Genetic Diversity of Ramie (*Boehmeria nivea* L. Gaudich.) Originating from Wonosobo and Malang Based on Simple Sequence Repeat (SSR) Molecular Markers

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Annisa*, Widya Nur Pangestu, Joko Kusmoro, Budi Irawan

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jalan Raya Bandung-Sumedang Km. 21, Jatinangor, Sumedang 45363, Indonesia

Corresponding email: annisa.annisa4@gmail.com

ABSTRACT

Ramie has been widely used as a fiber crop for over 4000 years. The fibers are durable, white in color, and smooth in texture. Information on genetic diversity is important for selecting good breeding materials to produce superior offspring. This study aimed at determining the genetic diversity of *Boehmeria nivea* (L.) Gaudich. from Wonosobo and Malang using SSR as molecular markers. Nineteen accessions of ramie were analyzed for genetic diversity using 9 SSRs located adjacent to the gene associated with fiber yield traits. This study included the DNA extraction, amplification, and visualization of amplification. Data analysis included the allele number, frequency, PIC value, heterozygosity, Shannon information index, and AMOVA analysis. The results showed 229 alleles, with an average polymorphic percentage of 68.67%, the average allele frequency ranging from 0.07 to 0.11, an average PIC value of 0.84, and Jaccard's similarity score of 0-0.18. The He and Ho values in both populations were 0.719 and 0.278, respectively. AMOVA analysis revealed that 88% of the observed molecular variance was due to genetic differences within the population, whereas 12% of genetic variation was partitioned between populations. The present study showed high genetic diversity between Wonosobo and Malang ramie. This finding might support further programs for the fiber and biomaterial industry.
Keywords: Diversity; DNA; Genetic; Ramie; SSR

INTRODUCTION

Boehmeria nivea (L.) Gaudich., also known as ramie or Chinese grass, comes from the Urticaceae family. This plant is naturally a diploid plant (2n=2x=28) (Ni et al., 2018) that has a very long ultimate fiber cell ranging from 120-150 mm, almost six times longer than cotton, ten times longer than linen and eight times longer than silk, resulting in excellent tensile strength (Yadav et al., 2022). The fiber can be processed into high-quality products, such as technical fabrics, personal clothing, and other textile fibers (Zhou et al., 2017). The fiber in this plant is found in the stem, especially the bark. The plant can reach 1 – 2.5 m in height with heart-shaped leaves covered with hairs underside. The most common plant propagation uses rhizome cuttings (Flora & Fauna Web, 2017) (Figure 1). This plant is an annual plant that has a life cycle of up to 20 years. Good ramie fiber is shiny, white, hygroscopic, colorless, and quick to dry. It also does not wrinkle in the sun. These qualities make this fiber a good cotton substitute (Murianingrum et al., 2019). Fiber production results are not always

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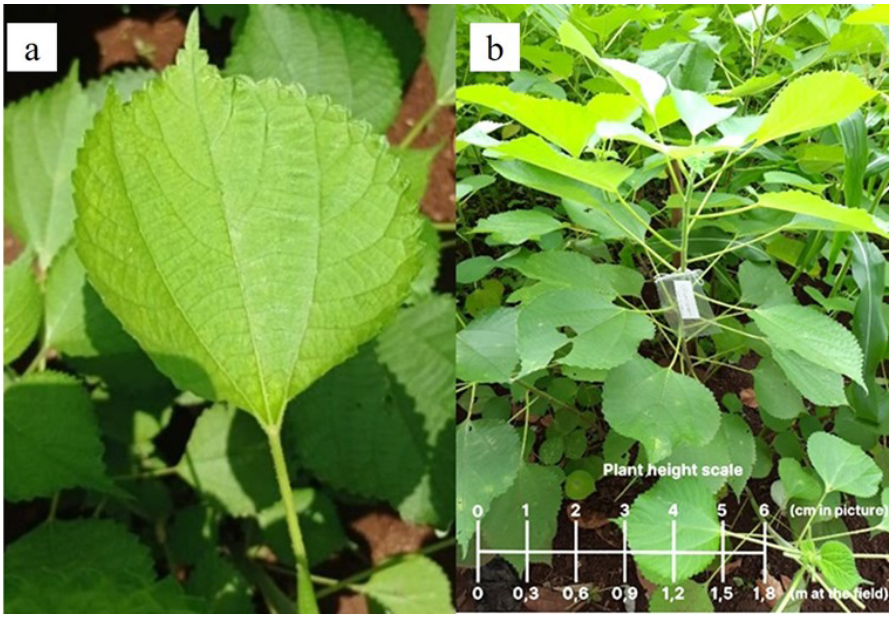

Figure 1. Leaf (a) and height (b) of ramie plant from Wonosobo

the same at every harvest despite being propagated vegetatively. This issue could make the quality of ramie fiber less standardized.

