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Molecular characterization and cultivar identification in *Bougainvillea* spp. using SSR markers

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ABSTRACT

The present study was undertaken to determine the genetic relatedness and molecular characterization of fifty bougainvillea cultivars that belong to four major species of bougainvillea namely *B. glabra, B. spectabilis, B.peruviana* and *Bougainvillea* × *buttiana*. Five microsatellite (simple sequence repeat; SSR) markers with high PIC values were used to characterize these bougainvillea cultivars. A total of 28 alleles were detected at an average number of alleles of 5.6 alleles /locus. The PIC values varied widely among primers and ranged from 0.364 to 0.891 with an average of 0.716 per locus and the size of the amplified products ranged from 90bp to 250bp. Primer BOUG-1 showed the highest polymorphism index content (0.891) thus reflecting it's ability to differentiate these cultivars much better at molecular level. A total of 18 rare alleles were identified among which the cultivar (Blondie) had maximum number of rare alleles (3). An unweighted pair group method cluster analysis (UPGMA) based on similarity values revealed five main clusters with Cluster I being the largest one encompassing 18 cultivars while cluster IV and V emerged as the smallest ones comprising 3 cultivars each. The pair wise estimates of genetic distance ranged from 0 (Cherry Blossom to Mary Palmer Special) to 1.0 (Blondie to Shubhra, Partha, Lady Hope, Gloriosus, Red September, Zakiriana, Lady Richards and Spledens). The present investigation is first of its kind in using microsatellite markers for phylogenetic analysis and molecular characterization in bougainvillea cultivars. The study proved the efficiency of SSR markers in documentation, identification and tracing out the molecular origin among unknown cultivars of bougainvillea.

Key words: Characterization, Cluster analysis, Genetic relationship, Principal Component Analysis

Bougainvilleas (*Bougainvillea* spp., family: Nyctanginaceae), are evergreen climbing ornamental shrubs which are extensively used in tropical and subtropical gardens. Its attractive foliage (variegated in some of the mutants), colourful bracts and wider adaptability to various soil and climatic conditions makes it one of the sought-after plant for diverse landscape uses, viz. shrub, climber, pot plant, hedge, specimen plant, topiary, cascade, bonsai, arches and pergolas. There are many bougainvillea cultivars which show only minor differences in leaf shape, variation in flower colour and are difficult to identify perfectly based on morphology. Traditionally, all efforts for improvement of bougainvillea were based on bud sports followed by use of chance seedlings. But now, efforts have been shifted to induced mutations and also crosses made among important species namely B. *glabra, B. spectabilis, B. buttiana* and *B. peruviana* which differ significantly from each other (Ohri and Zadoo 1980). It would be interesting to note the extent of contribution of parents in each such combination by using suitable molecular markers to assist bougainvillea improvement.

In recent years, molecular markers have been more frequently used to assess genetic diversity and correct identification of cultivars and it seems to be more effective rather than the morphological markers as they are easily influenced by the environment. Though several molecular markers are available, but the characterization work in bougainvillea is more dealt with RAPD markers, which are less reliable and known for their lower reproducibility (Chatterjee *et al.* 2007, Hammad 2009, Srivastava *et al.* 2009). Hence, they are not much preferred as suitable marker systems for diversity and evolutionary studies. Development of molecular markers profile for bougainvillea will help in reduction of duplications and also serve as a potential tool in cultivar identification. Therefore, use of more reliable and reproducible molecular markers like SSRs becomes indispensible in bougainvillea also like any other plant species. Reliable and reproducible molecular characterization is not only important for maintenance of

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cultivars but also for conservation and thereby avoiding the duplications.

Simple Sequence Repeats (SSRs or microsatellites) have become genetic markers of choice in many plant species due to their multi-allelic nature, high abundance, reproducibility, high degree of polymorphism, co-dominance inheritance and extensive genome coverage (Varshney *et al*. 2005). To understand the relationship among the different cultivars of bougainvillea, SSR markers were used in the present study to characterize the cultivars and to obtain information on genetic variability which will help in future breeding programme.

MATERIALS AND METHODS

The plant material utilized for the study comprised of fifty bougainvillea cultivars, available at the International Cultivar Registration Authority for Bougainvillea, Division of Floriculture and Landscaping, Indian Agricultural Research Institute, New Delhi. The list of cultivars used for the study is given in Table 1. The present study was conducted during 2012-14.

