RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
55 (3) 917-926, 2023
http://doi.org/10.54910/sabrao2023.55.3.26
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

IDENTIFICATION OF *PINUS MERKUSII* LANDRACE BELONGING TO KERINCI - WEST SUMATRA, INDONESIA, USING SEQUENCE-RELATED AMPLIFIED POLYMORPHISM (SRAP) TECHNIQUE

A.Z.S. RINALDI[1], NURAINAS[2], and SYAMSUARDI[1*]

[1]Department of Biology, University of Andalas, Padang, Indonesia
[2] Herbarium of ANDA, University of Andalas, Padang, Indonesia
*Corresponding author's email: anes82@gmail.com
Email addresses of co-authors: atiqarinaldi@gmail.com, nas_herb@yahoo.com

SUMMARY

Pinus merkusii is a potential wood that naturally grows in Central Sumatra (Kerinci) and North Sumatra (Aceh and Tapanuli), Indonesia. The Kerinci landrace has a different morphology from the other two Sumatran Pinus landraces, namely, Aceh and Tapanuli, but its placement is at the same taxonomic level. Hence, additional characters need evaluation to validate taxa on *P. merkusii* landrace belonging to Kerinci. This study aims to identify differences in genetic characters between Kerinci and Aceh (Rao and TAHURA [Taman Hutan Raya]) landraces using SRAP for long-term use and future conservation. DNA extraction ran from the leaves of 24 individual trees belonging to three populations of *P. merkusii,* i.e., one naturally occurring population in Kerinci and the two cultivated populaces found in Rao and TAHURA, Indonesia. Using seven SRAP combination primers, the analysis revealed an immense variety of alleles (bands). Overall, the amplification produced 62 bands, with nine, on average, per primary pair. However, most bands were polymorphic (91.79%), and only 0.57% were monomorphic. With 45% informative bands, the Kerinci landrace has the highest band variation. A combination of primer C (Me1 + Em3) at 900 bp and primer I (Me3 + Em1) at 1050 bp band size indicated specific bands that served as molecular characteristics. A crucial molecular characteristic distinguishes the landraces of *P. merkusii* found in Kerinci and other populations in Rao and TAHURA, Indonesia. The study revealed superior genetic variation among the *P. merkusii* populations evaluated.

Keywords: Sumatran pine *(Pinus merkusii*), molecular characterization, genetic conservation, Kerinci, landraces, SRAP

Key findings: Conservation and sustainable use of *P. merkusii* from Kerinci decline due to the reduction. The discovery of molecular characteristics in the form of a combination of primer C (Me1 + Em3) at 900 bp and primer I (Me3 + Em1) at 1050 bp is crucial for recognizing Kerinci pine tree populations and valuable for plant breeders to conserve and develop germplasm.

Communicating Editor: Dr. Gwen Iris Descalsota-Empleo

Citation: Rinaldi AZS, Nurainas, Syamsuardi (2023). Identification of *Pinus merkusii* landrace belonging to Kerinci - West Sumatra, Indonesia, using sequence-related amplified polymorphism (SRAP) technique. *SABRAO J. Breed. Genet.* 55(3): 917-926. http://doi.org/10.54910/sabrao2023.55.3.26.

Manuscript received: March 24, 2023; Accepted: May 26, 2023.

INTRODUCTION

Sumatran pine *(Pinus merkusii* Jungh. & de Vriese), also called Merkus pine, is naturally distributed on the island of Sumatra, specifically Aceh, Tapanuli (North Sumatra), and Kerinci (Central Sumatra). Different landraces of *P. merkusii* have distinct genetic, morphological, anatomical, and physiological attributes (Siregar and Hattemer, 1999; Sandri *et al.*, 2016). The *P. merkusii* landrace belongs to Kerinci (Kerinci landrace) with upright stems, thinner bark, and a scaly stem surface that lacks deep grooves, resembling more closely the stem shape of broadleaf plants, while the *P. merkusii* landrace belonging to the landrace Aceh with stems described as usually corkscrewed, thick-barked, dark-colored, and heavily fissured (Cooling, 1968; Andini *et al.*, 2022).

The *P. merkusii* is a monoecious plant, having male and female reproductive organs positioned at either end of a tree branch, with the female strobilus located slightly below the male strobili. The pollination system of *P. merkusii* is cross-pollination. Wind and rain help its pollen distribution and seed dispersal, as well as, the formation of pollen stores throughout the year, especially from March–June (Pousujja *et al*., 1986; Babayan, 2022; Saimova *et al*., 2022). Although *P. merkusii* is known as a plant that typically grows on high plains, it also grows on lowlands and in harsh soil conditions. *P. merkusii* has a broad range of ecological adaptations, frequently used as a pioneer plant in reforestation and rehabilitation programs (Imanuddin *et al.*, 2020).

