Crop Breeding and Applied Biotechnology 9: 154-163, 2009
Brazilian Society of Plant Breeding. Printed in Brazil

RAPD-based genetic relationships in different Bougainvillea cultivars

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Received 27 November 2008

Accepted 08 March 2009

ABSTRACT – *The present study deals with authenticating existing knowledge about 21 Bougainvillea cultivars comprising of 9 hybrids and their parents through RAPD analysis. The 19 degenerate primer sets generated 234 bands from which 158 (67.5%) were polymorphic. The UPGMA based dendrogram divided 21 cultivars into two major groups with Jaccard's similarity coefficient ranging from 0.51 to 0.942. Group A had three cultivars namely Trinidad, Formosa and Dr. H. B. Singh in which Dr. H.B. Singh was confirmed as a hybrid of other two cultivars. Group B was sub divided into 8 clusters. The parentages of 7 out of 8 hybirds have been confirmed based on clusters. The study concluded that the RAPD technique is suitable for confirmation of parent-hybrid relationship.*

Key words: Bougainvillea, RAPD, hybridity, genetic diversity, Principle Component Analysis.

INTRODUCTION

The domestication history of Bougainvillea (Nyctaginaceae family) is 250 years old (Pal and Swarup 1974). Bougainvillea is a genus of flowering plants native to South America from Brazil to Peru and to southern Argentina (Chubut Province). The name comes from Louis Antoine de Bougainville, an admiral in the French Navy who discovered the plant in Brazil in 1768. From its native tropical and sub tropical regions, Bougainvillea was introduced to temperate regions of European countries where they were grown in greenhouses. However, in warmer parts of the Mediterranean, the Canary Islands, African countries and India, the environment was akin to what they had in their native regions. In India, *B. spectabilis* was first introduced in Calcutta in 1860 from Europe and its improvement work started in the early 20th century, with the introduction of a few varieties by the Agricultural and Horticultural society of Calcutta and Madras. It achieved its popularity in 1920's with the introduction of 'Mrs. Butt' cultivar in Calcutta from the Royal Botanic Garden, Kew, England. Presently, Bougainvilleas are popular ornamental plants in most areas with warm climates, including India, Taiwan, Vietnam, Malaysia, Australia, the Mediterranean region, the Caribbean, Mexico, South Africa, and the United States in Arizona, California, Florida, Hawaii, and southern Texas. The existing cultivars of Bougainvillea are mostly diploid (2n=34) and generally sterile and multiply through vegetative propagation. Due to strong sporophytic self-incompatibility systems in the plant, only out-crossing persists which leads to a higher level of heterozygosity in its progenies. However, the rate of success of the

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crosses between related and unrelated cultivars remains very low. Nevertheless, breeding of Bougainvillea cultivars has been accomplished by traditional techniques (Khoshoo 1971, Choudhary and Singh 1981).

This documentation has allowed the introgression of new Bougainvillea cultivars with important characteristics such as bract color, vigorosity, resistance to pests and diseases, floral complexity etc. as in the case of rose (Martin et al. 2001). Traditionally the varietal identification was based only on morphological and agronomical features. In fact, it is difficult to identify cultivars based entirely on morphological and cytological markers as they are easily influenced by the environment (Roxas et al. 1993). The variation in such a large number of Bougainvillea cultivars is mainly due to the color of bracts aided by some other characteristics such as leaf and bract size, foliage variegation, floral tube, star, the pubescence of different parts, stamen positions and flowering behavior of the cultivars. All these factors have led to a lot of confusion in the identification of these cultivars (MacDaniels 1981) and the relationship between parents and their hybrids which is mostly based according to the new international checklist of Bougainvillea (Singh et al. 1999) or other check lists and/or personal information. Therefore, breeders and researchers exigently hope to establish an effective method to correctly identify these cultivars.

Molecular analysis aimed at identifying cultivars and determining diversity and genetic relationship significantly improve our present knowledge of Bougainvillea germplasm. In recent years, several molecular approaches have become available, among them RAPD is most commonly used for the identification of cultivar/variety due to its simplicity, rapidity and requirement of only a small quantity of DNA to generate numerous polymorphisms (Wight et al. 1993, Cheng et al. 1997). The RAPD assay has been successfully used for studying genetic diversity of many crop species such as kenaf (Zhou et al. 2002), rose (Debener et al. 1996, Martin 2001), chrysanthemum (Sheng et al. 2000) Amaranthus (Faseela and Joseph 2007) etc. Bougainvillea cultivars can be distinguished by several sets of degenerate primers used in RAPD. Bougainvillea, though being an important ornamental plant, has had no sincere attempt to try and authentically characterize the available genetic stock for future utilization in the development of new color varieties. Keeping this in view, the present study was undertaken with the objectives: 1) to confirm the parentage of the hybrids and 2) to characterize 21 cultivars of Bougainvillea on the basis of genetic diversity through molecular markers and morphological traits.

