BIODIVERSITAS
Volume 27, Number 2, February 2026
Pages: 7

ISSN: 1412-033X
E-ISSN: 2085-4722
https://doi.org/10.13057/biodiv/d270223

Genetic diversity and mutant detection of *Dendrobium bicaudatum* using SRAP markers

IDA AYU PUTRI DARMAWATI[1,♥], YUYUN FITRIANI[1], GEDE WIJANA[1], WAFA' NUR HANIFAH[1], RINDANG DWIYANI[2], NI LUH MADE PRADNYAWATHI[1], FITRIA DWI DARMAYATI[2]

[1]Department of Agroecotechnology, Faculty of Agriculture, Universitas Udayana. Jl. PB. Sudirman, Denpasar 80231, Bali, Indonesia.
Tel.: +62-361-702801, Fax.: +62-361-703602, ♥email: darmawati@unud.ac.id
[2]Graduate Program of Agroecotechnology, Faculty of Agriculture, Universitas Udayana. Jl. PB. Sudirman, Denpasar 80231, Bali, Indonesia

Manuscript received: 11 May 2025. Revision accepted: 20 February 2026.

Abstract. *Darmawati IAP, Fitriani Y, Wijana G, Hanifah WN, Dwiyani R, Pradnyawathi NLM, Darmayati FD. 2026. Genetic diversity and mutant detection of* Dendrobium bicaudatum *using SRAP markers. Biodiversitas 27 (2): d270223. https://doi.org/10.13057/biodiv/d270223*. Natural occurring mutations have been an important source of genetic diversity and new phenotypes for ornamental plants. However, the occurrence rate is relatively low for most plants, including *Dendrobium bicaudatum*. Mutation induction triggers mutations in plants and can be achieved through physical or chemical methods. One widely used chemical is Ethyl Methane Sulphonate (EMS). Molecular techniques can detect genetic changes in mutated orchids early, without the need for the plants to mature. Thus, this study aimed to investigate genetic variation and detect mutants in *D. bicaudatum* orchids following EMS treatment using Sequence Related Amplified Polymorphism (SRAP). This study used four SRAP primer combinations. The DNA concentration of sample B2.Chimera approaches purity, indicated by an A160/280 ratio of 1.67. The results indicated that the four primers exhibited high orchid polymorphism, ranging from 91% to 100%. A total of 47 loci and 258 DNA bands were generated using four primers, yielding a PIC value of 0.32-0.37. The high level of polymorphism detected by SRAP indicates genetic diversity in mutant plants, which importantly contributes to conservation. The analysis of genetic similarity via dendrograms revealed two primary clusters at a coefficient of 0.23: one comprising albino explants and the other consisting of non-albino explants. This result suggests that albino mutants possess genetic traits that significantly differ from those of non-albinos. Non-albino explants, including chimeras and normals, exhibited high similarity coefficients ranging from 0.63 to 0.96.

Keywords: Dendogram, EMS, mutation, orchid, SRAP

INTRODUCTION

Dendrobium bicaudatum Reinw. Ex Lindl. is one of the many endemic orchid species found in the lowland forests of Indonesia, specifically in the Sulawesi and Maluku region (Mustaqim and Astuti 2019; Puspaningtyas 2019). Other epiphytic orchid species, namely *Dendrobium concinnum* Miq., *D. Crumenatum* Sw., *D. bellatulum* Rolfe, *D. christyanum* Rchb.f., and *D. scabrilingue* Lindl., are commonly found in Southeast Asia, including Indonesia, Laos, Vietnam, and Cambodia (Averyanov et al. 2016; Setiawan et al. 2024). Although this species is widely distributed in lowland forests; rapid deforestation, habitat destruction, overexploitation, and poor seed characteristics of *D. bicaudatum* may reduce its natural population. The limited distribution of this species is due to the germination of *D. bicaudatum* seeds, which requires specific environmental conditions and habitats (Aprilianti et al. 2021). Several endemic *Dendrobium* species in mid-elevation areas of Sabah (Borneo) are currently endangered, requiring conservation efforts both in situ and ex situ (Juiling et al. 2020).

Dendrobium bicaudatum can serve as a genetic resource for producing diverse hybrid orchid varieties. The mutants that form can broaden genetic diversity and even create varieties with new desirable traits, such as high adaptability. Breeding orchids through crosses, such as hybrid *Dendrobium*, typically takes 3-5 years to develop new cultivars due to their extended juvenile period. Mutations in nature contribute to genetic diversity and the emergence of new phenotypes in ornamental plants; however, the mutation rate remains relatively low. Diversity enhancement and the development of new varieties can be achieved not only through conventional crosses but also through unconventional methods such as mutation induction.

Dendrobium bicaudatum can be used as a genetic resource to produce diverse hybrid orchid varieties. The mutants that are formed can widen genetic diversity, and even create varieties with new desirable traits such as high adaptability. Breeding orchids through crosses, such as hybrid *Dendrobium* orchids, requires a considerable amount of time, typically 3-5 years, to develop new cultivars due to their extended juvenile period. Mutations in nature contribute to genetic diversity and the emergence of new phenotypes in ornamental plants; however, the mutation rate remains relatively low. Diversity enhancement and the development of new varieties can be achieved not only through conventional crosses but also through unconventional methods such as mutation induction.

