

## Genetic variability of *Bougainvillea* varieties through molecular markers

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An investigation has been conducted on evaluation of genetic variability and relationships among 25 *bougainvillea* varieties by using two molecular markers i.e. DAMD and ISSR. There were 282 bands were amplified by using 5 DAMD primers and 10 ISSR primers wherein 253 bands (approximately 88.26%) were polymorphic and showed the significant level of genetic diversity. ISSR markers showed higher polymorphism (94.91%) compared to DAMD markers (81.61%). The PIC values ranged from 0.17 to 0.42 for DAMD and 0.10 to 0.19 for ISSR markers, exhibiting moderate to high genetic informativeness. Genetic dissimilarity coefficients varied from 0.37 to 0.78 for ISSR markers and 0.44 to 0.80 for DAMD markers. The maximum genetic distance (0.79) was recorded between the *bougainvillea* variety *Abhimanyu* and *Los Banos Variegata Silver Margin*, as well as between *Pallavi* and *Dr. P.V. Sane* whereas the lowest genetic distance (0.38) was observed between the *bougainvillea* varieties *Krumbiegel* and *Mary Palmer Special*. Cluster analysis based on UPGMA and Jaccard's similarity coefficient classified the genotypes into two primary clusters with several sub-clusters under each primary cluster. Moreover, the varieties *Abhimanyu* and *Los Banos Variegata Silver Margin* showed 100% bootstrap support and showing strong genetic similarity.

**Keywords:** Cluster analysis, Comparison, DAMD, ISSR

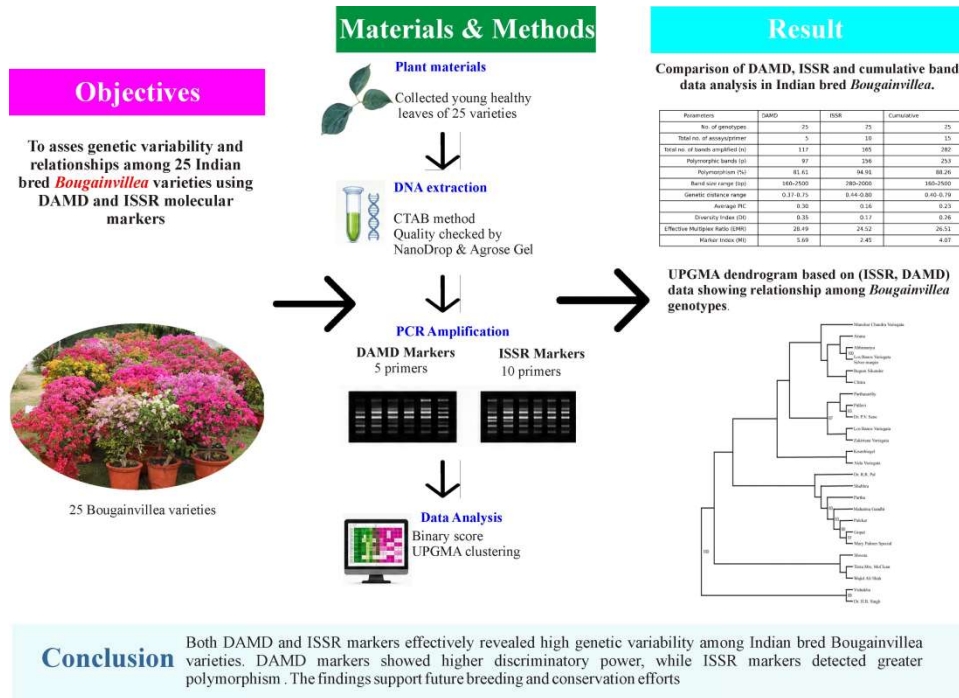
A vibrant and widely cultivated ornamental genus *Bougainvillea* belongs to the family Nyctaginaceae and renowned for its colourful bracts as well as adaptability to different climatic situations. *Bougainvillea* is originated from tropical and subtropical regions of South America<sup>1</sup>. It is found prominent in India as well as across the tropical regions of the world as a garden and landscape plant because of its wide range of coloured bracts viz. white, pink, mauve, purple, yellow etc. along with specific variegated foliage and multi-coloured bracts on a single plant. Due to its visual appeal and environmental resilience, it is also referred as "Glory of the Tropics"<sup>2</sup>.