Ramie grows well in the tropics and produces high yields if planted in lowlands or highlands areas with an altitude of 10 – 1500 m asl, rainfall of 1,500 – 2,500 mm/ year, and soil pH of 5.4 – 6.5 (Habibie et al., 2021). Wonosobo, Central Java, has suitable conditions where this plant can develop well, making it the center of the ramie development area in Indonesia (Hidayah et al., 2022). As the center for ramie development in Java, Wonosobo has the potential to become a home for plants with various genetic backgrounds that are very interesting to study. Balai Penelitian Tanaman Pemanis dan Serat (Balittas) Malang produces Ramindo 1 as a superior variety for ramie, which has productivity of 2 to 2.7 tons/ha/year with adaptability to wide altitudes (Suherman et al., 2017), making Wonosobo and Malang accessions suitable for genetic diversity study information is needed to select suitable breeding material to obtain superior hybrid offspring. Many markers, such as morphological, cytological, and molecular markers, are used to assess genetic diversity. A morphological marker is a marker that uses phenotypic properties, such as leaf shape and color, that external factors can easily influence. This is considered to be a limitation for morphological markers. Cytological markers can overcome external factors and problems in morphological markers but cannot distinguish species or individuals in a population if the number of chromosomes is the same. On the other hand, molecular markers allow direct analysis of genetic material effectively without being influenced by external factors and gene expression (Ni et al., 2018). Molecular marker studies have been widely used to measure the genetic relationship of ramie plants in China, Brazil, Indonesia, India, and Cuba populations (Ni et al., 2018; Shi et al., 2022; Wang et al., 2019).

Simple sequence repeat (SSR) markers are useful for various applications in genetics, especially in plant breeding, compared to other molecular markers due to their codominance, characterized by high polymorphism, and widely recognized as informative in plant species (Aiello et al., 2020). SSR

markers, such as diversity studies and genetic mapping, have been used for different purposes. SSR is a molecular marker characterized by a small DNA sequence consisting of one to six tandemly repeated base pairs (Kapoor et al., 2020). It is known that ramie harvest for use in industry is becoming important, especially in several countries that have ramie cultivars, such as China, Brazil, the Philippines, and Indonesia. Genetic diversity is an important factor in plant breeding programs to assist in obtaining desired varietal traits. Therefore, because of the importance of information about the genetic diversity of ramie, this study was conducted to examine the genetic diversity of ramie from Wonosobo and Malang using SSR molecular markers. The SSR locus used was chosen based on its proximity to the characteristics associated with ramie fiber so that the information from this study can be used to provide scientific information on the genetic diversity of ramie and to assist ramie breeding efforts to increase ramie production in Indonesia.

MATERIALS AND METHODS

This experiment was conducted at the Department of Biology, Faculty of Mathematics and Science, Padjadjaran University Jatinangor from January 2021 to April 2022. The research utilized 16 ramie leaf accessions from CV. Rabersa (Wonosobo, Central Java), and there were three accessions from the Balai Penelitian Tanaman Pemanis dan Serat (Balittas) in Malang. All accession samples were subsequently planted in Jatinangor.

Leaf Sample Collection

The leaves were dried at room temperature using silica gel with a sample and silica gel ratio of 1:10 for at least 48 hours without air (Chase & Hills, 1991). Each Wonosobo accession was marked with codes Wo1 to Wo16, and Malang accessions with codes MR2, MR4, and RMD1.

