

SABRAO Journal of Breeding and Genetics
 57 (1) 67-76, 2025
<http://doi.org/10.54910/sabrao2025.57.1.7>
<http://sabraojournal.org/>
 pISSN 1029-7073; eISSN 2224-8978



GENETIC DIVERSITY OF *PANDANUS* SPP. BASED ON ISSR MARKERS IN SUMATRA, INDONESIA

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SUMMARY

Extensive community exploitation caused a decline in *Pandanus* spp. population, with its natural habitats being converted into monoculture plantation areas, posing a threat to the species. Currently, limited information exists on the genetic diversity of *Pandanus* spp. from Sumatra, Indonesia. Therefore, the genetic diversity study is crucial for conservation and breeding purposes. The presented study aimed to assess the genetic diversity of *Pandanus* spp. germplasm from Sumatra using ISSR markers. Seventeen *Pandanus* species, collected from seven provinces, had their data analyzed using 10 ISSR primers, resulting in 50 total bands. The molecular analysis divided the *Pandanus* species into two main groups and five subgroups. The highest genetic distance (0.93) was evident between the species *P. helicopus*-1 from Riau Province and *P. helicopus*-2 from Bangka Belitung. However, the lowest genetic distance (0.20) appeared between the *Pandanus* species *P. helicopus*-2 and *P. stenophyllus*. Based on ISSR markers, *Pandanus* spp. from Sumatra can achieve clustering according to species diversity. The latest results provide valuable insights for planning the conservation strategies, optimal utilization, and future improvement of the *Pandanus* species.

Keywords: Pandanaceae, *Pandanus* species, genetic diversity, ISSR markers, molecular analysis, genetic distance, Sumatra

Key findings: Studying the genetic diversity of *Pandanus* spp. collected from various regions of Sumatra, Indonesia succeeded. Molecular analysis divided the species into two groups and five subgroups. Using ISSR markers, classification of *Pandanus* spp. from Sumatra can depend on species diversity. These findings can help in planning the conservation strategies, optimal utilization of the species, and future crop improvement programs.

Communicating Editor: Dr. Irma Jamaluddin

Manuscript received: June 26, 2024; Accepted: September 11, 2024.

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Citation: Hutasuhut MA, Pasaribu N, Siregar ES, Fitmawati (2025). Genetic diversity of *Pandanus* spp. based on ISSR markers in Sumatra, Indonesia. *SABRAO J. Breed. Genet.* 57(1): 67-76. <http://doi.org/10.54910/sabrao2025.57.1.7>.

INTRODUCTION

Pandanaceae is a family of flowering plants native to the tropics and subtropics of the Old World, from West Africa to the Pacific (Christenhusz and Byng, 2016). It contains five genera and 982 species, with the genus *Pandanus* as the most important, having species like *P. amaryllifolius* and *P. julianettii* as crucial sources of food. The family likely originated during the Late Cretaceous.

The Pandanaceae family is a widely distributed group across Indonesian islands, including Sumatra, Java, Bali, Kalimantan, Sulawesi, Maluku, and Papua (Keim, 2017). Pandanaceae genera, comprising *Pandanus*, *Benstonea*, *Freycinetia*, *Martellidendron*, and *Sararanga*, have a lowland to highland distribution (Callmander *et al.*, 2012). Sumatra has the highest genetic diversity of *Pandanus* spp. after Java Island, though, it is not well-documented (Keim, 2011). The genus *Pandanus* has trees, shrubs, and non-climbing plants, with thick and bony endocarp (Stone, 1976). *Pandanus* plants have supporting roots from the stem and leaf axis, which are aerial roots. The leaves are single, arranged in a three-plane spiral, hard, and finely spiny on the edges. Mostly, pollination occurs by insects and wind, which increased the fruit's and seed viability (Keim, 2011).