Mutation induction serves as an alternative method for rapidly generating new plant variants. This method enables

plant breeders to transform existing varieties into preferred ones. Mutation induction can be done physically or chemically. Physical mutation utilizes gamma or beta radiation. Common chemical mutagens include colchicine for chromosome doubling, as well as nitric acid (HNO_2), hydroxylamine (NH_2OH), Methyl Methane Sulphonate (MMS), and Ethyl Methane Sulphonate (EMS).

Chemical mutagens, such as EMS, are highly effective at inducing mutations in the plant genome without causing massive deletions (Martha et al. 2023; Türkoğlu et al. 2023). EMS induction has successfully increased plant genetic diversity in various crops. Some of the mutation studies utilizing EMS in ornamental plants are bougainvillea (Anitha et al. 2017), jasminum (Ghosh and Ganga 2019), gladiolus (Tirkey and Singh 2019), amaryllis (Xiong et al. 2020), chrysanthemum (Nasri et al. 2022), and marigold (Lenawaty et al. 2022). The application of EMS treatment to orchid plants is a widely utilized method for enhancing genetic diversity.

The diversity of orchids produced through chemical mutation techniques is evident in the plantlets, which may exhibit albino, chimera, or normal (unchanged) appearances. Despite the plantlets appearing normal, mutations may arise. The variation observed in plantlets primarily affects the flowers, but it takes up to 2 years to be apparent. The time required to observe phenotypic and genetic diversity underscores the need for molecular analysis to assess genetic diversity without waiting for plant maturation. Genetic diversity in *Dendrobium* mutants can be assessed through Polymerase Chain Reaction (PCR) employing various marking techniques, including Inter-Simple Sequence Repeat (ISSR) (Khairum et al. 2022), Sequence-Related Amplified Polymorphism (SRAP) (Chueakhunthod et al. 2023), Random Amplified Polymorphic DNA (RAPD) (Sherpa et al. 2022; Jyothsna et al. 2024), and Single-Nucleotide Polymorphism (SNP). SRAP marking systems are recognized for their speed, cost-effectiveness, and efficiency in analyzing genetic diversity, constructing maps, marking genes, and facilitating map-based cloning (Kaewpongumpai et al. 2016). SRAP markers are widely used in agronomy for Quantitative Trait Loci (QTL) studies and biodiversity assessments, including genetic diversity analyses, in germplasm collections, and are recognized as among the most effective molecular markers for these applications (Robarts and Wolfe 2014; Zheng et al. 2017). SRAP evaluates genetic diversity rapidly by amplifying multiple loci simultaneously, making it suitable for screening early generations of mutant populations (M1-M2). Based on these considerations, SRAP markers were selected for strategic reasons of cost efficiency and high throughput. This research aimed to improve the genetic diversity of *D. bicaudatum* through EMS treatment and to identify mutant variants using SRAP.

MATERIALS AND METHODS

Dendrobium mutation induction with EMS

Orchid protocorms were cultivated on MS media and treated with Ethyl Methane Sulphonate (EMS) at concentrations of 0, 2, 4, 6, and 8 mL^{-1}. This study employed a non-factorial, Completely Randomized Design (CRD) consisting of five replicates.

SRAP marker analysis of *D. bicaudatum* mutants

DNA isolation

DNA from orchids was isolated using the Wizard Genomic DNA Purification Kit (Promega 2015). Approximately 20 mg of the sample was ground with a pestle until a smooth consistency was achieved, then transferred to a 1.5 mL tube and 300 µL of Tail Lysis Buffer (TLA) was added. Samples were incubated at 65°C for 30 minutes. Subsequently, 300 µL of RNAse solution was added to the sample, and the mixture was vortexed for 10 minutes to ensure complete mixing. The next step was to incubate the sample at 37°C for 30 minutes. Subsequently, 200 µL of protein precipitation solution was added to the sample and mixed thoroughly using a vortex. The sample was cooled on ice for 5 minutes. Furthermore, the sample was centrifuged for 4 minutes at 13,000 rpm to precipitate. The supernatant formed was transferred to a new tube, and isopropanol was added. Isopropanol and supernatant were mixed by inverting. After mixing, the sample was centrifuged for 1 minute at 13,000 rpm. The supernatant formed was discarded. The sample was mixed with 600 µL of 70% ethanol, then re-centrifuged. The pellets were dried for 10 minutes and mixed with 20 µL of rehydration solution. Next, DNA rehydration was carried out at 65°C for 1 hour. The final stage involves storing the sample in the freezer. The concentration and purity of mutant orchid DNA were measured by quantifying the DNA stock with a Gene Quant 1300 spectrophotometer. DNA purity against contamination was measured by light absorbance at 260/280 nm (A260/280) and 260/230 nm (A260/230).

DNA amplification by PCR

DNA amplification was performed using PCR with four SRAP primer combinations: Me3-Em2, Me4-Em2, Me4-Em3, and Me4-Em4 (Table 1). PCR was performed on a total volume of 20 µL of mixture containing 12 µL master mix (consisting of 2 µL dNTPmix containing dATP, dTTP, dGTP, and dCTP; 2 µL Taq polymerase buffer; 1.5 µL MgCl2; 0.5 µL Taq polymerase; 0.5 µL glycerol; 5.8 µL ddH2O), 6 µL primers, and 2 µL DNA. The PCR reaction was performed for 35 cycles. First heating at 94°C for 5 minutes, followed by 35 cycles consisting of 1 minute denaturation at 94°C, 3 minutes annealing at 35°C, and 2 minutes extension at 72°C. After 35 cycles were completed, followed by 7 minutes final extension at 72°C and cooling for 30 minutes. The amplification results were evaluated by electrophoresis on a 2.0% agarose gel in TAE (Tris-Acetate EDTA) buffer for 50 minutes at 50 V. The sample was then immersed in an ethidium bromide solution at a final concentration of 15 µL per 100 mL for 10 minutes. The separation of DNA fragments was detected and visualized using a UV transluminator, followed by photography with a Nikon D3400 DSLR camera. As a standard, a 1 kb DNA ladder (Fermentas) was used to determine the band sizes of the DNA amplification results.

