes. However, 4 indigenous bred cultivars, namely 'Pusa Suhagin', 'Pusa Archana', 'Punjab Dawn' and 'Pusa Dhanvantari' were also present. This indigenous group of

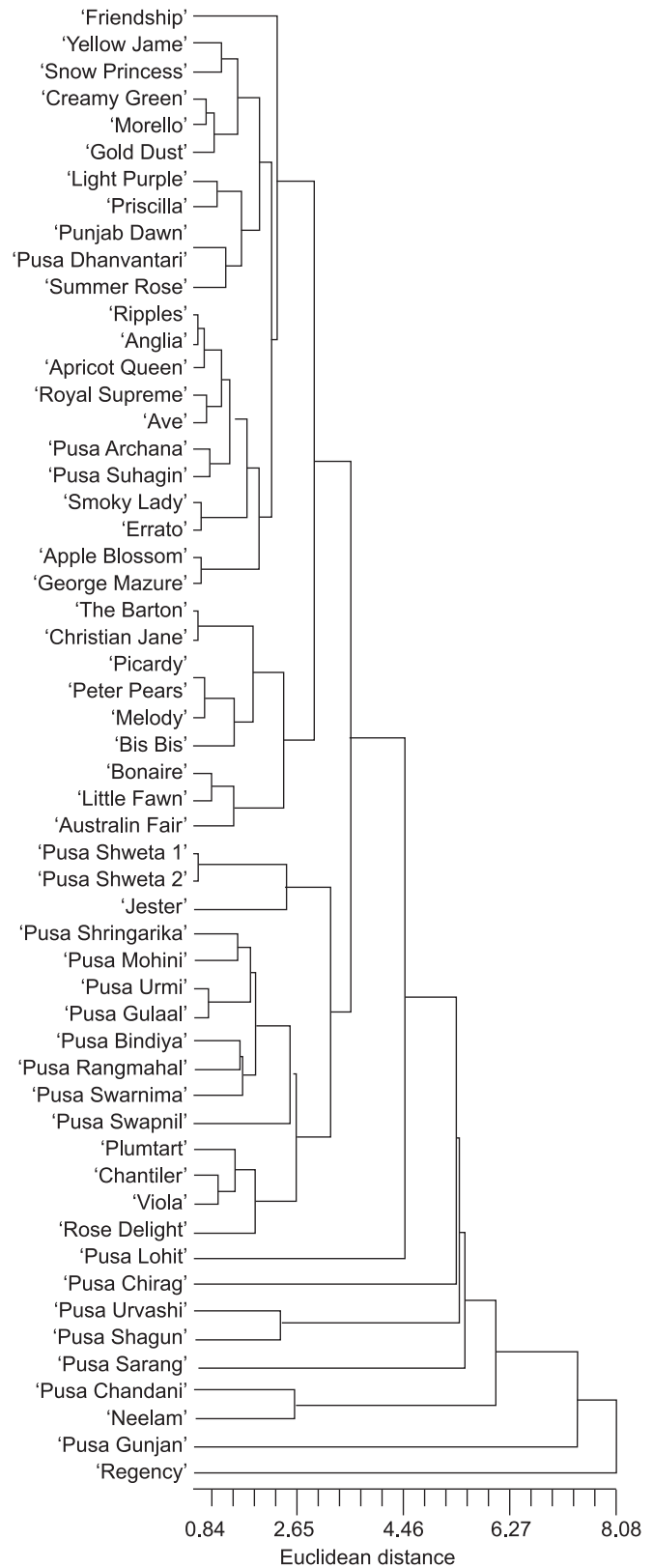


Fig 1 UPGMA dendrogram of 54 *Gladiolus* cultivars and a duplicate sample of 'Pusa Shweta' based on morphological characters

Table 2 Morphological characters of 54 *Gladiolus* cultivars and a duplicate sample of 'Pusa Shweta'

