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Morphological diversity and genetic structure of cultivated *Bougainvillea* germplasm collected from Vietnam, Laos, and Thailand

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Abstract. *Truong HTH, Nguyen NV, Ho NTH, Nguyen HHT, Ho HN, Huynh CV. 2026. Morphological diversity and genetic structure of cultivated* Bougainvillea *germplasm collected from Vietnam, Laos, and Thailand. Biodiversitas 27 (1): d270141. https://doi.org/10.13057/biodiv/d270141. Bougainvillea* is a major ornamental genus widely cultivated across tropical regions, yet germplasm from Southeast Asia remains poorly characterized. This study aimed to assess the genetic diversity and population structure of 33 *Bougainvillea* accessions from Vietnam, Laos, and Thailand using an integrated analysis of nuclear ribosomal ITS sequences, RAPD markers, and DUS-based morphological traits. ITS phylogeny clearly delimited two species, *B. glabra* and *B. spectabilis*, and revealed moderate sequence variation (π = 0.03299; Hd = 0.68; six haplotypes). RAPD profiling showed a high proportion of polymorphic bands (91.54%) and moderate genetic diversity, resolving the accessions into six genetic clusters, including a distinct singleton accession (T7). Morphological characterization based on 21 qualitative traits revealed substantial phenotypic variation, particularly in bract-related traits, and separated accessions into two major morphological groups, with consistent outliers (V1, V18, and V24). Overall, the combined morphological and molecular evidence demonstrated concordant patterns of species delimitation and cultivar-level differentiation, indicating that ITS is effective for resolving species boundaries. In contrast, RAPD provides higher resolution among cultivated materials. These results provide a practical basis for germplasm authentication, redundancy management, and the identification of genetically unique accessions, such as T7, V1, V18, and V24. This process will assist in conservation efforts and parent selection, ultimately supporting the breeding and sustainable use of *Bougainvillea* in Southeast Asia.

Keywords: *Bougainvillea*, DUS descriptors, genetic diversity, germplasm conservation, ITS

INTRODUCTION

Bougainvillea (*Bougainvillea* Comm. ex Juss.) is one of the most widely cultivated ornamental shrubs in tropical and subtropical regions, prized for its vivid bracts, extended blooming period, and high adaptability. In Southeast Asia, particularly in Vietnam, Laos, and Thailand, *Bougainvillea* is extensively used in urban landscaping, home gardens, and roadside greening, playing an important role in local horticultural industries (Kobayashi et al. 2007; Singh et al. 2016; Kumar et al. 2020). The popularity of this genus is further supported by its drought tolerance, ease of vegetative propagation, and the large number of cultivars generated through natural hybridization and long-term horticultural selection (Kobayashi et al. 2007; Kumar et al. 2015; Singh et al. 2016). However, this domestication history has also resulted in pronounced morphological variation and persistent taxonomic uncertainty, particularly among closely related species and cultivars (Chatterjee et al. 2007; Hammad 2009; Srivastava et al. 2009). The economic significance of *Bougainvillea* makes accurate germplasm characterization essential for its sustainable conservation and breeding (Kobayashi et al. 2007; Kumar et al. 2020). Challenges such as mislabeling, synonymy, and duplication among cultivars complicate germplasm management. Additionally, the loss of rare genotypes limited opportunities to enhance ornamental traits, including bract color, shape, and floral longevity (Nybom and Bartish 2000; Roy et al. 2013).

Morphological characterization is a fundamental method for varietal identification and classification. However, phenotypic traits are often influenced by environmental conditions, leading to potentially inaccurate assessments of genetic relationships. Consequently, molecular markers have been widely adopted to complement morphological assessment and to provide more stable estimates of genetic relationships. Early studies employing Random Amplified Polymorphic DNA (RAPD) markers demonstrated substantial intra- and interspecific variation among *Bougainvillea* cultivars (Chatterjee et al. 2007; Srivastava et al. 2009). Subsequently, co-dominant and more reproducible marker systems, such as Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR), and Directed Amplification of Minisatellite-region DNA (DAMD), have been used to further resolve genetic diversity and population structure (Kumar et al. 2014, 2020; Rastogi et al. 2019). In parallel, plastid DNA barcodes (e.g., *trnH-psbA*, *trnL-trnF*, *matK*) and nuclear ribosomal regions have been applied to clarify species-level relationships within the genus (Lin et al. 2023). Nevertheless, these studies were mostly restricted to germplasm from India or China and did not integrate both molecular data with standardized

morphological descriptors. Therefore, the genetic structure and morphological diversity of *Bougainvillea* germplasm from Southeast Asia remained poorly characterized. This lack of regional data limits our understanding of genetic variation within cultivated materials and its connection to observable phenotypic diversity, thereby constraining the development of region-specific conservation and breeding strategies.

Integrative approaches that combine molecular and morphological datasets offer a powerful framework for addressing these limitations. Nuclear ribosomal ITS sequences are particularly suitable for resolving interspecific boundaries and assessing species-level relationships because of their concerted evolution and relatively slow rate of change (Baldwin et al. 1995; Alvarez and Wendel 2003). In contrast, RAPD markers analyze multiple anonymous regions across the genome, making them useful for detecting polymorphisms at the intra-species and cultivar levels (Williams et al. 1990; Nybom and Bartish 2000). When these molecular tools are interpreted alongside DUS-based morphological descriptors, which are still the standard for varietal description and protection (Roy et al. 2013), they facilitate comprehensive germplasm identification, diversity assessment, and redundancy management.

Therefore, this study aimed to assess the morphological diversity and genetic structure of *Bougainvillea* germplasm collected from Vietnam, Laos, and Thailand using an integrated ITS-RAPD-morphological approach. The specific objectives were to: (i) quantify ITS diversity and delimit species; (ii) evaluate RAPD-based genetic diversity and clustering; (iii) characterize DUS morphological diversity and compare its concordance with molecular data. This study provides the first integrated molecular and morphological assessment of *Bougainvillea* germplasm from Southeast Asia, establishing a foundation for improved taxonomic resolution, germplasm conservation, and future ornamental breeding efforts.

MATERIALS AND METHODS

Plant materials

A total of 33 *Bougainvillea* accessions representing cultivated ornamental germplasm were collected from Vietnam (17 accessions), Thailand (12) and Laos (4 accessions) between December 2023 to January 2025 (Table 1). All materials were obtained from commercial flower shops and nurseries rather than from wild populations. For each accession, a single plant was established and treated as a representative genotype for morphological and molecular analyses. It is acknowledged that reliance on commercial sources may introduce a degree of preselection and potential mislabeling; however, the use of molecular markers in this study provides an independent means to partially verify genetic identity and relationships among accessions. ITS sequences generated in this study have been deposited in GenBank under submission number SUB15913123 and are available under accession numbers PX795088-PX795120.

Soil preparation and plant cultivation

The soil used for *Bougainvillea* cultivation was collected from Hue city, Vietnam (16°32′06.2″N, 107°31′39.7″E) and analyzed for its physicochemical properties as described by Truong et al. (2024). The soil was classified as loamy with approximately 31.5% limon, 22.1% clay, and 33.12% fine sand. It had moderate organic matter content (1.86%), and adequate macronutrient levels (total N = 0.09%, P_2O_5 = 3.0 mg/100 g, and K_2O = 15 mg/100 g). The original soil was slightly to moderately acidic (pH 3.78); therefore, it was amended with calcium carbonate ($CaCO_3$) to adjust acidity, resulting in an approximate working pH of 5.8-6.2, which is suitable for *Bougainvillea* growth.

For each accession, 30 kg of the same amended soil was mixed with 30 g of organic compost (Que Lam, Vietnam) and 30 g of calcium carbonate ($CaCO_3$). Plants were cultivated individually in pots (45 cm diameter × 60 cm height). All accessions were grown at a single experimental site under identical soil mixture, fertilization, and irrigation regimes, receiving full sunlight and daily watering to maintain moderate soil moisture. The average ambient temperature ranged from 22°C to 39°C, with relative humidity between 50% and 85%. All plants were grown for approximately six months until full vegetative maturity prior to morphological characterization.

Molecular analysis

Genomic DNA extraction

Genomic DNA was extracted from young leaves of a single plant of each accession using the CTAB (Cetyl Trimethyl Ammonium Bromide) method following the protocol of Rasphone et al. (2023). Approximately 50 mg of fresh leaf tissue was used per extraction. The DNA extraction process was repeated until an A260/A280 ratio of 1.8-2.0 was achieved, as confirmed by 1.0% agarose gel electrophoresis. The final DNA extracts were diluted to a working concentration of 20 ng/µL and stored at -20°C for PCR-based analyses.