According to the Kew Plant List, *Bougainvillea* genus consists of approximately 18 species but only four species have horticultural significance which are *Bougainvillea glabra*, *Bougainvillea* × *buttiana*, *Bougainvillea spectabilis* and *Bougainvillea peruviana*. However, over 500 cultivated varieties have been reported at world level<sup>3</sup>. *Bougainvillea* is a robust climber with thorny stem, simple leaves and white

flowers underlaid by coloured and persistent bracts arranged typically in three-flowered cymes. There are two main flowering periods observed in the northern regions of India, i.e. November-December and March-April.

Traditional varietal classification in *bougainvillea* has been done on the basis of morphological parameters wherein the overlapping vegetative and floral characters generally complicate the precise identification and differentiation of varieties, especially when dealing with large number of populations. Keeping in view the facts, there is a dire need of molecular approaches to study the molecular characterization of existing diversity for taxonomy and breeding programs. In this consequence, the DNA markers really offer a reliable, heritable means to analyze the genetic variation which is totally independent of environmental impacts. Among all the molecular approaches, Random Amplified Polymorphic DNA (RAPD) has been become more popular due to its simplicity and cost-effectiveness<sup>4</sup> whereas Inter Simple Sequence Repeat (ISSR) and Direct Amplification of Minisatellite DNA (DAMD) have found superior among the advanced markers based on reproducibility and resolution for assessing genetic diversity<sup>5-6</sup>.

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#### Graphical abstract

On the basis of above facts, an investigation has been conducted to characterize genetic variability of *Bougainvillea* varieties through DAMD and ISSR markers as well as to estimate the genetic relationships for future breeding efforts. Molecular characterization not only refines germplasm management but also contributes to the identification of parentage and the development of new ornamental cultivars suitable for changing climatic conditions and aesthetic demands due to urbanization.

#### Material and Methods

The present study was initiated at Botanic Garden and Biotechnology Laboratory, Floriculture and Landscaping Division, CSIR-National Botanical Research Institute (CSIR-NBRI), Lucknow. Twenty-five *Bougainvillea* cultivars were selected for genetic analysis which represents the four major indigenous species. The selected varieties were *Abhimanyu*, *Aida Variegata*, *Aruna*, *Begum Sikander*, *Chitra*, *Dr. H.B. Singh*, *Dr. P.V. Sane*, *Dr. R.R. Pal*, *Gopal*, *Krumbiegel*, *Los Banos Variegata*, *Los Banos Variegata Silver Margin*, *Mahatma Gandhi*, *Manohar Chandra Variegata*, *Mary Palmer Special*, *Palekar*, *Pallavi*, *Partha*, *Parthasarthy*, *Shubhra*, *Shweta*, *Tetra Mrs. Mc Clean*, *Vishakha*, *Wajid Ali Shah* and *Zakiriana Variegata*.

#### DNA Extraction

The collection of young and disease-free leaves was done for genomic DNA extraction. The DNA extraction was carried out by using a modified version of Cetyl Trimethyl Ammonium Bromide (CTAB) method. The assessment of DNA concentration and purity have been done assessed by using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA), while DNA quality and integrity have been verified by electrophoresis on 1.5% agarose gels stained with ethidium bromide. The results have been compared against a standard DNA ladder for accuracy.

#### DAMD and ISSR Analysis

A total of 120 primers (20 DAMD and 100 ISSR) have been procured from Sigma Aldrich (India), previously proven and effective in plant genomic studies. An initial screening has been done by using two randomly selected genotypes to identify primers that yielded clear, reproducible and polymorphic bands. On the basis of screening results, five DAMD and ten ISSR primers were selected for further analysis.

Polymerase chain reaction (PCR) amplifications were carried out in 20  $\mu$ L volumes, each containing 20 ng genomic DNA, 1.6  $\mu$ L of 10 mM dNTPs, 0.4  $\mu$ L of 25 mM  $MgCl_2$ , 2  $\mu$ L of 10X PCR buffer (with 15 mM  $MgCl_2$ ), 0.8  $\mu$ L of 20  $\mu$ M primer, and 1.2 U of Taq DNA polymerase (Merck Specialties Pvt. Ltd.,

India). Primer-specific thermal cycling conditions have been optimized. Amplification products were resolved using 1.5% agarose gel prepared in 0.5X TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.3). Electrophoresis has been carried out at a voltage of 5 V/cm. the staining of gels were done with ethidium bromide, visualized under UV illumination by using a gel documentation system (UV Tech, UK) and documented in the form of photographs.