Genomic DNA was extracted using CTAB method (Murray and Thompson, 1980) with minor modifications. For each cultivar, five grams of fresh young leaves rinsed with tap water was taken; frozen in liquid nitrogen and ground to a fine powder which served as the starting material for subsequent steps of DNA extraction. After DNA purification, DNA concentration was estimated using 1% agarose gel while quality was detected with NanoDrop (Thermo Scientific, USA). The extracted DNA was diluted to a final concentration of 30ng/ul.

Table 1 List of bougainvillea cultivars used for SSR analysis

Cultivar	Cultivar
Thimma	Pink Beauty
Mahatma Gandhi	Hawaiin White
Blondie	Tomato Red
Lady Mary Baring	Torch Glow
Zakiriana	Golden Glow
Dr Bhabha	Parthasarthy
Sweet Heart	Dream
Singapore Red	Flame
Shubhra	Radha
Chitra	Partha
Mary Palmer Special	Gloriosus
Cherry Blossom	Lady Richards
Roseville's Delight	Manohar Chandra
Los Banos Beauty	Splendens
Mahara	Meera
Summer Time	Sanderiana
Stanza	Poultoni Special
Sonnet	Filoman
Vishaka	Lady Hope
Spring Festival	Rosea Fuchsia
Dr H B Singh	Red September
Refulgens	Dr R R Pal
R S Bhatt	Abraham Kavoor
Mrs Butt	Sensation
Chandrabieri	Jawaharlal Nehru

A total of fifty SSR markers were identified in the laboratory from genomic sequences were used for the genetic diversity analysis and on the basis of contig sequences, SSR primers were synthesised. The sequence information, repeat motifs with amplified annealing temperatures were shown in Table 2.

SSR primers were used to amplify 50 bougainvillea cultivars using thermalcycler. PCR programme consisted of an initial denaturation at 94°C for 6 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50ºC - 58ºC (based on primer annealing temperature) for 1 min and 72°C for 2 min, with a final extension at 72°C for 10 min before cooling to 4°C. 25 µl of reaction cocktail was prepared containing 2 µl of template DNA, 2 µl of primer, 2 µl of 4-deoxy ribonucleotide triphosphate, 1.5 µl $MgCl_2$, 2.5 µl Taq DNA buffer and 0.33µl Taq DNA polymerase (Genei, India). The PCR products were separated on a 3% agarose gel by electrophoresis in 1 X TAE buffer.

Polymorphic bands of each SSR marker were scored as 1 for their presence and 0 for absence at each level of a particular locus. The resulting data were analyzed using NTSYS-pc Version 2.1 (Rohlf 2000). The binary matrix was converted to appropriate formats required for specific programs. The polymorphism information content (PIC) values for each SSR were estimated by determining the frequency of alleles per locus using the formula as given below.

$$PIC = 1 - \Sigma x_i^2$$

where, $x_{i,}$ relative frequency of the 'i' th allele of SSR loci.

The genetic associations among cultivars were estimated by calculating the simple matching coefficient which is based on pair wise comparisons of the proportion of bands shared among the cultivars. The dendrogram was generated by un-weighted pair-group method with arithmetic average (UPGMA) using the NTSYS-pc program Version. 2.1 (Rohlf 2000). Principal components analysis of the data was also performed using NTSYS-pc program.

RESULTS AND DISCUSSION

Screening of SSR markers

For preliminary screening work, six different cultivars of bougainvillea were screened using all the fifty SSR primers synthesised. Five SSR markers with good amplification and high level of polymorphism in different genotypes were selected and these were subsequently used in molecular characterization of all the cultivars under study.

Analysis of SSR Marker profiles

Allelic number and their size : Table 3 summarizes the analysis of SSR profiles of 50 cultivars of bougainvillea using 5 SSR markers. The minimum and maximum molecular weight among the alleles, number of rare alleles,

Table 2 SSR primer code, sequences, motifs and annealing temperature

Primer code	Primer sequence	Motifs	Annealing temperature (ºC)
BOUG-1	F: TTGCTCCTCTTCGCCTTCAG		
	R: GCCTGTACAGTGATCCCACC	(AAC)7	50
BOUG-2	F: AGGTTGCAGATGGACACCAA		
	R: TGAAAGCAGATGGACACCAA	(TA)8	53
BOUG-3	F: ACGGCTGTCAAAATGTTCGC		
	R: CCACGTCCACCTCCTAGTTG	(AT)8	50
BOUG-4	F: AGGTACCCACTCCTCTCTCT		
	R: TGACCGCTATTCACGGCATT	(TC)8	53
BOUG-5	F: TTAGCCACCCTAGAATCGGT		
	R: TTAGCCACCCTAGAATCGGT	(TTG)5	58