Pandanus spp. has various uses, including culinary seasoning, medicinal ingredients, and religious rituals (Tanzerina *et al.*, 2022). Species like *P. tectorius*, *P. odoratissimus*, *P. dubius*, and *P. furcatus*, have long, tracheid fibers in their leaves, making them flexible, durable, and suitable for handicrafts, such as, hats, bags, and mats (Purwanto and Munawaroh, 2010). *P. amaryllifolius* contains pandanin compounds with antiviral properties against herpes and influenza viruses (Ooi *et al.*, 2004). Some *Pandanus* species even serve as ornamental plants due to their unique shapes, such as, *P. dubius* and *P. utilis* (Wardah and Setyowati, 2009).

The existence of *Pandanus* spp. receives threats from massive exploitation of the community and habitat's conversion into monoculture plantation, leading to potential

endangerment. Furthermore, deforestation and land use changes reduced the population sizes, hindering allele exchange, and eventually, decreasing the genetic diversity. Information on genetic diversity is vital in the conservation for future and plant breeding activities (Salgotra and Chauhan, 2023). Therefore, the maximum genetic diversity is crucial for short-term ecological adaptation and long-term evolution in conservation strategies and for selection through breeding for further improvement (Rahayu and Handayani, 2010). Genetic diversity patterns are essential for designing conservation, management, and sustainable strategies for using plant species (Chung *et al.*, 2013; Ho and Tu, 2019; Liu *et al.*, 2019). Conservation approaches depend on geographical regions, habitat ecology, identified species, and threatened taxa (Pratami *et al.*, 2020; Yun *et al.*, 2020).

A molecular approach can better explore the genetic diversity using DNA analysis (Yang *et al.*, 2016; Guliyev *et al.*, 2018; Teixeira and Huber, 2021). The inter-simple sequence repeats (ISSR) markers are highly polymorphic and efficient for studying genetic diversity at the intraspecies level (Lu *et al.*, 2011; Zhao *et al.*, 2015; Alhasnawi *et al.*, 2019). ISSR markers have been functional to discriminate the *Benstonea thwaitesii*, Pandanaceae (Borse *et al.*, 2018) and assess genetic diversity among 13 *Pandanus* species and six *Freycinetia* species (Rahayu *et al.*, 2007). The ISSR markers have determined genetic similarity between 10 *Pandanus* species from West Java (Rahayu and Handayani, 2010). However, at present, no documentation existed on the allele diversity of *Pandanus* spp. in Sumatra, Indonesia.

Understanding the genetic diversity of *Pandanus* spp. is crucial for both conservation and breeding purposes, as it helps to determine the relationship among the species. The loss of genetic variation means a population cannot evolve in response to environmental changes, increasing the risk of extinction. Studying the genetic diversity of *Pandanus* spp. will aid in resolving the identity of species collected from various locations in Sumatra. The presented study aimed to develop a classification system for genetic

variations in *Pandanus* spp., using inter-simple sequence repeat (ISSR) markers to provide comprehensive information on the genetic diversity of *Pandanus* spp. in Sumatra, Indonesia.

MATERIALS AND METHODS

Breeding material

The collected 14 *Pandanus* spp. came from different locations of Sumatra, Indonesia. Sampling procedure used the explore method. The explored areas for the samples' collection were seven provinces, including Aceh, North Sumatra, Riau, Bengkulu, South Sumatra, and Bangka Belitung (Figure 1, Table 1). The selection of research locations relied on the distribution center of *Pandanus* spp. in Sumatra.

Molecular analysis

The DNA extraction of *Pandanus* spp. leaves employed the Plant Genomic DNA Mini Kit (Geneaid®, Taiwan). The DNA amplification used 10 ISSR primers (Table 2). Polymerase chain reaction (PCR) proceeded in 12 µl volume, which consisted of 1 µl DNA (0.5–2.0 ng), 1 µl primer ISSR, 5.5 µl GreenTaq MasterMix, and 4.5 µl free nuclease water. The PCR program consisted of initial denaturation at 94 °C for two minutes, followed by 35 cycles of denaturation at 93 °C for 30 seconds, annealing at 48 °C–54 °C for 60 seconds, extension at 72 °C for 30 seconds, and a final extension at 72 °C for five minutes. The amplified PCR products' separation utilized electrophoresis on 1% agarose gel in TBE IX buffer and stained with ViSafe Green Gel Stain (10,000× in water). Recording the results continued under the blue light Accuris Smart