DNA Extraction and Quantification

Sodium dodecyl sulfate (SDS) DNA extraction used in this study was based on the modified method of Amani et al. (2011). A total of 0.3 grams of leaf tissues were put into a sterile Eppendorf tube and crushed with 500 µl of extraction buffer [200 mM Tris-HCl (pH 7.5), 250 mM NaCl, 25 mM EDTA, 0.5% SDS], then the tube was mixed for 5 seconds with a vortex and placed in a water bath at a temperature of 60ºC for 30 minutes. Chloroform: iso-amyl alcohol (24:1) was added in equal volume to the tube, then mixed and centrifuged at 13,000 g for 8 minutes. The supernatant was transferred to a new tube, after which isopropanol was added with an amount equal to the volume in the tube, then mixed and incubated at -20ºC for 30 minutes. After that, the tubes were centrifuged at 13,000 g for 8 minutes, and the pellets were dried and dissolved in 25 µl TE buffer. The tube was placed on ice for 5 min and centrifuged at 13,000 g for 5 minutes. Each sample was repeated 2 times and put together in one tube. DNA quantification was carried out by measuring the absorbance value at 260 and 280 nm.

PCR Amplification and Visualization

The primer used was determined based on its proximity to the properties associated with ramie

Table 1. The primer sequences used in the amplification designed by Liu et al. (2013)

Marker	Forward primer (5'-3')	Reverse primer (5'-3')	Ta (°C)	Product size estimate (bp)
RAM144	TTCAACGGAACACAGTCAGC	AACACCTGAAATGGAATCGC	57.4	269
RAM263	GTTAGCTGCCGGAGGAAAG	CTCTCCACCATTCTCACCATC	59.5	113
RAM111	TCTTTGGCAGAGAGGAAGGA	TGCTGGGAGTTTGCTTTCTT	55	176
RAM179	TTGGCTCTGGCTCTGGTTAT	GAGGAGGAAGATGACGACGA	59.4	171
RAM181	CGTCAAGCTTCACAAATCCA	CTTCTACAACCGCTCCTTCG	57.4	151
RAM435	GATTTCCAAGGAAAGTGCCA	GCGACTTGCTCTCTGAGCTT	57.4	183
RAM579	ACAAGCCCATTGTCAGGAAG	TCGCTCTTGGGAACTTGTTT	57.4	229
RAM022	GCCGCCAAAGAGACCCAC	CAAGACATCAAGAGGCGTGA	59.5	264
RAM210	CCCATGCACATGATTCTCTG	CGACGGTAGAACAAAGGAGC	59.5	202
RAM053	GATGAACAACGCAGTGGAGA	GCATTGACAGGAGAGTGCAA	57.4	224

Remarks: Ta = annealing temperature
bp = base pairs

fiber based on molecular linkages indicating the position of Quantitative Trait Loci (QTL) for fiber yields per stem and genes related to ramie fiber yield (Liu et al., 2013; Liu et al., 2014; Luan et al., 2017). The list of the primers used is shown in Table 1. PCR was carried out with a total volume of 20 µl (20 ng extracted DNA, 2X GoTaq Green Master Mix, (Promega), 10 µM primer, 1.5 mM $MgCl_2$) using the following condition: 94°C for 3 minutes, followed by 35 cycles of 94°C for 10 seconds, specific annealing temperature for 20 seconds, 72°C for 1 minutes, and a final elongation step of 72°C for 5 minutes (Liao et al., 2014). From the PCR reactions, 5 µl samples were separated by electrophoresis in 2% Agarose: 2% MetaPhor agarose (Lonza) at 20 Volt powers for 2 hours (Mokrousov et al., 2018) and visualized by gel red (Biotin) staining. The band sizes were estimated by comparison with a 100 bp DNA ladder (Promega).

Data Analysis

The allele size was determined through Gel Analyzer 19.1 software. Alleles in the PCR results were made into 1-0 matrix (Alhariri et al., 2021), and the percentage of polymorphic alleles, allele frequency, and PIC values were calculated. GenAlEx 6.503 software was used to analyze genetic diversity parameters, including Shannon Information Index (I), observed and expected heterozygosity (Ho and He), and Analysis of Molecular Variance (AMOVA). The Dendrogram of the phylogenetic tree was created by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with Jaccard similarity coefficient through Palaeontological Statistics (PAST) 4 software (Clarke, 1993).

RESULTS AND DISCUSSION

The information on the genetic diversity of ramie [*Boehmeria nivea* (L.) Gaudich.] is important to help accelerate the development of breeding strategies. In addition, genetic diversity can also assist in conserving and utilizing ramie germplasm resources (Ni et al., 2018). Simple sequence repeat (SSR) molecular markers have been used to study the genetic diversity of germplasm resources due

to several advantages, such as being widely distributed in the genome, its high polymorphism, chromosome-specific, and frequently inherited in a Mendelian co-dominant fashion (Desai et al., 2021).

