The Genetic Diversity of *Moringa Oleifera* on Poteran Island-Madura Based on Petiole Colors Using ISSR (*Inter Simple Sequence Repeat*) Method

Wirdhatul Muslihatin[1,*] Triono Bagus Saputro[1] Nur Isma Latifah[1] Chusnul Eka Safitri Himayani[1]

[1]*Department of Biology, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Jl. Teknik Mesin No. 173, Keputih, East Java, Indonesia.,*
[*]*Corresponding author. Email: wirdhabioits@gmail.com*

ABSTRACT
Moringa oleifera, which is endemic of Pulau Poteran, Madura, is one of a genetic variation of Moringa. It is native Indonesian germplasm, which must be preserved to maintain the genetic biodiversity of Moringa. Known locally as *kelor*, the plant has four different color of petiole: red, white, purple, and green. The purpose of this study was to determine the genetic diversity of the plant with its four different colors of the petiole. This study used six ISSR molecular markers in the analysis. The stages of this research were the preparation of the leaf samples, the DNA extraction, the DNA quality and quantity observation, the PCR with ISSR (inter simple sequence repeat) primer, then the electrophoresis. The locus that shows bands was used for scoring: present (1) and is absent (0). Data analysis and the making of the dendrogram were done by using the unweighted pair group method arithmetic (UPGMA) via the MVSP program. The level of polymorphism generated by the ISSR marker was 97,2% with an average PIC of 0,46 and similarity value of 5%-66,7%.

Keywords: *Genetic diversity, ISSR (inter simple sequence repeat), Moringa oleifera, Petiole colors*

1. INTRODUCTION

Moringa oleifera or *kelor* in local language is reported to have many benefits in both nutritive importance and medicinal application [1]. It can be used as medicine, antidiabetic, anticancer, anti-atherosclerotic agents, neuroprotectant antioxidants, antimicrobial and anti-inflammatory agents [2,3,4,5,6,7]; bio-stimulant [8,9]; and wastewater treatment [10]. Some parts of *Moringa oleifera* i.e., the flowers, leaves, and young fruits are edible too. Indonesian people commonly consume *Moringa oleifera* as a vegetable. Since it is widely spread across the country, it is important to promote it as an alternative source of nutrients [11]. [11] mapped *M. oleifera* plants in Java and Lesser Sunda Island. They found that the plants are mostly distributed in the Jakarta area, West Java, Central Java, Yogyakarta, the northern part of East Java, Madura Island, and the Kangean Islands. In Lesser Sunda Island, they are distributed in the southern part of Bali, Lombok Island, Sumbawa, Kupang, Flores, Sumba and Alor Islands. From the study, it can be inferred that 44% of found in areas with an average annual rainfall of 1500-2000 mm, 20% in areas with an average annual rainfall of 2000-2500 mm. As many as 8% were found in the lowest average annual rainfall (500-1000 mm) and only 5% of the plant were found in the highest average annual rainfall (3000-3500 mm).

Plants are integral parts of biological diversity and are an essential source of human life. They balance the ecosystems as they are the main producers in the food chain. In many cases, they function as natural medicines that are valuable to humans. *Moringa oleifera*, commonly known as *kelor*, is a genetically diverse phenotypes such as multicolored petioles, wide leaves [12], and various shades of green in the leaves [13].

Research on the genetic diversity of *Moringa oleifera* has been carried out in different parts of the world. analyzed genetic diversity of *M. oleifera* in Nigeria [14], then The other researcher used 24 and 10 RAPD primers and obtained polymorphisms of 74% and 81.5% [13], and analyzed the genetic relationship among 20 *Moringa oleifera* from Malaysia by using 24 RAPD primers and obtained 32.7% polymorphism. The molecular markers for the characterization of *Moringa oleifera* germplasm are very important to determine the level of genetic diversity and the genetic relationship between genotypes. Molecular markers are variations in DNA sequences found in certain locations in a genome and can be used to identify an individual or species [15].

The selection of inter simple sequence repeat (ISSR) molecular markers in this study is because microsatellite primers are abundant in eukaryotic organisms such as plants so that they have a higher success rate of amplification, as well as the use of longer primers (16-25 nucleotide bases) compared to RAPD primers (10 bases of nucleotides). The analysis of genetic diversity using ISSR has been applied in *Murraya koenigii* [16], *Jatropa curcas* [17], *Punica granatum* [18], *Brassica napus* [19], *Moringa oleifera* in India [20]. The genetic diversity study of *M. oleifera* in India conducted by [20] using 6 ISSR primers found that there was 48.57% polymorphism. The study of the genetic diversity analysis of the genus of Moringa in Saudi Arabia conducted by [21] using 15 ISSR primers found that there was 75% polymorphism. Genetic characterization using molecular markers is the most effective way to determine the genetic distribution of different morphological characters in plants [13].