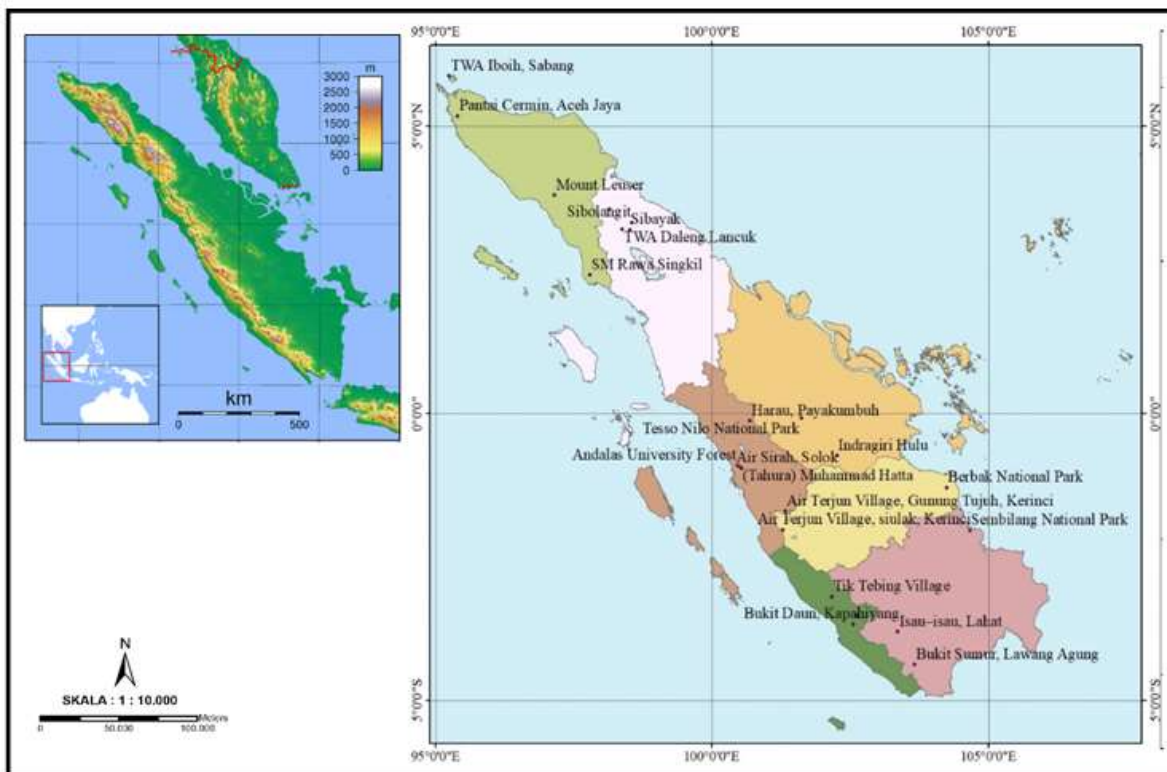


Figure 1. Map of the locations for collecting the *Pandanus* spp. in Sumatra, Indonesia.

Table 1. *Pandanus* species collections used for ISSR analysis.