Cultivar	Flower colour	Plant height (cm)	Spike length (cm)	Rachis length (cm)	Floret diameter (cm)	Florets / spike	Corms/ plant	Cormels/ plant	Weight of corms (g)
'Friendship'	Pinkish-orange	83.46	60.70	48.27	10.10	8.28	1.08	9.34	20.13
'Yellow Jame'	Yellow-orange	116.17	62.51	54.58	9.37	7.23	1.47	19.94	25.21
'Creamy Green'	Creamy-Green	111.56	58.67	44.78	8.01	7.40	1.07	19.48	22.50
'Light Purple'	Light-Purple	117.58	64.65	46.14	8.57	12.14	2.52	12.42	25.18
'Morello'	Orange	103.48	55.38	45.30	9.34	7.56	1.02	11.31	16.24
'Ripples'	Bluish-mauve	102.35	49.88	25.57	9.45	7.52	1.68	19.51	18.15
'Priscilla'	Light-yellow	119.37	57.63	45.24	8.25	13.09	2.54	23.41	17.47
'Apricot Queen'	Apricot	119.19	40.25	30.62	8.16	8.41	1.82	21.02	16.25
'Anglia'	Yellow	107.45	45.67	31.85	8.38	8.25	1.20	20.38	18.51
'Pusa Archana'	Red, throat yellow	139.26	48.82	35.19	9.57	9.26	1.88	14.44	19.42
'Gold Dust'	Yellow	109.41	58.78	50.38	8.10	9.50	1.89	8.30	17.82
'Punjab Dawn'	Orange-yellow	108.40	60.31	40.65	8.50	14.36	1.48	15.31	24.50
'Pusa Suhagin'	Maroon	120.11	56.37	39.74	9.31	10.12	1.39	15.72	22.16
'Smoky Lady'	Smoky rose violet	111.41	52.34	30.35	8.23	9.50	1.07	12.11	30.29
'The Barton'	Deep-red	120.36	68.61	47.39	10.88	9.19	1.30	10.11	25.72
'Christian Jane'	Orange-pink	119.56	69.20	48.17	11.76	8.04	1.46	11.43	30.95
'Errato'	Red	118.57	45.67	34.80	7.34	9.03	1.08	12.19	25.26
'Royal Supreme'	Light-peach	120.13	48.68	28.54	8.32	8.25	0.99	20.11	20.23
'Ave'	Light-orange	112.34	58.81	32.58	8.16	9.23	1.89	20.14	19.19
'Picardy'	Light-pink	110.66	60.25	50.10	8.31	8.19	1.20	12.98	29.18
'Summer Rose'	Pink	120.16	57.81	37.34	9.01	12.68	1.54	19.38	30.04
'Jester Gold'	Yellow	129.48	78.38	66.14	9.71	10.12	1.27	40.69	19.44
'Peter Pears'	Peach	116.44	68.59	54.85	8.14	10.03	1.20	18.18	32.34
'Pusa Dhanvantari'	Yellow	108.38	60.00	41.01	8.38	12.59	1.15	15.31	24.30
'Snow Princess'	White	124.12	65.56	56.26	8.45	11.34	0.99	16.84	23.05
'Pusa Shagun'	Dazzling-red	117.07	95.89	55.10	10.20	16.36	2.82	10.41	22.51
'Plumtart'	Purple-red	104.37	89.51	50.24	8.07	14.21	1.12	19.93	24.11
'Chantiler'	Purplish-orange	112.41	61.76	52.48	8.40	15.97	1.87	22.97	21.92
'Viola'	Violet-red	109.61	65.23	50.41	8.14	11.34	1.57	18.27	20.16
'Bonaire'	Peach	111.34	57.61	31.79	8.24	10.21	1.68	15.24	40.13
'Regency'	Deep-maroon	112.14	57.96	36.26	8.37	12.03	1.91	1609	29.57
'Australian Fair'	Scarlet-pink	119.99	72.51	44.88	10.77	11.24	1.61	19.62	40.12
'Little Fawn'	Buff orange	123.11	59.99	41.79	8.27	12.49	1.16	15.38	39.76
'Bis- Bis'	Red with white tip	129.11	69.76	60.10	9.52	11.38	1.89	12.62	29.08
'Melody'	Peach with red throat	114.25	65.35	51.95	8.11	8.40	1.57	21.45	28.67
'Apple Blossom'	Pink	121.01	49.69	30.28	8.60	9.23	1.72	17.46	18.98
'Rose Delight'	Rose	113.19	69.59	55.31	9.12	19.14	0.99	17.36	20.15
'Pusa Chandani'	White	121.84	64.66	35.34	7.43	12.44	1.50	16.27	38.45
'George Mazure'	Deep-orange	115.36	54.67	34.10	7.21	9.81	1.07	14.25	19.14
'Pusa Bindiya'	Light-orange	117.41	93.37	66.25	11.28	20.11	2.62	39.64	18.51
'Pusa Sarang'	Purple-red	140.11	124.20	82.70	13.32	22.14	1.98	10.21	28.20
'Neelam'	Bluish-mauve	120.73	84.40	68.39	10.25	12.31	1.60	20.16	19.45
'Pusa Mohini'	Red-purple	108.89	92.71	48.53	9.36	14.76	2.16	14.76	18.47
'Pusa Lohit'	Red	144.61	123.77	70.76	11.53	15.98	2.36	11.26	18.94
'Pusa Chirag'	Orange	123.04	99.34	70.19	10.65	18.04	3.40	39.74	22.35
'Pusa Rangmahal'	Red-purple	103.12	81.85	45.27	10.36	16.39	1.98	11.54	25.03
'Pusa Shweta 1'	White	115.49	94.90	64.66	10.80	16.73	2.41	15.07	32.39
'Pusa Shweta 2'	White	114.98	95.71	64.01	11.09	15.86	2.31	16.17	27.86
'Pusa Swarnima'	Coppery-yellow	126.88	101.50	55.98	11.12	16.27	3.66	6.18	24.13
'Pusa Urvashi'	Soft red-purple	102.65	80.75	54.12	10.94	17.10	1.92	13.28	26.05
'Pusa Shringarika'	Purple	110.27	94.31	50.50	12.20	14.32	1.84	10.22	38.56
'Pusa Urmi'	Red-purple	106.58	90.70	53.34	10.21	15.31	1.98	30.10	25.52
'Pusa Swapnil'	Red with white tip	129.36	109.84	68.33	11.16	17.30	2.48	14.31	36.58
'Pusa Gulaal'	Red-purple	105.38	84.35	51.82	11.04	17.31	2.01	23.46	24.89
'Pusa Gunjan'	Soft-orange	110.24	86.68	42.85	11.16	18.14	2.64	10.96	25.04
Mean		114.17	70.82	47.97	9.38	12.43	1.75	46.19	25.00
CD ($P=0.05$)		0.20	1.60	0.94	1.93	0.26	0.35	0.36	0.53