ITS characterization

PCR amplification of the ITS region was performed using the primer pair ITS_{u1}-ITS_{u4} under the conditions described by Rasphone et al. (2022) in a PCR system (Applied Biosystems, USA). The PCR mixture contained 20 ng/µL DNA, 7.5 µL of 2X MyTaq Mix (Meridian Bioscience, US) and 0.5 µM of primer, making a total volume of 15 µL. The thermocycling program was set as follows: 95°C for 5 minutes; followed by 35 cycles of 95°C for 15 seconds, 56°C for 15 seconds, and 72°C for 30 seconds; concluding with a final extension at 72°C for 5 minutes. ITS-PCR products were sequenced using the Sanger sequencing method at 1st BASE (Apical Scientific Sdn. Bhd., Malaysia) and analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignment was conducted with MEGA 12 software to create a phylogenetic tree (Kumar et al. 2024). Phylogenetic relationships among the accessions were determined using the Neighbor-Joining (NJ) method, based on the Tamura 3-parameter model. Node support was assessed using 1,000 bootstrap replicates. In this study, we included sequences

from our research along with reference ITS sequences of *Bougainvillea glabra* Choisy and *B. spectabilis* Willd. retrieved from GenBank for comparison. No external outgroup from other genera was used, and the tree was midpoint-rooted. Furthermore, DNAsp v6 software was utilized to evaluate various parameters of the sequences, including the number of variable (polymorphic) sites, the number of mutations, nucleotide diversity, average number of nucleotide differences, minimum number of recombination events, number of Haplotypes, Haplotype diversity, minimum number of recombination events, Fu's Fs statistic, Tajima's D, Fu and Li's D* test statistic, and Fu and Li's F* test statistic (Fu and Li 1993; Rozas et al. 2017).

RAPD analysis

Random Amplified Polymorphic DNA (RAPD) analysis was performed to assess genetic relationships among 33 *Bougainvillea* accessions. A preliminary screening of 100 RAPD primers (Bioneer, Korea) (Supplementary Table 1) was first conducted using genomic DNA from three representative *Bougainvillea* accessions such as T8 (*B. glabra*), L2 (*B. glabra*), and V3 (*B. spectabilis*), to identify primers capable of generating clear and polymorphic amplification patterns. To confirm that polymorphism patterns, two additional accessions (T3 and V2) representing different geographic origins and species were included in the secondary validation step. This two-phase screening approach ensured that the selected primers exhibited reproducible and representative polymorphism across the germplasm. Based on clarity, reproducibility, and band resolution, 15 primers showing consistent amplification were preselected, of which nine highly informative primers were finally retained for genotyping all 33 *Bougainvillea* accessions. Primers producing weak amplification, excessive smearing, ambiguous band resolution, or low polymorphism/near-monomorphic patterns were excluded. This selection process ensured that the resulting RAPD dataset accurately represented the genome-wide diversity of the entire germplasm collection, capturing both interspecific and intraspecific variation within the *Bougainvillea* germplasm.

Table 1. Cultivated *Bougainvillea* accessions from Vietnam, Laos, and Thailand used in this study, showing species assignment, origin, and voucher information for integrated morphological and molecular analyses

Accession code	Accession name	Genbank accession	Species	Place of collection	Coordinates
HUIB_B1	T1	PX795088	*Bougainvillea spectabilis* Willd.	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B2	T2	PX795089	*Bougainvillea glabra* Choisy	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B3	T3	PX795090	*Bougainvillea glabra* Choisy	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B4	T4	PX795091	*Bougainvillea spectabilis* Willd.	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B5	T5	PX795092	*Bougainvillea glabra* Choisy	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B6	T6	PX795093	*Bougainvillea glabra* Choisy	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B7	T7	PX795094	*Bougainvillea spectabilis* Willd.	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B8	T8	PX795095	*Bougainvillea glabra* Choisy	Mukdahan, Thailand	16°33'27"N 104°43'06"E
HUIB_B9	T9	PX795096	*Bougainvillea glabra* Choisy	Mukdahan, Thailand	16°33'27"N 104°43'06"E
HUIB_B10	T10	PX795097	*Bougainvillea spectabilis* Willd.	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B11	T11	PX795098	*Bougainvillea glabra* Choisy	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B12	T12	PX795099	*Bougainvillea glabra* Choisy	Mukdahan, Thailand	16°33'13"N 104°43'06"E
HUIB_B13	L1	PX795100	*Bougainvillea glabra* Choisy	Vientiane, Laos	19°09'39"N 102°13'18"E
HUIB_B14	L2	PX795101	*Bougainvillea glabra* Choisy	Vientiane, Laos	19°09'39"N 102°13'18"E
HUIB_B15	L4	PX795102	*Bougainvillea glabra* Choisy	Vientiane, Laos	18°29'28"N 102°23'51"E
HUIB_B16	L5	PX795103	*Bougainvillea glabra* Choisy	Vientiane, Laos	18°29'28"N 102°23'51"E
HUIB_B17	V1	PX795104	*Bougainvillea spectabilis* Willd.	Ha Noi, Viietnam	20°24'36"N 106°35'17"E
HUIB_B18	V2	PX795105	*Bougainvillea glabra* Choisy	Hue, Vietnam	16°29'38"N 107°36'19"E
HUIB_B19	V3	PX795106	*Bougainvillea spectabilis* Willd.	Hung Yen, Vietnam	20°24'36"N 106°35'17"E
HUIB_B20	V4	PX795107	*Bougainvillea glabra* Choisy	Hung Yen, Vietnam	10°16'23"N 105°48'50"E
HUIB_B21	V5	PX795108	*Bougainvillea glabra* Choisy	Hue, Vietnam	16°29'38"N 107°36'19"E
HUIB_B22	V6	PX795109	*Bougainvillea spectabilis* Willd.	Hue, Vietnam	16°29'36"N 107°36'23"E
HUIB_B23	V7	PX795110	*Bougainvillea glabra* Choisy	Dong Thap, Vietnam	10°16'24"N 105°48'50"E
HUIB_B24	V10	PX795111	*Bougainvillea spectabilis* Willd.	Hue, Vietnam	16°31'34"N 107°26'26"E
HUIB_B25	V11	PX795112	*Bougainvillea spectabilis* Willd.	Hue, Vietnam	16°26'14"N 107°36'36"E
HUIB_B26	V13	PX795113	*Bougainvillea spectabilis* Willd.	Hue, Vietnam	16°26'01"N 107°35'51"E
HUIB_B27	V18	PX795114	*Bougainvillea spectabilis* Willd.	Hue, Vietnam	16°29'12"N 107°37'32"E
HUIB_B28	V19	PX795115	*Bougainvillea spectabilis* Willd.	Dong Thap, Vietnam	10°16'25"N 105°48'50"E
HUIB_B29	V20	PX795116	*Bougainvillea spectabilis* Willd.	Dong Thap, Vietnam	10°16'25"N 105°48'50"E
HUIB_B30	V21	PX795117	*Bougainvillea spectabilis* Willd.	Hung Yen, Vietnam	20°58'08"N 105°55'01"E
HUIB_B31	V22	PX795118	*Bougainvillea spectabilis* Willd.	Phu Tho, Vietnam	21°20'49"N 105°34'36"E
HUIB_B32	V23	PX795119	*Bougainvillea spectabilis* Willd.	Dong Thap, Vietnam	10°16'25"N 105°48'50"E
HUIB_B33	V24	PX795120	*Bougainvillea spectabilis* Willd.	Hung Yen, Vietnam	20°58'08"N 105°55'01"E

The PCR reactions were performed with a total volume of 15 µL, consisting of 20 ng/µL of DNA, 7.5 µL of 2X MyTaq Mix (Meridian Bioscience, US), 1.675 mM $MgCl_2$, 0.67 µM of RAPD primer, and 167.5 µM of each dNTP. Amplifications were conducted in a thermal cycler following the protocol described by Truong et al. (2013) with the following program: initial denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 37°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 7 min. The PCR products were separated using a 1.5% agarose gel and visualized under UV light, then documented with a gel documentation system. Fragment sizes were estimated using an appropriate 100 bp DNA ladder. RAPD bands were scored manually as binary data across accessions, where presence was recorded as 1 and absence as 0. A defined band-intensity threshold was applied, and band scoring was visually confirmed across all accessions to maintain consistency among gels. To assess the informativeness and efficiency of the selected primers, several parameters were calculated, including the total number of bands (TB), number of Polymorphic Bands (PB), Percentage of Polymorphic Bands (PPB), Polymorphism Information Content (PIC), Marker Index (MI), Resolving power (Rp), and Effective Multiplex Ratio (EMR). Genetic diversity parameters, including observed Number of alleles (Na), Effective number of alleles (Ne), Nei's gene diversity (h), and Shannon's Information Index (I), were computed using POPGENE version 1.32, assuming all loci to be dominant and in Hardy-Weinberg equilibrium. Pairwise genetic similarity coefficients were calculated using Jaccard's coefficient, and cluster analysis was performed using the UPGMA method.

