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Research Article
Molecular and Morphological Analysis of Indonesian Drumstick Tree (*Moringa oleifera* Lam.)

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Abstract

Background and Objective: Moringa (*Moringa oleifera* Lam.) which also known as drumstick tree has been widely used for functional food in many countries and has spread out to almost all over the world including Indonesia. This study aimed to identify the genetic diversity of 30 genotypes from 10 populations of Moringa plants originating from several islands in Indonesia using SRAP molecular markers in combination with observations of morphological characters. **Materials and Methods:** In this experiment, the genetic diversity of Moringa from several islands in Indonesia was evaluated using Sequence Related Amplified Polymorphism (SRAP) molecular markers as well as morphological characters. A total of 30 genotypes of Moringa from 10 different islands in Indonesia were planted in 10 kg of polybags for 3-7 months to observe their molecular as well as morphological characters. For molecular analysis, 10 selected primers of SRAP were used. **Results:** The 70 polymorphic bands from 86 total bands (81.40%) were obtained and Polymorphic Information Content (PIC) values were 0.36 on average. The UPGMA analysis divided 10 accessions into 2 main groups which Java accession was grouped alone and separated from other accessions. The principle component analysis based on morphological characters divided them into 4 groups with Java accession consistently separated from others. **Conclusion:** Based on molecular and morphological analysis, accession of Java was the most distinct Indonesian Moringa accession suggesting the narrow distribution of this accession than others. Leaflets number, tuberous root number and color were among the most variables that influenced the distinction of Moringa accession from Indonesian archipelago.

Key words: *Moringa oleifera*, drumstick tree, genetic diversity, molecular analysis, SRAP

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Moringa oleifera Lam. or drumstick tree is a fast-growing tree with an average height can reach up to 12 m has a fragile trunk with a gray-whitish bark. The plant has compound leaves arranged in a spiral and consist of tens to hundreds of oblong or elliptical leaflets with small size, 1.2-2.0 cm long and 0.6-1.0 cm wide[1]. Moringa plant is known to have high nutritional content and bioactive compounds and therefore this plant has been widely used as food, medicine and even cosmetics[2]. This plant is indigenous to Himalayan foothill covering Pakistan, India, Nepal and Bangladesh[1] and has currently spread almost throughout the world, especially in the tropical region, including Indonesia[3,4].

Several studies on genetic diversity of moringa plants have been conducted including Silva *et al*.[5] using Moringa from Brazil, Shahzad and Khan[6] and Ganesan *et al*.[7] using Moringa from Pakistan, Ojuederie *et al*.[8] using Moringa from Nigeria, Rajalakshmi *et al*.[9] and Ravi *et al*.[10] using Moringa from India and Kleden *et al*.[11] using Moringa from Indonesia. Meanwhile, the genetic diversity studies of Moringa plants from the Indonesian archipelago are still very limited. Even though they use Moringa from Indonesia, Kleden *et al*.[11] only used Moringa from one island, namely Timor Island. So far, no one has studied the genetic diversity of Moringa plants from various islands in Indonesia. Information about the genetic diversity of Moringa plants is important for further development, breeding and cultivation on an industrial scale.

Observation of morphological characters can be carried out on vegetative organs and / or plant generative organs, as has been done by Salem *et al*.[12] on wheat, Cortese *et al*.[13] on Switchgrass plants and Ebrahimi *et al*.[14] on Persian Walnut (*Juglan regia* L.). Even, the minimum morphological characters of Moringa plants for genetic diversity studies have been formulated by Santhoshkumar *et al*.[15]. In addition to morphological analysis, plant genetic diversity can be studied using molecular approaches. Molecular markers that have been commonly used in plant genetic diversity study include RAPD (Random Amplified Polymorphic DNA)[5,11,16,17], ISSR (Inter Simple Sequence Repeat)[18-21], AFLP (Amplified Fragment Length Polymorphism)[22-25], SSR (Simple Sequence Repeat)[6,7,9] and SRAP (Sequence Related Amplified Polymorphism)[26-29]. From those molecular markers, SRAP is a molecular marker that has never been used to study the genetic diversity of Moringa plants, in spite of some advantages over other molecular markers. The RAPD and ISSR are reported to have low levels of accuracy, reproducibility and the ability to detect polymorphism, whereas AFLP is expensive and has more complicated procedure. The SRAP requires a relatively low cost with simpler techniques but has high accuracy, reproducibility and the ability to detect polymorphism[30]. SRAP has also been reported to be effectively successful in identifying the genetic diversity of tree plants such as bamboo[26], Dalbergia[29] and Ramin[31].

The purpose of this study was to identify the genetic diversity of 30 genotypes from 10 populations of Moringa plants originating from several islands in Indonesia using SRAP molecular markers in combination with observations of morphological characters.

MATERIALS AND METHODS

Time and location: The experiment was carried out from 6th April to 25th September, 2020, in the Glass House and the Laboratory of Plant Molecular Systematics and Laboratory of Genetic and Plant Breeding, Research Centre for Biology, Indonesian Institute of Sciences, Bogor, West Java, Indonesia.

