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Genetic divergence analysis of bougainvillea (*Bougainvillea* spp) cultivars using morphological markers

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ABSTRACT

A study was undertaken to analyze diversity among the bougainvillea (*Bougainvillea* spp.) cultivars using morphological markers. A total of 38 morphological descriptors of UPOV guidelines were recorded for hundred bougainvillea cultivars. Significant amount of variation was observed for all the descriptors, indicating that many of the cultivars were documented to be superior. The Neibour Joining (NJ) dendrogram based on morphological characters data separated the bougainvillea cultivars into 12 major clusters. Cluster-I exhibited as largest cluster compared to all other clusters comprising 49 cultivars. However, cluster II with 14 cultivars, cluster III with 12 cultivars, cluster XII with 7, cluster X with 6, cluster VII with 5 cluster IV with 2 and clusters no V, VI, VIII, IX, XI having only one cultivar in each case. It clearly grouped the cultivars of single and double type of bracts and also grouping based on pattern of variegation in leaves. PCA plot also grouped the cultivars as a fashion of NJ clusters however, it was unable to distinguish among the single bracted types in this method. This considerable amount of variation was due to the diversity in bougainvillea cultivars. On the basis of present investigation, these cultivars were grouped into different categories for different character, may be used effectively for identification of cultivars. Further, these cultivars can be used in crop improvement programme.

Key words: Bougainvillea, Genetic diversity, Morphological markers

The genus *Bougainvillea* contains about 14 species out of which three species namely B. *glabra, B. spectabilis* and *B. peruviana* possess colourful bracts and are of ornamental value (Zadoo *et al*. 1975). According to Khoshoo (1998), *Bougainvillea* also having three hybrid groups namely, *B.* × *buttiana* (*glabra* × *peruviana*), *B.* × *specto-peruviana* and *B.* × *specto-glabra*. The variation in bougainvillea cultivars is mainly due to the bract colour coupled with other morphological characters such as leaf and bract size, foliage variegation, floral tube, star, pubescence of different parts and flowering behavior of the cultivars. All these factors have led to lot of confusion in the identification of bougainvillea cultivars (MacDaniels 1981) and the relationship studies between parents and their hybrids are mostly based on the New International Checklist of Bougainvillea (Singh *et al*. 1999) so, it is difficult to distinguish all the bougainvillea cultivars. Therefore, breeders and researchers have to establish an effective method to properly identify the existing germplasm.

In India, characterization of ornamental crop cultivars based on DUS test has started and well succeeded in rose and chrysanthemum (Prasad *et al*. 2007). The Division of Floriculture and Landscaping, Indian Agricultural Research Institute (IARI), New Delhi has done pioneer work on most important ornamental crops and developed DUS test guidelines for rose and chrysanthemum in collaboration with other horticultural institutes. At present, the division is underway to validate DUS guidelines for bougainvillea as one of the centres with National Botanical Research Institute (NBRI), Lucknow. In the light of significant work done in bougainvillea improvement programs, IARI has been appointed as the International Crop Registration Authority for Bougainvillea by International Society for Horticultural Science, Belgium for registration of cultivars.

Morphological trait measurements are commonly used to quantifying genetic variation in plant species, since they provide a simple technique while, simultaneously assessing genotype performance under relevant growing environments (Fufa *et al*. 2005). Selection of genetically diverse cultivars used as parents is the prerequisite for the success of any breeding programme. Different methods are available to employ genetic diversity in crop plants. Traditionally, distance estimation and classification were entirely based

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on morphological markers and quantitative traits (Goodman 1972).

In Bougainvillea, to date, only few studies were conducted to assess the genetic variation including, growth behaviour studies (Gupta *et al*. 2006, Kumar *et al*. 2002), correlation studies (Singh *et al*. 2010, Singh *et al*. 2006). However, none of the above studies had taken to assess diversity and classify larger bougainvillea cultivars with huge number of morphological parameters. Hence, the present study was formulated to assess genetic diversity and relation among the large bougainvillea germplasm. Morphological characterization aimed at identifying cultivars, determining genetic diversity and relationship among them to significantly improve our understanding about the extent of genetic variability available in Indian and exotic bougainvillea germplasm.

MATERIALS AND METHODS

The study was carried out in the Research Farm of International Crop Registration Authority for Bougainvillea (ICRAB), Division of Floriculture and Landscaping (FLS), IARI, New Delhi which is one of the DUS validation centre for Bougainvillea approved by PPV&FRA. The experiment consists of one hundred bougainvillea cultivars and the detailed lists of cultivars are listed in Table 1. A total of 38 morphological traits including quantitative and qualitative characters were selected based on draft guidelines developed by UPOV for bougainvillea (Anon. 2011) are recorded for all the cultivars during 2012-13 and 2013-14. The data were recorded on 10 plants or parts of plants at peak flowering stage. The detailed descriptions of all the morphological characters were elaborated in the Table 2. Morphological data were subjected to interval analysis using NTSYS software version 2.02 (Rohlf 2000). Distance coefficient was used to develop the clusters using Neighbour Joining (NJ) method.