Morphological identification

Morphological characterization of *Bougainvillea* accessions was conducted following Distinctness, Uniformity, and Stability (DUS) guidelines (Roy et al. 2013) with minor modification. A total of twenty-one qualitative morphological traits related to vegetative growth, leaves, thorns, inflorescence, bracts, and flowers were recorded for each accession (trait codes and categories are provided in Table 5). These included Growth Habit (GH), Young Shoot Color (YSC), Thorn Curvature (TC), Thorn Strength (TS), Leaf Base Shape (LBS), Leaf Apex Shape (LAS), Leaf Main Color (LMC), Leaf Secondary Color (LSC), the Distribution of Secondary Color on Leaves (DSCL), Presence of Flowers in the Inflorescence (PFI), Petal Color (PC), Arrangement of Bract Clusters (ABC), Shape of Bract (SB), Type of Bract (TB), Density of Bract Cluster (DBC), Bract Apex Shape (BAS), Bract Base Shape (BBS), Bract Main Color (BMC), Bract Secondary Color (BSC). These traits were evaluated at the full foliage stage.

For each accession, one representative plant was evaluated. To minimize within-plant variation, measurements were based on 10 fully expanded leaves, five thorn pairs, and three representative bract clusters per plant, and modal states were used for scoring qualitative traits. All observations were made during a single growing season (from June to September 2025) under uniform cultivation conditions to minimize environmental variation. All traits were treated as categorical multistate variables and encoded numerically according to the scoring scheme in Table 5. A Euclidean similarity matrix, which is appropriate for qualitative and mixed categorical data, was calculated to estimate pairwise morphological distances among accessions. Cluster analysis was performed using the UPGMA method, and multivariate relationships were explored using Principal Coordinates Analysis (PCoA) and Principal Component Analysis (PCA) based on the same similarity matrix. All analyses were conducted using PAST version 4.03 (Hammer et al. 2001).

RESULTS AND DISCUSSION

ITS characterization

Sequencing of the nuclear ribosomal ITS region across 33 *Bougainvillea* accessions generated an aligned matrix containing 45 polymorphic sites (S = 45) (Table 2). Overall nucleotide diversity was moderate (π = 0.03299), with an average number of nucleotide differences of k = 22.30303. A total of six haplotypes (h = 6) were detected, and haplotype diversity (Hd) was 0.68, indicating the presence of multiple ITS sequence variants within the studied germplasm. This aligns with the established role of ITS as an effective marker for delineating species boundaries and hybrid complexes in angiosperms (Baldwin et al. 1995; Alvarez and Wendel 2003).

Neutrality tests indicated a significant deviation from the mutation-drift equilibrium in the ITS dataset. This was evidenced by strongly positive Fu's Fs (20.233; P<0.001), along with positive values for Tajima's D (3.69113; P<0.001), Fu and Li's D* (1.83551; P<0.02), and Fu and Li's F* (2.90597, P<0.02). Additionally, Fu's Fs (20.23, P<0.001) supports the idea that structured diversity is preserved through hybridization and limited recombination. Neutrality tests yielded a strongly positive Tajima's D value of 3.69113 (P<0.001). This unusually high value suggests substantial population subdivision and the presence of divergent lineages (Tajima 1989; Fu 1997; Meirmans and Van Tienderen 2004). As a result, deviations from neutrality are best understood as indicators of interspecific divergence and clonal structure (Alvarez and Wendel 2003; Feliner and Rosselló 2007).

The observation of a minimum recombination event (Rm = 1) was estimated across all 33 accessions, indicating a limited but detectable level of reticulate variation within the ITS dataset. This finding likely reflects historical hybridization between *B. glabra* and *B. spectabilis*. Such hybridization is well documented in *Bougainvillea* breeding and often produces mosaic ITS sequences through incomplete concerted evolution (Alvarez and Wendel 2003; Kobayashi et al. 2007; Lin et al. 2023). This finding is consistent with previous evidence of frequent interspecific hybridization between *B. glabra*, *B. spectabilis* (Lin et al. 2023).

Overall, ITS sequence variation in *Bougainvillea* germplasm indicated a moderately structured nuclear gene pool, primarily influenced by hybridization and population subdivision. This variation provides a strong phylogenetic

understanding of the interspecific relationships within this complex ornamental genus (Baldwin et al. 1995; Alvarez and Wendel 2003; Feliner and Rosselló 2007; Lin et al. 2023).

The phylogenetic tree based on the ITS region (Figure 1) clearly categorized the 33 *Bougainvillea* accessions into two main clades, corresponding to *B. glabra* and *B. spectabilis*. The *B. glabra* lineage showed high bootstrap support (≥99%), while *B. spectabilis* had moderate support (57-66%), with both lineages supported by high to moderate bootstrap values. All accessions grouped with either *B. glabra* or *B. spectabilis* references in the ITS tree, and no clear ITS intermediates were detected; however, given the frequent hybridization in *Bougainvillea*, more loci (e.g. plastid + low-copy nuclear markers) would be needed to fully resolve hybrid origin.

The *B. spectabilis* clade consisted of the majority of accessions, including T1, T4, T7, T10, V1, V3, V6, V10, V11, V13, and V18-V24, along with two reference sequences from GenBank corresponding to verified species (KJ161169 and KJ161670). Internal bootstrap support within this clade was moderate (57-66%) and accompanied by short branch lengths, indicating limited nucleotide divergence within the ITS region. The finding is consistent with the general properties of ITS in ornamental plants with recent diversification and extensive clonal propagation (Baldwin et al. 1995). RAPD-based analyses demonstrated high phenotypic diversity but weak genetic separation among *B. spectabilis* accessions (Chatterjee et al. 2007; Srivastava et al. 2009). In contrast, the *B. glabra* clade, which included accessions V2, V4, V5, V7, L1, L2, L4, L5, T2, T3, T5, T6, T8, T9, T11, and T12, demonstrated strong bootstrap support (99-100%) and short internal branches. These accessions clustered tightly with the reference sequence from GenBank EF079463.1, indicating high phylogenetic cohesion and low intraspecific divergence. The results are consistent with previous molecular studies of *Bougainvillea*, which consistently identified *B. glabra* as a genetically stable lineage, while *B. spectabilis* exhibited a broader clustering and weaker internal resolution (Chatterjee et al. 2007; Srivastava et al. 2009). Thus, ITS marker is effective for species-level discrimination in *Bougainvillea* but requires integration with morphological and more polymorphic molecular markers for cultivar-level and breeding-oriented analyses.