The wood of *P. merkusii* classifies as grades three and four based on its durability and strength. Moreover, the wood produced by *P. merkusii* from Kerinci is superior due to its large and perpendicular stem (Saputra *et al.*, 2014). In addition to pine trunks, *P. merkusii* also produces gondorukem resin sap. However, the Kerinci landrace is a more highly sap-producing plant than the other two indigenous pines. Numerous industries, including textile and paint, require resin products (Andini *et al.*, 2022).

However, before breeding and further management of the existence of the Kerinci landrace, its population has reduced due to the disruption of community activities, population fragmentation by the road, and its low regeneration (Istomo *et al*., 2000; Saputra *et al*., 2014; Imanuddin *et al*., 2020). The entry of Aceh landrace into the habitat of the population Kerinci can also lead to erasing the original genetic resources and existing germplasm. Therefore, collecting genetic data about the Kerinci landrace is necessary for directing plant conservation efforts and preserving the germplasm.

The sequence-related amplified polymorphism (SRAP) is one of the molecular primers developed by Li and Quiros (2001). SRAP also combines the advantages of the simple and efficient RAPD (randomly amplified polymorphic DNA) molecular techniques with the AFLP (amplified fragment length polymorphism), which produces more accurate sequences (Robarts and Wolfe, 2014). Several studies have shown that using SRAP for genetic characterization has proven effective in classifying or identifying, differentiating, and demonstrating plant phylogenetic relationships at the species and population levels (Han *et al*., 2008; Ding *et al*., 2010; Xie *et al*., 2015; Alghamdi *et al*., 2019; Wang *et al*., 2019; Zagorcheva *et al*., 2020). Therefore, the presented study aims to identify differences in genetic characteristics between Kerinci and Aceh landraces (Rao and TAHURA populations) using SRAP.

MATERIALS AND METHODS

Genetic material and research sites

The concerned research ran from August 2020 to October 2021, with sampling conducted at three different locations in West Sumatra, Indonesia, i.e., a) Lubuk Layang Rao Selatan,

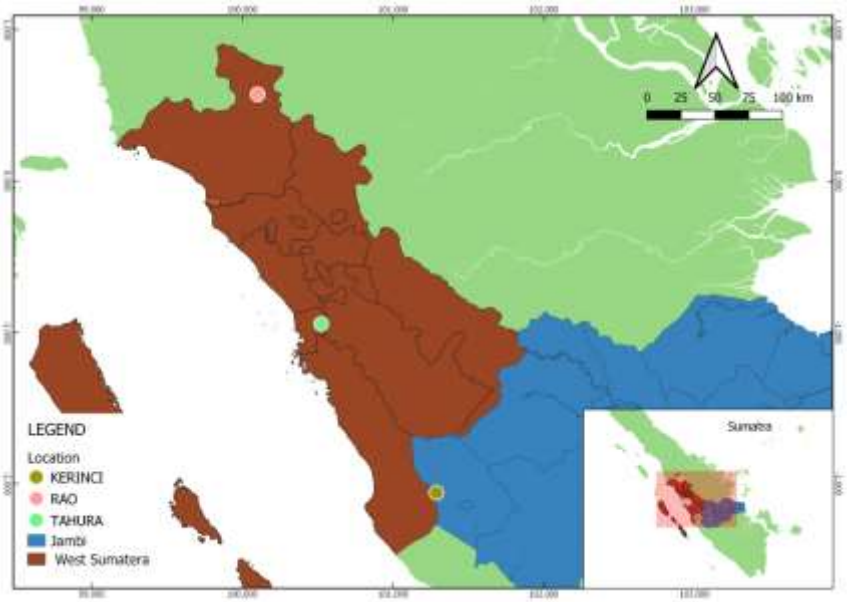

Figure 1. Collection sites of *P. merkusii*. Coordinate points: Lubuk Layang, Rao Selatan, Pasaman (0°34'25.70"N, 100° 6'3.40"E), Bukit Tapan Kerinci (2°3'38.75"S, 101°17'12.53"E), Forest park, Bung Hatta, Ladang Padi (0°56'41.81"N, 100°31'19.53"E).

b) Forest Park Bung Hatta Ladang Padi (TAHURA), and c) Bukit Tapan, Kerinci (Figure 1). Sample processing continued at the Herbarium and Genetics and Biomolecular Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia.

Working procedure

Sample collection and DNA isolation

In determining the sampling method, with provisions for large populations of >10 individuals of *P. merkusii*, the collected individuals were within a range of 150 m (Wang *et al*., 2019). This study used 14 individuals of *P. merkusii* from Aceh (Rao and TAHURA populations) and 10 individuals from Kerinci. The collection of molecular samples comprised fresh leaves of *P. merkusii*, placed into a tea bag earlier filled with silica gel, then labeled. The isolation of the DNA of *P. merkusii* samples employed the modified 2% CTAB method.