cultivars was morphologically closer to exotic group than other indigenous cultivars. Besides this, 'Pusa Suhagin' and 'Pusa Archana' and 'Punjab Dawn' and 'Pusa Dhanvantari' reflected close similarity in their morphological characters. However, their closeness could not be supported by their parental relationships. The II cluster was formed by exotic cultivars. However, cluster III and IV were constituted by both indigenous as well as exotic group of cultivars. In cluster II, 'Picardy' and 'Peter Pears' were found similar with respect to almost all morphological features including flower colour to some extent (Table 2).

RAPD analysis

A total of 225 RAPD bands (250–3000 bp) were obtained of which 211 (93.78%) were polymorphic. Number of bands generated per primer ranged from 4 (OPA-8) to 14 (OPO-4) with an average of 9 bands. The amplification products obtained with primer OPD-20 are illustrated in Fig 2. Pair-wise genetic distances based on RAPD data for 54 gladiolus cultivars ranged from 0.407 to 0.782 indicating high diversity.

Genetic relationships among cultivars

A wide range of analysis methods (UPGMA, bootstrapping and PCA) was used to provide detailed insights into the genetic relationships. UPGMA tree based on morphological traits (Fig 1) reflected 4 major clusters, while UPGMA tree based on RAPD data (Fig 3) showed 3 major clusters.

In both the cluster analyses, 'Pusa Lohit' branched out from the dendrograms, confirming that it is quite different from the other genotypes. However, at molecular level, 'Pusa Lohit' and 'Pusa Swarnima' clustered together ($J=0.74$), indicating a close genetic relationship. Nevertheless, 'Pusa Lohit' has red florets which is different from 'Pusa Swarnima' with coppery yellow florets. 'Pusa Lohit' has got 'Creamy Green' and 'American Beauty' as its parentage, whereas 'Pusa Swarnima' is a spontaneous mutant of 'Pusa Dhanvantari'. Thus, the genetic closeness could not be supported by their parental relationships. This could have happened due to highly heterozygous nature of this crop.

