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Genetic diversity and relationships among native *Dendrobium* species from Bali, Indonesia, using SSR markers

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Abstract. *Darmawati IAP, Dwiyani R, Pradnyawathi NLM, Fitriani Y, Listihani L, Kamuhi RH. 2025. Genetic diversity and relationships among native* Dendrobium *species from Bali, Indonesia, using SSR markers. Asian J Agric 9: 917-924.* Bali, Indonesia, has significant potential for the development of orchid cultivation, particularly through the production of hybrids that meet market preferences. Among various orchid genera, *Dendrobium* holds major economic and horticultural importance due to its wide adaptability, aesthetic value, and high demand in both domestic and international markets. Wild *Dendrobium* species represent an invaluable source of germplasm that can be used for interspecific hybridization and the development of new cultivars with desirable characteristics such as flower color, shape, and durability. However, the lack of information on the genetic diversity of native *Dendrobium* species in Bali limits their effective use in breeding and conservation programs. This study aimed to evaluate the genetic diversity and relationships among 24 native *Dendrobium* species collected from 12 forest areas across Bali using Simple Sequence Repeat (SSR) markers. The molecular analysis revealed high genetic variation, with 86.21 percent of loci showing polymorphism and moderate heterozygosity across populations. Genetic similarity coefficients among species ranged from 0.66 to 0.97. The highest genetic similarity was observed between *Dendrobium* sp. Wanagiri and *Dendrobium* sp. Sepang, while *D. fimbriatum* was identified as the most genetically distinct species. These findings demonstrate a broad genetic base among Bali's native *Dendrobium* species, indicating their strong potential as parental resources in hybrid breeding programs. The study also provides valuable molecular data that support the conservation and sustainable utilization of Bali's native orchid germplasm for future cultivation and breeding initiatives.

Keywords: Bali native *Dendrobium*, genetic distance, SSR

INTRODUCTION

Orchids of the genus *Dendrobium* represent one of the largest and most diverse groups within the family *Orchidaceae*, with an estimated 1,600 species distributed widely across Southeast Asia and extending to Australia (De et al. 2015). In Indonesia, approximately 275 species of *Dendrobium* have been recorded, including those naturally occurring on the island of Bali (Gandawidjaya and Sastrapraja 1980). These orchids are valued for their ecological diversity and high ornamental potential. In addition, *Dendrobium* is the dominant genus in Indonesia's orchid export market, which reflects its significant economic importance both nationally and internationally (Agricultural Data and Information Center 2015a).

Despite this potential, Bali's contribution to Indonesia's hybrid orchid production remains relatively low. Between 2010 and 2015, Bali contributed only about 6.62 percent of the total national hybrid orchid production, suggesting that the potential for local cultivation, hybrid development, and market-oriented breeding remains largely untapped (Agricultural Data and Information Center 2015b). Wild *Dendrobium* species are therefore essential genetic resources, serving as germplasm for the creation of new hybrids with superior traits.

However, the existence of wild orchid populations, including *Dendrobium* species, is increasingly threatened by habitat loss, deforestation, and uncontrolled collection. These pressures result in population decline and loss of genetic variation, which may limit future breeding and conservation efforts. As emphasized by Babu et al. (2021), the conservation and systematic documentation of native orchid germplasm are crucial to ensure the sustainability of genetic resources. Previous surveys reported 24 *Dendrobium* species distributed across 12 forest areas in Bali (Darmawati et al. 2018), indicating that the island still maintains diverse natural populations that have not yet been fully characterized.

To utilize these biological resources effectively, a comprehensive understanding of their genetic diversity and relationships is needed. Morphological identification alone is often unreliable because morphological traits can be influenced by environmental conditions. Therefore, molecular approaches are preferred to obtain more accurate and stable information. Molecular markers have become an essential tool in studying genetic diversity and phylogenetic relationships because they provide more consistent and reproducible results compared to morphological traits (Hashim and Al-Shuhaib 2019).

Among molecular markers, Simple Sequence Repeat (SSR) markers, also known as microsatellites, have several advantages. They are co-dominant, highly polymorphic, reproducible, and require only a small amount of DNA for analysis (Song et al. 2022). SSR markers have been successfully used to assess genetic variation and identify relationships among *Dendrobium* species in several studies, including those involving *D. huoshanense* (Wang et al. 2012) and other medicinally important orchids (Chattopadhyay et al. 2012). Compared to dominant markers such as RAPD and ISSR, SSR markers provide more detailed and reliable information on genetic diversity and allelic composition.