Primary amplification of SRAP

This study used seven primer mixtures previously selected from 16 combinations of SRAP by using the primary screening method BSA (Bulked Segregant Analysis) but modifying the stages and conditions of the PCR process (Wang *et al.*, 2019). Carrying out the PCR reactions comprised a total volume of 25.6 µl containing 5 µL genomic DNA (100 ng/µl), 12.5 µL of MyTaq HS Mix Red Master Mix (Bioline), 0.8 µl of each primer, and 6.5 µL nuclease-free water. Stages and conditions of the PCR process were initial denaturation at 94 °C for 5 min, followed by five cycles with three stages, i.e., denaturations at 94 °C for 1 min, annealing at 35 °C for 1 min, extension at 72 °C for 1 min, and further with 35 cycles including three stages: denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, extension at 72 °C for 1 min, with a final extension at 72 °C for 8 min. The separation of SRAP fragments from the amplification results employed the electrophoresis method on 2% agarose gel with EtBr staining.

Data analysis

Table 1. Analysis of SRAP-PCR amplification results.

No.	Primer	Forward/Reverse primer	Range Bands (pb or bp?)	Total Bands	Monomorphic Bands	PIC (%)
1	C (Me1 + Em3)	F:5′-TGAGTCCAAACCGGATA-3′ R:5′-GACTGCGTACGAATTGAC-3′	200–1370	11	0	100
2	E (Me2 + Em1)	F:5′-TGAGTCCAAACCGGAGC-3′ R: 5′-GACTGCGTACGAATTAAT-3′	260–800	4	1	75
3	I (Me3 + Em1)	F:5′-TGAGTCCAAACCGGAAT-3′ R: 5′-GACTGCGTACGAATTAAT-3′	170–1450	11	2	81,82
4	J (Me3 + Em2)	F:5′-TGAGTCCAAACCGGAAT-3′ R: 5′-GACTGCGTACGAATTTGC-3′	100–600	7	1	85,71
5	K (Me3 + Em3)	F:5′-TGAGTCCAAACCGGAAT-3′ R:5′-GACTGCGTACGAATTGAC-3′	175–1600	8	0	100
6	L (Me3 + Em4)	F:5′-TGAGTCCAAACCGGAAT-3′ R: 5′-GACTGCGTACGAATTTGA-3′	188–1600	10	0	100
7	N (Me4 + Em2)	F: 5′-TGAGTCCAAACCGGACC-3′ R: 5′-GACTGCGTACGAATTTGC-3′	100–1570	11	0	100
Total				62	4	642,53
Means				8,86	0.57	91,79

Scoring the obtained data gave a value of '1' with existing fragments and '0' with non-existing ones. The binary data of the matrix revealed the sum of polymorphic, monomorphic, and informative bands. The percentage of polymorphic bands (P, %), Shannon's information index (I), the observed number of alleles (Na), the effective number of alleles (Ne), Nei's gene diversity analysis (H), total gene diversity (Ht), genetic diversity within populations (Hs), the relative magnitude of genetic differentiation among populations (Gst), and gene flow (Nm) all helped estimate the genetic variation (Finkeldey, 2005). All genetic diversity parameters analysis used POPGENE software version 1.32 (Yeh *et al.*, 1999). Principal coordinate analysis (PcoA) clustering adopted the PAST 4.03 program (Hammer *et al*., 2001).

RESULTS

From the seven selected SRAP combination primers (Table 1), all 62 bands produced have average bands of 8.86 per primary pair. The primary combinations that constructed the most bands were C (Me1 + Em3), I (Me3 + Em1), and N (Me4 + Em2), with as many as 11 bands. However, the primary combination with the least number of bands was E (Me2 + Em1) with only four. Of the 62 total bands, 91.79% were polymorphic, while 0.57% were monomorphic, with monomorphic band sizes of 260 bp in primer E (Me2 + Em1), 170 and 200 bp in primer I (Me3 + Em1), and 100 bp in primer J (Me3 + Em2).

Numerous major combinations of the relevant band's features have surfaced for the three populations. A few distinct bands were also notable that were typical of each *P. merkusii* population, as shown in Table 2. A combination of primary C (Me1 + Em3), I (Me3 + Em1), and N (Me4 + Em2), with an occurrence frequency of 10%–50% bands, were evident in the Kerinci population. A combination of primary E (Me2 + Em1), K (Me3 + Em3), L (Me3 + Em4), and I (Me3 + Em1), with an occurrence frequency of 14.3% bands showed in two other populations, i.e., RAO and TAHURA. In addition, the combination of primers E and L did not show an informative band in the Kerinci population but emerged in the Rao and TAHURA populations. According to the results, the Kerinci landrace has more distinctive bands than the Aceh landrace (RAO and TAHURA). Additionally, two unique bands were apparent, with 40%–50% of each occurring in the combinations of primer C (Me1 + Em3) and primer I (Me3 + Em1), respectively, in the Kerinci population (Figure 2).