Table 2. Nucleotide polymorphism and haplotype diversity of ITS sequences in *Bougainvillea* accessions that support species-level differentiation

Indices	Population
No. of variable (polymorphic) sites, S	45
No. of mutations, Eta	45
Nucleotide diversity (per site), π ($\times 10^{-3}$)	32.99
Average number of nucleotide differences, k	22.30303
Number of haplotypes, h	6
Haplotype diversity, Hd	0.68
Minimum number of recombination events, Rm	1
Fu's Fs statistic	20.233
Tajima's D	3.69113***, P<0.001
Fu and Li's D* test statistic	1.83551**, P<0.02
Fu and Li's F* test statistic	2.90597**, P<0.02

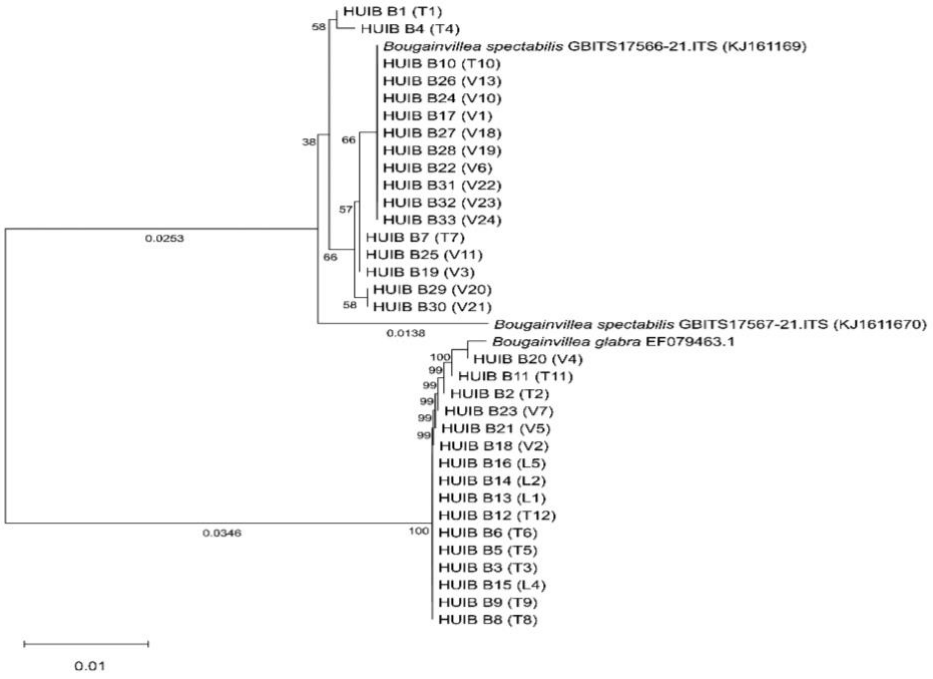

Figure 1. Neighbor-Joining phylogenetic tree based on ITS sequences showing separation of 33 *Bougainvillea* accessions into two major clades corresponding to *Bougainvillea glabra* and *Bougainvillea spectabilis*. All accessions grouped with their respective species reference sequences from GenBank, indicating clear species-level delimitation, while short internal branches reflect limited ITS divergence among cultivars. Bootstrap values (>50%) are shown at nodes. Scale bar indicates nucleotide substitutions per site

RAPD analysis

The nine validated RAPD primers (Table 3) identified during preliminary screening were used to genotype 33 *Bougainvillea* accessions collected from Vietnam, Laos, and Thailand. This comprehensive genotyping ensured that the resulting dataset reflected genome-wide polymorphism representative of the entire germplasm collection. The selected primers produced clear, reproducible, and highly polymorphic amplification profiles, confirming their suitability for assessing genetic diversity at both interspecific and cultivar levels (Figure 2). Across all accessions, the nine primers generated a total of 74 scorable DNA fragments, of which 91.54% were polymorphic (Table 3). The high percentage of polymorphic bands, together with moderate mean polymorphism information content (PIC = 0.291) and high resolving power (Rp = 3.596), demonstrated that the chosen primers were informative and capable of differentiating closely related genotypes. The strong reproducibility and informativeness of primers such as UBC#435 and UBC#500, validated the screening approach used, ensuring that primer polymorphism was representative across the germplasm. The overall results align with previous reports in *Bougainvillea* where RAPD markers have effectively detected genetic polymorphism among cultivars (Chatterjee et al. 2007; Srivastava et al. 2009; Kumar et al. 2020).

Cluster analysis using the UPGMA method based on Jaccard's similarity coefficients revealed clear genetic structuring among the 33 accessions (Figure 3). Similarity coefficients ranged from 0.51 to 1.00, indicating moderate overall genetic diversity within the collection. This range is typical of RAPD-based diversity assessments and highlights the ability of the marker system to distinguish both closely related and divergent genotypes (Williams et al. 1990; Powell et al. 1996), and is consistent with earlier studies on *Bougainvillea*, which frequently reported wide ranges of genetic similarity using RAPD markers (Chatterjee et al. 2007; Srivastava et al. 2009). The dendrogram delineated six major clusters, reflecting varying degrees of genetic relatedness among accessions. This clustering pattern corresponded well with species-level classification derived from ITS sequence analysis. Accessions belonging to *B. glabra* and *B. spectabilis* generally formed distinct but internally diverse clusters, consistent with previous findings that these species exhibit moderate intra-specific variation due to long-term clonal propagation and recurrent hybridization (Chatterjee et al. 2007; Hammad 2009). Cluster I included accessions T1, V10, V13, T4, T11, and V2, all of which displayed high genetic similarity (>0.88). This suggests that these genotypes may represent closely related clonal lineages that are maintained through vegetative propagation. Cluster II was the largest and most diverse group, contained accessions from Vietnam, Thailand, and Laos, with moderate similarity ranging from 0.76 to 0.88. The presence of distinct internal subclusters within this group indicates geographic differentiation, emerging from a largely shared genetic background. This pattern is commonly observed in ornamental crops that frequently exchange planting materials across regions (Gepts 1993; Nybom and Bartish 2000).

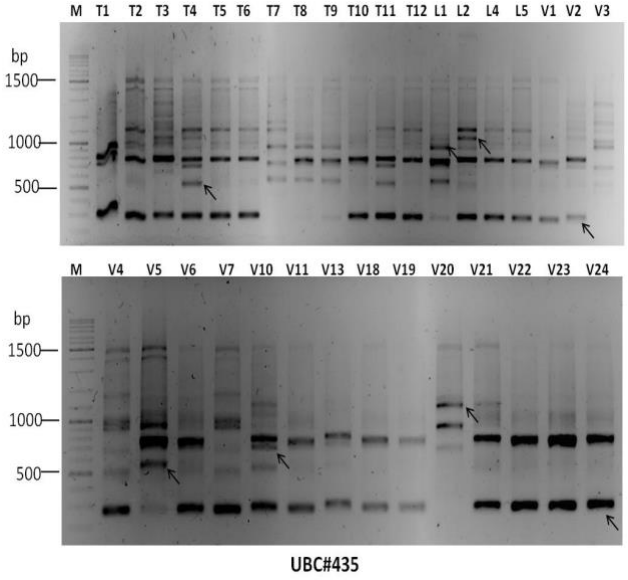

Figure 2. Representative RAPD amplification profiles of 33 *Bougainvillea* accessions generated using selected primers, illustrating reproducible and highly polymorphic banding patterns used for genetic diversity analysis. M: 100 bp DNA ladder; Arrows indicate polymorphic bands contributing to accession discrimination

Table 3. RAPD primers selected from initial screening and their amplification performance and informativeness were used for genetic diversity analysis of *Bougainvillea* accessions

Primer name	Size (bp)	TB	PB	PPB (%)	PIC	EMR	MI	Rp
UBC#412	350-2200	9	9	100	0.312	9.000	2.813	4.121
UBC#435	300-3000	11	11	100	0.355	11.000	3.905	6.121
UBC#446	300-1800	8	6	75	0.180	4.500	0.810	1.758
UBC#466	550-2250	8	8	100	0.274	8.000	2.193	2.849
UBC#475	350-2200	9	9	100	0.309	9.000	2.784	4.485
UBC#482	320-1500	5	3	60	0.247	1.800	0.446	1.939
UBC#493	400-1200	5	5	100	0.315	5.000	1.576	2.303
UBC#497	600-2500	9	8	88.89	0.280	7.100	1.991	3.576
UBC#500	600-2500	10	10	100	0.343	10.000	3.434	5.212
Mean	300-3000	8.22	7.67	91.54	0.291	7.267	2.217	3.596

Note: TB: Total number of bands, PB: Number of Polymorphic Bands, MB: Number of Monomorphic Bands, PPB: Percentage of Polymorphic Band, PIC: Polymorphic Information Content, EMR: Effective Multiplex Ratio, MI: Marker Index, Rp: Resolving power

Similar internal structuring has been documented in RAPD-based studies of *Bougainvillea*, where moderately diverse clusters were further divided into geographically or phenotypically coherent lineages (Chatterjee et al. 2007; Srivastava et al. 2009). The size and branching complexity of Cluster II suggest that multiple closely related lineages coexist, shaped by both gene flow and localized selection, rather than being a single clonal assemblage.