Although the application of SSR markers in *Dendrobium* studies has been widely reported in other countries, similar research on Indonesian *Dendrobium*, particularly native species from Bali, remains very limited. Most existing studies focus on morphological characterization or the use of dominant markers, which provide less detailed genetic data. This lack of SSR-based genetic information creates a significant gap in understanding the diversity and relationships among Bali's native *Dendrobium* species. Consequently, the absence of molecular data limits strategic planning for conservation and the identification of suitable parent species for breeding programs.

The present study aims to evaluate the genetic diversity and genetic relationships of 24 *Dendrobium* species collected from 12 forest areas in Bali using SSR markers. The main hypothesis is that genetic similarity coefficients can reveal potential compatibility for interspecific hybridization, with closely related species expected to serve as compatible parental lines. The specific objectives of this study are as follows: (i) to assess the level of genetic diversity among *Dendrobium* species in Bali; (ii) to construct a genetic relationship map among the species studied; (iii) to identify potential species for hybridization based on genetic compatibility; and (iv) to provide molecular data to support breeding and conservation programs.

By applying SSR markers to *Dendrobium* species from Bali for the first time, this study aims to fill an important knowledge gap and establish a molecular foundation for the sustainable utilization of local orchid biodiversity. The results are expected to contribute to more effective conservation efforts, guide the selection of compatible parents for hybrid breeding, and strengthen Bali's role in the national and international orchid industry.

MATERIALS AND METHODS

Sampling method

A total of 24 *Dendrobium* species were collected from 12 forest areas in Bali (Table 1). Species included *D. acuminatissimum*, *D. aloifolium*, *D. aphyllum*, *D. arcuatum*, *D. conspicuum*, *D. crumenatum*, *D. fimbriatum*, *D. heterocarpum*, *D. inflatum*, *D. linearifolium*, *D. macrophyllum*, *D. plicatile*, *D. rugosum*, *D. salaccense*, *D. secundum*, *Dendrobium* sp. Sepang, *Dendrobium* sp. Wanagiri, *D. spathilingue*, *D. stuplosum*, *D. stuartii*, *D. subulatum*, *D. spurium*, *D. tetraedre*, and *D. truncatum*. Collection was conducted with permission from local forest authorities, following ethical and conservation guidelines.

DNA extraction and SSR analysis

Genomic DNA was isolated from 0.5 g fresh leaf tissue using a modified CTAB method. DNA quality and concentration were verified by spectrophotometry and agarose gel electrophoresis. Five SSR primers previously developed by Chattopadhyay et al. (2012) were selected for amplification. PCR was performed under standard conditions, and amplified products were resolved by polyacrylamide gel electrophoresis and visualized with ethidium bromide under UV light. SSR markers were chosen due to their co-dominance, high polymorphism, reproducibility, and ability to distinguish homozygous and heterozygous genotypes.

Allele scoring and data analysis

Amplified bands were scored manually as present (1) or absent (0) to create a binary matrix. Genetic diversity parameters including observed alleles, effective alleles, heterozygosity, and percentage of polymorphic loci-were calculated using GenAlEx 6.5 (Peakall and Smouse 2012). Polymorphism Information Content (PIC) for each locus was determined using an online tool. Genetic relationships among species were analyzed using NTSYS 2.02 with UPGMA clustering, and dendrograms were constructed to visualize similarity coefficients. Principal Coordinate Analysis (PCoA) was also performed in GenAlEx 6.5 to further assess genetic structure. This streamlined methodology highlights essential procedures while detailed PCR programs and primer sequences are provided in the supplementary materials.

RESULTS AND DISCUSSION

Genetic diversity of Bali *Dendrobium* species based on SSR

SSR analysis of 24 native Bali *Dendrobium* species using four successful SSR primers (DO-03, DO-07, DO-09, and DO-12) revealed varying levels of genetic diversity. The highest PIC value was observed for primer DO-07 (0.707) with an expected heterozygosity (He) of 0.395, indicating that this primer was the most informative for detecting allelic variation. In contrast, primer DO-12 was monomorphic (He=0.000), although it still provided stable amplification. Primers DO-03 and DO-09 detected moderate variation, with PIC values of 0.418 and 0.661, respectively. On average, the four primers produced 1.725 alleles per locus with a PIC of 0.595, demonstrating that SSR markers are effective in capturing genetic variation in Bali *Dendrobium* species.