Moringa oleifera in Indonesia, especially on Poteran Island in Madura, shows four kinds of petiole colors, namely red, white, green, and purple. The color differences in petiole indicate a genetic difference. However, studies for the analysis of *Moringa oleifera* genetic diversity in this area have not been carried out. Therefore, it is necessary to analyze genetic diversity using inter simple sequence repeat (ISSR) molecular markers to determine the closeness of the interrelated relationship presented in the dendrogram.

2. METHOD

2.1. Plant material

Fresh leaf samples from green, white, red, and purple petiole were taken from Poteran in Madura. The island is located at coordinate 7.04 - 7.12 s and 113.92 - 114.08 E (Figure 1). Fresh young leaf samples of petioles are wrapped with wet cotton and put in a plastic bag and then put in a cool box to prevent loss of moisture.

2.2. DNA Extraction

The DNA extraction method used is CTAB (cetyl trimethyl ammonium bromide). After 80 mg of leaves were taken, each sample was crushed and 1.5 ml of the extract was put into a centrifuge tube with 3 % of CTAB 200 µl. This was then incubated in a Dry Block Heating Thermostat at 60°C for 30 minutes before being cooled at room temperature and added 500 µl of chlorophome: isoamil alcohol (24: 1). After that, it was inverted with vortex and centrifuged at 12,000 rpm for 15 minutes at 4°C. The solution at the top of the tube (supernatant) was taken and put into a new tube. This was then added with 7.5 mM cold ammonium acetate 2.8 µl and 200 µl of cold isopropanol. After being slowly inverted and incubated at 4°C overnight, it was centrifuged at 12,000 rpm for 5 minutes. The pellet was subsequently washed with 700 µl

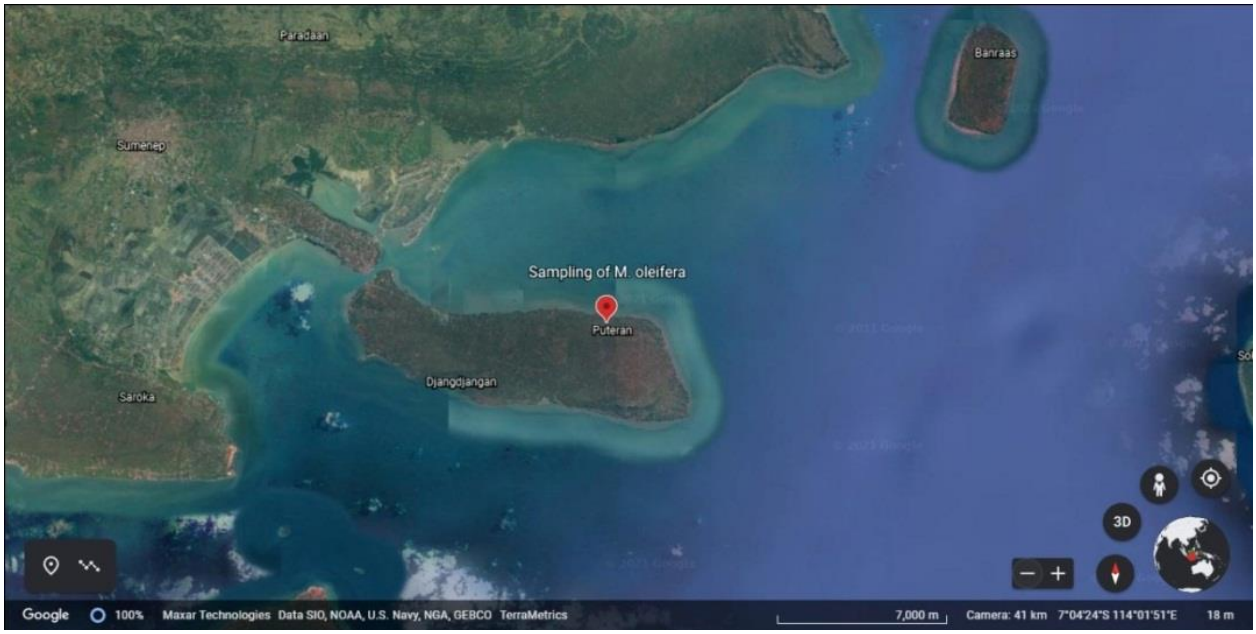

Figure 1. Location of sampling of *Moringa oleifera*.

ethanol 70%, then centrifuged for two minutes. The supernatant was discarded. Then, it was washed with absolute ethanol 700 µl and centrifuged for two minutes before the supernatant was removed. Then, it was air-dried and dissolved in TE 100 µl and stored at -20 °C.