MATERIAL AND METHOD

The experimental material comprised fresh young leaves of 21 Bougainvillea cultivars belonging to five species with one cultivar of the *B. spectabilis* species, five of *B. glabra*, four of *B. peruviana*, eight of *B. x buttiana* and three of *B. specto-glabra* collected from the Indian Agriculture Research Institute (IARI), New Delhi, Sunder nursery, CPWD and HUDA nursery of Gurgaon, New Delhi (Table 1). The collected leaves were washed and deep-frozen to –80ºC. During leaf sample collection, observations on habit, leaf-shape (elliptic, ovate, broadly ovate), size (large, small), apex (acute, acuminate), base (acute, obtuse, cordate, acuminate), vestitute (glabrous, puberulent), bract size (large, small), shape (ovate, elliptic), apex (acuminate, acute), base (cordate), persistent, non-persistent, floral tube (velutinous, tomentose, puberulent), and flowering (at the end of the branch or all along the branch) of these cultivars were also recorded. Out of nine hybrids, two hybrids had a parent common ('Dr. B. P. Pal') and in three hybrids both parents were common ('Thomasii' x 'Louise Wathen').

Hybrid Combinations

Through Planned Hybridization

1. 'Trinidad' x 'Formosa' '⟶`Dr. H.B. Singh' (intraspecific), (IIHR, Bangalore, India, 1979)

2. 'Princes Margaret Rose' x 'Dr. B.P. Pal''⟶Mary Palmer Special' (intraspecific), (Zadoo and Khoshoo, India, 1974)

3. 'Dr. B.P. Pal' x 'Tetra Mrs. McClean'' ⟶`Chitra' (interspecific), (Khoshoo, Ohri & Sharma, India, 1981)

Through Natural Hybridization

1. 'Thomasii' x 'Louise Wathen''⟶`Dr. R. R. Pal' (interspecific), (Dr.B.P. Pal, India 1959)

2. 'Thomasii' x 'Glabra''⟶'Tomato Red' (interspecific), (T. F. Turley, Australia)

3. 'Thomasii' x 'Louise Wathen''⟶'Spring Festival' (interspecific), (Dr.B. P. Pal, India, 1959)

4. 'Thomasii' x 'Louise Wathen`'⟶'Summer Time' (interspecific), (Dr.B. P. Pal, India, 1959)

5. 'Crimson Lake' x 'Sanderiana''⟶'Barbara Krast' (interspecific), (J.E.Hendary, USA 1927)

6.'Mrs. H. C. Buck' x 'Refulgens''⟶'Purple Gem'

Table1. Collection sites, origin, habits and their affinity with species of different Bougainvillea cultivars used in RAPD analysis

S.No.	Cultivars and their hybrids	Origin	Origin according to check lists	Collection Sites	Affinity (Check list)	Habit
1	Trinidad (TRI)	Bangalore, India	Seedling of glabra	Buddha Jayanti park, New Delhi	*B. glabra*	Vigorous growing
2	Formosa (FOR)	Brazil	——	CPWD, Gurgaon	*B. glabra*	——
3	Dr. H. B. Singh (HBS)	Bangalore, India	Seedling of Trinidad x Formosa	Buddha Jayanti park, New Delhi	*B. glabra*	Dwarf, drooping, growth restricted
4	Princes M. Rose (PMR)	Madras, India		CPWD, Gurgaon	*B. peruviana*	Tall, growth vigorous
5,7	Dr. B. P. Pal (BPP)	Lucknow India	Tetraploid hybrid	CPWD, Gurgaon	*B. peruviana*	Growth restricted
6	Mary Palmer Special (MPS)	Lucknow, India	Triploid hybrid	Buddha Jayanti Park, New Delhi	*B. peruviana*	Tall, growth vigorous
8	Tetra Mrs. McClean (TMM)	Lucknow, India	Tetraploid	Buddha Jayanti Park, New Delhi	*B.x buttiana*	Tall, growth vigorous
9	Chitra (CH)	Lucknow, India	Hybrid	Buddha Jayanti Park, New Delhi	*B.x buttiana*	Tall, growth vigorous
10,13,16	Thomasii (TH)	Australia,1905	——	Buddha Jayanti Park, New Delhi	*B x spectabilis*	Not so vigorous
11,17	Louise Wathen (LW)	England	Bud sport	CPWD, Gurgaon	*B. x buttiana*	Erect, vigorous
12	Dr. R. R. Pal (RRP)	New Delhi, India	Hybrid seedling	IARI, New Delhi	*B. x buttiana*	Vigorous
14	Glabra (GL)	Brazil	——	CPWD, Gurgaon	*B. glabra*	Vigorous, dwarf
15	Tomato Red (TR)	Queensland, Australia	Hybrid seedling	IARI, New Delhi	*B. spectabilis*	Less vigorous
18	Spring Festival (SF)	New Delhi, India	Hybrid seedling	IARI, New Delhi	*B. x buttiana*	Vigorous
19	Summer Time (ST)	New Delhi, India	Hybrid seedling	IARI, New Delhi	*B. x buttiana*	Vigorous
20	Crimson Lake (CL)	Calcutta, India	Hybrid seedling	IARI, New Delhi	*B. x buttiana*	Tall, growth vigorous
21	Sanderiana (SA)	Exact origin not known	——	NBRI, Luck now	*B. spectabilis*	Semi-erect, vigorous and compact
22	Barbara Krast (BK)	Florida, U.S.A	Hybrid seedling	CPWD, Gurgaon	——	——
23	Mrs. H. C Buck (HCB)	Madras, India	Hybrid seedling	IARI, New Delhi	*B. x peruviana*	Vigorous
24	Refulgens (REF)	Brazil	——	IARI, New Delhi	*B. spectabilis*	Climber with drooping branches
25	Purple Gem (PG)	Not known	Hybrid seedling	Buddha Jayanti Park, New Delhi	——	——