SSR Marker Polymorphism

A total of 229 alleles were detected from 9 SSR loci tested on 19 ramie samples from 2 populations, with a mean of 25 alleles per locus (Table 2). The selection of genes (DNA markers) that are closely linked (tightly-linked markers) with genes controlling fiber properties is based on the concept that the genes closer to each other are more likely to be inherited together than genes that are located far apart from each other (Greytak et al., 2019), so it is expected that the selected markers have the ability to control fiber properties in ramie plants. This is intended to assist in selecting a desired character with high precision, accuracy, and selection efficiency to help accelerate the development of new varieties.

Table 2. Ramie originating from Wonosobo and Malang polymorphism data based on SSR markers

Locus	Total Allele	Allele size range (bp)	Average allele frequency	Number of polymorphic alleles	Number of monomorphic alleles	Polymorphic %	PIC value
RAM179	19	136-175	0.09	12	7	63.15	0.87
RAM435	19	161-196	0.10	15	4	78.94	0.87
RAM022	19	243-266	0.11	15	4	78.94	0.85
RAM210	16	203-225	0.09	13	3	81.25	0.91
RAM181	20	203-225	0.07	10	10	50.00	0.89
RAM111	35	166-256	0.08	23	12	65.71	0.81
RAM263	19	91-119	0.09	14	5	73.68	0.88
RAM579	30	424-539	0.07	16	4	53.33	0.85
RAM053	52	351-486	0.09	38	14	73.07	0.67
Mean	25.44	294.02	0.08	17.33	7	68.67	0.84

Remarks: bp = base pairs

The RAM210 locus showed the lowest total allele, and the RAM053 locus showed the highest total allele, 16 and 52, respectively. Some samples could not be amplified for a certain locus, indicated by the absence of alleles in the electrophoresis results, known as null alleles. A null allele is any allele at the SSR locus that consistently fails to amplify and cannot be detected by PCR. One of the causes of null alleles is mutation in one or both primer binding sites, which inhibits primer binding and amplification (Wu et al., 2019).

The molecular markers' informativity can be determined based on the Polymorphic Information Content (PIC) value. The PIC value was calculated by the following formula (Smith et al., 1997):

$$PIC = 1-\sum fi^2 \quad (1)$$

Where fi^2 is the allele frequency, previous studies on the genetic diversity of ramie stated that the PIC value ranged from 0.1578 to 0.7603 (average of 0.5017) (Ni et al., 2018), while in this study, PIC value showed a higher value, which ranged from 0.67 (RAM053) to 0.91 (RAM210) with an average of 0.85. The high PIC value suggests the marker's usefulness for genetic polymorphism

study. All SSR markers used in this study had PIC values of more than 0.5, which indicates the markers are informative. Based on Botstein et al. (1980), markers with PIC values greater than 0.5 are considered to be very informative, values between 0.25 and 0.50 are informative, and values lower than 0.25 are not informative. This study showed all markers used were informative with PIC values greater than 0.5, so they are useful for the genetic diversity study of *Boehmeria nivea* (L.) Gaudich. originating from Wonosobo and Malang.

Molecular markers corresponding to a class of genetic markers are used to evaluate genetic differences between two or more individuals (Lemos et al., 2019). In this study, the markers were used to determine differences between individuals of the same species in two different populations. Polymorphic markers will show differences between genotypes (the pattern of DNA banding varies across individuals tested), while markers that do not differentiate between genotypes are called monomorphic markers (there is only one banding pattern that does not vary). Ramie is a naturally diploid species. Allelic relationships in diploid species can be easily established by considering single bands as homozygous and double bands as heterozygotes. This study showed that RAM111