2.3. DNA Quality Check

Observation of DNA quality was analyzed by 0.8% agarose gel. The gel was put on electrophoresis and then added 1x TBE on both sides until it sank. Then, 5 µl of DNA was mixed with dye 1 µl loading included in the gel carefully. Two agarose gels used in this study were 0.8% agarose gel and 2% agarose gel. The 0.8% agarose gel was used to observe the results of DNA extraction and 2% agarose gel was used to view the PCR products. Electrophoresis was carried out at 100V for 30 minutes. Then the gel was placed on the Bio step UV transilluminator to visualize DNA bands.

2.4. PCR Analysis with ISSR

ISSR analysis was done by mixing the reagents (12,5 µl My Taq Red Mix, 2 µl Primer ISSR, 9 µl ddH_2O, 1,5 µl DNA Template) with a total volume of 25µl. Amplification was used in the PCR Rotor-Gene Q machine. This study used six ISSR primers [20, 21] with concentrations of each primer being 0,4 µM. The six ISSR primers are as follows (Table 1). Initial denaturation at 95°C lasted for five minutes, and this was followed by 35 cycles (denaturation at 95°C for 30 seconds, annealing at 35°C to 45°C (depending on the primer) for 30 seconds, extension at 72°C for 30 seconds) and final extension at 72°C for 10 minutes. All PCR reactions are carried out with three replications to ensure that the amplification was stable. For negative controls, all substrates were amplified with a mix without adding DNA. The amplification product was separated in size on DNA electrophoresis Mupid-next submarine electrophoresis system with 0.8% agarose gel in 1x TBE buffer at 100V for 30 minutes at room temperature. The gel was colored with ethidium bromide and then photographed under the Bio step UV Light Transilluminator. The PCR results were analyzed by using a scoring system.

Table 1. ISSR primers

Number	Primer name	Primer sequence	Tm (°C)
1	ISSR–1	(CA)6GG	41
2	ISSR–2	(CT)8GC	45
3	ISSR–3	(GT)6CC	41
4	ISSR–4	(CT)6A	35
5	ISSR–5	(CA)6AG	38
6	ISSR–6	(CAC)3GC	35

2.5. Data Analysis

Genetic variations between *Moringa oleifera* plants can be seen through the images from the DNA visualization, namely in the form of visualized DNA bands on electrophoresis images. DNA band data were converted into binary data using a Microsoft Excel program with the value of 0 for no band and 1 for the presence of DNA bands in the same position of accession/type compared. Based on the ribbon pattern, the similarities between accessions/types are calculated based on the clustering data and the making of dendrograms by using the unweighted pair arithmetic group method (UPGMA) by using multivariate-statistical package (MVSP) program.

After that, the polymorphism presentation of the polymorphic band was calculated. Polymorphic bands are the number of ISSR bands that show variation, namely the presence of DNA bands in several samples and the absence of DNA bands in other samples. The percentage of polymorphism can be calculated by Equation (1) [22]:

$$\frac{\text{Number of Polymorphic bands}}{\text{Total number}} x100\% \quad (1)$$

Polymorphic Index Content (PIC) was calculated using the Equation (2), where fi a symbol is amplification band frequency. PIC of all ribbons of each primer is averaged to get the PIC for each primer. PIC is useful for showing marker information content of each experiment [22].

$$\text{Polymorphic Index Content (PIC)} = 2fi\,(1 - fi) \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. Inter Simple Sequence Repeat (ISSR) Analysis