Table 1. SRAP primers used for PCR

Primer	Sequences (5'-3')	Tm (°C)	Reference
Me3-Em2			
Forward	TGAGTCCAAACCGGAAT	50º	Cornea-Cipcigan et al. 2023
Reverse	GACTGCGTACGAATTTGC		
Me4-Em2			
Forward	TGAGTCCAAACCGGACC	50º	Cornea-Cipcigan et al. 2023
Reverse	GACTGCGTACGAATTTGC		
Me4-Em3			
Forward	TGAGTCCAAACCGGACC	50º	Cornea-Cipcigan et al. 2023
Reverse	GACTGCGTACGAATTGAC		
Me4-Em4			
Forward	TGAGTCCAAACCGGACC	50º	Cornea-Cipcigan et al. 2023
Reverse	GACTGCGTACGAATTTGA		

Data analysis

The analysis of data obtained from electrophoresis, represented by DNA banding patterns, commenced with data scoring. DNA banding profiles were converted into binary data, assigning a value of 0 for the absence of a band and a value of 1 for the presence of a DNA band at corresponding positions within the orchid type. The clustering analysis and dendrogram construction were performed using the Unweighted Pair-Group Method with Arithmetic means (UPGMA) via the Numerical Taxonomy and Multivariate System (NTSYS) program Version 1.80.

RESULTS AND DISCUSSION

DNA concentration and purity

Genetic diversity in *Dendrobium bicaudatum* mutants treated with EMS relative to the control was analyzed using SRAP markers. The results of DNA concentration (Table 2) indicate that the DNA concentration obtained in this study ranged from 100 ng/uL to 500 ng/uL. The concentration was adequate for dilution to achieve a working DNA concentration of 25 ng/uL. The purity of DNA can be assessed from the absorbance ratios A260/280 and A260/230, which range from 1.8 to 2.0-2.2 (Bunu et al. 2020). The A260 wavelength indicates nucleic acid concentration, A280 indicates protein concentration, and A230 indicates the concentration of contaminants (Faraldi et al. 2022).

Sample B2.Chimera exhibited the highest DNA purity, indicated by an A260/280 ratio of 1.67. This value is close to the ideal absorbance of 1.8 with a purity ratio between 1.6-2.0 (1.8±0.2). A value of 1.6 or less suggests the presence of contaminants, such as proteins (Lucena-Aguilar et al. 2016). The findings of this study indicate that samples B3.Chimera, B6.Normal, and B6.Albino exhibit purity level values exceeding 1.8. The A260/230 ratio is commonly used as an additional indicator to assess DNA purity (Lucena-Aguilar et al. 2016). The A260/230 DNA purity measurement indicated that all plants had values outside the optimal range of 2.0-2.2. Specifically, all samples had A260/230 ratios between 0.5 and 1.0, suggesting contamination. Lower A260/280 values may indicate the presence of protein or phenolic compounds, which are common in orchid tissues. The high polysaccharide content in *Dendrobium* is associated with contamination, as indicated by the low A260/230 ratio. The study by Wang et al. (2024) describes the high polysaccharide content and the presence of proteins with diverse profiles across eight *Dendrobium* species. Despite this, the extracted DNA was successfully amplified, indicating that residual contaminants did not inhibit PCR reactions.

Amplification products using SRAP primers

The bands generated from each orchid sample using the four primers generated a total of 47 loci and 258 DNA bands (Figure 1, Table 3. A total of 46 polymorphic loci were identified, indicating that nearly all loci exhibit genetic variation. The polymorphic loci percentage using SRAP markers on *D. bicaudatum* mutant orchids was 100% for three marker pairs (Me3-Em2, Me4-Em2, Me4-Em3) and 91% for one marker pair (Me4-Em4). A total of 11 loci were analyzed using Me4-Em4 primers, of which 10 were polymorphic, and one was monomorphic.

Table 2. DNA concentration and purity in orchid mutants

Sample name	Sample code	DNA concentration (ng/uL)	A260/280	A260/230
Dendrobium bicaudatum Control	B.Control	300	1.50	1.00
Dendrobium bicaudatum 1 Chimera	B1.Chimera	300	1.00	0.75
Dendrobium bicaudatum 1 Albino	B1.Albino	200	1.00	0.67
Dendrobium bicaudatum 2 Normal	B2.Normal	100	1.00	1.00
Dendrobium bicaudatum 2 Chimera	B2.Chimera	500	1.67	1.00
Dendrobium bicaudatum 2 Albino	B2.Albino	300	1.50	0.60
Dendrobium bicaudatum 3 Chimera	B3.Chimera	300	3.00	1.00
Dendrobium bicaudatum 3 Albino	B3.Albino	200	1.00	0.50
Dendrobium bicaudatum 4 Chimera	B4.Chimera	300	1.50	0.75
Dendrobium bicaudatum 6 Normal	B6.Normal	500	2.50	1.00
Dendrobium bicaudatum 6 Chimera	B6.Chimera	200	1.50	0.50
Dendrobium bicaudatum 6 Albino	B6.Albino	400	4.00	0.80
Dendrobium bicaudatum 8 Normal	B8.Normal	300	1.50	1.00
Dendrobium bicaudatum 8 Chimera	B8.Chimera	300	1.00	1.00