Table 1 List of cultivars used for morphological characterization

Cultivar	Cultivar	Cultivar
Abraham Kavoor	Hawaiine White	Philomina
Alick Lancaster	Isacbele Greensmith	Pink Beauty
Aruna	Jawaharlal Nehru	Pixi Variegata
B T Red	Jayalakshmi	Poultoni
Bangalore Variegata	Jubille	Poultoni Special
Begum Sikander	Killie Campbell	Queen Elizabeth
Blondie	Krumbigel	R S Bhatt
Cascade	Lady Hope	Radha
Chandrabieri	Lady Hudson	Red September
Cherry Blossom	Lady Mary Baring	Refulgens
Chitra	Lady Richards	Rose Ville Delight
Coleoptera	Lakshmi	Rosea Fuschia
Deep Red	Los Banos Beauty	Sanderiana
Dr B P Pal	Los Banos Variagata	Sensation
Dr Bhabha	Los Banos Variagata	Shubhra
Dr H B Singh	Jayanti	Singapore Red
Dr H C Buck	Louis Wathen	Sofia Mutant
Dr Hadu	Mahara	Sonnet
Dr Homibhabha	Mahatma Gandhi	Spectable
Dr PV Sane	Manohar Chandra	Splendens
Dr R R Pal	Mary Palmer Special	Spring Festival
Dr Rao	Mataji Agnihotri	Stanza
Dream	Meera	Summer Time
Elizabeth Angus	Mrs Backery	Superba
Fantasy	Mrs Butt	Sweet Heart
Filoman	Mrs Frasser	Swetha
Flame	Mrs McClean	Tetra Mrs McClean
Gangaswamy	Padmi	Thimma
Garnet Glory	Palekar	Tomato Red
Glabra	Pallavi	Torch Glow
Glady's Heburn	Partha	Versicolour
Gloriosus	Parthasarthy	Vishaka
Golden Glow	Perfusion	Zakiriana
Gopal	Phillips	

RESULTS AND DISCUSSION

Cluster analysis using Neighbour Joining (NJ) Method

The NJ dendrogram based on morphological characters data separated all the bougainvillea cultivars into 12 major clusters and several sub-clusters. Out of twelve major clusters, the cluster-I exhibited as largest cluster comprising 49 cultivars, followed by cluster II with 14 cultivars, cluster III with 12 cultivars, cluster XII with 7, cluster X with 6, cluster VII with 5, cluster IV with 2 and clusters V, VI, VIII, IX and XI having only one cultivar in each case (Fig 1 and Table 3).

Cluster I is further sub-divided into 2 sub-clusters, viz. Ia (43 cultivars) and Ib (6 cultivars). The first major cluster includes majority of the cultivars with most of all the cultivars covered with hybrid seedlings of *B. × buttiana, B. glabra* and *B. peruviana* species. It indicates that the hybrid cultivars of *B. glabra × B. peruviana* group cultivars had affinity towards *B. × buttiana* species or they are genetically similar with each other. Subcluster Ia was again divided into many more mini-clusters in which most of the mini clusters possessed identical coloured bracts like white to greenish white coloured bracts (Shubhra, Begum Sikander, Dr B P Pal), crimson red to magenta coloured bracts and other similar vegetative parameters. However, subcluster Ib consisting of yellow coloured to orange shaded bract cultivars (Abraham Kavoor, Golden Glow, Lady Mady Baring, Philomina) which were also having same leaf characters of subcluster Ia. The second major cluster (cluster II) contains 14 cultivars having morphological features like lanceolate to elliptical shaped leaves with attenuate shaped bases, margin of the leaves are strongly curved and bract clusters were sparsely arranged in plants. This second cluster further divided into 2 sub-clusters (IIa and IIb) in which subcluster IIa with twelve cultivars and subcluster IIb with two cultivars (Palekar and Perfusion).