Genetic clustering patterns

The UPGMA dendrogram (Figure 1) grouped the 24 species into four main clusters. Cluster II, which includes *D. crumenatum* and *D. plicatile*, represents the largest group, indicating similar genetic backgrounds and potential shared ecological adaptations or evolutionary history. Cluster IV, consisting solely of *D. fimbriatum*, exhibited

unique genetic characteristics likely resulting from isolation or distinct selective pressures.

PCoA analysis (Figure 2) supported these clustering patterns, with most individuals grouping according to their clusters, although some overlap was observed among subgroups. This indicates a continuum of genetic variation, which can be exploited in hybridization programs to combine desirable traits while maintaining heterozygosity.

Practical implications

High levels of genetic diversity enable the selection of parental plants for hybridization without reducing heterozygosity or adaptive potential. Species with similar genetic backgrounds (e.g., *Dondrobium* sp. Wanagiri and Sepang) are ideal for crossing due to high compatibility. Genetically distant species, such as *D. fimbriatum*, have the potential to introduce novel variation, but require careful hybrid management.

From a conservation perspective, populations with narrow genetic distances need protection to prevent loss of variation, while unique species should be prioritized to maintain natural diversity. SSR markers effectively reveal polymorphism, clustering patterns, and outlier species, supporting sustainable conservation and hybridization strategies for Bali *Dendrobium*.

Use of SSR primers in genetic diversity analysis of Bali *Dendrobium* species

The primers used in this study are presented in Table 2. Among the five pairs of primers tested, four (DO-03, DO-07, DO-09, and DO-12) successfully amplified DNA fragments, while DO-02 produced weak or inconsistent amplification. This indicates that not all primers are equally effective in targeting microsatellite regions in *Dendrobium* genomes. The successful primers demonstrated strong binding affinity and produced reproducible bands, making them suitable for assessing genetic diversity. Thus, the selection of these primers ensured reliable detection of polymorphisms across the 24 *Dendrobium* species.

PCR amplification program optimization for SSR primers

The PCR program for each primer is shown in Table 3. The annealing temperatures varied among primers, with DO-03 requiring the lowest (50°C), while DO-07, DO-09, and DO-12 required higher temperatures (56-57°C). These differences reflect variations in nucleotide composition and GC content of each primer sequence. In addition, elongation times ranged from 1 to 1.5 minutes, suggesting variability in amplicon length. Such optimization is essential to ensure specific amplification and to minimize non-specific products. Overall, the PCR programs applied in this study proved effective in producing clear and consistent SSR banding patterns for genetic diversity analysis of *Dendrobium*.

Table 1. Genetic diversity of Bali *Dendrobium* species based on four SSR primers

Primers	Na	Ne	He	PIC
DO-03	2.000	1.231	0.162	0.418
DO-07	2.000	1.698	0.395	0.707
DO-09	1.900	1.446	0.259	0.661
DO-12	1.000	1.000	0.000	0.593
Total	6.900	5.375	0.816	2.379
Average	1.725	1.344	0.204	0.595

Note: Na: Number of alleles per locus, Ne: Effective number of alleles, He: Expected heterozygosity, PIC: Polymorphic Information Content