#### Data Scoring and Genetic Analysis

The scoring of amplified fragments was done manually as binary data like presence of a band scored as '1' and absence as '0'. The position of each unique band was treated as a separate locus. Further, the resulting binary matrix was subjected to cluster analysis by using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) on the basis of Jaccard's similarity coefficient. Thereafter, a dendrogram was constructed depicting the genetic relationships by using the software *viz.* NTSYSpc and its reliability was confirmed through 1000 bootstrap replications.

#### Assessment of Genetic Diversity and Marker Efficiency

The genetic variability of the selected *Bougainvillea* varieties was evaluated by using various statistical parameters such as total number of bands, number of polymorphic bands, percentage of polymorphism as well as genetic distance. Moreover, molecular marker helps in estimation of Polymorphic Information Content (PIC), Resolving Power (Rp), Diversity Index (DI), Effective Multiplex Ratio (EMR) and Marker Index (MI) using standard protocols. PIC values were computed by this formula:

$$PIC=1-(p^2+q^2) \quad \text{PIC} = 1 - (p^2 + q^2) \quad PIC=1-(p^2+q^2)$$

Where,  $p$  represents the frequency of the band presence allele, and  $q$  denotes the frequency of the absence allele

#### Results

A detailed significant morphological diversity in both foliage and floral bract traits were presented in Table 1 which revealed that the shape of leaf blade varied primarily among medium ovate, elliptic, broadly ovate, circular and lanceolate forms. The predominant leaf shape across the selected genotypes was found medium ovate and observed in *Manohar Chandra Variegata*, *Aruna*, *Begum Sikander*, *Chitra*. However, unique forms of leaf blade such as circular were observed in *Chitra* and lanceolate in *Shweta*

which showed a clear difference. In case of leaf blade colour, the majority of *bougainvillea* genotypes exhibited shades within the Green group 137 and variants such as *Partha*, *Shubhra* and *Mahatma Gandhi* comes under Green group 143 and Yellow-green group 144, reflecting their variability in pigmentation. The data recorded on bract arrangement revealed that the single bracts was observed predominantly, though some of the genotypes such as *Los Banos Variegata silver margin*, *Pallavi*, and *Los Banos Variegata* have found with multiple bracts. This character indicates towards a potential ornamental advantage. The variability in bract shape was also recorded as medium ovate, broadly ovate, narrowly ovate and circular in which the most frequently recorded bract shape was medium ovate and observed consistently in the genotypes like *Dr. P.V. Sane*, *Partha*, *Vishakha* and *Gopal*. Moreover, the genotype *Tetra Mrs McClean* was observed with a distinct circular bract form. Under the observations recorded on bract colour, the most of the genotypes displayed the tones commonly from Red-purple colour group under group codes of 70 B, 72 B and 62 A while some of the genotypes observed with unique hues, such as *Shweta* showed bract colour in Yellow-white group 158-D and *Zakriana Variegata* featured in a Red group 48 B. Hence, data on bract colour highlighted the diversity in pigmentation and may be utilized for their ornamental appeal. Overall, the recorded data on morphological variability present in *Bougainvillea* genotypes, suggesting their rich potential for selection and breeding for improving the aesthetic value and wider adaptability.

#### DAMD Marker Analysis

Out of the 20 DAMD primers tested, five primers were selected for detailed analysis which exhibited clear and consistent amplification patterns. These five primers collectively amplified 117 loci, with fragment sizes varying with base pairs of 160 to 2500. The range for number of loci generated per primer varies between 17 (YST) to 28 (33.6) with an average of 23.4 bands per primer. The maximum number of amplified fragments was recorded with primer 33.6 (28 bands), followed by OGRB01 (26 bands) and URPIF (26 bands), whereas the minimum number of amplified fragments were recorded in case of primer YST (17 bands). There were 97 loci found to be polymorphic and the number of polymorphic loci per primer was ranged from 12 (YST) to 27 (33.6). The per cent polymorphism among the primers has been ranged from 57.69% to 100 % wherein