(interspecific), (M.S. Soundarya Nursery, Madras, India).

DNA Extraction and RAPDs

Genomic DNA was extracted from plant material (leaves) using a modified protocol of Doyle and Doyle (1990). The DNA amplification was performed in a gene amp PCR system 9700. Samples were screened for RAPD variations using standard 10-base primers supplied by Operon (USA). 20 µL of a reaction cocktail was prepared [10 X thermo stable PCR buffer 2.0 µL, dNTPs (0.2mM) 4 µL, $MgCl_2$ (25mM) 0.5 µL, 10-mer primers (Operon,

USA) (0.5mM) 1.0 µL, Taq polymerases (Genei, Bangalore) 1U/0.3 µL, water 7.2 µL, and sample DNA 5.0 µL (10ng μL^{-1})]. Thermal cycles were performed at: 2 min at 94ºC initial denaturation followed by 40 cycles of 1.0 min 94ºC; denaturation, 1.30 min 36ºC; annealing, 1.30 min 72ºC; extension, and 5 min 72ºC; final extension. The amplification products (17 µL) were size separated by gel electrophoresis in 1.5% wv^{-1} agarose gel with 0.5 X TBE (Tris-Borate-EDTA). The gel was stained in ethidium bromide solution (0.001µg mL^{-1}) for 20 min and visualized under UV illumination and photographed. All the reactions were run in duplicate. A primary survey was carried out and 35 primers of A&T series (Operon, USA) were screened to get a good profile. Finally, 19 primers were selected which gave reproducible, scorable bands on repeated trials and were used for further analysis (Table 2).

Data Scoring and Statistical Analysis

The size of resolved bands were determined by comparing with double digested λ DNA with EcoR I and Hind III molecular markers which was also run with the amplified products. Each reproducible band was scored '1' for presence and '0' for absence and was transferred into the binary matrix. The binary data obtained was subjected to statistical analysis using the software Windowstat, Hyderabad, India. Dendrogram was constructed from the distance matrix by the UPGMA (Unweighted Pair Group Method of Arithmetic Averages) using NTSYS pc v 2.0 to generate Jaccard's similarity coefficient (Rohlf 1998). To ascertain the identity of the three cultivars namely BPP, TH and LW, two, three and two different plants of each cultivar were taken, respectively, for the construction of the dendrogram. The dissimilarity matrix was developed using Squared Euclidean Distance (SED), which

Table 2. Description of 19 primers and clustering of 21 cultivars of *Bougainvillea* based on RAPD