Cluster I of both the dendrograms was formed by almost

same group of cultivars. A further comparison of morphological characters indicated that all the cultivars in this group had spikes of more than 40 cm with medium sized florets (>8.0 cm) and colours ranging from light-pink to pinkish-orange or yellow to orange. Cluster II consisted of all exotic cultivars. However, out of 11 genotypes genetic closeness of only 5 ('Picardy', 'Peter Pears', 'Melody', 'Bis-Bis' and 'Little Fawn') was supported by their phenotypic expressions. Majority of indigenous bred cultivars clustered to form III cluster. Furthermore, cultivar pairs 'Ripples' and 'Anglia', 'Friendship' and 'Yellow Jame', 'Smoky Lady' and 'Errato', 'Creamy Green' and 'Morello', 'Pusa Suhagin' and 'Pusa Archana', 'The Barton' and 'Christian Jane', 'Plumtart' and 'Chantiler' and 'Peter Pears' and 'Picardy' were very close in both the dendrograms indicating that they are quite similar to each other and this result was also supported by PCA analysis (Fig 4). Due to the lack of pedigree information of many of these cultivars the above said results could not be supported by their parental relationships. However, considering the highly heterozygous nature of the crop and consistency of morphological data with RAPD data, it can be concluded that both morphological traits and RAPD markers were suitable for analyzing the genetic relationships between gladiolus cultivars to some extent, which could further be helpful in deciding the parents for effective breeding programme. In addition, cultivars 'Pusa Suhagin', 'Pusa Archana' and 'Punjab Dawn' were observed in the cluster dominated with exotic cultivars in both the dendrograms. The parentages of these cultivars are different but of exotic origin. 'Pusa Archana' is a cross of 'Creamy Green' and 'American Beauty' and the female parents was found to present in the same group. Thus, its presence in this very cluster could be justified. However, 'Pusa Suhagin' is a selection from the seedlings of variety 'Sylvia'. Parents of 'Punjab Dawn' were not known. It might be possible that these parents have got similarity with the genotypes present in this particular group.

In RAPD based dendrogram, 'Pusa Swapnil', 'Pusa Urmi', 'Pusa Shringarika' and 'Pusa Gulaal' were clustered together in the same sub-cluster. Even in morphology-based

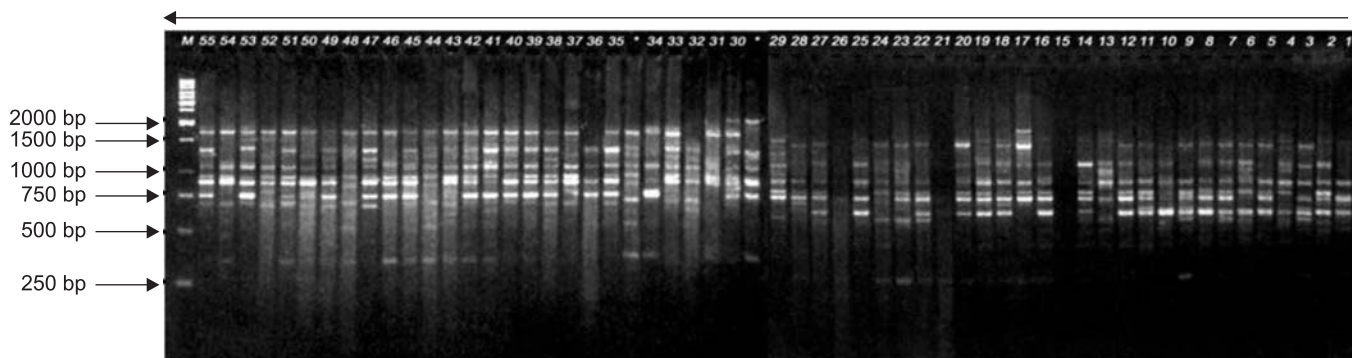


Fig 2 RAPD profile of 54 *Gladiolus* cultivars and a duplicate sample of 'Pusa Shweta' amplified with primer OPD-20. Refer Table 2 for identity code of these cultivars. Lane M, DNA marker