Table 1 — Morphological Appearance of flowers							
S. No.	Genotypes	Location	Leaf Blade		Type	Bracts	
			Shape	Main Colour		Shape	Main Colour
1.	Manohar Chandra Variegata	AHSI Kolkata	Medium Ovate	Green group 137 A	Single	Medium Ovate	Red Purple group
2.	Aruna	NBRI Lucknow	Medium Ovate	Green group 137 A	Single	Medium Ovate	Orange group 32 C
3.	Abhimanyu	NBRI Lucknow	Elliptic	Yellow green Group	Single	Narrow Ovate	Red-purple group 72 B
4.	Los Banos Variegata silver margin	NBRI Lucknow	Broadly Ovate	Green group 137 C	Multiple	Narrowly ovate	Red-purple group 68 B
5.	Begum sikander	NBRI Lucknow	Medium Ovate	Green Group 137 A	Single	Broadly Ovate	Red-purple group 65 B
6.	Chitra	NBRI Lucknow	Circular	Green Group 137 A	Single	Broadly Ovate	Red-purple group 70 B
7.	Parthasarthy	NBRI Lucknow	Elliptic	Green group 137 C	Single	Medium Ovate	Red-purple group 70 B
8.	Pallavi	NBRI Lucknow	Medium Ovate	Green group 138 B	Multiple	Narrowly ovate	Red-purple group 62 A
9.	Dr. P.V. Sane	NBRI Lucknow	Medium Ovate	Green group 137 C	Single	Medium ovate	Red-purple group 71 B
10.	Los Banos Variegata	NBRI Lucknow	Broadly Ovate	Green group 138 A	Multiple	Medium ovate	Red-purple group 70 C
11.	Zakriana Variegata	AHSI Kolkata	Medium Ovate	Green group 137 C	Single	Medium ovate	Red group 48 B Fan-1
12.	Krumbiegel	LBG Bangalore	Elliptic	Green Group 137 C	Single	Broadly ovate	Red-purple group 72 B
13.	Aida Variegata	NBRI Lucknow	Medium Ovate	Green group 137 A	Single	Medium ovate	Red-purple group 64 A
14.	Dr. R. R. Pal	IARI Delhi	Medium ovate	Green Group 137 B	Single	Medium Ovate	Red-purple group 61 B
15.	Shubhra	NBRI Lucknow	Medium ovate	Green group 143 A	Single	Medium Ovate	Yellow-white group 158-D Fan-4
16.	Partha	LBG Bangalore	Medium ovate	Yellow-green group 144 A Fan-3	Single	Medium Ovate	Red-purple group 72 B
17.	Mahatma Gandhi	BARC Mumbai	Medium ovate	Green group 143 A	Single	Medium Ovate	Red-purple group 72 B
18.	Palekar	BARC Mumbai	Elliptic	Green Group 137 A	Single	Medium Ovate	Red-purple group 64B
19.	Gopal	LBG Bangalore	Medium Ovate	Green group 137 B	Single	Medium Ovate	Red-purple group 71 B
20.	Mary Palmer Special	NBRI Lucknow	Broadly ovate	Green group 138 A	Single	Broadly ovate	Red-purple group 70 B
21.	Shweta	NBRI Lucknow	Lanceolate	Yellow-green group 144 A	Single	Narrowly Ovate	Green-yellow group 157-D
22.	Tetra Mrs McClean	NBRI Lucknow	Medium ovate	Green Group 137 A	Single	Circular	Red-purple group 62 A
23.	Wajid Ali Shah	NBRI Lucknow	Medium ovate	Green Group 137 C	Single	Medium Ovate	Red-purple group 70 B
24.	Vishakha	IARI Delhi	Elliptic	Yellow-green group 147 A	Single	Medium ovate	Red-purple group 60 B
25.	Dr. H. B. Singh	LBG Bangalore	Elliptic	Green Group 137 C	Single	Medium ovate	Purple group 75 A

Table 2 — Details of DAMD primers and the extent of polymorphism determined with these primers

Primer	Sequence 5'-3'	Amplified Loci	Polymorphic Loci	Percentage Polymorphism	Mean PIC	Rp value	Approx. band size range
YST	CCTTTCCCGAG	17	12	70.59	0.22	20.72	200-2000
FVIIex8	TGCACACACACAGG	20	19	95	0.42	23.04	300-1200
OGRB01	GGGCTGGAGGAGGGC	26	15	57.69	0.17	41.36	160-1500
URP 1F	TCCAAGGTCCGAGACAAC	26	24	100	0.36	33.76	200-2500
33.6	GGGCTGGAGG	28	27	96.43	0.32	40.64	210-2500
Total		117	97	83.94	0.30	31.9	160-2500

Table 3 — Details of ISSR primers and the extent of polymorphism determined with these primers