Primer	Sequence	Band No.	Amplicon size	Polymorphism (%)
OPT-2	GGAGAGACTC	5	1500-500	80
OPT-4	CACAGAGGGA	12	3500-300	83.3
OPT-5	GGGTTTGGCA	2	4000-3000	68.4
OPT-6	CAAGGGCAGA	19	3700-160	62.6
OPT-7	GGCAGGGTGT	8	3600-500	62.5
OPT-8	AACGGCGACA	18	3600-200	69.6
OPT-9	CACCCCTGAG	15	4000-800	58.8
OPT-11	TTCCCCGCGA	15	3500-500	63.6
OPT-12	GGGTGTGTAG	8	2000-200	87.5
OPT-13	AGGACTGCAA	4	5100-1300	70.6
OPT-14	AATGCCGCAG	15	3500-500	100
OPT-15	GGATGCCACT	8	1400-300	65.5
OPT-16	GGTGAACGCT	23	3700-200	61.8
OPT-17	CCAACGTCGT	15	3000-500	63.6
OPT-18	GATGCCAGAC	15	3700-1000	58.6
OPT-19	GTCCGTATGG	17	3600-200	49.8
OPA-9	GGGTAACGCC	18	3400-125	68.6
OPA-20	GTTGCGATCC	9	2000-900	69.8
OPD-5	TGAGCGGACA	8	1000-200	68.6
Group/clusters	RAPD data			
Group A 3	(TRI, FOR, HSB)			
Group B I	3 (PMR,BPP,BPP,MPS)			
II	2 (TMM, CH)			
III	2 (TH,TH,TH,LW,LW)			
IV	2 (RPP,ST)			
V	2 (SF, HCB)			
VI	3 (CL,BK, PG,)			
VII	2 (GL, SA)			
VIII	2 (TR, REF)			

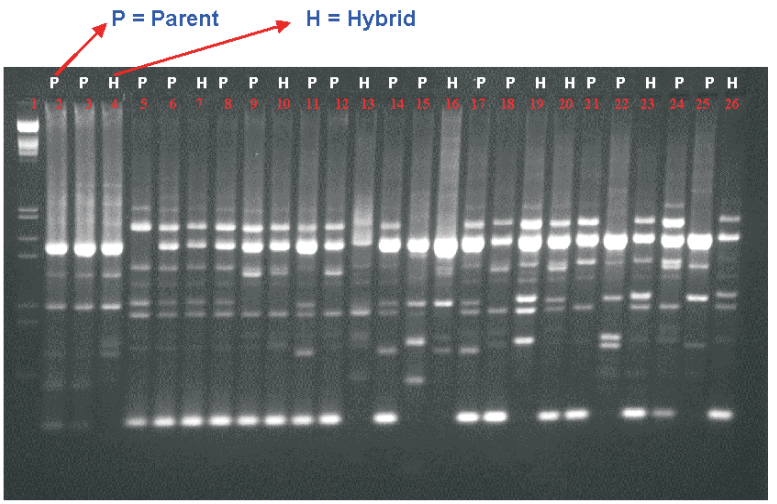
estimated all pair wise differences in the amplification products (Sokal and Sneath 1963).

RESULTS AND DISCUSSION

RAPD markers have the potential to measure variations accurately utilizing the entire genome and can produce reliable data for authentic characterization of genetic diversity. But it requires a properly standardized protocol, replication of amplification reaction and conservative criterion of band selection (Faseela and Joseph 2007). So in the present study, optimization of various experimental steps was done carefully in order to overcome the problem of sensitivity in the RAPD technique.

RAPD Profile Analysis

Selected primers detected an average polymorphism of 67.54% over all cultivars and 234 bands by a set of 19 primer pairs. One of the major factors contributing to the high degree of polymorphism might be due to the available genetic divergence in current sets of germplasm analyzed. The number of bands per primer set ranged from 2 (OPT-5) - 23 (OPT-16) with an average of 12.3 scorable bands per primer (Figure 1). Out of 234 bands, 158 were polymorphic (67.4%) and scorable that showed high level of polymorphism, which indicated the efficiency of the selected primers to characterize the germplasm. A high level of polymorphism (84.4%) was also observed in Bougainvillea by the person who studied the genetic diversity to trace out the origin of some unknown group of Bougainvillea cultivars. However, the present study aims to confirm both the parentage of hybrids and genetic diversity among some different selected cultivars other than the study of Chatterjee et al. (2007). The highest level of polymorphism evident with OPT-16 followed by OPT-6, OPT-8 and OPA-9 could be explained as the capability of RAPD primers to amplify less conserved and highly repeated regions of DNA. However, the high level of polymorphism exhibited by OPT-14 might also be due to the capability of RAPD primers to amplify the non conserved and highly repeated regions of the DNA. The high level of probability of amplified fragments containing repeated sequence was also reported (Prasannalatha et al. 1999, Faseela and Joseph 2007). Percentages of polymorphism was the highest with primer T-14 (100%) and the lowest with OPT-19 (49.8%) (Table 2). Three primer sets showed no polymorphism (OPT-2, OPT-5, and OPT-13) and were amplified in a range of monomorphic bands. The high level of polymorphism observed in the present study confirms that much diversity exists within this germplasm. The number of co-migrating bands exhibits the reproducibility of amplification patterns, which provide the key to genotype identification, as observed for rice (Mathew et al. 2000). The molecular weight of amplicons ranged from 125bp (OPA-9) to 5100bp (OPT-