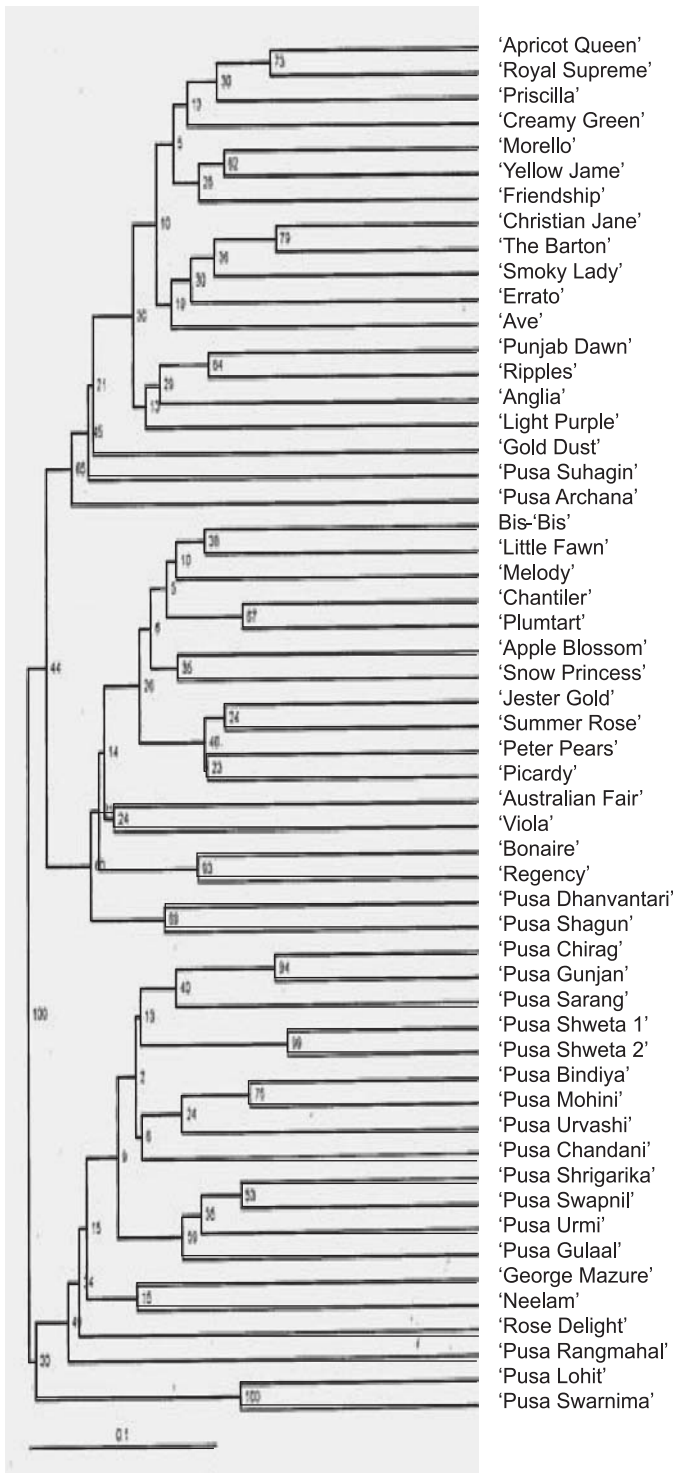


Fig 3 UPGMA dendrogram of 54 *Gladiolus* cultivars and a duplicate sample of 'Pusa Shweta' based on RAPD data. Numbers at each node represent their respective bootstrap values

dendrogram, all of these were present in the same group. This might be due to similarity in the genotypes of their parentages. 'Pusa Swapnil' had been developed through the

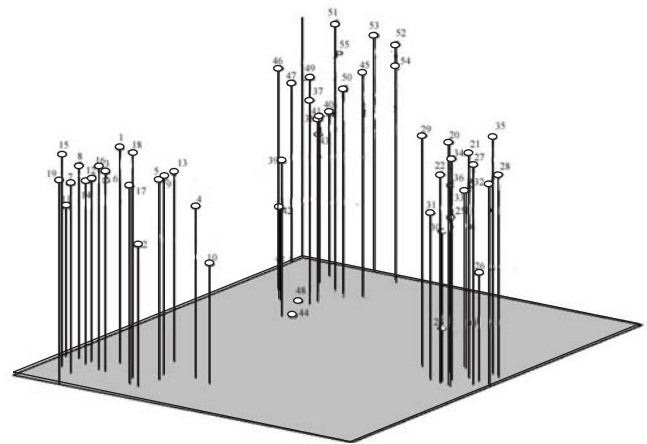


Fig 4 Three-dimensional graph based on PCA using RAPD markers. The three axes represent the first 3 principal components (PC) which accounted for 23.2% of the total variation. Refer Table 2 for identity code of cultivars