Primer	Sequence 5'-3'	Loci Amplified	Polymorphic Loci	Percentage Polymorphism	Mean PIC	Rp value	Approx. band size range
UBC 807	(GA) <sub>7</sub> GT	12	12	100	0.19	16.8	280-1600
UBC 808	(GA) <sub>7</sub> GC	16	15	93.75	0.18	18.8	320-2000
UBC 809	(GA) <sub>7</sub> GG	17	17	100	0.19	21.2	280-1700
UBC 810	(AG) <sub>7</sub> AT	14	14	100	0.14	8.4	300-2100
UBC 835	(AG) <sub>7</sub> GYC	21	20	95.23	0.15	21.2	290-2000
UBC 836	(GA) <sub>7</sub> GYA	17	13	76.47	0.10	21.52	280-1800
UBC 842	(AG) <sub>8</sub> AYG	24	23	95.83	0.17	22.96	200-2000
UBC 880	(CTC) <sub>6</sub>	16	15	93.75	0.11	13.92	350-2000
UBC 883	(TA) <sub>7</sub> BVB	17	16	94.11	0.16	22.4	300-1300
UBC 886	(CT) <sub>7</sub> DV	11	11	100	0.19	11.36	300-1200
		165	156	94.91	0.16	17.86	280-2100

the maximum polymorphism was observed in URP1F and minimum in OGRB01. A keen observation of the Table 2 revealed that the DAMD markers produced 83.44% polymorphism across all the selected varieties. In case of the polymorphic information content (PIC) for the DAMD primers, the average value was recorded at 0.30 and yielded the lowest PIC value with OGRB01 primer. This level of polymorphism highlights the effectiveness of DAMD markers in revealing genetic variability present among all the selected *Bougainvillea* accessions.

#### ISSR Marker Analysis

In case of ISSR markers, 100 ISSR primers were screened wherein 10 primers were selected as per production of well-defined and reproducible bands, for further analysis. These primers collectively amplified 165 loci ranging from 280 bp to 2100 bp. The number of amplified bands per primer varied between 11 (UBC 886) and 24 (UBC 842), with an average of 16.5 bands per primer. Among these, 156 loci were polymorphic, translating to a high polymorphism rate of 94.91%.

It seems that some of the primer names were miss assigned from the DAMD list whereas the primers UBC 842 generated the most bands and UBC 886 produced the fewest in case of ISSR markers. The

values observed on polymorphism percentage showed that it ranged from 57.69% to 100%, similar to the trend observed in DAMD markers, too. The mean PIC value for the ISSR primers was recorded lower with UBC880 primer i.e. 0.11 (Table 3). Although the ISSR markers revealed higher polymorphism, yet the individual discriminatory power of each primer was less than that of DAMD markers.

#### Genetic Diversity and Clustering Analysis

The data on combined analysis of the DAMD and ISSR marker have been presented in (Table 4). There were 253 polymorphic loci detected. Between the two marker systems, ISSR markers have showed the greater polymorphism (94.91%) as compared to DAMD markers (81.61%). However, the cumulative polymorphism was found to be 88.26%, underlining the high level of genetic diversity among the selected *Bougainvillea* genotypes.

The genetic dissimilarity coefficients derived from binary data and ranged from 0.37 to 0.75 in case of ISSR markers whereas it was found between the ranges of 0.44-0.80 for DAMD markers. On the basis of UPGMA and Jaccard's similarity coefficients, the cluster analysis have been done and revealed congruent patterns in both marker systems. Subsequently, the dendrogram have been constructed

Table 4 — Comparison of DAMD, ISSR and cumulative band data analysis in Indian bred *Bougainvillea*

Markers	DAMD	ISSR	*Cumulative
No. of genotypes	25	25	25
Total no of assays /primer	5	10	15
Total no. of bands amplified (n)	117	165	282
Polymorphic bands (p)	97	156	253
Polymorphism (%)	81.61	94.91	88.26
Band size range (bp)	160-2500	280-2000	160-2500
Genetic distance range	0.37-0.75	0.44-0.80	0.40-0.79
Average PIC	0.30	0.16	0.23
Diversity Index (DI)	0.35	0.17	0.26
Effective Multiplex ratio (EMR)	28.49	24.52	26.51
Marker Index (MI)	5.69	2.45	4.07

\*Cumulative: combined data of DAMD and ISSR

according to the combined data presented in (Table 4) which classified the 25 *Bougainvillea* varieties into two main clusters viz. Cluster I and Cluster II. Further, these clusters were divided into six sub-clusters.