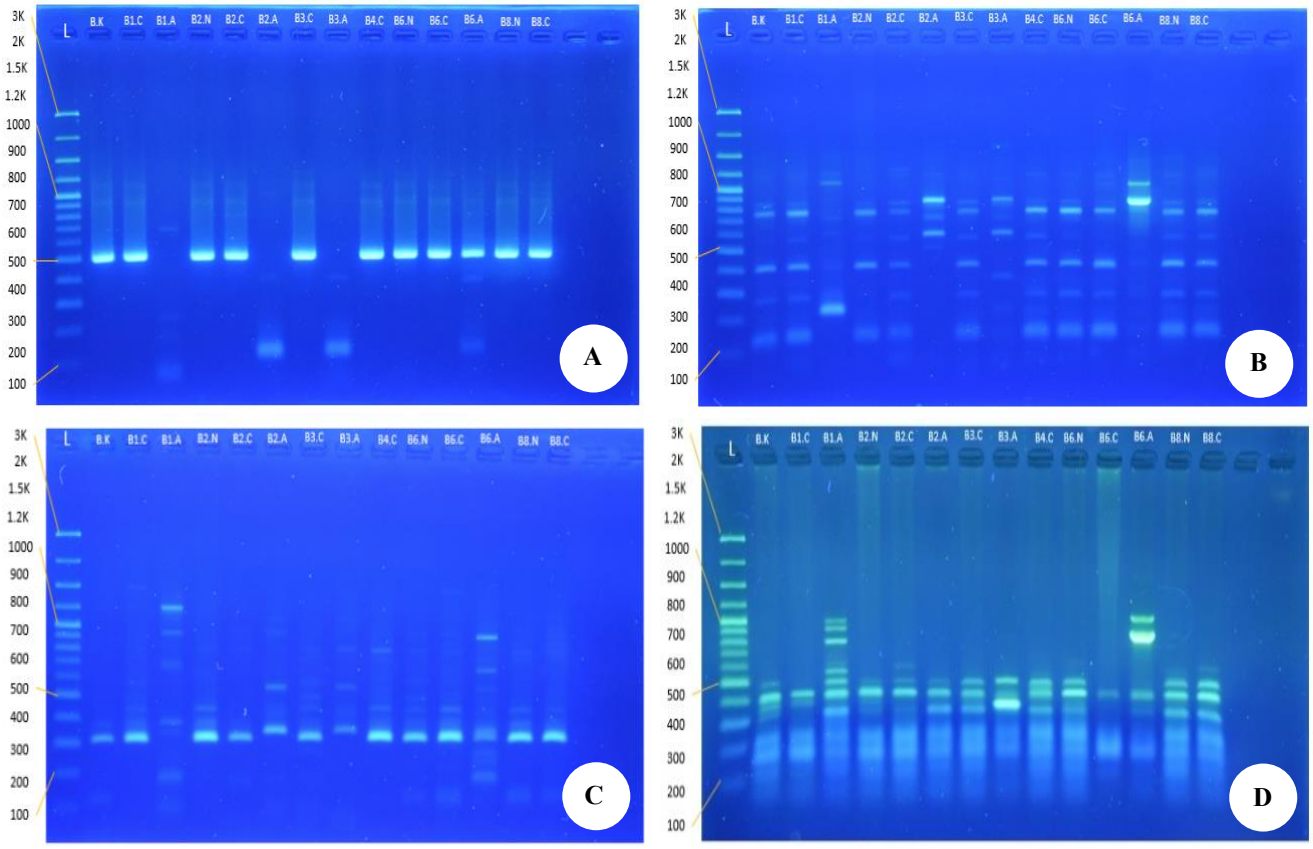

Figure 1. DNA profile of *Dendrobium bicaudatum* mutants using 4 SRAP primers. A. Me3-Em2, B. Me4-Em2, C. Me4-Em3, D. Me4-Em4

Table 3. Number of loci, percentage of polymorphic loci, number of DNA bands, and PIC in *Dendrobium bicaudatum* mutants resulting from SRAP amplification

Primer	Number of loci	Number of polymorphic loci	Percentage of polymorphic loci	Number of DNA bands	PIC
Me3-Em2	7	7	100	40	0,37
Me4-Em2	17	17	100	79	0,35
Me4-Em3	12	12	100	48	0,32
Me4-Em4	11	10	91	91	0,37

This finding aligns with the research by Türkoğlu et al. (2023), which demonstrated that EMS influences the production level of DNA polymorphism through mutations caused by the loss or deletion of chromosomal regions. High levels of polymorphism indicate segregation and genomic variation in mutant plants (Arumingtyas et al. 2023). EMS can induce G/C to A/T transition variably depending on the genetic composition and/or genomic features of different species (Yan et al. 2021). Genomic changes arise from the emergence of new alleles or the loss of existing ones, leading to DNA fragment damage, chromosomal rearrangements, and nucleotide modifications (Keawsaard et al. 2025).

This study demonstrates that SRAP primers can reveal substantial polymorphism. The SRAP markers employed in the research by Purwantoro et al. (2023) successfully identified genetic variations in hybrid orchids, demonstrating a polymorphism level of 96.24%. This result differs from the research by Khairum et al. (2022) on the *Dendrobium* 'Earsakul' mutant, which showed an average polymorphism percentage of 28.94% and a Polymorphism Information Content (PIC) of 0.19 using ISSR markers. Keawsaard et al. (2025) investigated Toothbrush Orchid mutants and found a polymorphism rate of only 50% using SSR markers. SRAP markers provide genome-wide coverage and are non-specific, making them complementary to markers that target one or a few loci, such as SSR and SNP markers (Zagorcheva et al. 2020).