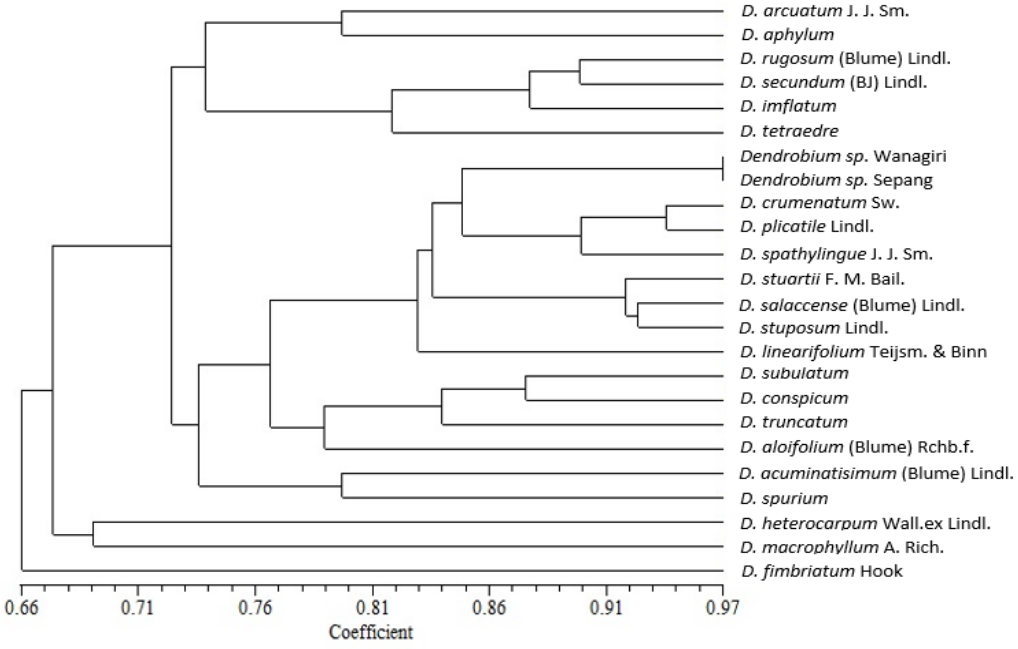


Figure 1. Dendrogram of the twenty four Bali *Dendrobium* species based on 4 SSR primers genotyping

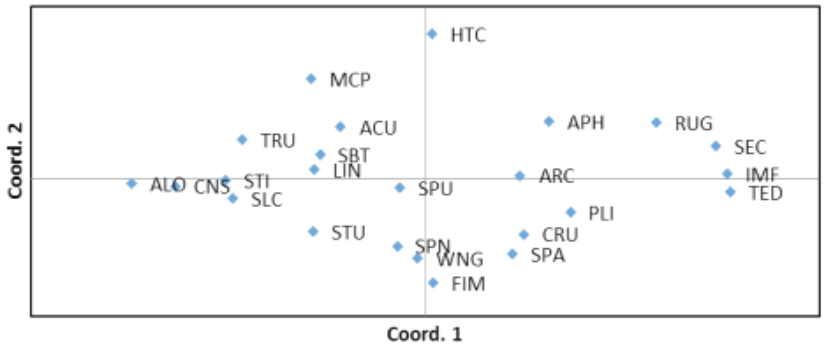


Figure 2. Distribution of the twenty four Bali *Dendrobium* species based on 4 SSR primers in one dimensional space

Characteristics of SSR primers used in *Dendrobium* analysis

The identity of each SSR primer is detailed in Table 4. Primer DO-07 showed the longest repeat motif, (CT)26, with allele sizes ranging from 110 to 180 bp, which produced a relatively high degree of specificity. Meanwhile, DO-03 and DO-09 amplified wider allele size ranges (130-550 bp and 110-550 bp, respectively), indicating their ability to detect higher levels of polymorphism. Primer DO-12, although containing a simple repeat motif (GT)7, consistently amplified products within a narrow allele range (160-190 bp). These results demonstrate that primers with different repeat motifs contribute complementary information, with some providing broader genetic variability and others offering more stable amplification.

Visualization of SSR polymorphism and genetic distribution of *Dendrobium* species in Bali

Figure 3 is SSR electrophoresis results of 24 Bali *Dendrobium* species using four primers (DO-03, DO-07, DO-09, and DO-12). Distinct bands indicate allelic variation among species, with DO-07 exhibiting the highest polymorphism, while DO-12 is monomorphic. This visualization illustrates the genetic distribution and intra-species diversity, which can be utilized for clustering analysis and parental selection in hybridization programs.

Discussion

Genetic diversity levels and global comparison

SSR analysis of 24 native Bali *Dendrobium* species revealed a high level of genetic diversity. The successful use of four SSR primers (DO-03, DO-07, DO-09, and DO-12) in detecting clear polymorphic variation underscores the effectiveness of these markers in capturing genetic differences among species (Table 1 and Figure 3). Primer DO-07 was the most informative, with an expected heterozygosity of 0.395 and a Polymorphic Information Content value of 0.707, whereas DO-12 was monomorphic. On average, the four primers produced 1.725 alleles per locus with a PIC value of 0.595, reflecting substantial allelic richness in the Bali *Dendrobium* population. This high allelic variation is crucial for supporting conservation and breeding programs, as populations with high genetic diversity tend to be more adaptable to environmental changes and natural selective pressures.