No.	Species	Location	Sample Code
1	<i>P. yvanii</i>	Aceh	MA2
2	<i>P. borneensis</i>	Aceh	MA3
3	<i>P. labyrinthicus</i>	North Sumatra	MS3
4	<i>P. albifrons</i>	North Sumatra	MS4
5	<i>P. discostigma</i>	North Sumatra	MS5
6	<i>P. sumatranus</i>	North Sumatra	MS6
7	<i>P. forbesii</i>	North Sumatra	MS7
8	<i>P. leram</i>	North Sumatra	MS8
9	<i>P. helicopus1</i>	Riau	MR2
10	<i>P. helicopus2</i>	Bangka Belitung	MB1
11	<i>P. tectorius1</i>	West Sumatra	MP2
12	<i>P. tectorius2</i>	South Sumatra	MG2
13	<i>P. tectorius3</i>	Bengkulu	MU3
14	<i>P. stenophyllus</i>	Bengkulu	MU1
15	<i>P. furcatus</i>	South Sumatra	MG1
16	<i>P. dubius</i>	Bangka Belitung	MB2
17	<i>P. rostratus</i>	Bangka Belitung	MB3

Table 2. The primers used in this study.

No.	Primer	Sequences	Annealing temperature (°C)
1	ISSR-3	(AG) ₈ T	48
2	ISSR-4	(AG) ₈ AA	48
3	ISSR-5	(AG) ₈ TT	48
4	UBC-808	(AG) ₈ C	52
5	UBC-841	(GA) ₈ YC	52
6	UBC-855	(AC) ₈ YT	50
7	UBC-827	(CA) ₈ RC	50
8	UBC-891	HVH(TG) ₇	54
9	UBC-886	VDV(CT) ₇	54
10	UBC-811	(GA) ₈ C	54

Y= Pyrimidine (C,T); R= Purine (A,G); V= A, G, C; H= A, T, C.

Blue Transilluminator, with documentation using Smart Doc Enclosure with the Smartphone.

Data analysis

Scoring the banding pattern obtained for each ISSR primer used the Gel Pro Analyzer program 3.1 version. The score of polymorphic bands was zero (0) if no band appeared, and one (1) if a band occurred in the same position. The binary data served to calculate the genetic similarity matrix using the SIMQUAL (Similarity for Quality Data) procedure. Based on the genetic similarity index, cluster analysis construction utilized the SAHN (Sequential Agglomerative Hierarchical

and Nested Clustering) procedure. The similarity coefficient with the SM (Simple Matching) method and clustering with Unweighted Pair Group Method Arithmetic Average (UPGMA) method proceeded using the NTSYS PC version 2.01 (Numerical Taxonomy and Multivariate System) (Rohlf, 2000).

RESULTS AND DISCUSSION

ISSR polymorphism

Based on the primers' capability, selected primers totaled 10 primers, which produced clear and polymorphic banding patterns and explored the genetic diversity of 17 *Pandanus*

Table 3. ISSR primer sequences and amplified band profiles.

No.	Primer	Fragment size (bp)	Band total	Polymorphic band total	Percentage polymorphic band (%)
1	ISSR-3	100-1500	8	7	87.5
2	ISSR-4	300-1500	5	5	100
3	ISSR-5	250-2000	7	6	85.7
4	UBC-808	250-1500	5	5	100
5	UBC-841	250-1000	5	5	100
6	UBC-855	250-1500	6	5	83.3
7	UBC-827	100-600	4	4	100
8	UBC-891	250-750	3	3	100
9	UBC-886	250-750	4	4	100
10	UBC-811	250-1000	3	3	100
Total			50	47	
Average					95.65

species. The patterns, number, and sizes of bands varied based on the species and primers. A total of 50 bands reached amplification, 47 of which were polymorphic, and the polymorphism for each primer ranged from 83.3% to 100%, averaging 95.65%. This authenticated a considerable genetic diversity among the *Pandanus* spp. from Sumatra. Each primer amplified between three and eight bands, with lengths ranging from 100 to 2000 base pairs (bp) (Table 3).

The primer ISSR-5 produced the largest band size at 2000 bp, while the primers ISSR-3 and UBC-827 produced the smallest bands at 100 bp. Consistent band sizes of 250 and 500 bp appeared across all the primers, indicating as common genetic markers in *Pandanus* spp. The primers UBC-891 and UBC-811 produced the fewest bands (three bands each), whereas the primer ISSR-3 formed the most bands (eight bands) (Table 3). Genetic polymorphism signified a closer link to how plants adapt to variations in climate, temperature, and water availability (Henderson and Salt, 2017). Plants undergo allelic mutations in response to environmental adaptation, leading to variations in genetic material (Chung *et al.*, 2023). Additionally, genetic diversity incurred influences from the genetic background of ancestors, plant migration, and human domestication practices (Li *et al.*, 2018; Napier *et al.*, 2023).