Table 3 Primer, number of alleles, PIC content and rare alleles

Primer	Min bp	Max bp	No. of alleles	Rare alleles	PIC content
BOUG-1	160	210	6	2	0.8910
BOUG-2	150	250	8	3	0.8861
BOUG-3	140	200	6	6	0.8122
BOUG-4	90	140	4	6	0.6298
BOUG-5	100	130	4	1	0.3640

total number of alleles and PIC values for each marker are given in Table-3. The five markers showed high polymorphism and a total of 28 alleles were identified from the experimental set of bougainvillea cultivars. The number of alleles per locus ranged from 4 (BOUG-5) to 8 (BOUG-2) with an average of 5.6 alleles per locus. The smallest number of alleles identified was 4 each in the primer BOUG-4 and BOUG-5. The highest number of alleles in this category was 8 which were detected from the profile of the marker BOUG 2. The overall size of the amplified products ranged from 90bp to 250bp which represents alleles of molecular weight ranging from 59400 to 165000. The differences in SSR product size between the smallest and largest allele for a given SSR locus varied from Primer BOUG-4 (90bp in Thimma, Shubhra etc.) to Primer BOUG-2 (250bp in Blondie, Dr H B Singh). Maximum variation in allele size was observed in Primer BOUG-2 (100bp difference). So the SSR marker with greater size difference between the alleles would be relatively better for characterization and diversity analysis. This preliminary investigation reports greater average number of alleles due to inclusion of diverse species of bougainvillea cultivars in the study which was lacking in earlier studies.

The inherent genetic diversity of this set of bougainvillea cultivars is apparent from the analysis of the SSR profiles. Stutter bands, which were minor products amplified in PCR and had lower intensity than the main allele because they normally lacked or had extra repeat units; (Walsh *et al*. 1996) were also present in the profiles of most of the markers used.

The Polymorphism Index Content (PIC)

The PIC values denote allelic diversity and frequency among the cultivars under study. PIC value of the each marker was calculated on the basis of its allele's occurrence and it varied for all the SSR loci tested. The level of polymorphism among 50 bougainvillea cultivars was evaluated by calculating PIC values for each of SSR loci. The average PIC value was 0.716 per locus while the range of PIC values was 0.364 in Primer BOUG-5 to 0.891 in BOUG-1. High PIC value of BOUG-1 is reflecting its potential to better differentiate the cultivars at molecular level. From the PIC values it is evident that the allelic diversity is quite high among the bougainvillea cultivars under study.

Rare alleles

As per the definition of rare alleles, a total of 18 rare alleles were identified from 5 polymorphic markers with an average of 3.6 rare alleles per loci. The highest number of rare alleles (6 rare alleles) was identified in the profile of BOUG 3 and BOUG 4. Among the bougainvillea cultivars studies, Blondie had the maximum number of rare alleles (3). This was followed by Singapore Red and Chitra cultivars that possessed 2 rare alleles.

Cluster analysis using UPGMA method

The dendrogram grouped the 50 cultivars into five major clusters. Out of 5 clusters, the largest cluster was Cluster I which comprised 18 cultivars, whereas, Cluster IV (Vishaka, Dr H B Singh and Sensation) and Cluster-V (Blondie, R S Bhatt and Tomato Red) emerged as a smallest clusters with 3 cultivars in each case (Fig 1 and Table 4).

The cluster I which is further sub-divided into 2 sub-clusters, viz. Ia (15 cultivars) and Ib (5 cultivars). The first major cluster includes majority cultivars with leaf variegation which belong to *Bougainvillea glabra* and *B. spectabilis* and also few species of unknown origin. The cluster II possess 17 cultivars, which is again divided into 4 sub-clusters (IIa, IIb, IIc and IId) with almost 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. IId sub-cluster was again divided into two more mini clusters out of which one mini cluster

Table 4 Clustering based on Jacquard's similarity co-efficient of 50 bougainvilleas revealed through SSR analysis