Cluster III included the accessions T8, T9, L1, and V5, which were grouped at a similarity level of approximately 0.85, forming a compact yet clearly distinct cluster. Similar clustering behavior has been reported in plants, in which cultivar groups or breeding lines form discrete clusters despite sharing the same species using RAPD markers (Nybom and Bartish 2000). Cluster IV included accessions V3 and V20, which exhibited a very high mutual similarity (>0.90) suggesting they were closely related to each other but genetically distinct from the rest of the germplasm. Similar paired clusters have been reported in RAPD and RAPD-isozyme studies of *Bougainvillea* (Hammad 2009). Cluster V included only accession T7, which was positioned on a long, independent branch and showed low similarity (<0.63) with all other accessions. This genetic distinctiveness may reflect (i) a unique evolutionary background, (ii) a different domestication or introduction route, or (iii) limited gene flow with other materials in the panel. From a breeding perspective, such an outlier represents a valuable genetic resource because it may harbor rare alleles not present in the major clusters. This finding aligns with previous research by Srivastava et al. (2009).

In previous studies, singleton clusters are widely recognized as valuable genetic resources due to their potential to broaden the genetic base in breeding programs (Gepts 1993; Nybom and Bartish 2000). Cluster VI comprised accessions V4 and V7, which showed nearly identical RAPD profiles, with similarity coefficients approaching 1.00. This is likely because both accessions were collected from the same province but different flower shops. This finding indicates that V4 and V7 may represent identical or extremely closely related genetic materials. This phenomenon has been repeatedly documented in *Bougainvillea*, where RAPD markers efficiently identify genetically redundant accessions later confirmed by other marker systems such as SSRs (Hammad 2009; Kumar et al. 2020). Thus, T7 appears genetically distinct in the RAPD dendrogram and therefore is a promising candidate for broadening the genetic base in breeding. Likewise, V4 and V7 show nearly identical RAPD profiles and may represent redundant material in the collection, which should be confirmed by detailed morphological and pedigree data.

The diversity indices derived from RAPD data (Na = 1.932, Ne = 1.492, h = 0.295, I = 0.450) indicated moderate genetic variability within the studied population (Table 4). Such moderate diversity is typical of clonally propagated ornamental crops, where somatic mutation, hybridization, and human-mediated selection maintain variation despite restricted sexual recombination. The high polymorphism rate and well-resolved clusters demonstrate the ability of RAPD markers to detect subtle genetic differences even among vegetatively propagated cultivars.

Thus, the RAPD results confirmed that the 33 *Bougainvillea* germplasm collected from Vietnam, Laos, and Thailand exhibited structured yet interconnected genetic diversity, shaped by hybridization, clonal propagation, and regional germplasm exchange. These findings are consistent with previous RAPD and SSR studies, highlighting the usefulness of RAPD for initial germplasm characterization (Chatterjee et al. 2007; Hammad 2009; Kumar et al. 2020). The alignment of primer performance metrics, genetic diversity indices, and dendrogram topology indicates that the RAPD markers used in this study effectively capture intra-specific genetic variation in *Bougainvillea*. When considered alongside ITS-based species classification, the RAPD dendrogram offers complementary insights at the cultivar level, supporting an integrative molecular framework for germplasm characterization and breeding selection.

Morphological identification

Morphological characterization, based on 21 qualitative traits, revealed significant phenotypic variation among 33 *Bougainvillea* accessions, particularly in bract-related characteristics and inflorescence architecture (see Figure 6). Table 5 outlines the key DUS-based qualitative morphological traits that exhibited considerable to significant variation. However, the traits related to flower color, leaf secondary color, the distribution of secondary color on leaves, and bract apex shape showed minimal to no variation and are not included. This broad phenotypic variability aligns with the well-documented taxonomic complexity of *Bougainvillea*, which is largely influenced by extensive hybridization, clonal propagation, and long-term ornamental selection (Kobayashi et al. 2007). The growth habit was predominantly spreading, with only a few accessions displaying a semi-upright architecture. Young shoot color varied from light green to pinkish white and green, reflecting differences in anthocyanin expression. Thorn traits, such as curvature (ranging from straight to slightly curved) and strength (from weak to strong), exhibited moderate variability and remain useful distinguishing features for species-level differentiation, as previously noted for *B. glabra*, *B. spectabilis*, and their hybrids (Kobayashi et al. 2007).

Table 4. Genetic diversity parameters of *Bougainvillea* accessions based on RAPD markers, indicating moderate genome-wide polymorphism and structured variation

Indices	na	ne	h	I
SD	1.932	1.492	0.295	0.450
	0.253	0.331	0.161	0.211

Note: na: Observed number of alleles, ne: Effective number of alleles, h: Nei's gene diversity, I: Shannon's information index, SD: Standard Deviation

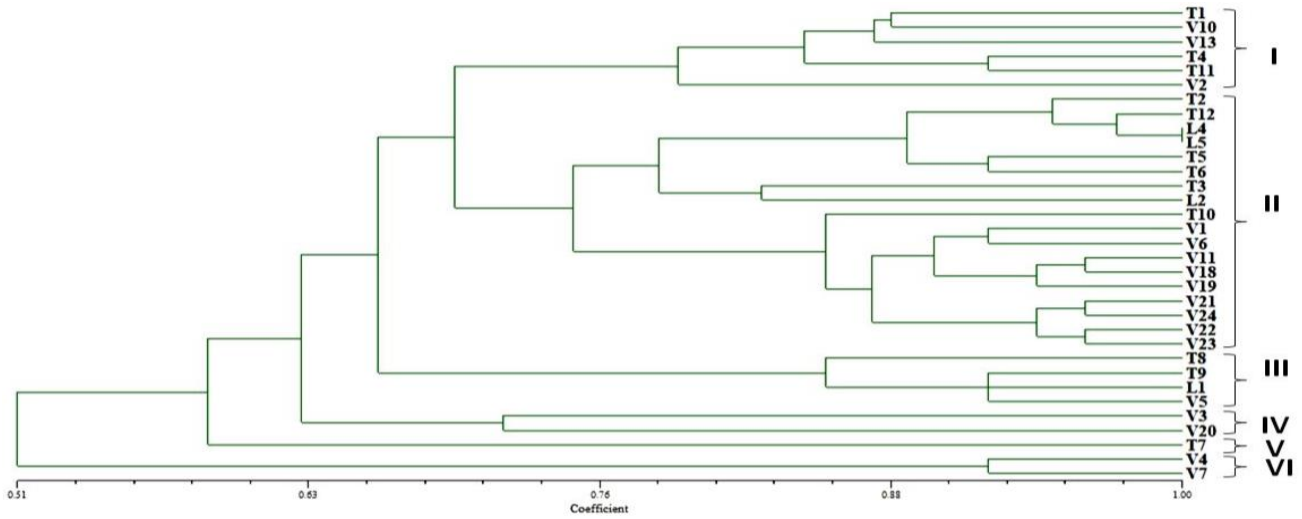

Figure 3. UPGMA dendrogram of 33 *Bougainvillea* accessions based on Jaccard's similarity coefficients derived from RAPD markers, resolving six major clusters (I-VI). Accession T7 forms a distinct singleton cluster (V), while V4 and V7 (VI) show nearly identical profiles, suggesting probable redundancy

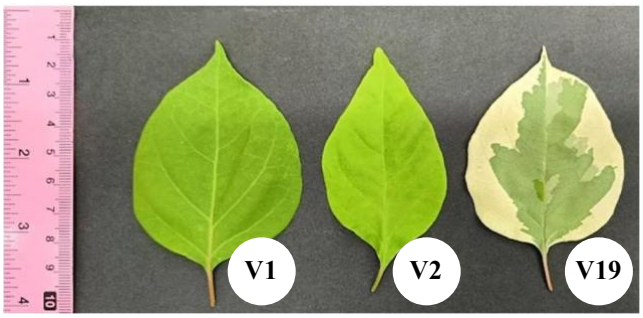

Figure 4. Representative variation in leaf morphology among *Bougainvillea* accessions, including typical non-variegated leaves and rare variegated forms (e.g., V19), illustrating that leaf traits are largely conserved while variegation is restricted to a few cultivars

Leaf traits were largely conserved, with most accessions showing acute or acuminate bases (23 and 10 accessions, respectively) and acuminate or acute apex (21 and 12 accessions, respectively). Leaf color was predominantly medium green (26 accessions), with only four accessions displaying darker green foliage. Leaf morphology was largely conserved across accessions, with all materials showing no secondary leaf color, except for T6 (white and broad-marginal, respectively) and V19 (pinkish white and white and broad-marginal, respectively) (Figure 4). The largely uniform distribution of secondary color on leaves suggests limited variegation among the studied materials.