Lane 1 EcoRI and Hind III digested λ DNA marker
Lane 2-26 RAPD Profiles of different cultivars of *Bougainvillea*

Figure1. RAPD profile of Bougainvillea cultivars i.e. {(2) TRI, (3) FOR, (4) HBS, (5) PMR, (6) BPP, (7) MPS, (8) BPP, (9) TMM, (10) CH, (11) TH, (12) LW, (13) RRP, (14) TH, (15) GL, (16) TR, (17) TH, (18) LW, (19) SF, (20) ST, (21) CL, (22) SA, (23) BK, (24) HCB (25) REF and (26) PG}generated using OPT-14 random primer

13) (Table 2). In the present study the genetic diversity assessed through PCR amplicons using 19 (10-mer) primers was found satisfactory up to 100%.

Genetic Diversity Analysis

The clustering pattern of the cultivars was estimated by the UPGMA method based on a genetic similarity matrix which is well suited for diversity analysis and is presented in Figure 2. The similarity coefficient between all possible pairs of cultivars ranged between 0.51-1.00. Such a wide range of dissimilarity values suggests that the Bougainvillea cultivars represent a genetically diverse population. Cluster analysis of Bougainvillea employing the UPGMA method led to the classification of cultivars into two major groups. The resultant dendrogram grouped 18 cultivars in one group, while three cultivars of *B. glabra* were placed in a separate group and were clearly discernable from the other species except that one cultivar of *B. glabra* which fell into another group might be due to different geographical origins or selection pressure (Figure 1). At a 0.51 similarity level all the cultivars combined to form two major groups, 'A' and 'B', which clearly demarcated the cultivars into six species (Zadoo et al. 1975) and into four species (according to the new international checklist of Bougainvillea (Singh et al. 1999) respectively. The major group 'B' was further divided into eight sub clusters which was comprised of 18 cultivars of different species showing diversity up to 0.656 (Table 2, Figure 2). Group 'B' contained cultivars from *B. spectabilis, B. glabra, B. peruviana, B. x buttiana B. specto-glabra* and *B. specto-peruviana.* Cluster analysis revealed that all cultivars were not clustered on the same branch, suggesting variations within them. The association among the cultivars of different species may be attributed to the broad genetic base, which enables them to maintain and exist in different gene combinations.

Group 'A' had three cultivars i.e. 'Trinidad', 'Formosa' and 'Dr. H. B. Singh' which all showed affinity to the *B. glabra* species and also had similarity in morphological characteristics i.e. leaves (elliptical with short hairs, smooth to touch), bract (elliptical, color phlox purple, mauve, old bracts persistent), floral tube (swollen at base, angled), flowering all along the branch and sets seeds. 'Dr. H. B. Singh' is an interspecific hybrid of cultivars 'Trinidad' and 'Formosa' produced through planned hybridization (raised by IIHR, Bangalore, 1979). Our study also confirms the parents and hybrid relationship of these cultivars. Both parents are closely related to each other with the second highest value at 0.930 in the similarity matrix. However, parents and hybrids were not much similar morphologically, but proved to be genetically similar through RAPD band data sharing (Figure 2). The morphological dissimilarities might be due to the long history of domestication under different climatic conditions. The lack of agreement in RAPD and morphological data was also noticed earlier by Kundun and Park (2002).

Group 'B' was further divided into sub clusters. Sub cluster I was comprised of three cultivars; 'Princes Margaret Rose', 'Dr. B. P. Pal' and 'Mary Palmer Special'. Cultivars 'Dr. B. P. Pal' and 'Mary Palmer Special' are closely related, as evident through a similarity matrix which had the highest similarity value of 0.942 in sub cluster I. Both of these showed affinity to the *B. peruviana* species and also share the morphological characteristics of this species, but differs only in the