There were six varieties found in Sub-cluster I(a) namely *Manohar Chandra Variegata*, *Mahatma Gandhi*, *Partha*, *Pallavi*, *Parthasarthy* and *Shubhra*, five varieties in Sub-cluster I(b) namely *Aruna*, *Aida Variegata*, *Abhimanyu*, *Begum Sikander* and *Chitra*, two variety in Sub-cluster I(c) namely *Wajid Ali Shah* and *Dr. P.V. Sane*, while only one variety in Sub cluster I(d) namely *Tetra Mrs McClean*. In addition to this, there were four genotypes grouped under Cluster II(a) viz. *Los Banos Variegata*, *Los Banos Variegata Silver Margin*, *Mary Palmer Special* and *Palekar* and Cluster II(b) included five genotypes namely *Krumbiegel*, *Dr. R.R. Pal*, *Gopal*, *Dr. H.B. Singh* and *Zakiriana* (Fig. 1).

#### Comparison of Marker Systems

A comparative evaluation for the two marker systems have been carried out for the assessment of their effectiveness during characterization of the genetic diversity. During the study of all the genetic parameters, the DAMD markers depicted the superior values over ISSR markers. The average PIC value was recorded higher in DAMD markers (0.30) as compared to PIC value found in ISSR markers (0.16). Similarly, DAMD markers have been exhibited higher Diversity Index (0.35) as compared to ISSR markers (0.17). Moreover, the Effective Multiplex Ratio (EMR), which incorporates multiple factors such as

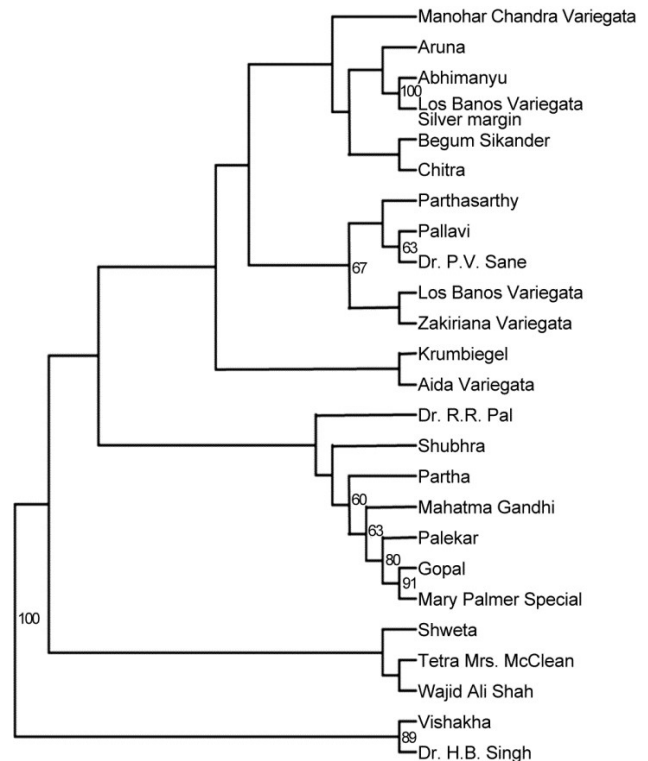


Fig. 1 — UPGMA dendrogram based on (ISSR, DAMD) data showing relationship among *Bougainvillea* genotypes

polymorphism and primer efficiency, was also found higher for DAMD (28.49) as compared to ISSR (24.52). Correspondingly, the Marker Index (MI), representing the overall utility of the marker system, was also recorded the value of 5.69 for DAMD markers and 2.45 for ISSR markers (Table 4).

#### Discussion

The effect of medium ovate leaves in most *Bougainvillea* genotypes reflects the typical vegetative growth pattern. Similarly, the occurrence of diverse leaf forms *i.e.* elliptic, lanceolate and circular form of leaf indicates existence of substantial morphological variability among the accessions. Assessment of genetic variability is important for breeding because plant ideotype contributes not only to ornamental appearance but also for plant adaptation under varying environment<sup>7</sup>. The presence of contrasting leaf traits among genotypes provides genetic resources for future improvement programmes for developing aesthetically superior and climate-resilient cultivars<sup>8</sup>.