All accessions displayed flowers within the inflorescence, except for accessions V1, V18 and V24, with bract clusters predominantly arranged terminally. This confirmed their reproductive competence and is consistent with established botanical descriptions of *Bougainvillea* (Kobayashi et al. 2007). Most accessions displayed white flower color, but variations in petal color were observed among the accessions. The clusters of bracts were primarily located at the terminal ends. The absence of axillary bract clusters in the germplasm further reinforced the taxonomic stability of inflorescence position in cultivated *Bougainvillea*, as previously emphasized by Kobayashi et al. (2007).

Bract-related characters displayed the highest degree of variation among accessions, particularly in color, density of bract clusters, and bract shape (Figure 5). The wide spectrum of bract colors corresponded well with earlier reports identifying bract pigmentation as the most polymorphic and horticulturally significant trait in *Bougainvillea* (Singh et al. 2016; Liu et al. 2025). Bract density and cluster arrangement varied markedly, consistent with previous studies highlighting their value for *Bougainvillea* cultivar discrimination (Kumar et al. 2015). In contrast, the shape of the bract apex (acute) was relatively stable across the accessions. Most accessions had a single type of bract, while only a few accessions exhibited double bracts (V1 and V18) and multiple bracts (V22, V23, and V24). Variation in bract shape was observed, ranging from narrowly ovate to circular, while the bract base shape remained consistently acute.

The variation in morphological traits of 33 *Bougainvillea* accessions was illustrated using a UPGMA dendrogram (Figure 6), which was based on morphological distance matrix. The results revealed a clear hierarchical structure, highlighting significant phenotypic diversity within the examined germplasm. The clustering pattern indicated that the morphological variation was distinctly organized rather than continuous, reflecting both common ancestry and diverse selection histories. At a distance of 7.0, the accessions separated into two major groups. The first group (I) contained the majority of accessions, which cluster at moderate distances, indicating a closer morphological similarity among them. In contrast, the second group (II) included highly divergent accessions, such as V1, V18, and V24, which only connect with the rest of the dendrogram at

distances of 11. This highlighted their substantial phenotypic uniqueness. Notably, accessions V1, V18, and V24 were consistently isolated across all analyses. Their extreme positions in both Principal Coordinate Analysis (PCoA) (Figure 7) and Principal Components Analysis (PCA) (Figure 8). Therefore, morphological variation among accessions was summarized using UPGMA, PCoA, and PCA, which yielded broadly consistent grouping patterns and highlighted several distinct phenotypes. In the PCoA ordination, Coordinate 1 and Coordinate 2 explained 37.399% and 25.031% of the total variation, respectively, with V1, V18, and V24 clearly separated from the main assemblage along Coordinate 1 (data not shown). PCA confirmed the same structure, where PC1 (37.399%) was primarily associated with floral and bract architecture (presence of flowers in the inflorescence, bract shape, and bract base shape), whereas the negative PC1 direction reflected bract cluster traits (density, bract type, and cluster arrangement). PC2 (25.031%) was dominated by pigmentation traits, especially bract main color, with petal color contributing to separation in the opposite direction. Together, these results indicate that phenotypic differentiation in the germplasm panel is mainly driven by bract-related architecture and color traits, consistent with the distinct clustering of V1, V18, and V24 across analyses.

Such outlying behavior is consistent with the known complex hybrid origin of many cultivated *Bougainvillea* forms. This broad separation revealed a high overall morphological diversity, a characteristic often observed in cultivated *Bougainvillea*. The observed concordance between clustering and ordination is consistent with previously published studies on morphological diversity in *Bougainvillea* (Kobayashi et al. 2007; Kumar et al. 2015; Singh et al. 2016). Thus, integrating morphological traits, *ITS* phylogeny, and RAPD diversity patterns provided a robust framework for accurate germplasm characterization, conservation planning, and breeding-oriented selection in *Bougainvillea.*

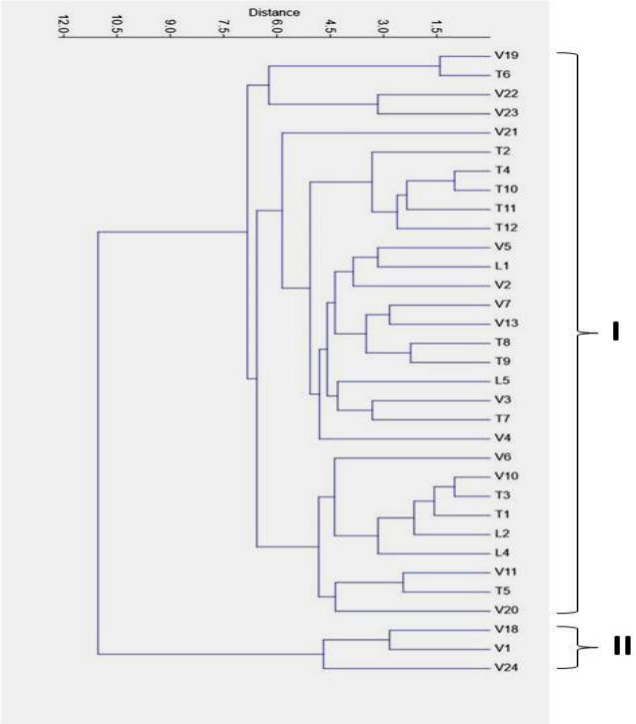

Figure 6. UPGMA dendrogram based on Euclidean distance calculated from 21 categorical DUS-based morphological traits, showing two major morphological groups. Accessions V1, V18, and V24 form a divergent subgroup, consistent with their distinct bract architecture and pigmentation, whereas most accessions cluster at moderate distances, indicating shared phenotypic backgrounds

Figure 5. Representative variation in bract color, shape, and inflorescence density among *Bougainvillea* accessions, highlighting bract-related traits as the primary source of phenotypic differentiation within the germplasm

Table 5. DUS-based qualitative morphological traits revealed variability among the 33 *Bougainvillea* accessions

Accession code	GH	YSC	TC	TS	LBS	LAS	LMC	PFI	PC	ABC	SB	TB	DBC	BBS	BMC	BSC
V1	3	3	1	1	2	1	1	1	1	1	1	5	7	2	4	1
V2	3	1	3	3	1	2	1	9	3	1	3	1	3	2	5	1
V3	3	1	3	3	1	1	3	9	4	1	4	1	3	3	3	2
V4	3	3	3	3	2	2	1	9	5	1	1	1	3	2	3	4
V5	2	3	3	3	1	1	2	9	3	3	2	1	5	3	4	1
V6	2	3	3	3	1	1	2	9	7	1	4	1	3	3	6	1
V7	3	3	3	2	2	2	2	9	5	1	1	1	5	2	2	1
V10	3	1	1	3	1	1	2	9	4	1	3	1	3	3	7	1
V11	3	1	3	3	1	1	2	9	5	1	3	1	5	2	9	3
V13	3	1	3	2	2	1	2	9	6	1	2	1	5	2	1	1
V18	3	1	1	2	1	1	2	1	1	1	2	5	7	2	4	1
V19	3	5	1	3	2	1	3	9	2	1	3	1	5	3	4	1
V20	3	1	1	3	2	2	3	9	4	1	2	1	5	2	7	5
V21	3	3	3	3	1	1	2	9	8	1	4	1	5	3	3	4
V22	3	3	3	1	2	2	2	9	1	1	2	3	5	2	4	4
V23	3	3	3	1	2	2	2	9	1	3	3	3	7	1	4	4
V24	3	3	3	3	1	1	2	1	1	3	1	3	7	2	2	1
L1	2	2	3	2	2	2	2	9	2	1	2	1	5	2	4	1
L2	3	3	1	2	2	1	2	9	4	1	3	1	3	3	7	1
L4	3	3	1	3	2	1	2	9	5	1	4	1	5	3	8	1
L5	3	3	1	2	1	1	2	9	4	3	3	1	3	3	3	1
T1	3	2	1	3	2	2	2	9	4	1	3	1	3	3	7	1
T2	3	2	1	3	2	2	2	9	8	1	3	1	3	2	1	3
T3	3	1	1	3	2	1	2	9	4	1	3	1	3	3	7	1
T4	3	1	1	2	2	1	2	9	6	1	2	1	3	2	1	1
T5	3	3	3	2	2	1	2	9	5	1	3	1	5	2	9	3
T6	3	4	1	3	2	1	3	9	2	1	3	1	5	3	4	1
T7	3	2	3	3	2	1	2	9	3	1	3	1	3	2	1	3
T8	2	2	1	3	2	2	2	9	5	1	2	1	5	2	4	1
T9	2	2	3	2	2	2	2	9	5	1	2	1	5	2	4	1
T10	3	1	1	3	2	1	2	9	6	1	2	1	3	2	1	1
T11	3	1	1	2	2	2	2	9	6	3	2	1	3	2	1	1
T12	3	1	1	2	2	1	2	9	6	1	3	1	3	2	1	3