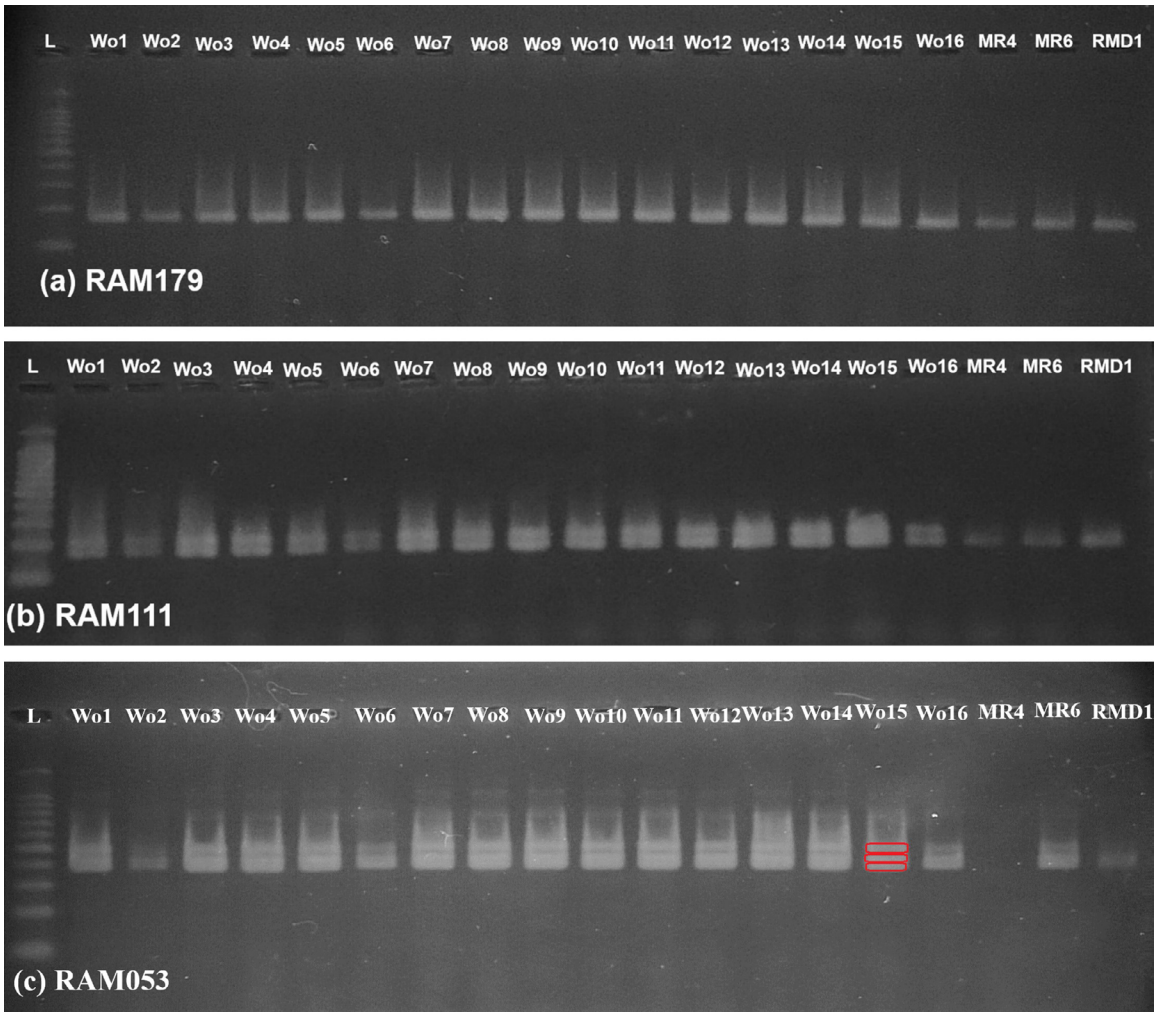

Figure 2. Visualization of electrophoresis results (a) locus showing one DNA band in each sample, (b) RAM111 locus showing two DNA bands, (c) RAM053 locus showing three DNA bands

and RAM579 produced two alleles, and RAM053 produced three alleles in each individual (Figure 2). RAM181 produced two alleles in two accessions of the ramie plant from Malang. The two alleles that appear in individuals amplified by RAM111, RAM579, and RAM181 indicate that these individuals are thought to have heterozygous alleles where both alleles are expressed when occurring together in an individual (Treuren, 2021). The three alleles that appeared in individuals amplified by RAM053 suggested that the locus was likely to be duplicated (Berry et al., 2005). When the SSR locus duplicates in a diploid organism, the PCR primer pair can amplify 3 to 4 alleles. The large amount of variability in SSRs has the potential to provide information about duplication, which is also observed in other plants such as in *Gossypium* (Gutiérrez et al., 2009), *Coffea* (Mishra et al., 2011), and *Lactuca* (Sochor et al., 2019). Plant accessions tested with five other markers (RAM263, RAM210, RAM435, RAM179, and RAM022) showed that these loci were homozygous alleles because they only showed one DNA array in each individual.