open pollination of 'Viola', while 'Pusa Gulaal' is a result of crossing 'Snow Princess' and 'Viola', ie one of the parentages is same for these 2 cultivars. 'Pusa Shringarika' and 'Pusa Urmi' share the common male parentage, ie 'Heady Wine'. Thus their presence in the same group could be justified.

Despite wide variation in flower colour, most of the indigenous bred cultivars clustered together in both the analyses. Breeders more often select a genotype based on commercial characters like novel flower colour, spike length, number of florets/spike, floret diameter and vase-life. That is why, all the genotypes with superior commercial characters clustered together in morphology-based dendrogram. In RAPD-based dendrogram, cluster III was again divided into two sub-clusters. The first sub-cluster was dominated with soft coloured genotypes, whereas second sub-cluster was found to be dominated with red to red-purple colour range. Although the parentages of these genotypes are morphologically quite different, they are found to be closely related at genotypic level. Here, the necessity of molecular tools in broadening the genetic bases for effective breeding programme could be realized. Two morphologically distinct genotypes might not have distinct genetic constitution. Thus, for creation of any new variety, selection of parents based on morphology should always be supplemented with an efficient molecular marker.

Morphological vs molecular data

The genetic relationships between the selected genotypes on the basis of morphological and molecular tools revealed some controversy. Compared with the other genotypes, 'Punjab Dawn' showed close similarity to 'Pusa Dhanvantari' in morphology including flower colour to some extent. 'Punjab Dawn' has orange florets, while 'Pusa Dhanvantari' has got light-yellow florets. However, RAPD analysis

reflected a different picture. These two genotypes were quite distant in the UPGMA tree based on RAPD data. Molecular characterization has always been known to discriminate between even morphologically similar genotypes. On the basis of morphological characters, 'Pusa Chirag' was present as out group. However, it demonstrated a very close relatedness with 'Pusa Gunjan' in RAPD-based dendrogram. It could also be confirmed by a robust bootstrap support for the branches (Fig 4). Although when all the morphological characters were considered, it was found as a distinct genotype, but when we observed different characters separately, they showed similarity in one of the most important morphological features for discrimination of varieties in ornamentals, ie flower colour. 'Pusa Chirag' has orange florets while 'Gunjan' has got soft-orange. Floret size was also found at par (10.65 and 11.04 cm, respectively) for these genotypes (Table 2). This could be because of the reason that morphological characters like plant height and spike length are highly affected by environment while flower colour is least affected. However, this finding could not be supported by their parental relationship as 'Pusa Chirag' is a cross between 'Cygnet' and 'Little Fawn' while 'Pusa Gunjan' is a selection from the seedlings of 'White Oak'. Chance similarity between these parents might have created this situation. Here, the reliability of morphological traits could also be illustrated as phenotypes are the expressions of genotypes nevertheless it is affected by many factors. In contrast to this, the genotype pair 'Apple Blossom' and 'George Mazure' were found very close for phenotypes but placed on different branches of RAPD-based dendrograms. These differences might have arisen due to several reasons, but the most important is that the genetic (or structural) origin of each RAPD marker is different, while morphological expression (phenotype) is conditioned by the state of the plant, agricultural management and environmental conditions. Thus, phenotypic differences are not necessarily concordant with the number of underlying gene mutations, and differences in phenotypic characters do not necessarily reflect different genetic events (Bachmann 1992). On the other hand, it is possible that the dominant nature of RAPD markers and limitation of RAPD technique to detect polymorphism in cases of heterozygosity (Parker *et al.* 1998), may account for the inconsistencies between the analyses. The discrepancy cases between DNA-based and morphology-based classification also have been documented in many plants (Persson *et al.* 2000, Wang and Bao 2005). To ensure the exactness of evaluation, only variables with strong genetic control should be quantified in morphological traits analysis and other more effective molecular markers such as AFLP, SSR or ISSR should be used (Wang and Bao 2005).

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