Phenetic analysis

In Sumatra regions, the *Pandanus* species found were *P. yvanii*, *P. borneensis*, *P. leram*, *P. albifrons*, *P. labyrinthicus*, *P. discostigma*, *P. sumatranus*, *P. forbesii*, *P. helicopus*, *P. tectorius*, *P. stenophyllus*, *P. furcatus*, *P. rostratus*, and *P. dubius*. Backer and Bakhuizen (1968) reported 15 species of *Pandanus* exist in Java, namely, *P. andamanensium* (W. Southern Coast of Nusakambangan), *P. labyrinthicus* (W. Coast of Sumatra; might also occur in Java), *P. faviger* (Lamongan and Bali), *P. pygmaeus*, *P. amaryllifolius*, *P. vandermeeschii*, *P. utilis*, *P. boninensis*, *P. kurzii* Merr., *P. tectorius* Soland ex Park, *P. polycephalus* Lamk, *P. furcatus* Roxb., *P. bidur* Jungh ex Miq, *P. nitidus* Kurz, and *P. hasskarlii* Merr.

The grouping based on the 50 bands of primary amplification of ISSR using the UPGMA method and simple matching coefficient merged 17 species into two main groups, with a similarity coefficient of 53%–93% (Figure 2). Group I consisted of two subgroups (A and B). Group II has four subgroups (C, D, E, and F). Group A consisted of the same species, but different accessions, *P. helicopus-1* and *P. helicopus-2*. For group B, it comprised three accessions of the species *P. tectorius* and *P. albifrons*. Based on their morphological characteristics, these two species revealed

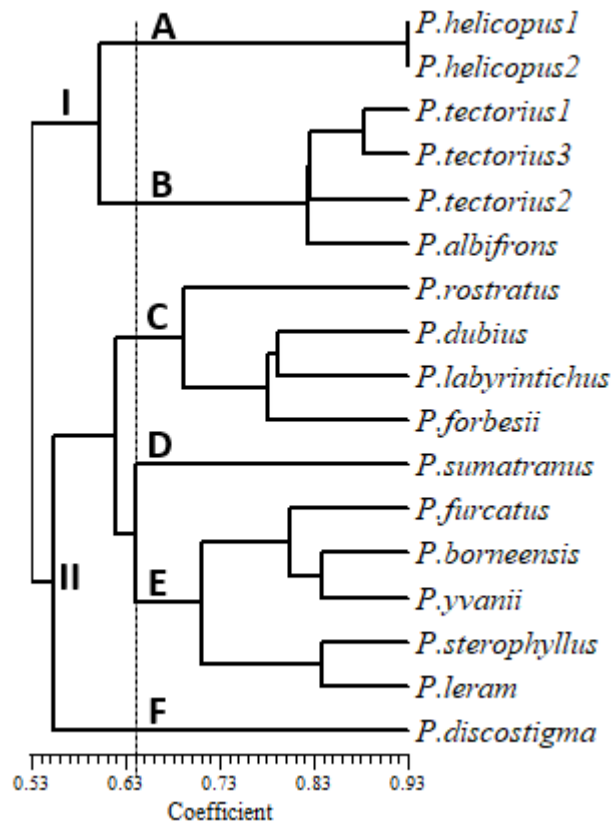


Figure 2. Dendrogram showing the similarity coefficient among 17 *Pandanus* spp. constructed using the *Unweighted Pair Group Method with Arithmetic Mean* (UPGMA) method based on 50 ISSR bands.