Technical factors in molecular marker assays also affect amplification results. SRAP markers amplify the Open Reading Frames (ORFs) region of the genome using paired primers (forward and reverse), in contrast to SSR and ISSR markers that target the microsatellite region. This approach yields a greater variety of functional gene information and

polymorphism characterization in SRAP (Kaewpongumpai et al. 2016). However, the SRAP marker is also sensitive to plants with low levels of polymorphic loci and can detect genetic diversity at the genome level (Feng et al. 2014). All primers yield more DNA bands than loci (Table 3), indicating polymorphism or genetic diversity at each locus. PIC serves as an indicator that quantifies the frequency of alleles at a single locus or across multiple loci (Zheng et al. 2017). The standard PIC value for assessing genetic markers is categorized into three groups: PIC<0.25 (low), 0.25<PIC<0.5 (medium), and PIC>0.5 (very informative) (Dalimunthe et al. 2020).

SRAP, as a dominant marker type, can detect only dominant alleles and cannot differentiate between homozygous and heterozygous genotypes (Purwantoro et al. 2023). This dominant marker type has a maximum PIC value of 0.5 (Chesnokov and Artemyeva 2015). The PIC values for the four SRAP markers in *D. bicaudatum* ranged from 0.32 to 0.37, indicating that the primers have sufficient informativeness to serve as molecular markers. The SRAP markers that provided the highest genetic information were Me3-Em2 and Me4-Em4 primers. Chueakhunthod et al. (2023) suggest that the elevated PIC value may result from the characteristics of SRAP markers, which specifically identify ORFs. This indicates the markers' suitability for plant breeding, particularly in mutant selection. The SRAP method applied to 31 *Dendrobium* specimens demonstrated significant genotypic diversity, yielding an average PIC value of 0.987 (Feng et al. 2014). Many polymorphic SRAP fragments or markers can be used as a cost-effective and efficient tool for developing preliminary genetic maps and initiating selection-based breeding programs (Zagorcheva et al. 2020). High variability within species indicates genetic diversity, offering significant opportunities for plant breeding and contributing to in situ and ex situ conservation. Genetic diversity in a population can help improve a species' adaptation to environmental changes and pathogen infections. DeWoody et al. (2021) highlighted that genetic diversity assessment is important for identifying species on the brink of extinction that require conservation action.

Morphological characteristics

This observed genetic diversity underlies the physiological changes in mutants. Various concentrations of EMS can influence the growth and developmental responses of the explants, including chloroplast development. Certain mutant explants exhibit normal growth, producing green leaves and shoots, while others display abnormal growth and fail to generate new shoots (Martha et al. 2023). The physiological changes manifested from these molecular variations are the formation of albino and chimera phenotypes (Figure 2). Plant albinism is characterized by a partial or complete lack of pigmentation in the leaves or other plant tissues, caused by several factors, including deletions in plastid DNA, nuclear-plastid genome incompatibility, or mutations in genes related to chlorophyll biogenesis (Kumari et al. 2009). This mutation inhibits chlorophyll synthesis, resulting in the absence of green pigment in the leaves.

Chimera plants in this study exhibit a combination of green and albino leaf tissue within a single individual. Chimeras form when mutations occur in cells of the shoot apical meristem (SAM), which consists of three layers: L1 (epidermis), L2 (sub-epidermis), and L3 (corpus). These changes then appear as distinctive color patterns on plants (Amundson et al. 2025). Based on the structure of the mutated cell layers, chimeras are classified into sectoral, mericlinal, and periclinal types (Frank and Chitwood 2016). The results of this study indicate the presence of sectoral chimeric mutations in plant tissues (Figure 2.B). A study by Park et al. (2023) shows the mutation types in chimera plants from the Orchidaceae family, namely *Cymbidium hybrid* (sectorial) and *Cymbidium sinense* (periclinal), as well as in several ornamental plants.

Genetic similarity analysis through dendrogram

The four SRAP markers used in the study can detect genetic diversity among mutant samples, especially in albino samples. Dendrogram analysis (Figure 3) visualizes genetic similarity among the samples. The Jaccard coefficient was used as the similarity measure, along with the UPGMA clustering method. Clustering of samples was seen from the branching formed. A smaller coefficient at the branching point (close to zero) indicates a lower similarity of the sample to other samples. The coefficient range was from 0.00 to 1.00. A higher coefficient value between samples, approaching 1, indicates greater similarity.