The allele frequency is determined by the formula (Hartl, 2020):

$$f(A) = {}^{A}/_{2n} \quad (2)$$

where f(A) = i^{th} allele frequency, A = number of i^{th} alleles at the locus, n = total individuals. The lowest average allele frequency is 0.07, and the highest is 0.11. This allele frequency value is lower than Ni et al.'s (2018) study, with frequency values ranging from 0.53 to 0.94. The low allele frequency is suspected to happen because of the latest mutation, so it has not been spread to all individuals in the population. The frequency of alleles that appear can be a measure of the polymorphism of a locus.

Polymorphism or allele diversity resulting from a marker is a picture of the diversity or variation that occurs in each individual gene. This study showed the presence of monomorphic alleles and polymorphic alleles. If the DNA sequence at a given locus is identical among all tested individuals in which only one banding pattern is observed, it is called a monomorphic marker. Whereas if the DNA varies, the pattern of marker bands will vary across DNA pathways from different individuals and have polymorphic marker loci.

This study showed the lowest percentage of polymorphisms was in the RAM181 locus (50%), and the highest was in RAM210 (81.25%). This suggested that the loci used in this study are polymorphic, so they are good for use as molecular markers. The size of the alleles obtained in this study varied between 91 to 539 base pairs (bp). The allele sizes at the RAM179, RAM435, RAM022, RAM111, and RAM263 loci were similar to those in previous studies (Liu et al., 2014; Luan et al., 2017). Four loci (RAM210, RAM181, RAM579, and RAM053) produced alleles with size outside the study range, producing alleles that were larger than the estimated product size. The difference in product size is probably because the ramie from Wonosobo and Malang do not have the same product size as the ramie plants used by Liu et al. (2014) and Luan et al. (2017) in China.

Genetic Diversity

Parameters of the genetic diversity of each population are listed in Table 3. Almost all loci showed an expected heterozygosity (He) value greater than the observed heterozygosity (Ho) at all loci, show-

Table 3. SSR markers data assayed in the characterization of ramie accessions

Pop.	Locus	N	Na	Ne	I	Ho	He	F
Wo	RAM179	16	10.00	7.11	2.13	0.00	0.85	1.00
	RAM435	16	8.00	6.40	1.96	0.00	0.84	1.00
	RAM022	16	8.00	5.33	1.85	0.00	0.81	1.00
	RAM210	15	9.00	7.75	2.11	0.00	0.87	1.00
	RAM181	16	11.00	9.14	2.30	0.00	0.89	1.00
	RAM111	16	21.00	17.65	2.95	1.00	0.94	-0.06
	RAM263	16	8.00	6.73	1.99	0.00	0.85	1.00
	RAM579	14	20.00	17.04	2.91	1.00	0.94	-0.06
	RAM053	16	20.00	15.51	2.87	1.00	0.93	-0.06
MR	RAM179	3	3.00	3.00	1.09	0.00	0.66	1.00
	RAM435	3	3.00	3.00	1.09	0.00	0.67	1.00
	RAM022	3	3.00	3.00	1.09	0.00	0.67	1.00
	RAM210	1	1.00	1.00	0.00	0.00	0.00	#N/A
	RAM181	2	4.00	4.00	1.38	1.00	0.75	-0.33
	RAM111	3	2.00	1.80	0.63	0.00	0.44	1.00
	RAM263	3	3.00	3.00	1.09	0.00	0.66	1.00
	RAM579	2	2.00	2.00	0.69	0.00	0.50	1.00
	RAM053	2	3.00	2.67	1.04	1.00	0.62	-0.60
Mean						0.28	0.72	