Table 2. Primary used in this study

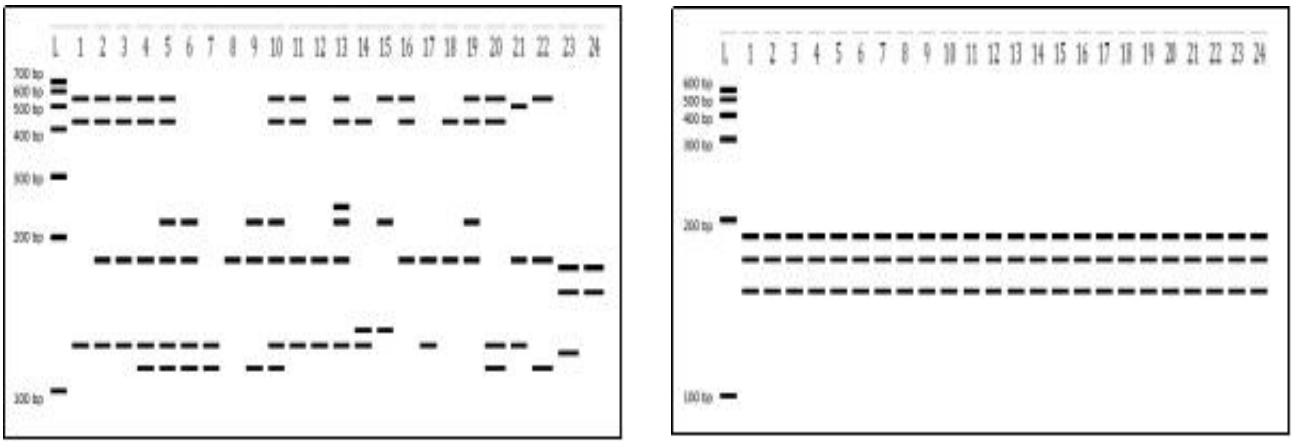
Number	Primers	Nucleotide base pairs
1	DO-02 (F)	5'-CTCCACGCATGAACATTAG-3'
	DO-02 (R)	5'-TTTGACCATACTGTGGGC-3'
2	DO-03 (F)	5'-GCCCGACTACATCCAAAC-3'
	DO-03 (R)	5'-GGTGTTGCTTCCGATCTAA-3'
3	DO-07 (F)	5'-AGGGCTTTCTTGGGTTCG-3'
	DO-07 (R)	5'-TCGCTCGCTGTGAAGTTG-3'
4	DO-09 (F)	5'-GGGAAGGTGGGTGCATGTC-3'
	DO-09 (R)	5'-GTATAGCGGCACGTGCAA-3'
5	DO-12 (F)	5'-CGGATGATGTGACCAAAA-3'
	DO-12 (R)	5'-GCTCAAGATGGGTAGACT-3'

Table 3. The PCR program for each SSR primer

Stages	DO-03		DO-07		DO-09		DO-12	
	Temperature	Time	Temperature	Time	Temperature	Time	Temperature	Time
Pre-heating	94°C	04:00	95°C	05:00	95°C	05:00	95°C	05:00
Denaturation	94°C	00:40	95°C	00:45	95°C	00:45	95°C	00:45
Annealing	50°C	00:40	57°C	01:00	56°C	01:00	56°C	01:00
Elongation	72°C	01:00	72°C	01:30	72°C	01:30	72°C	01:30
Final elongation	72°C	08:00	72°C	07:00	72°C	07:00	72°C	07:00
Storage	10°C	~	10°C	~	10°C	~	10°C	~

Table 4. SSR primer identity

Primers	Nucleotide sequences (5'- 3')	MT (°C)	Sequence repetition motif	Allele size (bp)
DO-03	F:GCCCGACTACATCCAAAC R:GGTGTTGCTTCCGATCTAA	50	$(GA)_{10}$	130-550
DO-07	F:AGGGCTTTCTTGGGTTCG R:TCGCTCGCTGTGAAGTTG	57	$(CT)_{26}$	110-180
DO-09	F:GGGAAGGTGGGTGCATGTC R:GTATAGCGGCACGTGCAA	56	$(TG)_4A(GT)_4T(TG)_5TTCG(TG)_7TT(TG)_8$	110-550
DO-12	F:CGGATGATGTGACCAAAA R:GCTCAAGATGGGTAGACT	56	$(GT)_7$	160-190