Table 2. Percentage of informative bands in the three populations of *Pinus merkusii* using seven SRAP primary combinations.

No.	Locus	Band	Population (%)		
			Rao (n=7)	Kerinci (n=10)	TAHURA (n=7)
1.	C (Me1 + Em3)	300	28,6	60	14,3
		900	-	50	-
		990	-	10	-
		1010	-	60	28,6
		1110	-	10	-
		1260	-	10	-
		1370	-	10	-
2.	E (Me2 + Em1)	600	-	-	14,3
		800	-	-	14,3
3.	I (Me3 + Em1)	1050	-	40	-
		1310	14,3	-	-
		1450	-	10	-
4.	J (Me3 + Em2)	225	14,3	20	-
		270	71,4	60	-
5.	K (Me3 + Em3)	630	-	-	14,3
		1000	-	70	28,6
		1600	-	-	14,3
6.	L (Me3 + Em4)	300	-	-	14,3
		850	14,3	-	-
		1000	14,3	-	-
		1150	14,3	-	-
		1200	-	-	14,3
		1300	14,3	-	-
		1600	14,3	-	-
7.	N (Me4 + Em2)	160	100	90	14,3
		250	-	40	14,3
		740	14,3	50	14,3
		800	-	10	-
		1070	-	40	14,3
		1130	-	10	-
		1300	-	10	-
		1570	-	10	-

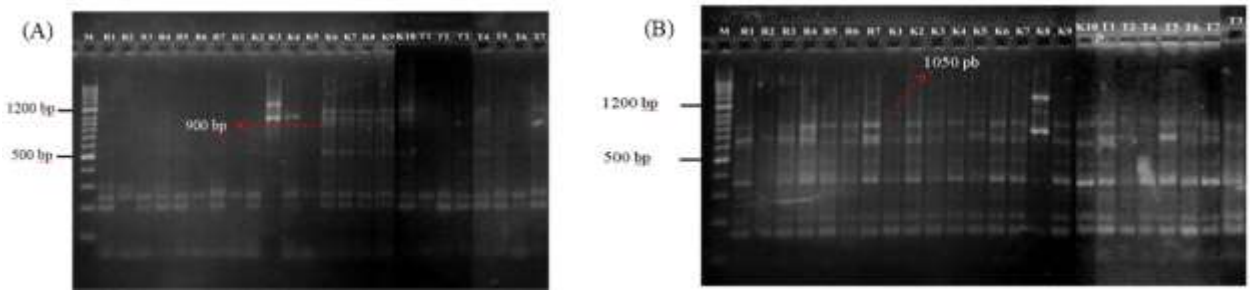

Figure 2. Profile bands resulting in amplification, Primer C (Me1 + Em3) (A) and Primer I (Me3 +Em1) (B) from the three populations of *Pinus merkusii* (R = Populasi RAO, K = Populasi Kerinci, T = Populasi TAHURA).

Table 3. Genetic diversity of three populations of *P. merkusii.*

No.	Pop.	Na	Ne	P	H	I	Ht	Hs	Gst	Nm
1	Rao	1.4839	1.2959	48.39%	0.1693	0.2523				
2	Kerinci	1.6290	1.3695	62.90%	0.2105	0.3142				
3	TAHURA	1.5161	1.2706	51.61%	0.1592	0.2433				
	Average	1.543	1.312	54.30%	0.1796	0.2699				
	Total	1.9355	1.3559	93.55%	0.2165	0.3414	0.2126	0.1797	0.1550	0.1797

Notes: Na, observed number of alleles; Ne, the effective number of alleles; P, percentage of polymorphic loci; H, Nei's gene diversity; I, Shannon's information indices; Ht, total genetic diversity; Hs, genetic diversity within populations; Gst, the relative magnitude of genetic differentiation among populations; Nm, gene flow among populations.