Major cluster	No. of cultivars	Cultivars
I	18	Thimma, Radha, Parthasarthy, Gloriosus, Filoman, Poultoni Special, Sanderiana, Dream, Flame, Jawaharlal Nehru, Singapore Red, Meera, Lady Richards, Mahatma Gandhi, Zakiriana, Spring Festival, Sweet Heart, Shubhra
II	17	Mary Palmer Special, Cherry Blossom, Summer Time, Pink Beauty, Sonnet, Stanza, Partha, Torch Glow, Splendens, Rosea Fuchsia, Lady Hope, Red September, Dr R R Pal, Hawaiin White, Golden Glow, Mrs Bhatt, Chandrabieri
III	9	Lady Mary Baring, Dr. Bhabha, Chitra, Abraham Kavoor, Roseville's Delight, Los Banos Beauty, Mahara, Refulgens, Manohar Chandra
IV	3	Vishaka, Dr H B Singh, Sensation
V	3	Blondie, R S Bhatt, Tomato Red

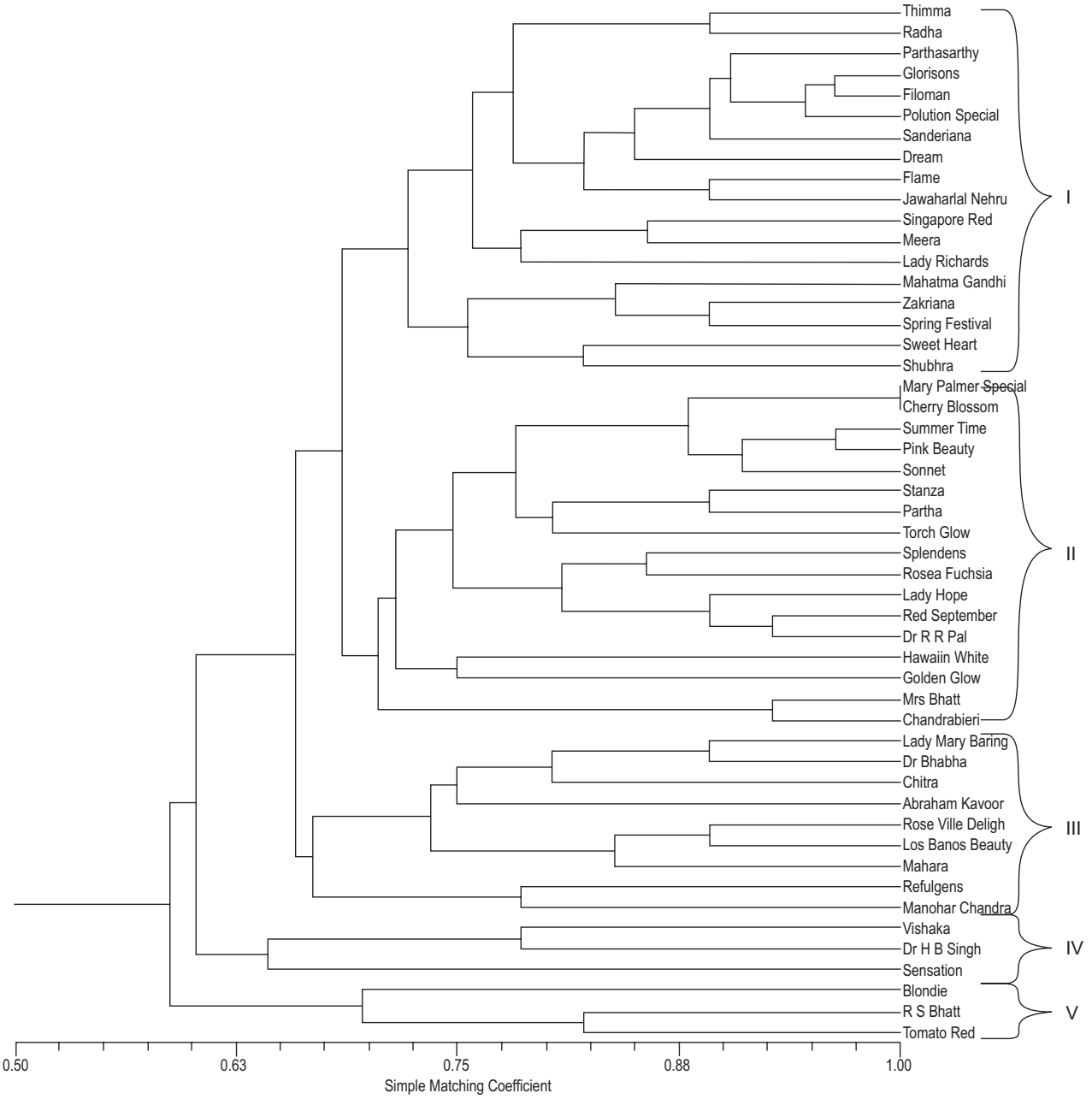

Fig 1 Genetic relatedness among the bougainvillea cultivars deciphered by SSR markers using NTSYS-pc program Version. 2.1