Cluster III consists of 12 cultivars in which few of them were hybrid seedlings of *peruviana* species and other

Table 2 List of descriptors used for morphological characterization

Characteristics	Characteristics
Plant: Growth habit (VG)	Peduncle: Length (cm) (MG/MS)
Young shoot: colour (VG)	Inflorescence: Arrangement of Bract Cluster (VG)
Plant : Length of internodes (cm) VG/MS)	Inflorescence: Number of Bract Cluster (VG)
Stem: Thorns (VG)	Inflorescence: Density of bract clusters (VG)
Number of thorns	Inflorescence: Presence of flower (VG)
Thorn: Length (cm) (VG)	Inflorescence: Type of bract (VG)
Thorn colour	Bract: Length (cm) (MG/MS)
Thorn: Curvature (VG)	Bract: Width (cm) (MG/MS)
Leaf blade: Length (cm) (MG/MS)	Bract: Shape (VG)
Leaf blade: Width (cm) (MG/MS)	Bract: Shape of base (VG)
Leaf blade: Shape (VG)	Bract tip
Leaf blade: Shape of base (VG)	Bract Margin
Leaf blade: Main colour (VG)	Calyx lobes: colour of upper side (VG)
Leaf blade: Secondary colour (VG)	Small young bract: main colour of outer side (VG)
Leaf Blade: Distribution of Secondary Colour (VG)	Young bract: main colour of inner side (calyx lobe not open) (VG)
Leaf blade: Tertiary colour (VG)	Young bract: main colour of inner side (calyx lobe open) (VG)
Leaf blade: Undulation of margin (VG)	Bract main colour of inner side (calyx bole wilted)(VG)
Leaf texture	Young outer bract: main colour of inner side (VG)
Petiole: Length (cm) MG/MS	For double bracted cultivars : Young inner bract: main colour of inner side (VG)

Table 3 Clustering based on Jaccard's similarity co-efficient of 100 cultivars as revealed through morphological marker analysis

Major cluster	No. of cultivars	Cultivars
I	49	Abraham Kavoor, Golden Glow, Lady Mary Baring, Aruna, Sonnet, Philomina, Begum Sikander, Shubhra, Dr B P Pal, Mahatma Gandhi, Poultoni Special, Flame, Stanza, Dr H B Singh, Glabra, Dr R R Pal, Gopal, Dr H C Buck, Chandrabieri, Singapore Red, Chitra, Tetra Mrs McClean, Mrs Butt, Alick Lancaster, Cascade, Poultoni, R S Bhatt, Filoman, Meera, Coleoptera, Elizabeth Angus, Spectable, Radha, Summer Time, Fantasy, Mrs McClean, Garnet Glory, Refulgens, Glady's Heburn, Killie Campbell, Sanderiana, Sensation, Splendens, B T Red, Lady Richards, Queen Elizabeth, Gloriosus, Mrs Backery, Zakiriana
II	12	Blondie, Krumbigel, Versicolour, Dream, Swetha, Tomato Red, Padmi, Deep Red, Isacbele Greensmith, Lady Hudson, Sweet Heart, Mataji Agnihotri, Palekar, Perfusion
III	12	Dr Hadu, Parthasarthy, Vishaka, Jubille, Lady Hope, Mrs Frasser, Rosea Fuschia, Lakshmi, Partha, Mary Palmer Special, Manohar Chandra, Phillips
IV	2	Hawaiine White, Jayalakshmi
V	1	Spring Festival
VI	1	Superba
VII	5	Dr PV Sane, Sofia, Louis Wathen, Jawaharlal Nehru, Thimma
VIII	1	Red September
IX	1	Dr Rao
X	6	Bangalore Variegata, Pixi Variegata, Gangaswamy, Dr Bhabha, Pink Beauty, Torch Glow
XI	1	Dr Homibhabha
XII	7	Cherry Blossom, Rose Ville Delight, Los Banos Beauty, Los Banos Variagata, Pallavi, Mahara, Los Banos Variagata Jayanti

cultivars of unknown origin with special identical features of spinal red to purple red coloured bracts. This cluster was again sub divided into 2 subclusters; subcluster IIIa consisting of three cultivars (Dr Hadu, Parthasarthy and Vishaka) which have medium sized internodes, less thorns, shorter and narrower leaves, bracts with acute tips. Subcluster IIIb comprised of nine cultivars having dark green, glabrous, acute tipped leaves and larger purple coloured bracts. Cluster IV represented by only 2 cultivars, viz. Hawaiine White and Jayalakshmi, in which both the cultivars were sharing a same parameters like semi upright growth habit, short and glabrous leaves, broad ovate with cordate shaped bracts. Cluster V contains only solo cultivar (Spring Festival) in which it is characterized by spreading in habit, red thorns,

shorter petioles, profuse flowering and many bract clusters. Similar ways, the Cluster VI also hold a single cultivar namely Superba with glabrous light- green coloured leaves and shoots.