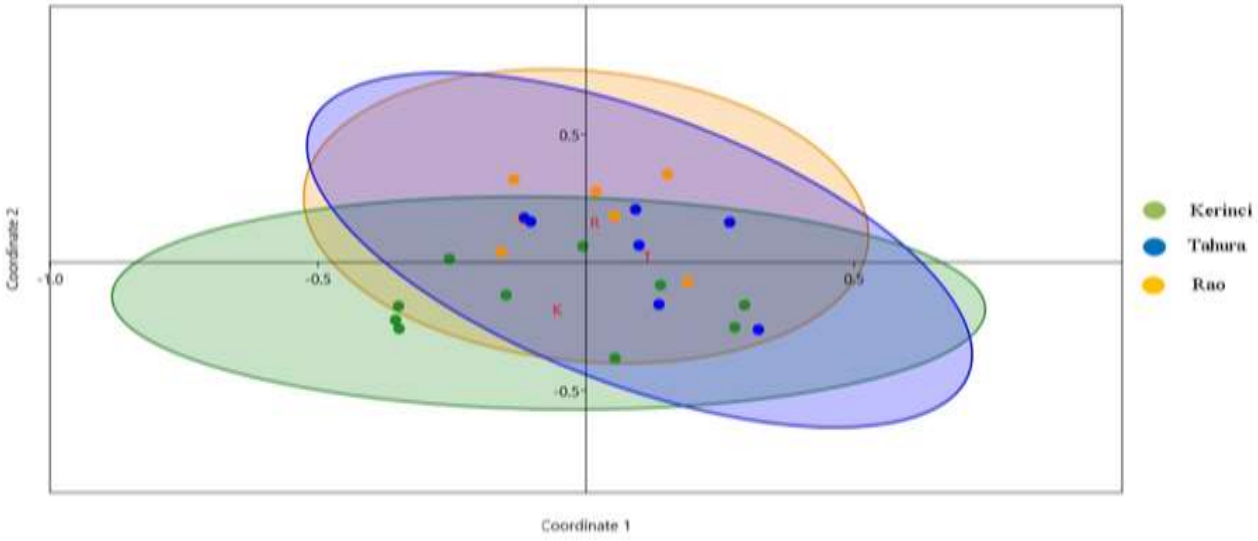

Figure 3. Distribution of 24 individuals (three populations of *Pinus merkusii*) by PCoA analysis.

Several genetic diversity parameters among the populations of one species and the individuals within one populace can be seen (Table 3). At the population level, the percentage of obtained polymorphic loci (PLP) varied between 48.39% and 62.90%, with the Kerinci population having the highest percentage, and the population from Rao with the lowest. The observed number of average alleles (Na) ranged from 1.4839 to 1.6290, while the means for an effective number of alleles (Ne) varied from 1.2706 to 1.3695. Nei's genetic diversity (H) differed from 0.1592 to 0.2105, with an average of 0.1796, and Shannon's information indices (I) ranged from 0.2433 to 0.3142, with an average of 0.2699., Nei's genetic diversity and Shannon's information indices were the same at 0.2165 at the species level.

The total genetic diversity (Ht) of the species and the genetic diversity within populations (Hs) were 0.2126 and 0.1797, respectively (Table 3). The average obtained Nm was 2.7250, suggesting the existence of a certain degree of gene flow among the *P. merkusii* populations. The *P. merkusii* population's genetic differentiation (Gst) value was 0.1550, indicating poor genetic divergence between populations. The genetic distance analysis revealed a range of 0.0367–0.0604, supporting the low value of genetic differentiation. Results also confirmed the Kerinci and TAHURA populations had the maximum genetic distance (0.0604), while the Rao and TAHURA with the lowest (0.0367).

Principal coordinate analysis (PCoA) results showed overlapping areas between the three populations (Figure 3), but a grouping of

four individuals from the Kerinci population occurred in one plot. All individuals in the TAHURA and Rao populations have overlapping grouping areas, thus showing that all individuals in the Rao and TAHURA populations have identical genetic characteristics. These results support the tendency for the Kerinci landrace to have different genetic features from the Aceh landrace (Rao and TAHURA populations). As a result, assumably, *P. merkusii* from Kerinci and Aceh share a common ancestor. However, the number of informative bands in the Kerinci landrace showed a tendency for differences in genetic characteristics with the Aceh landrace, as represented by the Rao and TAHURA populations.

DISCUSSION

Molecular characterization and identification play a vital role in distinguishing plants at the level of individuals, populations, races, and plant species (Huda *et al*., 2019; Nuratika and Aseny, 2020). However, various molecular techniques applied to distinguish between plant groups were generally DNA sequencing or RAPD and SRAP techniques. The observed key characters were very relevant in breeding programs to study the evolutionary potential of the taxa. In the case of pine found in West Sumatra, Indonesia, three populations have different distribution histories. Although morphologically, these pine populations exhibited as the natural population in Kerinci, two non-natural populaces originating from Aceh (Rao and TAHURA), can be prominent. However, discovering molecular characteristics of the primary combination of SRAP is a breakthrough that will strengthen the uniqueness of the pine landrace originating from Kerinci. Still, until now, taxonomically positioning the Kerinci landrace needs implementation. Therefore, combining morphological and molecular traits will provide more accurate data to place pine from Kerinci as a taxonomic variety because the factors of variety delimitation have been fulfilled (Syamsuardi *et al*. 2002; Syamsuardi, 2013).

Eleven specific bands appeared in the Kerinci population, whereas only six showed in the Rao and TAHURA populations (Table 2). Specific bands in the Kerinci population occurred mainly in the primer combinations C and N. In addition to the Kerinci landrace, the other two obtained produced a high percentage of specific band frequencies of 40%–50% contained in the primer combination C (Me1 + Em3) with a band size of 900 bp and primer I (Me3 + Em1) with 1050 bp (Figure 2). Hence, the substantial number of specific bands and their high frequency of appearance in the Kerinci landrace can be a distinguishing trait from the Aceh landrace (Rao and TAHURA populations).