In this study, the *Moringa oleifera* DNA isolation technique uses CTAB extraction buffer (*Cetyl Trimethyl Ammonium Bromide*), which functions as an extraction buffer that can lyse the membrane into a complex solution containing DNA [23]. This method is commonly used in the extraction of a plant's DNA which contains many polysaccharides and/or polyphenol compounds and produces more nucleic acids. Aquifoliaceae, Aspleniaceae, Cactaceae, Fabaceae, Lamiaceae, Oxalidaceae, Poaceae, Simaroubaceae, Typhaceae, and Vitaceae are examples of families that can be extracted using the CTAB method with good DNA quality results [24]. Molecular markers have several advantages in the determination of genetic diversity, including being free from environmental influences and requiring fewer

Table 2. Result of *Inter Simple Sequence Repeat* (ISSR) analysis

Primer	Sequence	Tm (°C)	Number of DNA Ribbon	Number of Polymorphism	% Polymorfism	PIC
ISSR–1	(CA)6GG	41	16	12	83	0,44
ISSR–2	(CT)8GC	45	9	9	100	0,38
ISSR–3	(GT)6CC	41	10	10	100	0,47
ISSR–4	(CT)6AG	35	7	7	100	0,49
ISSR–5	(CA)6AG	38	7	7	100	0,5
ISSR–6	(CAC)3GC	35	17	17	100	0,5
Total			67	62	583	2,77
Average			11	10	97,2	0,46

sample sizes when compared to plant differentiator morphologically and chemical markers. The molecular markers of ISSR can be used effectively to predict genetic diversity in closely related individuals present in a population [20]. Molecular markers of ISSR perform genetic detection of polymorphisms without first needing to know the base arrangement (sequence) of plant genomics between repetitive base arrangements, which are represented widely and spread throughout the genome. Such base arrangements are the characteristic of nuclear genomes, which show the variations between species or populations. The polymorphism of the ISSR amplification is indicated by the presence of a band in a sample and the difference in the size of the band produced by each sample.

In this study, six ISSR primers (ISSR1, ISSR2, ISSR3, ISSR4, ISSR5, and ISSR6) were used in the *Moringa oleifera* leaf samples in the PCR-ISSR reaction. The primer selection was based on the previous research [20,21], DNA analysis was carried out to determine the genetic diversity of *Moringa oleifera* with four different colors of petiole in Poteran, Madura. The results of polymorphism and PIC (Polymorphic Information Content) from six ISSR primers on the four *Moringa oleifera* plants are shown in Table 2.

The results of DNA amplification using ISSR primers in the table above show a high percentage of polymorphism with an average of 97.2%. The lowest percentage of polymorphism is at the ISSR-1 primer of 83%. While other primers (ISSR2, ISSR3, ISSR4, ISSR5, and ISSR6) show high polymorphisms of 100%. The average PIC value in the results of this study is 0.46. The highest PIC value is in the ISSR 5 and ISSR 6 primers of 0.5. While the lowest PIC value is found in the ISSR 2 primer of 0.38. The average number of bands amplified by each ISSR primer is 11. In the ISSR method, the number of bands amplified on average is small, it is less than 20 patterns of DNA fragments. The use of ISSR 1 primer (Figure 1) produced 16 DNA fragments, 12 polymorphic DNA, and 4 monomorphic DNA. All of the types in this study amplify the DNA fragments. The white petiole only produces 1 DNA fragment with a length of 500bp. The red and purple petiole Moringa produce the same 6 DNA fragments. The longest amplification results of the DNA fragment were 2500bp. Based on *M. oleifera* studies in Saudi Arabia using ISSR conducted [21], DNA fragments were obtained with a long range of 250bp-2510bp.

The ISSR 2 primer (Figure 2) produces 9 polymorphic DNA fragments with a long-range <500bp-1000bp. The white petiole does not amplify DNA fragments. The amplification of DNA fragments is very dependent on how the primer knows its homolog in the desired DNA template. ISSR 3 primer (Figure 2C) produces 10 polymorphic DNA fragments with a length of <500bp-<1500bp. The white petiole does not amplify DNA fragments. The ISSR 4 primer (Figure 2D) produces 7 polymorphic DNA fragments with a length of 500bp-1000bp. The white petiole does not amplify DNA fragments. The ISSR 5 Primer (Figure 2E) produces 7 polymorphic DNA fragments with a length of 500bp - 1000bp. The white and purple petiole do not amplify DNA fragments. The ISSR 6 primer (Figure 2F) produces 17 polymorphic DNA fragments with a length of <500bp-<2000bp. All amplify the DNA fragments. The primer amplifies the most ISSR 6 DNA fragments, while the primers in all are the ISSR 1 and the ISSR 6.