Figure 2. Morphological characteristics of *Dendrobium bicaudatum*: A. Normal, B. Chimera, and C. Albino

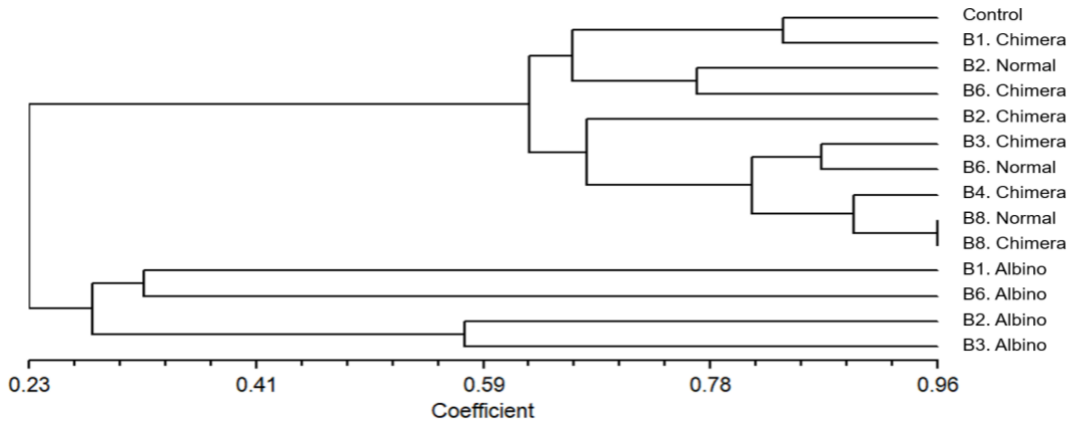

Figure 3. Dendrogram of *Dendrobium bicaudatum* using 4 SRAP primers

Dendrobium bicaudatum mutants exhibit three distinct morphological characteristics: normal, chimera, and albino (Figure 2). The diversity of characteristics is not only evident in morphology but also at the molecular level. The genetic similarity cluster analysis of mutants reveals two primary clusters: one comprising albino mutants and the other non-albino mutants, with a genetic similarity coefficient of 0.23. Clusters I and II had similarity coefficients of 0.27 and 0.63, respectively. Four albino *D. bicaudatum* explants, out of ten non-albino explants, demonstrated that the EMS-mutated albino explants exhibited a significant genetic divergence from the non-albino mutant explants. Ten non-albino explants exhibited clustering at various positions, without correlation with the EMS treatment administered. This result aligns with the statement by Chueakhunthod et al. (2023) that EMS enhances genetic diversity under in vitro conditions, which is advantageous for *Dendrobium* breeding programs. The application of SRAP primers facilitates the production of high polymorphism, which is beneficial for identifying genetic differences among the samples analyzed in this study (Zheng et al. 2017). The explants exhibiting the closest genetic fingerprint profiles were B8. Normal and B8.Chimera, with a similarity coefficient of 0.96. However, these two explants were already separated from the non-mutant explants at a coefficient of 0.63. This demonstrates that SRAP can identify nucleotide base changes present in sample sequences.

In conclusion, *D. bicaudatum* mutants induced by EMS treatment exhibited phenotypic variations, particularly in plantlet coloration: green (normal), chimera, and albino. Molecular analysis identified phenotypic and genotypic differences between mutant plant samples. The results of this study indicate that molecular analysis of *D. bicaudatum* mutants using SRAP markers effectively provides information on genetic diversity among plant samples, as evidenced by high polymorphism levels and PIC values. This can serve as a valuable resource in plant breeding. High genetic diversity can increase germplasm and conservation opportunities, as well as create species that are adaptable to environmental change. The dendrogram also shows two main clusters: cluster I, comprising albino mutants, and cluster II, comprising non-albino mutants (normal and chimera), with a distance coefficient of 0.23. This indicates that the genetic characteristics of albinos are significantly different from those of non-albinos.

ACKNOWLEDGEMENTS

The author acknowledges LP2M UNUD for providing research funding through DIPA PNBP Universitas Udayana, Denpasar, Bali, Indonesia, Year 2023, as per the Research Implementation Assignment Agreement Letter B/1.471/ UN14.4.A/PT.01.03/2023, dated May 2, 2023.

REFERENCES

Anitha K, Surendranath R, Jawaharlal M, Ganga M. 2017. Mutagenic effectiveness and efficiency of gamma (γ) rays and ethyl methane sulphonate on *Bougainvillea spectabilis* Willd. (cv. Lalbagh). Intl J Bio-Resour Stress Manag 8 (2): 247-256.

Aprilianti P, Handini E, Puspaningtyas DM. 2021. A seed morphometry study of selected species of *Bulbophyllum* and *Dendrobium* (Orchidaceae) in relation to their dispersals. Biodiversitas 22 (12): 5564-5571. https://doi.org/10.13057/biodiv/d221241.

Amundson KR, Marimuthu MPA, Nguyen O, Sarika K, DeMarco IJ, Phan A, Henry IM, Comai L. 2025. Spatial variation in the mutation rate within the plant shoot apical meristem. Proc Nat Acad Sci 122 (46): 1-9. https://doi.org/ 10.1073/pnas.2514507122.

Arumingtyas EL, Atiaturrochmah, Kusnadi J. 2023. Confirmation of mutation and genetic stability of the M4 generation of chili pepper's (*Capsicum frutescens* L.) Ethyl Methane Sulfonate (EMS) mutant based on morphological, physiological and molecular characters. Biodiversitas 24 (1): 531-538. https://doi.org/10.13057/biodiv/d240162.

Averyanov LV, Ponert J, Nguyen PT, Duy NV, Khang NS, Nguyen VC. 2016. A survey of *Dendrobium* Sw. sect. Formosae (Benth. & Hook.f.) Hook.f. in Cambodia, Laos and Vietnam. Adansonia 38 (2): 199-217. https://doi.org/10.5252/a2016n2a5.

Bunu SJ, Otele D, Alade T, Dodoru RT. 2020. Determination of serum DNA purity among patients undergoing antiretroviral therapy using Nanodrop-1000 Spectrophotometer and Polymerase Chain Reaction. Biomed Biotechnol Res J 4 (3): 214-219. https://doi.org/10.4103/bbrj.bbrj_68_20.