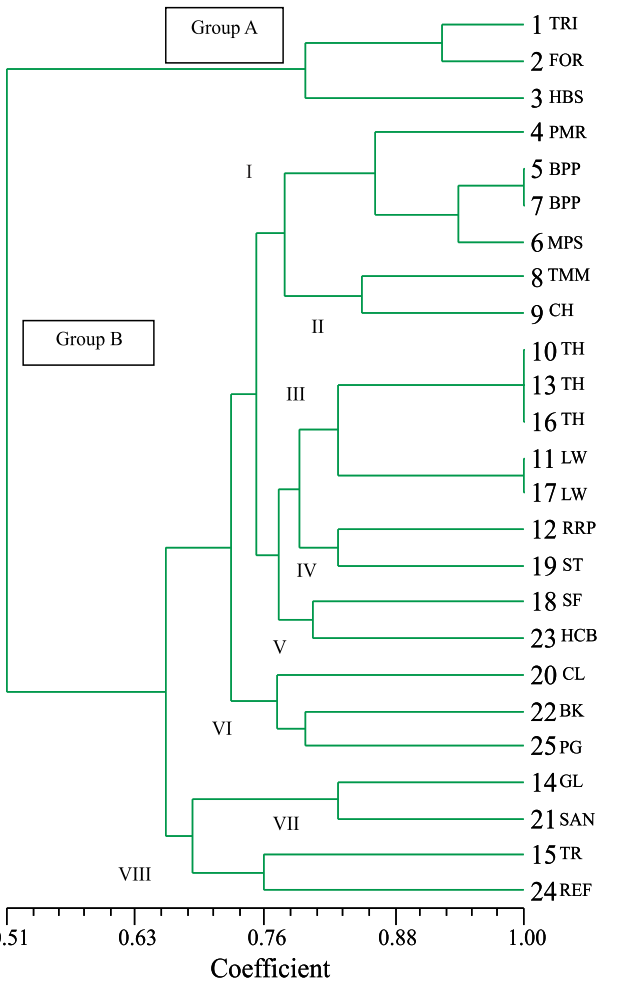


Figure 2. UPGMA dendrogram showing genetic relationship among 21 Bougainvillea cultivars based on RAPD data

white bract color in 'Dr. B. P. Pal' and three types of color patterns in 'Mary Palmer Special' i.e. parchment white, magenta 27/1 and parchment white in magenta 27/1. However, all three belong to *B. peruviana* and showed common characteristics like leaves (broadly ovate and glabrate), bracts (crinkled, color magenta or parchment white), flowering (near the end of the branches) and blooming season (winter and summer). 'Mary Palmer Special' is an interspecific hybrid (planned hybridization) of 'Princes Margaret Rose' and 'Dr. B. P. Pal' (raised by Zadoo et al. 1975).

On the basis of the present study, 'Mary Palmer Special' confirmed the hybridity with their parents. Sub cluster II comprised of two cultivars 'Tetra Mrs. McClean' and 'Chitra'. 'Tetra Mrs. McClean' belongs to the species *B. x buttiana* (a hybrid of *B. peruviana x B. glabra*). The essential features of habit and morphology of the 'Tetra Mrs. McClean' cultivar resemble *B. peruviana*. 'Chitra' is the interspecific hybrid (planned hybridization) of 'Dr.B. P. Pal' and 'Tetra Mrs. McClean' (raised by Khoshoo and Sharma 1981**). This hybrid was genetically very similar to one of the parent 'Tetra Mrs. McClean' with a similarity value of 0.832. However, morphologically this hybrid was also similar to 'Tetra Mrs. McClean' except for the bract color and floral tube. Thus 'Chitra' confirmed the hybridity between 'Tetra Mrs. McClean' and 'Dr.B. P. Pal'. One parent 'Dr.B. P. Pal' was common in two hybrids; 'Mary Palmer Special' (intraspecific) and 'Chitra' (interspecific). Sub cluster III comprised two cultivars; 'Thomasii' (species *B. spectabilis*) and 'Louise Wathen' (*B. x buttiana*). Genetically, 'Thomasii' and 'Louise Wathen' showed a similarity of less than 80% with each other but due to species-specific amplified products both were grouped in the same sub cluster. After natural hybridization between them, two interspecific hybrids 'Dr. R. R. Pal' and 'Summer Time' (raised by Dr. B. P. Pal, 1959***) were produced, which showed similarity to *B. x buttiana* and formed the separate sub cluster IV. However, both parents and their hybrids had a similarity value of 0.830. Morphologically, both hybrids differ from each other in characteristics like leaves (shape and size), bract (shape, size and color) and bract color (fuschia purple 28/1 in 'Dr. R. R. Pal' and cardinal red (822/3) in 'Summer Time') while maintaining similarities in some characteristics like vigorous growth and free flowering etc. The closeness and similarities between these hybrids were due to the same parentage, which confirmed the hybridity. Sub cluster V was comprised of 'Spring Festival', another natural interspecific hybrid of 'Thomasii' and 'Louise Wathen' (raised by Dr.B. P. Pal, 1959***) belonging to *B. x buttiana* (Singh et al. 1999) and related to their parents with a similarity value of 0.805. It also shared similarities with the 'Mrs. H. C. Buck' (*B. peruviana*) cultivar. Both 'Spring Festival' and 'Mrs. H. C. Buck' showed similarity in bract (rounded) and thorn (short and fairly straight).