The number of DNA fragments produced by 6 primers is 67. Different primers can also produce different DNA banding patterns. The more sites of attachment from the primer used, the greater number of DNA fragments produced; hence the greater number of banding patterns produced.

3.2. Analysis of polymorphism

In this study, 67 DNA alleles were obtained in which 97.2% of DNA alleles were polymorphic bands. A gene is called polymorphic if the frequency of one allele is less than or equal to 0.95 or 0.99. Polymorphism occurs

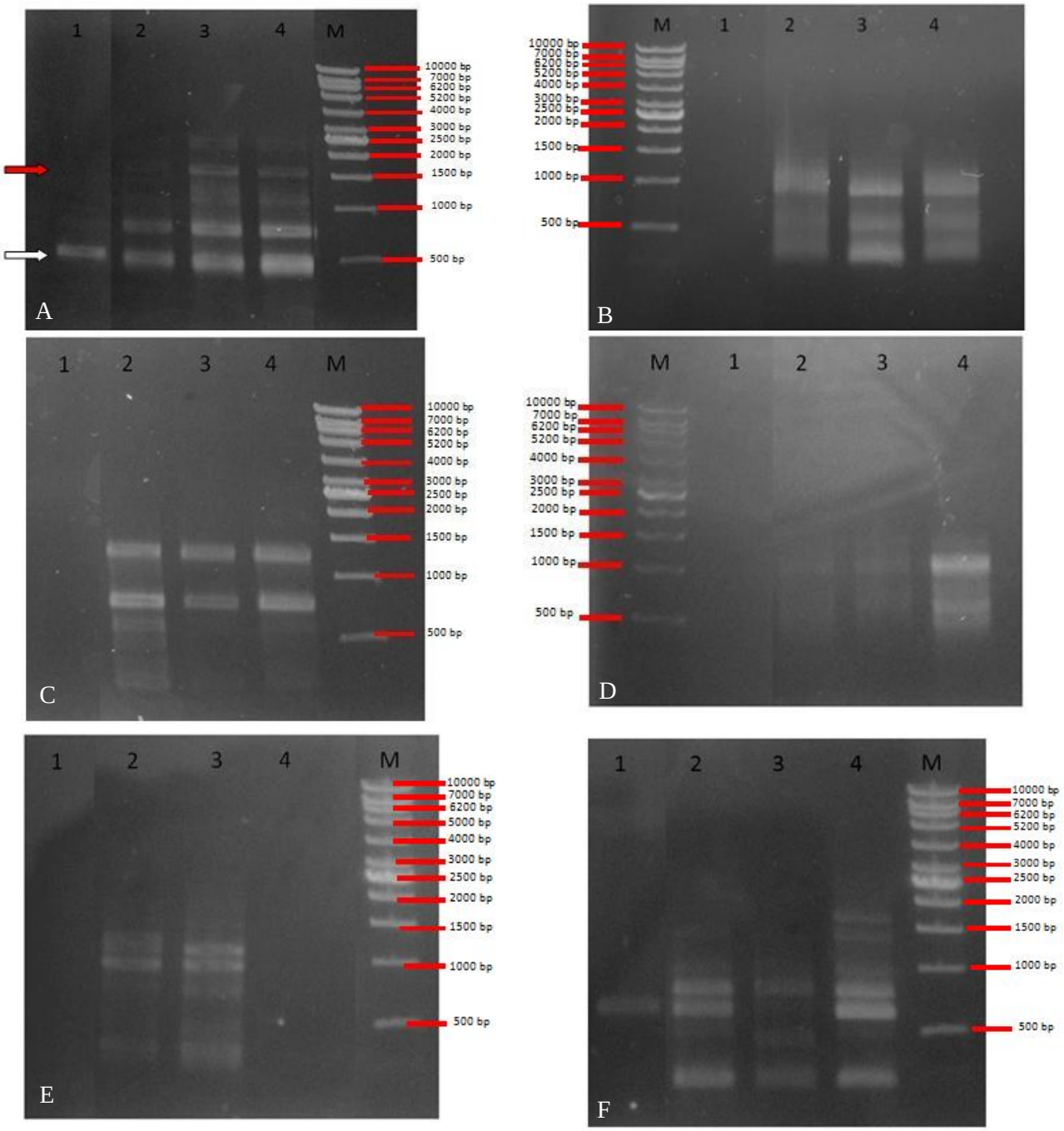

Figure 2. Visualization of amplified DNA bands on *Moringa oleifera* with different primers. Marker (M); Color of the petiole, white (1), green (2), red (3). purple (4). **A.** ISSR 1, **B.** ISSR 2, **C.** ISSR 3, **D.** ISSR 4, **E.** ISSR 5, **F.** ISSR 6.