Chesnokov YV, Artemyeva AM. 2015. Evaluation of the measure of polymorphism information of genetic diversity. Agric Biol 50 (5): 571-578. https://doi.org/ 10.15389/agrobiology.2015.5.571eng.

Chueakhunthod W, Khairum A, Jaukwon P, Chantakot T, Woramit T, Hualsawat T, Tantasawat PA. 2023. Sequence-related amplified polymorphism and target region amplification polymorphism markers-based profiling of sodium azide and ethyl methanesulfonate-derived

black rot-resistant *Dendrobium* sp. 'Earsakul' mutants from in vitro mutagenesis and selection. HortScience 58 (12): 1465-1473. https://doi.org/10.21273/hortsci17350-23.

Cornea-Cipcigan M, Pamfil D, Sisea CR, Margaoan R. 2023. Characterization of *Cyclamen* genotypes using morphological descriptors and DNA molecular markers in a multivariate analysis. Front Plant Sci 14: 1100099. https:// 10.3389/fpls.2023.1100099.

Dalimunthe SR, Siregar LAM, Putri LAP, Chairunnisa T, Hairmansis A. 2020. Polymorphism levels of some SSR markers (Simple Sequence Repeat) for parental line identification on low temperature tolerance. IOP Conf Ser: Earth Environ Sci 454: 012165. https://doi.org/10.1088/1755-1315/454/1/012165.

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. https://doi.org/10.13057/asianjagric/g090200.

DeWoody JA, Harder AM, Mathur S, Willoughby JR. 2021. The long-standing significance of genetic diversity in conservation. Mol Ecol 30 (17): 4147-4154. https://doi.org/10.1111/mec.16051.

Faraldi M, Mangiavini L, Conte C, Banfi G, Napoli N, Lombardi G. 2022. A novel methodological approach to simultaneously extract high-quality total RNA and proteins from cortical and trabecular bone. Open Biol 12 (5): 210387. https://doi.org/10.1098/rsob.210387.

Feng S-G, Lu J-J, Gao L, Liu J-J, Wang H-Z. 2014. Molecular phylogeny analysis and species identification of *Dendrobium* (Orchidaceae) in China. Biochem Genet 52: 127-136. https://doi.org/10.1007/s10528-013-9633-6.

Frank MH, Chitwood DH. 2016. Plant chimeras: The good, the bad, and the 'Bizzaria'. Develop Biol 419 (1): 41-53. https://doi.org/10.1016/j.ydbio.2016.07.003.

Ghosh S, Ganga M. 2019. Determination of lethal dose for ethyl methane sulphonate induced mutagenesis in jasmine. Chem Sci Rev Lett 8 (29): 6-10.

Juiling S, Leon SK, Jumian J, Tsen STL, Lee YL, Khoo E, Sugau JB, Nilus R, Pereira JT, Damit A, Tanggaraju S, O'Byrne P, Sumail S, Mujih H, Maycock CR. 2020. Conservation assessment and spatial distribution of endemic orchids in Sabah, Borneo. Nat Conserv Res 5: 136-144. https://doi.org/10.24189/ncr.2020.053.

Jyothsna BS, Dey S, Venkataramanan S, Hallur RLS, Srivastava D. 2024. Molecular, morphological, and biomolecular characterization of ethyl methanesulfonate-induced mutations in *Aerides odoratum*, an orchid. J Appl Biol Biotechnol 12 (4): 136-143. https://doi.org/10.7324/jabb.2024.178195.

Kaewpongumpai S, Poeaim S, Vanijajiva O. 2016. Sequence-Related Amplified Polymorphism (SRAP) analysis for studying genetic characterization of *Bouea macrophylla*. Biodiversitas 17 (2): 539-543. https://doi.org/10.13057/biodiv/d170224.

Keawsaard Y, Pentem P, Rotduang P, Weeraphong M, Ramasoot S. 2025. Induction of mutation in yoothbrush orchids using Ethyl Methane Sulphonate (EMS) and detection of genetic variation by SSR (Simple Sequence Repeat) marker. Asean J Sci Technol Rep 28 (1): e255125. https://doi.org/10.55164/ajstr.v28i1.255125.

Khairum A, Hualsawat S, Chueakhunthod W, Tharapreuksapong A, Tomsantia B, Yenchon S, Papan P, Tantasawat PA. 2022. Selection and characterization of in vitro-induced mutants of *Dendrobium* 'Earsakul' resistant to black rot. In Vitro Cell Dev Biol Plant 58: 577-592. https://doi.org/10.1007/s11627-022-10261-0.

Kumari M, Clarke HJ, Small I, Siddique KHM. 2009. Albinism in plants: A major bottleneck in wide hybridization, androgenesis and doubled haploid culture. Critical Rev Plant Sci 28 (6): 393-409. https://doi.org/10.1080/07352680903133252.

Lenawaty DY, Sukma D, Syukur M, Suprapta DN, Nurcholis W, Aisyah SI. 2022. Increasing the diversity of marigold (*Tagetes* sp.) by acute and chronic chemical induced mutation of EMS (Ethyl Methane Sulfonate). Biodiversitas 23 (3): 1399-1407. https://doi.org/10.13057/biodiv/d230326.

Lucena-Aguilar G, Sánchez-López AM, Barberán-Aceituno C, Carrillo-Ávila JA, López-Guerrero JA, Aguilar-Quesada R. 2016. DNA source selection for downstream applications based on DNA quality indicators analysis. Biopreserv Biobank 14 (4): 264-270. https://doi.org/10.1089/bio.2015.0064.