SRAP is an effective technique for special molecular features to distinguish several levels of taxa, such as the level of section, species, and cultivars (Hao *et al.*, 2008; Xie *et al.*, 2015), and in identifying sex in dioecious plants (Kumar *et al.*, 2019; Aseny *et al.*, 2021). SRAP can also recognize whether the variation in the suspected intersectional hybridization is a new variation resulting from a cross between two sections or not, and further, it proved effective in identifying 14 cultivars of Peony (*Paeonia officinalis* L.) by showing the specific band parameters in the primary combinations (Hao *et al.*, 2008). The primary SRAP was effective in tracing genetic characteristics and mapping plant cultivars among and within the populations of *P. officinalis* L. (Gao *et al.*, 2020) and lavender (*Lavandula angustifolia* L.) (Zagorcheva *et al.*, 2020).

A specific pattern of bands showing informative ones appeared in the two populations of *P. merkusii*, but non-appearing in any other population (Table 2). The primary combinations C (Me1 + Em3) with a band size of 1010 bp, K (Me3 + Em3) with a band size of 1000 bp, and N (Me4 + Em2) with band sizes of 250 bp and 1070 bp, manifested in the pine populations of TAHURA and Kerinci, yet not evident in the Rao. In the primary combination J (Me3 + Em2), band sizes of 225 bp and 270 bp are non-existent in the TAHURA population; however, they appeared in the pine populations of Rao and Kerinci. The five

informative bands always appear in the population of Kerinci, although they were not evident in any other populaces of Aceh in Rao and TAHURA, Indonesia.

Furthermore, the Kerinci population also showed to own 45% of the most informative bands, while the Rao population had only 25% of the total. It demonstrates the potential for genetic differences across the populations of Aceh and Kerinci, which may have resulted due to evolutionary stages in the Kerinci landrace. Therefore, it is assumable the Kerinci landrace is the originating species of *P. merkusii* in Sumatra, where the basis for evolutionary process dividing is on geographical location and the high polymorphic and particular bands on the plant.

The Kerinci population can be isolated from the two others of origin (Aceh and Tapanuli); since the distribution region of the Kerinci origin exceeds 2° South latitude on the equator, a rare habitat for the genus *Pinus* and the *P. merkusii* from Kerinci tends to be unique (Cooling, 1968). The existing fragmentation of population distribution, migration history, and phylogeographic structure affects the species' genetic characteristics that have been left for a long time from other populations (Shuvaev and Ibe, 2021; Chertov et *al.*, 2022). Another possible cause is that the Kerinci population is a longer-living stand than the other two, which will also affect genetic variation in each populace (Xie *et al*., 2015).

The genetic diversity analysis of the three *P. merkusii* populations is in Table 3. Indicators of the level of genetic diversity in a taxon are the percentage of polymorphic loci (P), heterozygosity values (H), and Shannon index (I) (Xie *et al*., 2015). In this study, the heterozygosity (H) and Shannon index (I) values were approximately the same as those of the endemic pine found in China at the genus level (H = 0.2165, I = 0.3414, respectively) (Xie *et al*., 2015). However, they were lower than the Chinese *Pinus keysia* population (H = 0.4567, I = 0.6484, respectively) (Wang *et al*., 2019). Generating the genetic parameter values of the *P. merkusii* in Sumatra demonstrated the highest genetic diversity.

Further discovery revealed that *P. merkusii* from Kerinci had a higher genetic diversity than *P. merkusii* populations found in the Rao and TAHURA at the population level (Table 3). Although, these findings were different from previous studies regarding the genetic diversity of the three populaces of *P. merkusii* (Aceh, Kerinci, and Java) using isozyme markers showing that the values of genetic, allele, and gamete diversity and heterozygosity between the two landraces of *P. merkusii,* i.e., Aceh and Kerinci, were very different, where the value of genetic diversity in *P. merkusii* from Kerinci was least, as indicated by a heterozygosity value of 0 in both seed and embryo samples (Siregar and Hattemer, 1999).

It suggests that the SRAP marker indicated more promising in identifying a molecular characteristic that differentiates the *P. merkusii* variants. Moreover, SRAP has sensitivity in identifying species even when the genome composition of the species is unknown, providing for its rapid identification (Wang *et al.*, 2019). *P. merkusii* showed a total heterozygosity (Ht) value of 0.2126 which was higher than the said value in the population HS (0.1797). These results were following Wang *et al*. (2019), who obtained the results for Ht = 0.4580 and Hs = 0.3801. In contrast, these findings were different from the study of Xie *et al.* (2015), who reported that the total genetic variation (Ht) was lower (0.2134) than the genetic variation in the population Hs (0.3426).