because each primer has its attachment site. Consequently, the polymorphic DNA bands produced by each primer are different, both in the size of the number of base pairs and the number of DNA bands. The number of polymorphic bands in the analysis of genetic diversity greatly determines the level of genetic diversity of a population [23]. The genetic diversity of *M. oleifera* plants gives a picture of its condition in nature. Previous research on *M. oleifera* genetic diversity in Flores, Indonesia conducted [12], showed lower levels of polymorphism with RAPD amplification of 55.9%. Research on *M. oleifera* genetic diversity in India conducted [20] also showed lower levels of polymorphism with ISSR amplification of 48.57%.

The PIC used in this study ranges from 0.38 to 0.5. This indicates that the primer is capable of detecting polymorphisms 38-50%. The PIC value for a primer depends on the frequency and distribution of alleles found [25]. The PIC value> 0.25 indicates that the primer is more sensitive and can be used as a molecular marker to determine the similarity and proximity of the organism. Based on the DNA band profile amplification

Table 3. Similarity Matrix

Petiole color	White	Green	Red	Purple
White (P)	100%			
Green (H)	11,1%	100%		
Red (M)	5%	48%	100%	
Purple (U)	7,4%	66,7%	58,6%	100%

using six primers, a similarity matrix was determined to determine the genetic relationship of the four *M. oleifera* with different petiole colors (white, green, red, and purple). Each genotype shows the close genetic relationship of the observed genotype.

The results of the similarity analysis using the MVSP program by using the UPGMA method in Table 3 show that the four Moringa with different petiole colors (white (W), green (G), red (R), and purple (P)) can be divided into two groups. Group 1 consists of *M. oleifera* with red, purple, and green petiole colors while group 2 consists of *M. oleifera* with white petiole color. The white Moringa's similarity value with the red one is 5%. The white and purple Moringa's similarity value is 7.4%. The white and green Moringa's similarity value is 11%. The red and purple Moringa's similarity value is 58.6%. While the red and green's Moringa similarity value is 48%. The purple and green Moringa's similarity value is 66.7%. The Moringa dendrogram with Jaccard's coefficient is shown in Figure 3.

The *M. oleifera* plant's similarity values in this study ranged from 5% to 66.7%. The greater the genetic similarity coefficient, the greater the success rate of crosses. The cophenetic correlation index obtained was

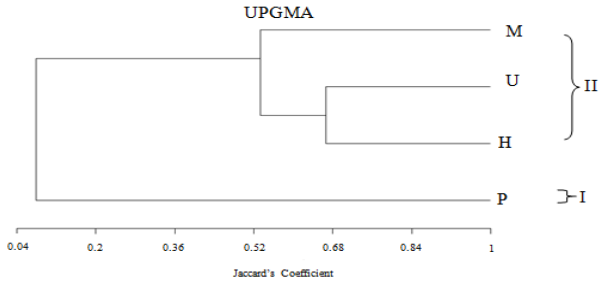

Figure 3. Dendrogram of four *Moringa oleifera* with four different petiole colors based on the banding pattern of 6 ISSR primers with UPGMA method and Jaccard's coefficient.

0.98. The correlation index received was 70.7 or ≥70%. The low correlation value means that the dendrogram formed increasingly depart from the similarity matrix, which is the basis of the construction

Analysis of the genetic relationship of the four *M. oleifera* plants with different petiole colors showed that the plant samples can be divided into two groups. Group 1 is Moringa with white petiole color, while group 2 is Moringa with petiole red, green, and purple. The group 1 and 2 similarity values are 7.8%. The white has a similarity value with red at a considerable distance of 5%. The green and purple have the closest similarity value of 66.7%.

The results of the dendrogram analysis showed the *M. oleifera* plants with different petiole colors in Poteran, Madura. The relationship between green and purple is very close compared to white. The red petiole is quite close to the green and purple petiole. The method used in this cluster analysis is UPGMA with the Jaccard coefficient similarity index formula. UPGMA is a simple method for constructing trees that assumes the average change along a tree is constant and its distance is roughly ultrametric, The Jaccard coefficient can be used to compare data similarities and group them based on those similarities [26].