DO-09 **DO-12**

Figure 3. Electrophoresis visualization of 24 Bali *Dendrobium* species using 4 SSR primer pairs

Compared to global studies, the PIC values observed in Bali *Dendrobium* are consistent with those reported for wild and cultivated *Dendrobium* populations in China, which ranged from 0.35 to 0.72 (Wang et al. 2012; Liu et al. 2022). In India, Chattopadhyay et al. (2012) reported moderate polymorphism in five *Dendrobium* species, which is likely lower than that observed in Bali. These differences are primarily influenced by the level of human intervention. Wild populations in Bali experience minimal disturbance, allowing allelic variation to remain high, whereas intensive cultivation in other regions may reduce genetic diversity.

Within the Southeast Asian context, similar patterns have been observed in wild *Dendrobium* populations in Thailand and Malaysia, where wild populations maintain higher genetic variation than cultivated ones. These findings emphasize that Bali harbors unique and valuable natural genetic resources, which can be utilized for both conservation and the development of new hybrids. High genetic diversity not only facilitates the selection of potential parental plants for hybridization aimed at improving aesthetic traits or environmental resilience, but also supports genetic-based conservation strategies to ensure the long-term sustainability of Bali's unique wild species (Babu et al. 2021; Liu et al. 2022).

Moreover, high genetic variation provides flexibility in breeding programs, allowing the combination of superior traits from multiple species while maintaining heterozygosity, which is essential for long-term population stability. This genetic diversity enhances the adaptability of species and increases the chances of producing hybrids with desirable ornamental and adaptive characteristics (Li et al. 2024). Molecular studies have shown that high heterozygosity in plant populations contributes to hybrid vigor or heterosis, which is a key factor for the success of many breeding programs (Chou et al. 2017). In orchid breeding, genetic variation within and among Dendrobium species provides valuable parental material for interspecific hybridization, allowing the creation of hybrids that combine unique floral traits, fragrance, and stress tolerance (Wegadara et al. 2022; Dwiyani et al. 2024). Furthermore, SSR markers have been proven to be efficient in evaluating such diversity, offering co-dominant inheritance, high reproducibility, and resolution in detecting polymorphisms across individuals and populations (Song et al. 2022).

Therefore, SSR data from Bali Dendrobium provide a solid scientific basis for supporting both in situ conservation and the development of hybrid varieties that meet global and local market preferences. The application of SSR-based molecular characterization ensures that conservation strategies are guided by accurate assessments

of genetic relationships, avoiding redundancy in germplasm preservation and maximizing the genetic potential for hybrid production (Dwiyani et al. 2024). Ultimately, this research contributes to the sustainable utilization of Bali's native orchid resources, bridging the gap between molecular data and applied breeding for the enhancement of Indonesia's orchid industry.

Genetic clustering patterns and ecological or geographic influence

UPGMA dendrogram analysis of 24 native Bali *Dendrobium* species grouped the species into four main clusters (Figure 1). Cluster II, which includes *D. crumenatum* and *D. plicatile*, is the largest group, encompassing the majority of the analyzed species. This pattern indicates a high level of genetic similarity among species, likely resulting from shared evolutionary history or ecological adaptation to similar habitat conditions. These findings are consistent with Qi et al. (2023), who emphasized that environmental factors such as light availability, humidity, and substrate type shape population structure and genetic distribution in wild orchids. The high genetic similarity within this cluster also suggests that interspecific hybridization is likely to be successful, as close genetic relationships generally facilitate crossing and produce stable progeny (Yin et al. 2023).

In contrast, Cluster IV contains only *D. fimbriatum*, which exhibits a unique genetic profile distinct from the other clusters. This uniqueness is likely due to geographic isolation or specific selective pressures experienced by this population. The outlier status of this species is important from a conservation perspective, as genetically unique species carry alleles not found in other species. Loss of this species could significantly reduce local genetic diversity (Zakiyah et al. 2019; Hartati et al. 2023). The presence of unique species highlights the importance of conservation strategies that focus on protecting rare or isolated populations while also providing new genetic resources for breeding and hybridization programs.