similar, and both have a single cephalia and a few fruits on each cephalia (38–82). Group C, comprising four species, included *P. rostratus*, *P. dubius*, *P. labyrinthicus*, and *P. forbesii*. Concerning morphological characteristics, these species were also similar, with a single cephalia and moderate fruits on each cephalia (105–387). Group D only had one species, *P. sumatranus*. Group E consisted of five species, viz., *P. furcatus*, *P. borneensis*, *P. yvanii*, *P. stenophyllus*, and *P. leram*. Similar with group D, group F has only one species, *P. discostigma*, and became separated from other species. Based on its morphology, *P. discostigma* has similarities with *Benstonea ornata* for morphological traits. These include no supporting roots, stems without spines, absence of inverted spines at the base of the leaves, old leaves remain attached, single cephalia, and brown stigma.

Based on molecular characters, the species grouping patterns often do not align

with morphological features in the genotypes. The ISSR primer is a short DNA sequence, with a length of 15–30 base pairs, scattered randomly throughout the genome. Primers of 20 base pairs can complement DNA sequences, as much as, 9×10^{-13} . With 3×10^9 base pairs per haploid genome, the use of primers in taxonomic analysis does not fully represent the actual state of the genome (Cha and Thilly, 1993). According to Zietkiewicz et al. (1994), the ISSR markers are only a small part of the genome, necessitating the use of more primers for accurate representation.

The collection of the *Pandanus* species, *P. tectorius*, came from three different locations. Despite being the same species, these landraces of the same species were genetically distinct and showed separate branches in the dendrogram. Environmental variations cause plants to adapt to their habitats, leading to physiological and genetic variations, affecting the plant population

diversity (Juliantari *et al.*, 2021). Genetic polymorphism signified as closely related to plant adaptation to variations in climate, temperature, and water availability (Henderson

and Salt, 2017). Plants adapt to their existing environment through allelic mutations, leading to sustainable variations in the genetic material (Ni *et al.*, 2018).

Table 4. Genetic distance among the 17 *Pandanus* spp. collected from Sumatra, Indonesia.

Species	MR2	MB1	MP1	MG2	MU3	MS4	MG1	MB3	MB2	MU1	MS5	MS6	MS8	MA7	MA3	MA2	MS7
MR2	1.00																
MB1	0.93	1.00															
MP1	0.69	0.67	1.00														
MG2	0.51	0.48	0.81	1.00													
MU3	0.62	0.60	0.88	0.83	1.00												
MS4	0.60	0.58	0.81	0.81	0.83	1.00											
MG1	0.48	0.46	0.65	0.74	0.67	0.69	1.00										
MB3	0.65	0.58	0.48	0.39	0.51	0.53	0.55	1.00									
MB2	0.55	0.48	0.62	0.53	0.65	0.67	0.65	0.72	1.00								
MU1	0.27	<i>0.20</i>	0.39	0.53	0.46	0.48	0.69	0.48	0.58	1.00							
MS5	0.48	0.41	0.60	0.51	0.62	0.60	0.67	0.69	0.79	0.69	1.00						
MS6	0.39	0.41	0.65	0.46	0.58	0.51	0.53	0.46	0.51	0.69	0.62	1.00					
MS8	0.48	0.41	0.60	0.65	0.53	0.46	0.62	0.51	0.60	0.60	0.72	0.48	1.00				
MA7	0.34	0.32	0.41	0.46	0.44	0.46	0.72	0.46	0.55	0.83	0.58	0.67	0.58	1.00			
MA3	0.46	0.44	0.58	0.58	0.55	0.53	0.79	0.67	0.67	0.58	0.65	0.51	0.69	0.74	1.00		
MA2	0.48	0.46	0.55	0.60	0.58	0.55	0.81	0.55	0.65	0.69	0.67	0.53	0.67	0.81	0.83	1.00	
MS7	0.53	0.51	0.65	0.55	0.62	0.69	0.67	0.65	0.79	0.46	0.76	0.44	0.62	0.58	0.74	0.72	1.00