Three cultivars 'Dr. R. R. Pal', 'Summer Time', and 'Spring Festival' according to the checklist, belong to *B. x buttiana* but according to Zadoo et al. (1975) and Ohri and Zadoo (1979), they are together with 'Mrs. H. C. Buck' from sub cluster III belonging to *B. specto-peruviana*. According to Zadoo et al. (1975) these cultivars exhibit morphological characteristics of both species such as rampant and floriferous characteristics, branched, main shoot such as the *B. spectabilis* type, recurrent blooming and division of flowering cyme more than twice the *B. peruviana* type (Zadoo et al. 1975). Still, according to Zadoo et al. (1975), the 'Thomasii' (*B. spectabilis*) cultivar and sub cluster III resembled the 'Dr. R. R. Pal', 'Summer Time', 'Spring Festival' and 'Mrs. H. C. Buck' cultivars and were closely related with nearly identical similarity values: 0.830 and 0.805, respectively. However, 'Thomasii' showed similarities with these cultivars in characteristics such as flowering behavior. According to the bougainvillea check list (Singh et al. 1999) the 'Mrs. H.C. Buck' cultivar shows an affinity to *B. peruviana* in characteristics like leaves (ovate, glabrous), bract (magenta) and blooming (winter and summer) etc. but according to Zadoo et al. (1975) and Ohri and Zadoo (1979) it shows affinity to *B. specto-peruviana*. Sub cluster VI comprises three cultivars; 'Crimson Lake', 'Barbara Krast' and 'Purple Gem'. 'Crimson lake' showed affinity to *B.x buttiana* as per the dendrogram and is closely related to the 'Barbara Krast' cultivar. According to Gorden Braswell, a nursery owner, 'Barbara Krast' belongs to *B. x buttiana* while according to the Master Gardner of the University of Arizona Pima country co-operative extension; it shows affinity to *B. glabra*. In our study 'Crimson Lake' showed similarities with *B. glabra* only for a single characteristic i.e. floral tube swollen at the base. The dendrogram states that 'Crimson Lake' seems genetically similar to 'Barbara Krast' and 'Purple Gem'. 'Barbara Krast' *(B. x buttiana* or *B. glabra)* and 'Purple Gem' (*B. glabra*) are closely related with a 0.795 similarity value

which proves that 'Barbara Krast' and Purple Gem' are genetically close. However 'Barbara Krast' shows affinity to *B. glabra*. 'Barbara Krast' is an interspecific natural hybrid of 'Crimson Lake' and 'Sanderiana' (raised by J.E. Hendry, USA 1927***). The dendrogram shows that the hybrid 'Barbara Krast' is similar to one of its parents, 'Crimson Lake'. 'Purple Gem', 'Crimson Lake' and 'Barbara Krast' are genetically dissimilar (similarity value of 0.025), although they were morphologically similar in leaf (broadly ovate, glabrous), bract (non-persistent, color crimson), floral tube (puberulent), and flowering (profuse). Our study confirms the hybridity of 'Barbara Krast' with only one of its parents, 'Crimson Lake' and not with 'Sanderiana'. Another interspecific natural hybrid 'Tomato Red' of 'Thomasii' and 'Glabra' (T.F. Turley, Australia) shows affinity to *B. spectabilis* according to the bougainvillea checklist (Singh et al. 1999), but according to Zadoo et al. (1975) it belongs to *B. specto-glabra*. 'Thomasii' and 'Glabra' are genetically related to each other with a similarity value of 0.830. The hybrid 'Tomato Red' of these parents shows morphological similarities with one of the parent 'Thomosii' with some characteristics; the lack of growth vigor and profuse seed setting. But hybrid 'Tomato Red' does not share similarities with both parents as all three fell in separate clusters, failing to confirm hybridity of 'Tomato Red'.

Sub cluster VII comprises of two cultivars 'Glabra' (*B. glabra*) and 'Sanderiana' (*B. glabra* or *B. specto-glabra*) with similarities in leaves (glabrous), thorns (curved at tips), bracts (purple), flowers (cream) and blooming (recurrent). According to the checklist, it belongs to *B. glabra*, but according to Zadoo et al. (1976) it belongs to *B. specto-glabra*. The degree of hairiness and the shape of the floral tube in 'Sanderiana' was intermediate between *B. spectabilis* and *B. glabra* (Zadoo et al. 1976). That's why 'Sanderiana' was kept as the sub-species *B. specto-glabra*.