Principal Coordinates Analysis (PCoA) supports the dendrogram results, showing that most individuals cluster according to their respective groups, although some overlap occurs among subclusters (Figure 2). This overlap indicates a continuum of genetic variation among species, which can be utilized in selective hybridization programs. By combining alleles from genetically related but distinct individuals, breeders can produce hybrids that combine superior traits such as environmental stress tolerance, flower morphology, and color while maintaining high levels of heterozygosity (Basavaraj et al. 2022).

Regional comparisons reveal similar patterns in wild *Dendrobium* populations in Thailand and Malaysia, where geographic distance, habitat isolation, and ecological conditions strongly influence genetic similarity among populations (Katengam et al. 2023). These findings underscore the critical role of sampling locations in Bali in shaping local genetic structure. Therefore, population collection for conservation or hybridization programs should consider ecological and geographic distribution to maintain optimal genetic variation.

High genetic similarity coefficients, such as those observed between *Dendrobium* sp. Wanagiri and Sepang at 0.97, indicate a high probability of successful interspecific crosses, as genetic proximity facilitates meiotic compatibility and progeny stability. Conversely, genetically distant species such as *D. fimbriatum* have the potential to introduce novel traits or unique alleles into hybrid populations, although such crosses require careful hybrid management to avoid genetic incompatibility or reduced fertility. This approach is important for developing new varieties with superior agronomic and aesthetic traits while preserving the original genetic diversity of Bali orchids.

Practical implications for conservation and hybridization

The high genetic diversity observed in 24 Bali *Dendrobium* species, as revealed by SSR analysis (Table 1) and visualization of SSR band polymorphism (Figure 3), has important implications for both conservation and hybrid breeding. High genetic diversity allows breeders to improve aesthetic traits, environmental stress tolerance, and adaptive capacity of Bali orchids without reducing heterozygosity (Yuhanna et al. 2021). Genetically diverse populations can be selected as potential parental candidates for hybrid crosses.

From a breeding perspective, species with high genetic similarity, such as *Dendrobium* sp. Wanagiri and Sepang, are likely to be more compatible in interspecific crosses (Nguyen et al. 2020). Such crosses minimize the risk of genetic incompatibility and allow the production of stable progeny with superior traits, including flower morphology and color, stress resilience, and longer flower longevity. Conversely, genetically distant species, such as *D. fimbriatum*, have the potential to introduce new alleles or traits, but careful hybrid management is required to avoid incompatibility or reduced fertility (Tikendra et al. 2021). This approach is important for expanding the genetic base of hybrids, increasing phenotypic variation, and developing new varieties that are both adaptive and commercially attractive.

From a conservation perspective, populations with narrow genetic distances require special protection to prevent the loss of genetic variation, while unique or isolated species should be prioritized to preserve natural diversity (Tran et al. 2022). Genetic-based conservation strategies are essential for maintaining long-term adaptive potential and ecosystem resilience.

Overall, SSR genotyping has proven effective in revealing polymorphism, clustering patterns, and identifying outlier species. These findings support the integration of conservation and breeding strategies, enabling the development of new hybrids from diverse populations while protecting unique species. Consequently, Bali orchids can be utilized sustainably while enhancing their competitiveness in both national and international markets.

In conclusion, this study revealed that Bali's 24 native *Dendrobium* species exhibit high genetic diversity, with SSR markers detecting substantial polymorphism and allelic richness. The closest genetic relationship was

observed between *Dendrobium* sp. Sepang and *Dendrobium* sp. Wanagiri, while *D. fimbriatum* was the most genetically distinct. These patterns highlight both the potential for successful interspecific hybridization among closely related species and the opportunity to introduce novel traits from genetically distant species. The findings underscore the practical value of SSR markers for guiding breeding programs and selecting compatible parental species to develop superior hybrids with enhanced adaptability and commercial appeal. Moreover, the observed genetic variation emphasizes the importance of conserving wild populations, particularly unique or isolated species, to maintain a resilient germplasm pool. Future efforts should focus on integrating molecular data with physiological and ecological studies, as well as conducting multi-location trials to ensure stable hybrid performance and effective conservation strategies. Overall, this research provides a solid foundation for both sustainable management of Bali's *Dendrobium* biodiversity and the advancement of orchid breeding programs.

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