Note: GH: Growth Habit: 1: Upright, 2: Semi-upright, 3: Spreading. YSC: Young Shoot Color: 1: Light green, 2: Medium green, 3: Reddish green, 4: White and green, 5: Pinkish white and green. TC: Thorn Curvature: 1: Straight, 3: Slightly curved, 5: Fully curved. TS: Thorn Strength:1: Weak, 2: Medium, 3: Strong. LBS: Leaf Base Shape: 1: Acuminate, 2: Acute, 3: Obtuse. LAS: Leaf Apex Shape: 1: Acuminate, 2: Acute, 3: Obtuse. LMC: Leaf Main Color: 1: Light green, 2: Medium green, 3: Dark green. PFI: Presence of Flowers in the Inflorescence: 1: Absent, 9: Present. PC: Petal Color: 1: None, 2: Magenta, 3: Magenta and light green, 4: Purple, 5: Orange, 6: Light Green, 7: Red, 8: Greenish Pink. ABC: Arrangement of Bract Clusters: 1: Terminal, 2: Axillary, 3: Axillary and terminal. SB: Shape of Bract: 1: Narrowly Ovate, 2: Medium Ovate, 3: Broadly Ovate, 4: Circular. TB: Type of Bract: 1: Single, 3: Multiple, 5: Double. DBC: Density of Bract Cluster: 3: Sparse, 5: Medium, 7: Dense. BBS: Bract Base Shape: 1: Acute, 2: Obtuse, 3: Cordate. BMC: Bract Main Color: 1: White, 2: Orange, 3: Pink, 4: Magenta, 5: Magenta and white, 6: Red, 7: Mauve, 8: Orangish mauve, 9: Yellow. BSC: Bract Secondary Color: 1: None, 2: Purple, 3: Mauve, 4: Orange, 5: White

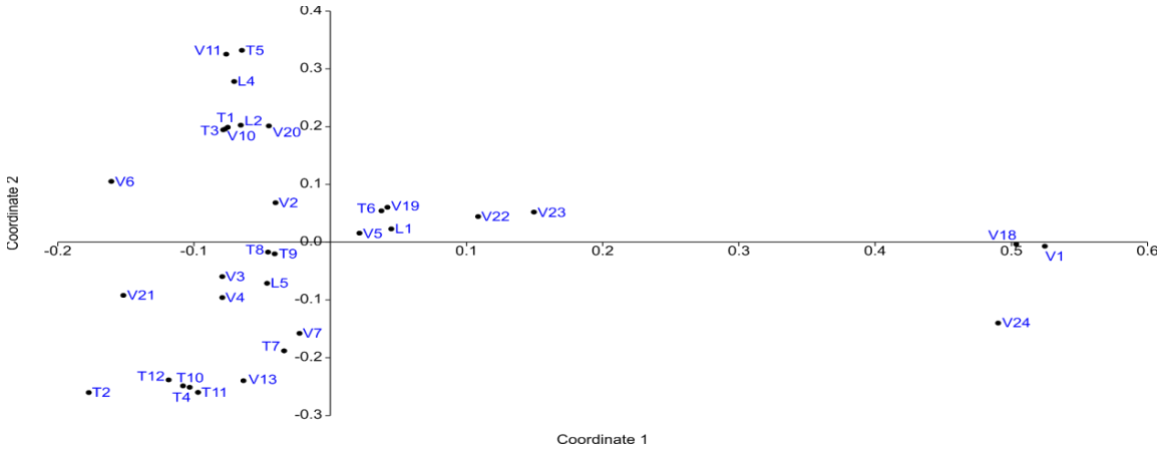

Figure 7. Principal Coordinate Analysis (PCoA) of 33 *Bougainvillea* accessions based on 21 categorical morphological traits. Coordinate 1 and Coordinate 2 explained 37.399% and 25.031% of the total phenotypic variation, respectively. Accessions V1, V18, and V24 are clearly separated along Coordinate 1, indicating strong phenotypic divergence from the main assemblage

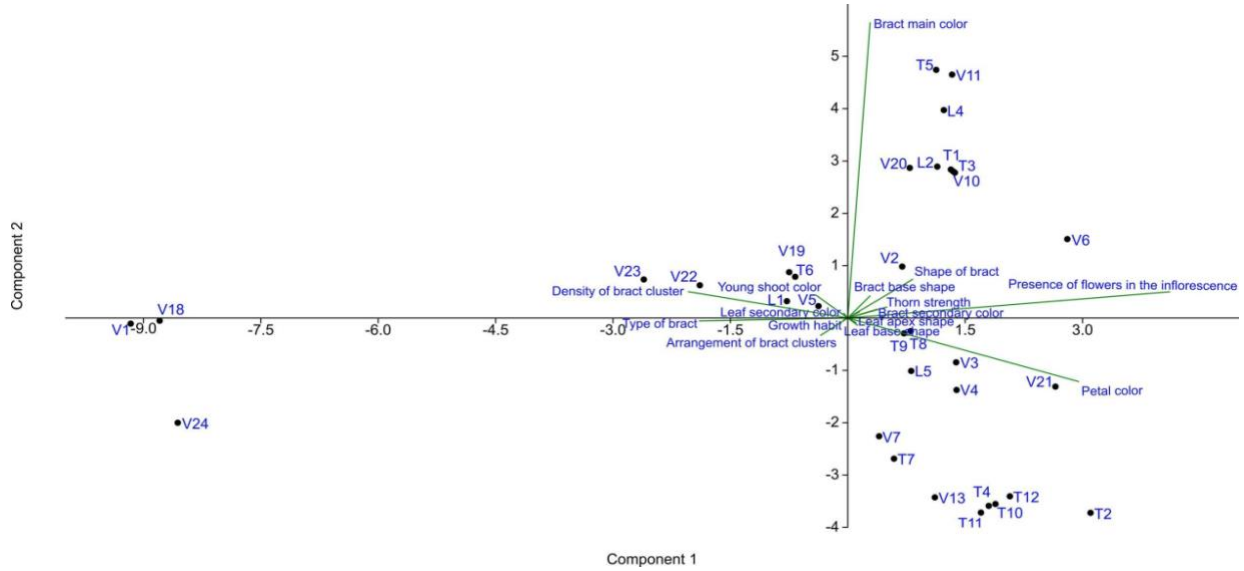

Figure 8. Principal Components Analysis (PCA) based on 21 morphological traits of 33 *Bougainvillea* accessions. PC1 (37.399%) is mainly associated with floral and bract architecture (presence of flowers in the inflorescence, bract shape, bract base shape), whereas PC2 (25.031%) is dominated by pigmentation traits, particularly bract main color, with petal color contributing in the opposite direction

Integration of morphological and molecular characterization

The primary strength of this study lies in the integration of ITS phylogeny, RAPD fingerprinting, and DUS-based morphological characterization, which together provide complementary perspectives on *Bougainvillea* diversity from species delimitation to cultivar-level differentiation. While each dataset alone captures only part of the underlying variation, their combined interpretation yields a coherent and biologically meaningful picture of genetic and phenotypic structure.

At the species level, ITS sequences consistently separated 33 accessions into two major clades corresponding to *B. glabra* and *B. spectabilis*. RAPD clustering broadly mirrored this division, while additionally resolving multiple subclusters within each species, reflecting finer-scale genomic differentiation. Morphological clustering likewise separated accessions into two major groups largely driven by bract architecture and pigmentation.