In the genetic diversity analysis of the two landraces, a.k.a., Kerinci and Aceh (Rao and TAHURA populations) of *P. merkusii*, it is apparent that Sumatran *P. merkusii* reveals the highest genetic diversity, particularly in the Kerinci landrace. The Kerinci landrace has a high genetic diversity, suggesting that it is a better source of germplasm and the parental population of *P. merkusii* in Sumatra. In some cases*,* a high genetic diversity value can allow researchers to determine and track the origins of plant groups, including both natural and artificial populations (Zhou *et al.*, 2023).

CONCLUSIONS

Based on the DNA characteristics of the SRAP polymorphism, there were 45% informative bands that could serve as distinguishing characteristics for *P. merkusii* landrace belonging to Kerinci, specifically the combination of primers C (Me1 + Em3) with the band size of 900 bp, and primer I (Me3 + Em1) at a band size of 1050 bp. The percentage value of polymorphic loci was 93.55% in the three populations of *P. merkusii*, with the heterozygosity and Shannon index values at 0.2165 and 0.3414, respectively, which were the indicators of greater genetic variety. However, it has a low genetic divergence (Gst) between the populations, with a score of 0.1550. The Kerinci landrace has higher genetic variation than the two others of Aceh, and thus, assumed as the origin species of *Pinus merkusii* in Sumatra.

ACKNOWLEDGMENTS

Thanks to Dr. Djong Hon Tjong for the advice given, as well as, to team members of ANDA Herbarium and the Genetics and Molecular Laboratory, Univeristas Andalas and Maythesya Octavioni M. Biotech, for the provided advice and assistance during the research process. This research received funding from the indexed publication Research Scheme (RPT), with Number T/133/UN16.17/PT.01.03/2022, year 2022.

REFERENCES

Alghamdi SS, Khan MA, Migdadi HM, El-Harty EH, Afzal M, Farooq M (2019). Biochemical and molecular characterization of cowpea landraces using seed storage proteins and SRAP marker patterns. *Saudi J. Biol. Sci*. 26(1): 74-82.

Andini R, Melinda V, Pardede E, Yanti LA, Hmon K, Moulana R, Indrioko S (2022). Morphological variation of Aceh Pinus (*Pinus merkusii*). *IOP Conference Series: Earth Environ. Sci*. 951(1): 012091. http://doi.org/10.1088/1755-1315/951/1/012091.

Aseny N, Syamsuardi S, Nurainas N (2021). Molecular characterization of Andalas tree dioecious plant [Morus macroura Miq.] using SRAP marker. *IOP Conference Series: Earth Environ. Sci*. 741(1): 012050. http://doi.org/10.1088/1755-1315/951/1/012091.

Babayan AH (2022). Pest species composition of fragrant trees in the parks and gardens of Yerevan City, Armenia. *SABRAO J. Breed. Genet.* 54(1): 201-209. http://doi.org/10.54910/sabrao2022.54.1.19.

Chertov N, Nechaeva Y, Zhulanov A, Pystogova N, Danilova M, Boronnikova S, Kalendar R (2022). Genetic structure of Pinus populations in the Urals. *Forests* 13(8): 1278. https://doi.org/10.3390/f13081278.

Cooling ENG (1968). Fast-growing timber trees of the lowland tropics No. 4 *Pinus merkusii.* Commonwealth Forestry Institute, Department of Forestry, University of Oxford, Inggris.

Ding WD, Cao ZM, Cao LP (2010). Molecular analysis of grass carp (*Ctenopharyngodon idella*) by SRAP and SCAR molecular markers. *Aquacul. Int*. 18: 575-587. https://doi.org/10.1007/s00122-003-1428-4.

Finkeldey R (2005). An Introduction to Tropical Forest Genetiks: Molekuler Basic the Gene as a Function Unit. Institute of Forest Genetiks and Forest Tree Breeding, Germany.

Gao S, Cong R, Gao L, Zhu Y, Meng Y, Zhou Y (2020). Genetic diversity analysis of phenotypic character and SRAP molecular markers in 45 tree peony cultivars. *Brazilian J. Bot*. 43(2): 291-302.

Hammer Ø DAT, Harper, Ryan PD (2001). PAST: Paleontological Statistics Software Package For Education and Data Analysis. *Palaeontologia Electr*. 4 (1):9.

Han XY, Wang LS, Shu QY, Liu ZA, Xu SX, Tetsumura T (2008). Molecular characterization of tree peony germplasm using sequence-related amplified polymorphism markers. *Biochem. Genet*. 46: 162-179.

Hao Q, Liu ZA, Shu QY, Zhang R, Rick JD, Wang LS (2008). Studies on Paeonia cultivars and hybrids identification based on SRAP analysis. *Hereditas* 145: 38-47.

Huda M, Murni P, Maulidah R (2019). Genetic divergence landrace of langsat (Lansium parasiticum) from Siberut Island based on ITS and MatK markers. *Indian J. Agric. Res*. 53(3): 338-342.