Based on the grouping, the *Moringa oleifera* on Poteran Island has high genetic diversity. The plant has a high source of diversity based on phenotypes, growth, production, and quality. The diversity of the phenotype, namely the color of the petiole can be determined by three factors: genetic diversity, environment, and environmental interactions with genotypes. As conventional as possible, genetic diversity can be obtained through cross-pollination from existing germplasm collections and *Moringa oleifera* is a plant with high cross-pollination [20]. If this happens, a new type will emerge that can adapt to its environment naturally and in the long term.

The collection of all the samples was at once and from the same location. However, the *M. oleifera* with white petiole shows quite a genetic distance. Individuals being in one region but having different groups indicate the existence of genetic diversity caused by genetic recombination. Populations from the same habitat do not necessarily have a closer kinship. The closeness of relationships is also found in genotypes of different origins. This is influenced by environmental factors or the presence of genotypic interactions with the environment. Information on genetic relationships among individuals and species has important uses for germplasm management, food safety, selection of plants with high productivity, plant breeding, identification of cultivars, and minimizing the number of individual samples [20].

The ISSR molecular markers can be used to determine the genetic diversity of *Moringa oleifera* with 4 different petiole colors. The highest similarity value was 66.7%, which was between green and purple. While the smallest similarity value was 5%, it was between red and white.

AUTHORS' CONTRIBUTIONS

W. Muslihatin designed experiments, analyzed results of the experiments, and prepared article manuscript. T.B.Saputro analyzed the results of the experiments. N.I. Latifat and C.E.C Himayani prepared the experiments and prepared the article manuscript.

ACKNOWLEDGMENTS

We would like to thank the Biology Department Institut Teknologi Sepuluh Nopember (ITS) Surabaya for granting the funding to support this research.

REFERENCES

[1] L.B. Gopalakrishnan, D.A. Kruthi, S.K. Devarai, *Moringa oleifera*: A review on nutritive importance and its medicinal application, Sciencedirect Food Science and Human Wellness vol. 5, 2016, pp. 49–56.

[2] J.L. Rockwood, B.G. Anderson, D.A. Casamatta, Potential uses of *Moringa oleifera* and an examination of antibiotic efficacy conferred by *M. oleifera* seed and leaf extracts using crude extraction techniques available to underserved indigenous populations, Int. J. Phytothearpy Res. vol. 3, 2013, pp. 61–71.

[3] M. Mbikay, Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review, Front. Pharmacol vol. 3, 2012, pp. 1–12.

[4] O.S. Ijarotimi, O. Adeoti, O. Ariyo, Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented *Moringa oleifera* seed flour, Food Sci. Nutr, 2013, pp. 452–463.

[5] I.L. Jung, Soluble extract from *Moringa oleifera* leaves with a new anticancer activity, PLOS ONE vol. 9, 2014, pp. 1–10.

[6] C. Sutalangka, J. Wattanathorn, S. Muchimapura, W. Thukham-mee, *Moringa oleifera* mitigates memory impairment and neurodegeneration in an animal model of age-related dementia, Oxid. Med. Cell. Longev, 2013, pp. 1–9.

[7] M.K. Choudhary, S.H. Bodakhe, S.K. Gupta, Assessment of the antiulcer potential of *Moringa oleifera* root-bark extract in rats, JAMS J. Acupunct. Meridian Stud. vol. 6, 2013, pp. 214–220.

[8] M. Culver, T. Fanuel, A. Chiteka, Effect of moringa extract on growth and yield of tomato. Greener Journal of Agricultural Sciences vol.2 , 2012, pp. 207-211.

[9] M.M. Abdalla, The potential of *Moringa oleifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in the rocket (*eruca vesicaria* subsp. *Sativa*) plants, Int. Jour. of Plant Physiol. and Chem. vol.5 , 2013, pp. 42-49.

[10] R. Januardi, T.R. Setyawati, Mukarlina, Pengolahan limbah cair tahu menggunakan kombinasi serbuk kelor (*Moringa oleifera*) dan asam jawa (*Tamarindus indica*). Protobiont vol. 3, 2014, pp. 41-45.

[11] I. Riastiwi, P. I Putu Gede, Damayanto, Ridwan, T. Handayani, L. Aryani, *Moringa oleifera* Distribution in Java and Lesser Sunda Islands Attributed with Annual Rainfall, Biosaintifika vol. 10, 2018, pp. 613-621.