Several accessions showed strong concordance across all three approaches. For example, accessions V18 and V24 grouped together in ITS (Figure 1), RAPD (Figure 3), and morphological analyses (Figure 6) and shared similar bract forms, colors, and inflorescence traits, indicating a stable genetic background underlying their phenotypes (Table 5). Likewise, accessions such as V4 and V7 clustered tightly in both RAPD and morphological datasets, suggesting probable duplicate or near-duplicate germplasm. These cases demonstrate how integration can confirm true genetic similarity and assist redundancy management in collections.

In contrast, informative discordances were also detected. Accessions V3 and V20 clustered with *B. spectabilis* in the ITS tree (Figure 1) and remained similar in RAPD clustering (Figure 3) but have distinct morphological clusters (Figure 6). These discordances highlight the importance of integrating multiple evidence layers, while also acknowledging that limited sampling per region may constrain inference about geographic differentiation or the frequency of admixture events. Similarly, V1 was consistently positioned as an outlier in morphological analyses (Figure 6) but genetically similar to accessions V21 and V23 (Figures 1 and 3), indicating a greater divergence. These cases highlight that while most morphological variation in *Bougainvillea* correlates with genetic differentiation, environmental or epigenetic factors may influence phenotypic plasticity (Liu et al. 2025).

Importantly, the integrated evidence highlighted both unique and redundant germplasm. The singleton T7 formed an independent cluster in the RAPD dendrogram with low similarity (<0.63) to other accessions (Figure 3). This indicates that T7 represents a rare or ancestral lineage, characterized by its compact growth and distinctive bract coloration. Additionally, accessions V1, V18, and V24 consistently appeared as morphological and molecular outliers across analyses (Figures 1, 3, and 6). This suggests that they may be valuable as genetically divergent parents for breeding purposes. In contrast, the highly similar accessions V4 and V7 clustered closely together in both morphological and molecular datasets, indicating probable duplication. This underscores the importance of molecular validation in collection management. The agreement among morphological descriptors (Figure 6), multilocus RAPD fingerprints (Figure 3), and ITS phylogeny (Figure 1) provides strong and complementary evidence for the genetic and phenotypic organization of cultivated *Bougainvillea*.

These concordant and discordant patterns emphasize that ITS is most informative for species-level delimitation, whereas RAPD captures genome-wide polymorphism relevant to cultivar differentiation, and morphology reflects the expressed outcome of both genetic background and phenotypic plasticity. When interpreted together, the three

datasets provide stronger inference than any single approach. This integrated analysis represents the primary contribution of the study, demonstrating how combining molecular and morphological datasets can improve germplasm authentication, reveal unique and redundant accessions, and guide conservation and breeding decisions for *Bougainvillea* in Southeast Asia. These combined results directly inform the following conclusions by identifying: (i) key sources of phenotypic variation, (ii) candidate accessions for expanding breeding pools, and (iii) materials that may be redundant within the collection.

In conclusion, this study demonstrates that an integrated framework combining ITS phylogeny, RAPD markers, and DUS-based morphology is sufficient to resolve species identity and characterize diversity in cultivated *Bougainvillea* from Vietnam, Laos, and Thailand. The results confirm clear species boundaries between *B. glabra* and *B. spectabilis* and reveal a moderately structured pool of genetic variation shaped by clonal propagation and horticultural selection. Notably, the concordant identification of genetically and phenotypically distinctive accessions (T7, V1, V18, and V24), together with the recognition of likely redundant materials (e.g., V4/V7), illustrates the practical value of this approach for germplasm curation. These findings provide a concrete basis for prioritizing unique accessions for conservation, rationalizing collections, and selecting divergent parents to broaden breeding populations. Collectively, this study informs more strategic conservation and breeding efforts for *Bougainvillea* in Southeast Asia and establishes a transferable model for managing ornamental germplasm with complex domestication histories.

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Supplementary Table 1. List of RAPD primers used for preliminary screening in this study

No.	Primer name	Primer sequence	No.	Primer name	Primer sequence	No.	Primer name	Primer sequence	No.	Primer name	Primer sequence
1	UBC#401	TAGGACAGTC	26	UBC#426	TCTCCCGGTG	51	UBC#451	CTAATCTCGC	76	UBC#476	TTGAGGCCCT
2	UBC#402	CCCGCCGTTG	27	UBC#427	GTAATCGACG	52	UBC#452	CTAATCACGG	77	UBC#477	TGTTGTGCCC
3	UBC#403	GGAAGGCTGT	28	UBC#428	GGCTGCGGTA	53	UBC#453	AGTACAAGGG	78	UBC#478	CGAGCTGGTC
4	UBC#404	TCTCTACGAC	29	UBC#429	AAACCTGGAC	54	UBC#454	GCTTACGGCA	79	UBC#479	CTCATACGCG
5	UBC#405	CTCTCGTGCG	30	UBC#430	AGTCGGCACC	55	UBC#455	AGCAAGCCGG	80	UBC#480	GGAGGGGGGA
6	UBC#406	GCCACCTCCT	31	UBC#431	CTGCGGGTCA	56	UBC#456	GCGGAGGTCC	81	UBC#481	GTAATTGCGC
7	UBC#407	TGGTCCTGGC	32	UBC#432	AGCGTCGACT	57	UBC#457	CGACGCCCTG	82	UBC#482	CTATAGGCCG
8	UBC#408	CCGTCTCTTT	33	UBC#433	TCACGTGCCT	58	UBC#458	CTCACATGCC	83	UBC#483	GCACTAAGAC
9	UBC#409	TAGGCGGCGG	34	UBC#434	TCGCTAGTCC	59	UBC#459	GCGTCGAGGG	84	UBC#484	CTGGCAAGGA
10	UBC#410	CGTCACAGAG	35	UBC#435	CTAGTAGGGG	60	UBC#460	ACTGACCGGC	85	UBC#485	AGAATAGGGC
11	UBC#411	GAGGCCCGTT	36	UBC#436	GAGGGGGCCA	61	UBC#461	CCCGTATGTC	86	UBC#486	CCAGCATCAG
12	UBC#412	TGCGCCGGTG	37	UBC#437	AGTCCGCTGC	62	UBC#462	CATAGCGGCA	87	UBC#487	GTGGCTAGGT
13	UBC#413	GAGGCGGCGA	38	UBC#438	AGACGGCCGG	63	UBC#463	AGGCGGAAGC	88	UBC#488	TTCGCTTCTC
14	UBC#414	AAGGCACCAG	39	UBC#439	GCCCCTTGAC	64	UBC#464	CACAAGCCTG	89	UBC#489	CGCACGCACA
15	UBC#415	GTTCCAGCAG	40	UBC#440	CTGTCGAACC	65	UBC#465	GGTCAGGGCT	90	UBC#490	AGTCGACCTT
16	UBC#416	GTGTTTCCGG	41	UBC#441	CTGCGTTCTT	66	UBC#466	TTCTTAGCGG	91	UBC#491	TCCTGTCAAG
17	UBC#417	GACAGGCCAA	42	UBC#442	CTACTCGGTT	67	UBC#467	AGCACGGGCA	92	UBC#492	GTGACTGCTC
18	UBC#418	GAGGAAGCTT	43	UBC#443	TGATTGCTCG	68	UBC#468	ACGGAAGCGC	93	UBC#493	CCGAATCACT
19	UBC#419	TACGTGCCCG	44	UBC#444	GCAGCCCCAT	69	UBC#469	CTCCAGCAAA	94	UBC#494	TGATGCTGTC
20	UBC#420	GCAGGGTTCG	45	UBC#445	TAGCAGCTTG	70	UBC#470	AGGAGCTGGG	95	UBC#495	CTTTCCTTCC
21	UBC#421	ACGGCCCACC	46	UBC#446	GCCAGCGTTC	71	UBC#471	CCGACCGGAA	96	UBC#496	CCTTTCAAGG
22	UBC#422	CACCTGCGGG	47	UBC#447	CAGGCTCTAG	72	UBC#472	AGGCGTGCAA	97	UBC#497	GCATAGTGCG
23	UBC#423	GGGTCTCGAA	48	UBC#448	GTTGTGCCTG	73	UBC#473	ATCCCCAAGA	98	UBC#498	GACAGTCCTG
24	UBC#424	ACGGAGGTTC	49	UBC#449	GAGGTTCAAC	74	UBC#474	AGGCGGGAAC	99	UBC#499	GGCCGATGAT
25	UBC#425	CGTCGGGCCT	50	UBC#450	CGGAGAGCCC	75	UBC#475	CCAGCGTATT	100	UBC#500	TTGCGTCATG