The cluster VII contains 5 cultivars with most of the cultivars which were originated as bud sports of × *buttiana* cv. Mrs Butt possessing variegated leaves with secondary colour distributed in an irregular pattern as well as surrounded al around the midrib. These cultivars also having the distinguishable character like bract cluster had only very few to medium bracts. Cluster VIII had one cultivar; Red September which is of unknown origin and special feature of spreading growth habit, few bract cluster arrangement, small green colour thorns, dark green coloured leaves with yellow patches distributed all along with the margins which is highly distighishable with other variegated cultivars. Cluster IX also represented a solo cultivar Dr Rao, bud sport Mrs. Butt, characterized as upright in growth habit, light green coloured shoots, broad ovate with variegated leaves and cordate based ovate bracts. Cluster X consists of 6 cultivars out of which most of the cultivars were bud sports of × *buttiana* sharing similar features, viz. variegated leaves, very shorter internodes, petiole and peduncles, narrow leaves with yellowish white secondary leaf colour distributed in a leaves with a broader margin to speckled pattern type of colour distribution.

Dr Homibhabha cultivar grouped separately in cluster XI owing to the outstanding unique characters like light green coloured circular leaves with obtuse base, longer intermodal length, yellowish white secondary colour distributed in a narrow margin in the leaves, bract clusters were arranged in both axiilary and terminal manner. Cluster XII consisted of 7 cultivars which are further divided into two subgroups, viz. XIIa and XIIb. Subcluster XIIa consisted of six cultivars (Cherry Blossom, Rose Ville Delight, Los Banos Beauty, Los Banos Variagata, Pallavi, Mahara), all of them had double bracted cultivars, devoid of perianth tube and also sharing some of the morphological features like reddish green coloured shoot, semi upright growth habit, glabrous- medium pubescent medium ovate leaves and dense bract cluster. However, one cultivar of the second subcluster XIIb (Los Banos Variegata Jayanti) was characterized to have yellowish green pubescent leaves with higher thorns in branches and yellowish white colour was distributed in a broader margin in the leaves. Similarly, Panwar *et al*. (2012) also grouped thirty two rose genotypes into three groups based on flower type; Single, Semi Double and Double type of flower. However, in Bougainvillea bract type was categorized into two types, i.e. single and double. Single type included 93 cultivars, whereas Cherry Blossom, Mahara, Rose Ville Delight, Pallavi, Los Banos Beauty, Los Banos Beauty Variagata, and Los Banos Variagata Jayanthi were observed to have double to multiple types of bracts. Almost all the single type cultivars having perianth tube but all multibracted cultivars were devoid of perianth tube. So, all these multibracted cultivars are clustered together in Cluster XII and showing maximum diversity compared with remaining clusters. Cluster analysis also revealed that all the single bracted cultivars were not assembled in the same clusters, indicating a lot of variation within those cultivars.

Cluster analysis using principal component analysis (PCA)

Data were subjected to principal component analysis (PCA), was constructed to demonstrated for morphological relationship. The PCA scatter plot provides the spatial representations of genetic distances among cultivars. The direct three principal components, viz. PCA I, II and III accounted for 14.92, 10.58 and 8.29% variation, respectively were used to compute PCA scatter plot. The 2D diagram representation of PCA grouped these 100 cultivars into four clusters. The grouping in broader sense was based on the most important morphological parameters such as bract type and variegation in the leaves. It clearly separated the cultivars of single and double type of bracts and grouping was also based on pattern of variegation in leaves in a fashion corresponding to the grouping obtained in NJ clustering. In contrast, the PCA plot was unable to distinguish among the cultivars of single bracted types as distinguished in NJ clusters. The first cluster based on PCA comprises all the cultivars of clusters I, II, III, IV, V, VI, VII, VIII, IX and XI as generated by NJ method. However, second PCA cluster consists of two cultivars out of five cultivars of NJ cluster VII except three cultivars (Louise Wathen, Jawaharlal Nehru and Thimma) which were found in the PCA cluster I. Similarly, PCA cluster III holds all the cultivars of Cluster X generated by NJ method in which all these cultivars are exhibited as single bracted with variegated leaves. In addition, PCA cluster IV containing all seven multibracted cultivars of the cluster XII grouped under NJ method.

The aesthetic value of bougainvillea comes from the diverse morphological characters and the study has reported presence of significant variation for all the characters studied. The present study classified the bougainvillea cultivars into character based groups and the helps to identify the reference genotype which can be deployed for characterization of bougainvillea cultivars across locations and years in future. This quantification of existing genetic variation and identification of cultivar for each economic character and grouping the popular cultivars based on genetic divergence will help breeders to select an appropriate cultivar and utilize them for future bougainvillea improvement through systematic and scientific breeding approaches.

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