had 2 cultivars, viz. Cherry Blossom and Mary Palmer Special which were not distinguished. The third cluster included 9 cultivars, which is again divided into 2 sub-clusters contained most of the bud sports of *B.* × *buttiana* and also multibracted cultivars with exception of cultivar Cherry Blossom. The cluster IV and cluster V included only 3 cultivars each. Cluster IV is further divided into 2 sub-clusters, viz. IVa and IVb which includes one sub-cluster having 2 cultivars belongs to the *B. peruviana*. The fifth and small cluster included only 3 cultivars, which is again divided into 2 sub-clusters in which cultivars of unknown origin were noticed. It may be due to widespread history of introduction of cultivars in new locations.

As of today, the availability of SSR markers in bougainvillea sp. is limited. This may be cited as one of reasons for failure of earlier studies to differentiate all the cultivars belonging to different species of bougainvillea. But still the affinity and origin of many unknown cultivars can be interpreted to some extent in this study. Authentic identification and documentation of these cultivars is possible only if we include many more primers in the screening process. Cluster analysis also reveals that all the cultivars were not assembled in the same clusters, indicating a lot of variation within those cultivars.

Cluster analysis using principal component analysis

The PCA scatter plot which gives the spatial representations of genetic distances among cultivars, grouped the 50 cultivars under study into six clusters. The direct three principal components I, II and III which accounted for 13.87, 11.44 and 9.73% variation, respectively were used to compute PCA scatter plot. Generally the PCA scatter plot, groups the cultivars akin to the hierarchical clustering as illustrated in the dendrogram. The first cluster based on PCA comprises of all the cultivars of cluster-I generated using UPGMA method; except, Radha, Spring Festival and Sweet Heart. However, second PCA cluster consists of all the cultivars of UPGMA cluster II. With the exception of Lady Mary Baring and Dr Bhabha third PCA cluster holds all the cultivars of cluster-III. Similarly, PCA clusters IV and V excluded Sensation and Blondie cultivars found in the Cluster IV and V based on UPGMA. In addition, PCA grouped the cultivar Blondie separately thereby suggesting it to be the most divergent one from all other cultivar under investigation. Moreover, the two cultivars Mary Palmer Special and Cherry Blossom were placed at same point in PCA scatter plot which corresponds to the second cluster in UPGMA method. The possible reasons for this may be due to the common characters shared by the two cultivars or the requisition to include additional molecular markers for further discrimination (Table 5).

Table 5 Clustering based on principal component analysis of 50 bougainvillea cultivars

Major cluster	No. of cultivars	Cultivars
I	17	Thimma, Parthasarthy, Gloriosus, Filoman, Poultoni Special, Sanderiana, Dream, Flame, Jawaharlal Nehru, Singapore Red, Meera, Lady Richards, Mahatma Gandhi, Zakiriana, Shubhra, Lady Mary Baring, Dr Bhabha
II	21	Mary Palmer Special, Cherry Blossom, Summer Time, Pink Beauty, Sonnet, Stanza, Partha, Torch Glow, Splendens, Rosea Fuchsia, Lady Hope, Red September, Dr R R Pal, Hawaiin White, Golden Glow, Mrs. Bhatt, Chandrabieri, Radha, Spring Festival, Sweet Heart, Sensation
III	7	Chitra, Abraham Kavoor, Roseville's Delight, Los Banos Beauty, Mahara, Refulgens, Manohar Chandra
IV	2	Vishaka, Dr H B Singh
V	2	R S Bhatt, Tomato Red
VI	1	Blondie