Remarks: N = total allele
Na = average number of alleles per locus
Ne = the effective alleles
I = Shannon information index
Ho = observed heterozygosity
He = expected heterozygosity
F = fixation index

ing a divergence from Hardy Weinberg equilibrium (HWE) and the possibility of inbreeding (Sharma et al., 2016). He and Ho values in the Wonosobo population ranged from 0.813 – 0.844 (average 0.883) and 0-1 (average 0.333), respectively. Meanwhile, the He and Ho values in the Malang population ranged from 0-0.750 (average 0.554) and 0 -1 (average 0.222), respectively. The He value obtained in the Wonosobo population was greater than the study found in the Chinese population. This high value suggests the richness of alleles in the germplasm so that it can be used in breeding programs that target the plant's commercial culture. The He value in the Malang population was lower than in the previous study (Ni et al., 2018). This can happen because the number of plant samples used in the Malang population was lower. A heterozygosity value greater than 0.5 is believed to be suitable for genetic diversity studies (Sheriff & Alemayehu, 2018). The F fixation index is a measure of the heterozygosity of an individual due to inbreeding. The RAM111, RAM579, RAM053 and RAM179, RAM181, and RAM053 showed Ho values that were greater than the He values of the Wonosobo and Malang population, respectively, which leads to the fixation index (F) value being negative. An F value approaching 1 indicates that the population leads to homozygosity, while an F value close to negative 1 indicates a heterozygous population (Gan et al., 2021).

The Shannon (I) information index value of the two populations ranged from 0 to 2.956 (aver-

age of 1.626). The Shannon index value in the population of Wonosobo ranged from 1.858 to 2.956 (average of 2.346), while the value of I in the population of Malang ranged from 0 to 1.386 (average of 0.906). This value was greater than previous studies found in the Chinese population (Luan et al., 2017). Molecular Variance (AMOVA) was analyzed to investigate genetic variation among populations. AMOVA analysis revealed that 88% of the observed molecular variance was due to genetic differences within the population, whereas 12% of genetic variation was partitioned between populations.

Kinship

The phylogenetic tree construction was carried out using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) on PAST4 software to determine the groups and relationships of ramie plants in the two populations tested. The grouping results are based on the Jaccard similarity coefficient. The grouping results are presented in the form of a dendrogram (Figure 3). Five groups were formed based on the UPGMA. Group I (marked with red color) consists of MR6 and RMD1; Group II (marked with orange color) consists of Wo14, Wo15, and MR4; group III (marked with yellow color) consists of Wo7, Wo13, Wo16, and Wo1; group IV (marked with green color) consists of Wo3, Wo6, Wo8, Wo9, Wo10, Wo11, and Wo12; and group V (marked with blue color) consists of Wo2, Wo4, and Wo5. The genetic distance calculated based on the similarity of Jaccard from the