'Purple Gem' is an interspecific natural hybrid of 'Mrs. H.C. Buck' and 'Refulgens' (raised by M.S. Soundarya Nursery, Madras, India). The hybrid 'Purple Gem' (*B. glabra*) belongs to sub cluster VI; Mrs. H. C. Buck, (*B. peruviana*) one of the parents, falls into sub cluster V; and 'Refulgens' is another parent (*B. spectabilis*) falling into sub-cluster VIII. 'Mrs. H.C. Buck' and 'Purple Gem' show similarity levels up to 0.805 and also have morphological similarities in leaf shape (glabrous) and floral tube (puberulent and creamish). However, both the parents and hybrids fall into different sub clusters. 'Mrs. H. C. Buck' shows affinity to *B. peruviana* and lies in between cultivar of *B. buttiana* ('Spring Festival' and 'Crimson Lake'). However, it shows genetic similarity to *B. buttiana*. It is considered to be a natural hybrid of *B. peruviana* and *B. glabra*. Thus, 'Mrs. H.C. Buck' shows close affinity to *B. peruviana of B. x buttiana* than *B. glabra*. 'Thomasii' (*B. spectabilis*) and 'Louise Wathen' (*B. buttiana*) through natural hybridization produced three interspecific hybrids i.e. 'Dr R. R. Pal' (fuschia purple 28/1), 'Spring Festival' (solferino purple 31/1) and 'Summer Time' (Cardinal Red 822/3); all of these belong to *B. buttiana*. Sub cluster VII and VIII comprise 'Glabra' (*B. glabra*) and 'Sanderiana', 'Tomato Red' and 'Refulgens' (*B.* specto-glabra) (Zadoo et al. 1975) cultivars, forming a separate cluster 'B' branch. *B. specto-glabra* has a hybrid group of *B. glabra* and *B. spectabilis* as they have similarities in essential morphological features. The only major difference is the villous character of the latter. The degree of hairiness and shape of floral tube in these cultivars is intermediate between *B. spectabilis* and *B. glabra*. Interestingly, the 'Glabra' cultivar and the 'Trinidad', 'Formosa' and 'Dr. H.B. Singh' cultivars all belong to *B. glabra* but they fell into different topologies: sub-clusters VII and clade A, respectively. 'Glabra' is near the *B. specto-glabra* viz. 'Sanderiana', 'Tomato Red' and 'Refulgens' cultivars due to morphological similarities with *B. spectabilis*, such as bract (persistent), floral tube (swollen at base) and flowering (all along the branch). 'Mrs. H. C. Buck' and its bud sport 'Princess Margaret Rose' fell in different sub-clusters due to a different genetic constitution with respect to allelic incompatibility (Zadoo et al. 1976). 'Barbara Krast' either belongs to *B. glabra* or *B. buttiana* (according to different experts), but in our study, it fell within the species *B. glabra*.

CONCLUSION

We conclude from the present study that to obtain reliable identification, tracing genetic relationships and characterization of the Bougainvillea germplasm, the molecular approach based on RAPD profile is a powerful technique. The resolution of the molecular markers is much higher than morpho-agronomic characters to identify individual cultivars. The information obtained will facilitate choosing the appropriate breeding program

to incorporate beneficial genes in desirable genotypes lacking the particular trait. Through the study, parentages of some of the hybrids of Bougainvillea have been confirmed on one hand, and the groupings of the cultivars based on their diversity have been successfully carried out on the other hand. In day-to-day management of the germplasm collection, RAPD allows identification of redundancy and provides additional cultivar verification methods. The genetic diversity analysis in the Bougainvillea germplasm collection will provide useful information for proper management and its future utilization in basic and applied studies.

Relação genética em diferentes cultivares de Bougainvillea baseado em RAPD

RESUMO – *O objetivo deste estudo foi atualizar o conhecimento existente sobre 21 cultivares de Bougainvillea, composto de nove híbridos e seus parentais por meio de marcadores RAPD. Um conjunto de 19 primers degenerados geraram 234 bandas das quais 158 (67,5%) foram polimórficas. O dendrograma baseado no método UPGMA dividiu as 21 cultivares dentro de dois grandes grupos, com o coeficiente de similaridade de Jaccard variando de 0,51 a 0,942. O grupo A têm três cultivares chamadas Trinidad, Formosa e Dr. H. B. Singh, sendo esta última confirmada como um híbrido entra as outras duas cultivares. O grupo B foi subdividido em oito grupos. O parentesco de sete dos oito híbridos foram confirmados baseados nos grupos formados. Este estudo concluiu que a técnica de RAPD é viável para confirmação da relação genética entre híbridos e seus parentais.*

Palavras chave: Bougainvillea, RAPD, hibridação, diversidade genética, Análise de Componentes Principais.

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