Genetic relationship and distance

To elucidate the genetic relationships among different cultivars, genetic distances were calculated for each pair of cultivars using the UPGMA method. The pair wise estimates of genetic distance ranged from 0.0-1.0 with an average of 0.56 for all the fifty bougainvillea cultivars. The lowest genetic distance was observed between the cultivar pairs Cherry Blossom to Mary Palmer Special (0.0) followed by Summer Time to Pink Beauty (0.07), Filoman to Gloriosus (0.08), Dr R R Pal to Lady Hope (0.11), Mrs Butt to Chandrabieri (0.11), Sonnet to Summer Time (0.13) Gloriosus to Poultoni Special (0.14), Flame to Sanderiana (0.14), Los Banos Beauty to Roseville's Delight (0.16), Los Banos Beauty to Mahara (0.18), and Jawaharlal Nehru to Poultoni Special (0.20). All these are evidenced by their presence in same sub clusters. The lowest genetic distance values between the cultivars indicated that these cultivars were less distantly related to each other. In addition to this, these cultivars were morphologically similar in vegetative or flowering traits which supported a closer relationship among them.

The genetic distance between Summer Time and Pink Beauty was very less (0.07) as they are sharing most of the common morphological features, viz. hybrid seedlings, bracts with acute tips, flower tube and stars were prominent. Another pair of cultivars namely Filoman and Gloriosus expressed lesser genetic distance (0.08) where the cultivars origin and ancestry are unknown. However, based on our SSR marker analysis, we have found that both are closely related to each other and clustered together in the same group. This is further supported by morphological characters young shoot leaves are coppery in nature, bracts are medium ovate with cordate shape at the base.. It may be hypothesised that one of the putative parents of these seedlings was of the same origin. Dr R R Pal and Lady Hope also exhibited close linkage with each other (0.11). Even though origin of both the cultivars were different *viz*. Dr R R Pal belongs to *B* × *buttiana* and Lady Hope belongs to *B. peruviana* but

morphologically both varieties possess same bract colour (Red purple group-67A) and also pink strips in flower tube. It reveals that some cultivars of *B. peruviana* have affinity towards the hybrid group of *B. buttiana*. Mrs Butt and Chandrabieri share common morphological features, *viz*. glabrous leaves, drooping branches and profuse flowering habit which are related to *B.* × *buttiana* group. Both the cultivars were closely related and clustered in the same sub-cluster, as also documented by Banerji and Dwivedi, 2013 who postulated that cultivar Chandrabieri evolved by hybrid seedling of Mrs Butt.

Los Banos Beauty, Mahara and Roseville's Delight were grouped in the same subgroup with an average genetic distance of 0.16. Additionally these cultivars shared some of the common morphological characters like vigorous growth type, multibracted flowers, free flowering nature and absence of perianth tube.

Lady Mary Baring was closely related to the Abraham Kavoor (0.33) and was grouped in the same sub-cluster. It is interesting to note that these cultivars shared similar morphological traits like bracts are medium ovate, grey orange coloured (N167A) with greenish veins, flower tube slender tinged with orange colour. Available data indicates that cultivar Lady Mary Baring is a bud sport of *B* × *buttiana* var. Golden Glow but the origin of Abraham Kavoor is still unknown (Singh *et al*. 1999). The present study therefore proves the affinity of Abraham Kavoor towards *B.* × *buttiana* species. Parthasarathy, a bud sport of cultivar 'Partha' is closely related to Partha (only 0.38 genetic distance). These cultivars also proved to be similar in morphological parameters, viz., same bract colour (Fuchsia Purple) with only difference in the variegation pattern on the margins of young and matured leaves. Similar kinds of results have been also reported by Chattarjee *et al*. (2007) by using the RAPD markers.

Shubhra and Thimma are two bud sports of cultivar Mary Palmer but considerable amount of diversity (0.42) exists between them which is also clearly evident in the bract colour and leaf variegation. Shubhra has white coloured bracts while Thimma produces white to magenta colour bracts and yellowish white variegation around the midribs of leaves. Since the cultivars Dr R R Pal and Summer Time share same parentage (Singh *et al*. 1999), they possess higher similarity percent (genetic distance: 0.29). This finding is in accordance with the findings of Srivastava *et al*. (2009) who also recorded lowest genetic distance in bougainvillea using RAPD markers. Lady Mary Baring a bud sport of Golden Glow possesses most of the morphological traits present in its parent but at molecular level they do differ (0.56 genetic distance). Presence of substantial molecular diversity (36%) among Bougainvillea cultivars when screened with RAPD was reported (Chattarjee *et al*. 2007).