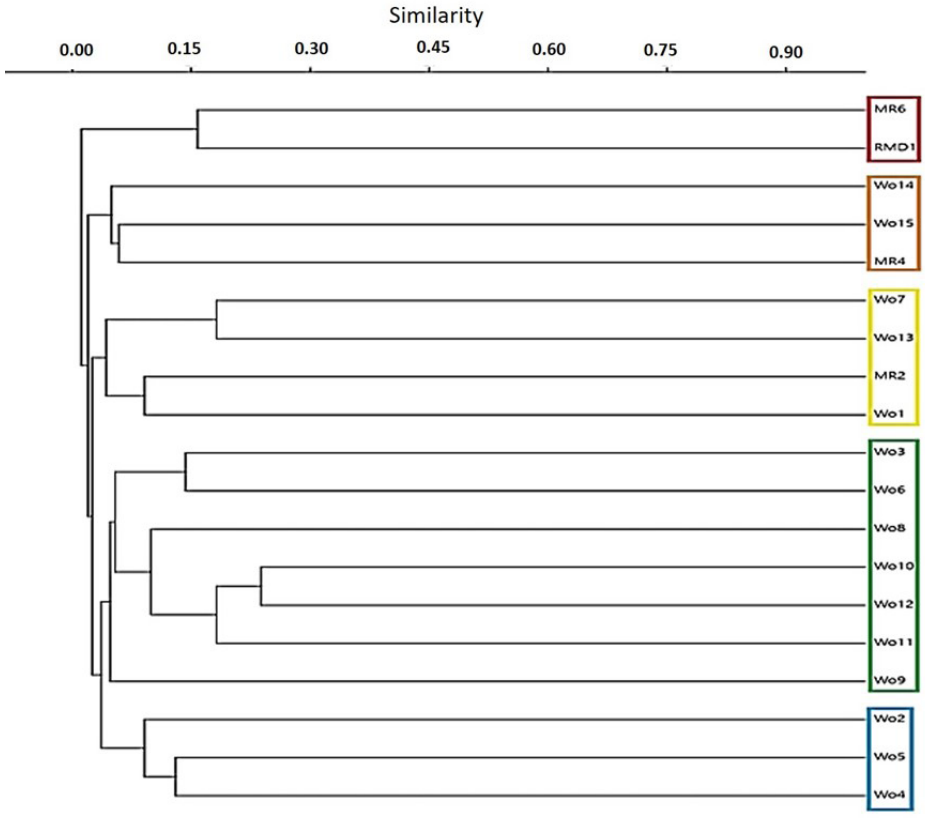

Figure 3. Dendogram based on SSR markers (Remarks: Wo = Wonosobo accession; MR = Malang accession. Group I marked with red box, Group II marked with orange box, Group III marked with yellow box, Group IV marked with green box, Group V marked with blue box)

Table 4. Jaccard similarity

	Wo1	Wo2	Wo3	Wo4	Wo5	Wo6	Wo7	Wo8	Wo9	Wo10	Wo11	Wo12	Wo13	Wo14	Wo15	Wo16	MR4	MR6	RMD1
Wo1	1																		
Wo2	0	1																	
Wo3	0	0.09	1																
Wo4	0	0.09	0.13	1															
Wo5	0.08	0.09	0	0.13	1														
Wo6	0	0	0.14	0.04	0	1													
Wo7	0.13	0	0	0.08	0	0.04	1												
Wo8	0	0.04	0.08	0	0.08	0.09	0.04	1											
Wo9	0	0.04	0.04	0.04	0.04	0.04	0	0.04	1										
Wo10	0.08	0	0.04	0	0.04	0.09	0.04	0.13	0.08	1									
Wo11	0.04	0.09	0	0.04	0.04	0	0.04	0.08	0.04	0.18	1								
Wo12	0.04	0	0.08	0	0.04	0.04	0.04	0.08	0.04	0.23	0.18	1							
Wo13	0.04	0.04	0.04	0.08	0	0.04	0.18	0.04	0.08	0	0.04	0	1						
Wo14	0	0	0	0.04	0.04	0.04	0.08	0.08	0	0.04	0	0.04	0	1					
Wo15	0	0	0	0.04	0	0.09	0	0.04	0.04	0	0.04	0	0	0.04	1				
Wo16	0.09	0.04	0	0	0	0	0	0	0	0	0	0.04	0	0.04	0.04	1			
MR4	0	0	0	0	0	0	0	0.05	0	0	0	0	0.05	0.05	0.05	0	1		
MR6	0	0	0.04	0.04	0.04	0	0.04	0	0	0	0	0.04	0	0.04	0	0	0	1	
RMD1	0	0	0.04	0.04	0	0	0	0	0	0	0	0	0	0	0	0.04	0	0.15	1

ramie plant originating from Wonosobo and Malang populations showed a distant kinship relationship (average = 0.13) (Table 4). The Jaccard similarity coefficient close to 1 indicates that the individuals are closely related, while the similarity coefficient close to 0 indicates the distant relationship between individuals (Zach, 2020). Wo7 and Wo13, Wo10 and Wo11, and Wo11 and Wo12 (0.18) showed a distant relationship with the Jaccard similarity coefficient close to 1 (0.18).

Group I consisted of plant accessions originating from the Malang population with a Jaccard similarity value of 0.15. Group I also showed the most distant relationship with all other plant accessions based on the family tree; this was supported by the low Jaccard similarity score between group I and other groups (0-0.4). One accession from Malang was merged with accession Wonosobo in Group II based on the family tree; MR4 was closely related to Wo15. Overall, the ramie plant relationship between these two populations is closely related, with most of the Jaccard similarity values being 0. Crosses between these highly related genotypes will most likely produce highly heterotic individuals and can be used in heterosis breeding. Heterosis refers to the offspring that exhibit superior performance over their parents that will be beneficial for their reproduction and adaptation (Liu et al., 2020).

CONCLUSION

Based on the SSR markers used in Wonosobo and Malang, five groups were formed. The study showed high genetic diversity between Wonosobo and Malang ramie. Our study determined that SSR markers in this study could differentiate which plant with all markers that link to fiber and which one does not. This suggests that these markers have great potential to be used as marked assisted

selection in ramie. This study demonstrates the importance of ramie genetic diversity to support the breeding of this plant.

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