The value in bold is the highest value and the value in bold italics is the lowest value. MR2: *P. helicopus1*, MB1: *P. helicopus2*, MP1: *P. tectorius1*, MG2: *P. tectorius2*, MU3: *P. tectorius3*, MS4: *P. albifrons*, MG1: *P. furcatus*, MB3: *P. rostratus*, MB2: *P. dubius*, MU1: *P. stenophyllus*, MS3: *P. labyrinthicus*, MS5: *P. discostigma*, MS6: *P. sumatranus*, MS8: *P. leram* MA3: *P. borneensis*, MA2: *P. yvanii*, and MS7: *P. forbesii*.

Furthermore, genetic diversity acquires influences from genetic ancestry, plant migration, and human domestication (Li *et al.*, 2018). This study demonstrated ISSR markers were effective in genetic fingerprinting, gene tagging, identification, and grouping of the different accessions. Amom and Nongdam (2017) confirmed ISSR markers can identify plant species and varieties and study genetic diversity. However, ISSR markers can group *Pandanus* spp. based on morphological characteristics, which has not been fully proven for all branches in the dendrogram. The results further revealed the highest genetic distance (0.93) appeared between the species *P. helicopus-1*, collected from Riau Province, and *P. helicopus-2*, procured from Bangka Belitung. Meanwhile, the lowest genetic distance (0.20) emerged between the species, *P. helicopus-2* and *P. Stenophyllus* (Table 4). The maximum similarity values among the landraces from different origins were due to their relationship

with the same species (*P. helicopus*) and the same morphological characters. The species *P. helicopus* was prevalent in swampy areas, while *P. stenophyllus* was evident in mountain forests, exhibiting distinct morphological traits, such as, branching patterns, spine color, inflorescence stalks, and fruit counts.

The *Pandanus* from Java enunciated significant genetic variations, which might be due to the highest genetic distance values (0.29–0.89) (Rahayu *et al.*, 2007). Several evolutionary factors, including mating systems, gene flow, seed dispersal, geographic range, and natural selection, also collectively influence genetic diversity. Of these factors, mating systems and geographic range most notably affect the genetic variations in a species (Sork, 2015). High similarity indices among the species from different locations might refer to similar habitats and environmental conditions, which reduce the need for adaptation and genetic changes (Ni *et al.*, 2018; Gunawan *et*

al., 2019). According to Ratnam (2009), natural selection and sexual mating within the same habitat lead to similar genes and phenotypes in the population. The similarity index can identify genetically similar *Pandanus* species. According to Kuwi *et al.* (2018), greater genetic distances among the accessions correspond to lower genetic characteristic similarity. For the future development of *Pandanus*, conservation is necessary considering the utilization of *Pandanus* has increased, while its habitat conditions remained threatened by population growth and modernization activities. The prohibition of exploiting *Pandanus* and habitat destruction should persist until the establishment of effective in-situ and ex-situ conservation strategies. This will prevent further reduction of population size and conserve the overall genetic base and structure of the *Pandanus* species. Sensitization of locals about the prevailing genetic scenario of alarming *Pandanus* populations and involvement of local communities in framing protection policies will be a highly effective approach to *Pandanus* conservation.

CONCLUSIONS

The genetic diversity analysis of 17 *Pandanus* species collected from seven provinces in Sumatra used 10 ISSR primers, resulting in 50 polymorphic bands. Molecular analysis categorized the species into two main groups and five subgroups. The highest genetic distance (0.93) was evident between the species *P. helicopus-1* from Riau and *P. helicopus-2* from Bangka Belitung. Meanwhile, the lowest (0.20) occurred between *P. helicopus-2* and *P. stenophyllus*. These findings demonstrated substantial allele diversity among the *Pandanus* spp. in Sumatra, which can help in planning for conservation strategies, optimal species utilization, and future crop improvement programs.

ACKNOWLEDGMENTS

The authors express their highest gratitude to the sampling team for their aid during the exploration and collection of *Pandanus* spp. in some locations of Sumatra.

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