In the present investigation, Cherry Blossom and Mary Palmer Special cultivars could not be distinguished when molecular markers were used, while morphologically both the cultivars are quite different (type of bract, bract colour and leaf characters). Therefore, we need to add more number of markers for further molecular characterization of these cultivars in order to observe the polymorphism.

The highest genetic distance was observed between the cvs. Blondie to Shubhra, Partha, Lady Hope, Gloriosus, Red September, Zakiriana, Lady Richards and Spledens (1.0); Vishaka to Singapore Red, Parthasarathy, Thimma, Gloriosus, Zakiriana and Filoman (1.0); Tomato Red to Sweet Heart and Shubhra (1.0); Dr H B Singh to R S Bhatt (1.0). One of the reasons for this high level of genetic distance recorded could be due to inter-specific variation. The highest genetic distance value between the cultivars reveals that presence of strong genetic diversity between these cultivars and significant differences exist in the genotypic diversity among themselves.

The genetic distance between Blondie with other cultivars like Shubhra, Partha, Lady Hope, Gloriosus, Red September and Zakiriana was more (1.0). These results are in concordance with the available pedigree information (Singh *et al*. 1999), as cv Blondie belongs to *B.* × *buttiana* cultivars and cvs Shubhra, Partha and Lady Hope belong to *B. peruviana* group, however, the pedigree information of cultivars Gloriosus, Red September, Zakiriana were not known. Cultivar Blondie differs from Lady Richards and Splendens which also belong to the *B. glabra* group. Further it also exhibits considerable variation with B. *glabra* and *B.* × *buttiana* cultivars, indicating the possibility of using them in future breeding programmes. If we compare Blondie with Splendens, they are highly diverse with each other (1.0 genetic distance) which is also clearly evidenced with pedigree and morphological traits (Blondie belongs to *B.* × *buttiana*, possessing glabrous, elliptical, light green leaves with amaranthus coloured bracts that are elliptic in shape while Splendens belongs to *B. glabra* group with, pubescencet leaves and large ovate bracts that are Magenta rose in coloured).

Cultivar Vishaka shows greater genetic distance with Singapore Red, Parthasarathy, Thimma, Gloriosus, Zakiriana and Filoman, which is a bud sport of *B. peruviana* and all other cultivars origin is not well known. Tomato Red is highly divergent with the cultivars like Sweet Heart and Shubhra. Tomato Red belongs to *B. spectabilis* species and produces solferino coloured bracts while Sweet Heart has a Fuschina pink coloured bracts and it is a hybrid of *B.* × *buttiana*. Similarly, cultivar Shubhra produces pure white bracts and belongs to *B. peruviana* group. Use of SSR markers proved to be an excellent tool to bring to the fore lot of diversity among the *B. peruviana, B. spectabilis* and *B.* × *buttiana* species in bougainvillea. Cultivar Dr H B Singh is distantly related to the cultivar R S Bhatt ant their origin of both the cultivars is still unknown. Similarly they differ significantly in terms of their morphological traits. Dr H B Singh has glabrous dark green leaves with light violet-purple bracts compared to crimson red bracts of R S Bhatt. Cultivar Sweet Heart and Mahara also recorded considerable f diversity (1.0) which is evidenced by its presence in different clusters and also a distinct

morphological profile. Cultivar Sweet Heart, a hybrid seedling of *buttiana* produces medium green leaves, single type of bracts with fuchsia pink coloured bracts, perianth tubers are star shaped and are prominent. While, cv Mahara produces coppery leaves at young stage and change to light green, double to multi-bracted rhodamine purple coloured bracts and is devoid of perianth tube.

To our knowledge use of SSR markers for characterisation and cultivar identification is first of its kind in Bougainvillea. Earlier reports are based on RAPD markers. The investigation further proved the fact that the SSR markers are robust in detecting a high level of molecular polymorphism to characterize and grouping the bougainvillea cultivars besides establishing the genetic relationship and diversity. The SSR marker based dendrogram clearly grouped the cultivars into distinct groups and can serve as a reliable method for identification of varieties in addition to morphological characters. Present investigation with microsatellite marker analysis was found to be helpful for documentation, identification and also to trace out the molecular affinity of origin of unknown cultivars to a certain extent. This study will also be helpful for selection of specific traits in cultivars to improve bougainvillea in future breeding programmes. This study would also pave the way for exploring the use of SSR markers to distinguish closely related cultivars to settle IPR related issues.

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