Most of the genotypes exhibited a single bract arrangement, which represents the normal floral architecture of *Bougainvillea*. However, certain

accessions, including *Los Banos Variegata Silver Margin* and *Pallavi* produced multiple bracts, showed its rich floral appearance. It may be important traits for ornamental breeding programmes for enhancing visual attractiveness and market value. Considerable variation was recorded in bract shape, from narrowly ovate to nearly circular forms. Since bracts are the primary ornamental component in *Bougainvillea*, such diversity greatly influences cultivar preference and landscape utility. Variation in bract colour emphasized wide phenotypic diversity within the germplasm<sup>9,10</sup>. Most of the genotypes belong to the red-purple colour group, *i.e.* generally preferred in ornamental horticulture due to bright and attractive appearance. Nevertheless, unique colour expressions in *Shweta* genotype which exhibited yellow-white bracts and *Zakriana Variegata*, characterized by red bracts. These distinct colour forms broaden the ornamental spectrum for commercial cultivation and landscaping. The occurrence of diverse morphological and colour traits demonstrates the rich genetic base maintained within Indian *Bougainvillea* collections and highlights their importance for future breeding and conservation.

*Bougainvillea* has attained immense popularity as an ornamental plant due to its profuse flowering habit, adaptability to diverse agro-climatic conditions and ease of propagation. In India, the crop occupies a prominent position in landscape horticulture because of wide range of plant forms, foliage patterns, bract colours and flower character. The extensive phenotypic variation observed within the genus provides significant opportunities for the development of novel cultivars with improved ornamental attributes<sup>9</sup>.

Molecular marker like ISSR (Inter-Simple Sequence Repeat) and DAMD (Directed Amplification of Minisatellite DNA) helps in evaluation of genetic diversity in ornamental crops. These PCR-based marker are reliable because of their reproducibility and its ability to detect high levels of polymorphism<sup>10</sup>. In this study, both markers proved effective in assessing the genetic diversity among Indian *Bougainvillea* genotypes. The polymorphism (88.26%) percentage indicated the presence of substantial genetic variability within the material, confirming its broad genetic base.

ISSR markers generated a comparatively higher percentage of polymorphism than DAMD markers, suggesting that ISSR primers amplified variable genomic regions which indicates that efficiency of ISSR markers in detecting genetic variation among

*Bougainvillea* accessions<sup>11-12</sup>. Conversely, DAMD markers showed comparatively higher values for polymorphic information content and resolving power, reflecting their stronger ability to discriminate among closely related genotypes. Although ISSR markers produced a larger number of polymorphic bands, DAMD markers appeared more informative for varietal differentiation and genetic characterization<sup>13</sup>. The contrasting performance demonstrates that these marker target different genomic regions and complement each other in diversity analysis<sup>14</sup>.

Cluster analysis based on UPGMA dendrograms categories the genotypes into two clusters with strong clustering pattern indicates their reliability of genetic relationships. Genotypes like *Dr. R.R. Pal* and *Gopal*, consistently clustered together, suggesting close genetic affinity, whereas genotypes like *Abhimanyu*, *Los Banos Variegata*, *Silver Margin* exhibited the maximum genetic divergence. Such genetically distant accessions may serve as promising parental lines in hybridization programmes for creating broad variability and developing superior ornamental types<sup>15-18</sup>. The cluster pattern did not show a clear association with morphological characters or pedigree information, indicates that phenotypic similarity may not always correspond to molecular diversity.

The results clearly demonstrate the importance of both DAMD and ISSR markers for characterization of *Bougainvillea* germplasm. The combined application of these markers provides a reliable approach for assessing genetic diversity for identifying unique genotypes and promotes conservation strategies<sup>19-25</sup>. The data generated through molecular characterization can assist breeders in selecting diverse parents for hybridization programmes for developing improved *Bougainvillea* cultivars with desirable ornamental traits<sup>26-28</sup>. Overall, the study highlights the importance of integrating morphological evaluation with molecular techniques for germplasm conservation and genetic improvement of *Bougainvillea* genetic resources.

### Conclusion

Both DAMD and ISSR markers effective in analyzing genetic diversity among Indian *Bougainvillea* varieties. DAMD markers showed higher discriminatory power, while ISSR markers detected greater polymorphism. The molecular analysis provides information about genetic relationships and breeding programme for developing superior ornamental *Bougainvillea* cultivars according to diverse agro-climatic conditions.

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### Conflict of interest

All authors declare no conflict of interest.

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