Imanuddin R, Hidayat A, Rachmat HH, Turjaman M, Nurfatriani F, Indrajaya Y, Susilowati A

(2020). Reforestation and sustainable management of *Pinus merkusii* forest plantation in Indonesia: A Review. *Forests* 11(12): 1235. https://doi.org/10.3390/f11121235.

Istomo I, Kusmana C, Roswandi S (2000). Kajian faktor lingkungan fisik *Pinus merkusii* Jungh Et De Vries Ras Kerinci di Resort KSDA Bukit Tapan, Kawasan Taman Nasional Kerinci Seblat, Jambi. *Media Konserv.* 7 : 231487. https://doi.org/10.29244/medkon.7.1.%25p. (in Indonesian).

Kumar J, Heikrujam M, Sharma K, Agrawal V (2019). SRAP and SSR marker-assisted genetic diversity, population structure analysis and sex identification in Jojoba (*Simmondsia chinensis). Ind. Crops Prod.* 133: 118-132.

Li G, Quiros CF (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. *Theor. Appl. Genet*. 103(2): 455-461.

Nuratika E, Aseny N (2020). Clarification of Sumatran mulberry (*Morus macroura* var. macroura, Moraceae) from West Sumatra, Indonesia using nucleus ribosomal ITS (Internal Transcribed Spacer) gene. *Indian J. Agric. Res*. 54(5): 635-640.

Pousujja R, Granhof J, Willan RL (1986). Pinus merkusii Jungh. & De Vriese. *Seedleaflets*.

Robarts DWH, Wolfe AD (2014). Sequence-related amplified polymorphism (SRAP) markers: A potential resource for studies in plant molecular biology. *App in Plant Sci*. 2(7): 1400017. https://doi.org/10.3732/apps.1400017.

Saimova RU, Batyrova KI, Bekenova NA, Kauynbaeva E, Esimov BK (2022). Ground beetles (Coleoptera: Carabidae) in different agroecosystems of Southeast Kazakhstan. *SABRAO J. Breed. Genet*. 54(2): 458-468. http://doi.org/10.54910/sabrao2022.54.2.21.

Sandri Y, Maideliza T, Mansyurdin, Febriamansyah R (2016). Kajian anatomi kayu pada tiga ekotipe *pinus merkusii* sumatera dan potensinya sebagai indikator perubahan Iklim. *Metamorfosa* 3(2): 103-111. (in Indonesian).

Saputra MH, Suhada DA, dan-Edy D (2014). Potensi Pinus merkusii Strain Kerinci Sebagai Penghasil Getah Hasil Hutan Bukan Kayu. Medan: Prosiding Ekspose Hasil Penelitian Tahun 2014 Balai Penelitian Kehutanan Aek Nauli, pp. 154-160. (in Indonesian).

Shuvaev DN, Ibe AA (2021). Genetic structure and postglacial recolonization of *Pinus sibirica* Du Tour in the West Siberian Plain, inferred from nuclear microsatellite markers. *Silvae Genet*. 70(1): 99-107.

Siregar IZ, Hattemer HH (1999). Genetic variation of *pinus merkusii* jungh et de vriese in Indonesia. Biodiversity and Development of Plant Genetic Resources, Deutscher Tropentag, Berlin, Germany.

Syamsuardi (2013). Genetik dan Potensial Evolusi Beberapa Jenis Tumbuhan Sumatera. In Mukhtar E, Syamsuardi, Partomohardjo T, Munir E, Prosiding Seminar Nasional Biodiversitas dan Ekologi Topika Indonesia. Jurusan Biologi FMIPA Universitas Andalas. Padang, Indonesia. (in Indonesian).

Syamsuardi S, Okada H, Ogawa M (2002). A new variety of *Ranunculus japonicus* (ranunculaceae) and its genetic relationships to the related species of sect. Acris in Japan. *Acta Phytotaxon. et Geobot*. 53(2): 121-132.

Wang D, Shen B, Gong H (2019). Genetic diversity of Simao pine in China revealed by SRAP markers. *Peer J*. 7: 6529. http://doi.org/10.7717/peerj.6529.

Xie Q, Liu Z-H, Wang S-H, Li Z-Q (2015). Genetic diversity and phylogenetic relationships among five endemic Pinus taxa (Pinaceae) of China as revealed by SRAP markers. *Biochem. System. Ecol.* 62: 115-120.

Yeh FC, Yang RC, Boyle T (1999). POPGENE, the user-friendly shareware for population genetik analysis, Molecular Biology and Biotechnology Centre University of Alberta, Canada.

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.

Zhou B, Zhang Z, Li Y, Ma Y, Zhang S, Niu S, Li Y (2023). Genetic diversity, genetic structure, and germplasm source of Chinese pine in North China. *Eur. J. For. Res*. 142: 183-195.