[12] Kleden, M. Markus, S. Hendrawan, Kusmartono, Kuswanto, Genetic Diversity Evaluation of *Moringa oleifera*, *Lam* from East Flores Regency Using Marker Random Amplified Polymorphic DNA (RAPD) and Its Relationship to Chemical Composition and In Vitro Gas Production. AGRIVITA, Journal of Agricultural Science vol. 39, 2017, pp. 219-23.

[13] Ojuederie, B. Omena, O.I. David, I.O. Somiame, F. Benjamin, Assessment of Genetic Diversity in some *Moringa oleifera* Lam. Landraces from Western Nigeria using RAPD Markers, African Journal of Plant Science and Biotechnology vol. 7, 2013, pp. 15-20.

[14] B.Y. Abubakar, R. Wusirika, S. Mua'zu, A.U. Khan, A.K. Adamu, Detection of genetic variability using random amplified polymorphic DNA marker in some accession of *Moringa oleifera* Lam from Northern Nigeria, International Journal of Botany vol. 7, 2011, pp. 237–242.

[15] P.S. Hendre and R.K. Aggarwal, DNA markers: Development and application for genetic improvement of coffee. In R.K. Varshney & R. Tuberosa (Eds.), Genomics Assisted Crop Improvement, Genomics Applications in Crops, Springer, 2007, pp. 399-434.

[16] S. Verma, T.S. Rana, Genetic diversity within and among the wild populations of *Murraya koenigii* (L.) Spreng., as revealed by ISSR analysis," Biochemical Systematics and Ecology vol. 39, no. 2, 2011, pp. 139–144.

[17] I.W. Arolu, M.Y. Rafii, M.M. Hanafi, T.M.M. Mahmud, M.A. Latif, Molecular characterization of *Jatropha curcas* germplasm using inter simple sequence repeat (ISSR) markers in Peninsular Malaysia, Australian Journal of Crop Science, 2012, pp. 1666–1673.

[18] M. Parvaresh, M. Talebi, B.E. Sayed-Tabatabaei, Molecular diversity and genetic relationship of pomegranate (*Punica granatum* L.) genotypes using microsatellite markers, Scientia Horticulturae, 2012, pp. 244–252.

[19] H.M. Abdelmigid, Efficiency of random amplified polymorphic DNA, (RAPD) and inter-simple sequence repeats (ISSR) markers for genotype fingerprinting and genetic diversity studies in canola (Brassica napus), African Journal of Biotechnology, 2012, pp. 6409–6419.

[20] R.K. Saini, K.R. Saad, G.A. Ravishnakar, P. Giridhar, N.P. Shetty, Genetic diversity of commercially grown *Moringa oleifera* Lam. cultivars from India by RAPD, ISSR and cytochrome P450-based markers. Plant Syst Evol. vol. 299, 2013, pp. 1205-1213.

[21] I. Robiansyah, A. Ramadan, M.A. Al-Kordy, A.S. Shushash, S.H. Abdulrahaman, Characterization of a New Biotype Moringa of Saudi Arabia Using Rapd And Issr Markers. Buletin Kebun Raya vol. 18, 2015, pp. 99-110.

[22] W.L. Ng, S.G. Tan, Inter-Simple Sequence Repeat (ISSR) Markers: Are We Doing It Right?, ASM Sci. J. vol.9, 2015, pp., 30–39.

[23] N. Carsono, N.L. Pradna, D. Farida, S. Untung, S. Santika, Identifikasi Polimorfis Marka-Marka Molekuler yang Diduga Berkaitan Dengan Karakter Daya Hasil Tinggi pada 30 Genotip Padi, Chimica et Natura Acta vol. 2, 2014, pp. 91-95.

[24] Siegel, S. Chloe, O. Florence, Stevenson, A.Z. Elizabeth, Evaluation and comparison of FTA card and CTAB dna Extraction methods for non-agricultural taxa, Applications in Plant Sciences vol. 2, 2017, pp. 1-7.

[25] P. Anantyarta, Identifkasi Variasi Genetik Kerbau (*Bubalus bubalis*) Pacitan aan Tuban Berbasis Mikrosatelit. Bioeksperimen vol. 3, 2017, pp. 1.

[26] S. Niwattanakul, S. Jatsada, N. Ekkachai, W. Supachanun, Using of Jaccard Cpefficient for Keywords Similarity, Proceedings of the International Multi Conference of Engineers and Computer Scientists vol. 1, 2013 pp. 13-15.