Martha KM, Dewanti P, Restanto DP, Ratnasari T, Alfian FN. 2023. In vitro mutagenesis of *Dendrobium gabriella suryajaya* using Ethyl Methane Sulfonate (EMS) and plantlet regeneration. Biosaintifika: J Biol Biol Educ 15 (1): 87-96. https://doi.org/10.15294/biosaintifika.v15i1.40997.

Mustaqim WA, Astuti IP. 2019. New and noteworthy orchid records from Buru Island, Maluku Archipelago. Gard Bull Singapore 71 (1): 167-174. https://doi.org/10.26492/gbs71(1).2019-10.

Nasri F, Zakizadeh H, Vafaee Y, Mozafari AA. 2022. In vitro mutagenesis of *Chrysanthemum morifolium* cultivars using Ethyl Methane Sulphonate (EMS) and mutation assessment by ISSR and IRAP markers. Plant Cell Tiss Organ Cult 149: 657-673. https://doi.org/10.1007/s11240-021-02163-7.

Park HS, Jeon JH, Cho W, Lee Y, Park JY, Kim J, Park YS, Koo HJ, Kang JH, Lee TJ, Kim SH, Kim JB, Kwon HY, Kim SH, Paek NC, Jang G, Suh JY, Yang TJ. 2023. High-throughput discovery of plastid genes causing albino phenotypes in ornamental chimeric plants. Hortic Res 10 (1): uhac246. https://doi.org/10.1093/hr/uhac246.

Promega Corp. 2015. DNA/RNA purification. www.worldwide.promega.com.

Purwantoro A, Setiawan AB, Nugraha RS, Mujtaba SB, Setyadi AH. 2023. Genetic variation and genomic constitution in orchid *Dendrobium* hybrid section Spatulata derived from interspecific hybridization based on sequence related amplified polymorphism marker. Indonesian J Biotechnol 28 (2): 119-126. https://doi.org/10.22146/ijbiotech.83595.

Puspaningtyas DM. 2019. Inventory and exploration of orchid in Polewali Mandar, West Sulawesi, Indonesia. Biodiversitas 20 (7): 1887-1896. https://doi.org/10.13057/biodiv/d200714.

Robarts DWH, Wolfe AD. 2014. Sequence-Related Amplified Polymorphism (SRAP) markers: A potential resource for studies in plant molecular biology. Appl Plant Sci 2 (7): 1400017. https://doi.org/10.3732/apps.1400017.

Setiawan RB, Handayani M, Nanda AR, Sukma D, Rahmi A, Syahputra A. 2024. Diversity of orchids (Orchidaceae) and host trees at Padang, West Sumatra, Indonesia: A preliminary step towards germplasm conservation. Jurnal Agronomi Indonesia 52 (2): 253-263. https://doi.org/10.24831/jai.v52i2.56159.

Sherpa R, Devadas R, Bolbhat SN, Nikam TD, Penna S. 2022. Gamma radiation induced in-vitro mutagenesis and isolation of mutants for early flowering and phytomorphological variations in *Dendrobium* 'Emma White'. Plants 11 (22): 3168. https://doi.org/10.3390/plants11223168.

Tirkey P, Singh D. 2019. Effect of induced mutagenesis on different characters of gladiolus (*Gladiolus grandifloras* L.). J Pharmacogn Phytochem 8 (6): 650-654.

Türkoğlu A, Haliloğlu K, Tosun M, Bujak H, Eren B, Demirel F, Szulc P, Karagöz H, Selwet M, Özkan G, Niedbala G. 2023. Ethyl Methane Sulfonate (EMS) mutagen toxicity-induced DNA damage, cytosine methylation alteration, and iPBS-retrotransposon polymorphisms in wheat (*Triticum aestivum* L.). Agronomy 13 (7): 1767. https://doi.org/10.3390/agronomy13071767.

Wang H, Wang Y, Liu Y, Xie J, Zhang Y, Jin H, Wei F, Ma S. 2024. Study on the structural features of eight *Dendrobium* polysaccharides and their protective effects on gastric mucosa. Foods 13 (18): 3011. https://doi.org/10.3390/foods13183011.

Xiong M, Wang Y, Chen D, Wang X, Zhou D, Wei Z. 2020. Assessment of genetic diversity and identification of core germplasm in single-flowered amaryllis (*Hippeastrum hybridum*) using SRAP markers. Biotechnol Biotechnol Equip 34 (1): 966-974. https://doi.org/10.1080/13102818.2020.1814865.

Yan W, Deng XQ, Yang C, Tang X. 2021. The genome-wide EMS mutagenesis bias correlates with sequence context and chromatin structure in rice. Front Plant Sci 12: 579675. https://doi.org/10.3389/fpls.2021.579675.

Zagorcheva T, Stanev S, Rusanov K, Atanassov I. 2020. SRAP markers for genetic diversity assessment of lavender (*Lavandula angustifolia* Mill.) varieties and breeding lines. Biotechnol Biotechnol Equip 34 (1): 303-308. https://doi.org/10.1080/13102818.2020.1742788.

Zheng Y, Xu S, Liu J, Zhao Y, Liu J. 2017. Genetic diversity and population structure of Chinese natural bermudagrass [*Cynodon dactylon* (L.) Pers.] germplasm based on SRAP markers. PLoS One 12 (5): e0177508. https://doi.org/10.1371/journal.pone.0177508.