Plant materials: The plant materials were obtained from Research Center for Biology, Indonesian Institute of Sciences consisted of 30 Moringa genotypes from 10 accessions/ population collected from 10 different islands in Indonesian archipelago, namely Sumatra (Deli Serdang), Java (Bogor), Madura (Sumenep), Bali (Tabanan), Lombok (Kuta-Mandalika), Sumbawa (North Moyo), Sumba (Southwest Sumba), Borneo (Mempawah), Celebes (Enrekang) and Papua (Jayapura) (Fig. 1). The 10 accessions of Moringa seeds were washed in running water and then soaked in clean water for 1 hr to induce water imbibition. The seeds were then soaked in solution contained fungicides and bactericides 2 g L^{-1} water for 30 min before drained. A total of 3 seeds for each accession were planted on a 35×45 cm polybag containing a mixture of soil, manure and roasted husk with a ratio of 2:1:1 (v/v/v) for a total weight of 10 kg. The humidity of the media was maintained by watering every 2 days. The plants were grown for 7 months to provide the adequate samples for molecular analysis as well as morphological observation.

Molecular analysis: Analysis of genetic diversity was carried out using Sequence-Related Amplified Polymorphism (SRAP) molecular markers. DNA was isolated from young leaf samples using Genomic DNA Mini Kit (Plant) from Gene Aid. Genomic DNA amplification of Moringa plants was performed using 10 SRAP primers combinations (Table 1) which had been screened before from 25 pairs of primers developed by Li and Quiros[32]. The PCR reaction consisted of 1×PCR master mix (Promega), 10 ng templates DNA, 2 µM for each primer with a total volume of 15 µL. The PCR amplification was performed at

Fig. 1: Map of the location of plant material collection
1: Sumatera, 2: Java, 3: Madura, 4: Bali, 5: Lombok, 6: Sumbawa, 7: Sumba, 8: Borneo, 9: Celebes and 10: Papua

Table 1: List of SRAP primer pairs and its sequences used in the study

Primer pairs	Sequences
me 1F and em 1R	TGAGTCCAAACCCGATA and GACTGCGTACGAATTAAT
me 1F and em 4R	TGAGTCCAAACCCGATA and GACTGCGTACGAATTTGA
me 1F and em 5R	TGAGTCCAAACCCGATA and GACTGCGCACGAATTGCA
me 2F and em 1R	TGAGTCCAAACCGGAGC and GACTGCGTACGAATTAAT
me 2F and em 2R	TGAGTCCAAACCGGAGC and GACTGCGTACGAATTTGC
me 2F and em 4R	TGAGTCCAAACCGGAGC and GACTGCGTACGAATTTGA
me 3F and em 1R	TGAGTCCAAACCGGAAT and GACTGCGTACGAATTAAT
me 3F and em 2R	TGAGTCCAAACCGGAAT and GACTGCGTACGAATTTGC
me 3F and em 3R	TGAGTCCAAACCGGAAT and GACTGCGTACGAATTGAC
me 3F and em 4R	TGAGTCCAAACCGGAAT and GACTGCGTACGAATTTGA

Table 2: Description keys of *Moringa oleifera* qualitative characters

Characters	Descriptions
Leaflets shape	Elliptical, Oblong[7]
Leaflets color	Based on RHS mini color chart
Stem bark shape	Striped, warty, spotting[36]
Stem bark color	Based on RHS mini color chart
Tuberous root color	Based on RHS mini color chart

the following optimum conditions: 94°C for 5 min, 50°C for 45 sec and 72°C for 2 min. The reaction was stopped by extension at 72°C for 5 min. Amplification was visualized with 1.5% agarose gel and stained with GelRed (Biotium) before being photographed using the gel documentation system (Atto Bioinstrument). The scoring for each sample was carried out by observing the results of electrophoresis, where a score of 1 was given for the present of band and 0 for the absent of band.

AMOVA analysis: AMOVA analysis and the calculation of the genetic distance of accessions were carried out using GenAlEx software[13]. UPGMA grouping analysis based on a genetic distance matrix was carried out using the Mega X software[33]. Polymorphism Information Content (PIC) was calculated using Microsoft Excel with the formula used by De Riek *et al.*[34]. PCA clustering analysis was performed using MetaboAnalyst 4.0[35]. Quantitative morphological data were analyzed with ANOVA using SPSS for Windows version 16 software at $\alpha = 0.05$. *Post hoc* was carried out with Duncan Multiple Ranges Test (DMRT) at $\alpha = 0.05$.

Morphological characters analysis: Morphological characters were observed by analyzing the leaves, stems and roots, qualitatively and quantitatively. The characters and the descriptions of qualitative observations are presented in Table 2. The quantitative characters were observed by measuring plant height, stem diameter, canopy diameter, compound leaves number and sizes, leaflets number and area, tuberous root number, diameter and length. Plant height was measured from the soil surface to the top of the youngest shoot. Stem diameter was observed at a height of 5 cm from the surface of the medium. Canopy diameter was measured on the widest part of the canopy from 2 perpendicular sides. The number of compound leaves was calculated based on the number of leaves still attached to an individual plant. The length and width of compound leaves as well as the number and the area of leaflets were measured to the 4th compound leaves calculated from the shoot apex. The length of compound leaves was measured from the base of the petiole to the tip of the leaves, while the width of the leaves was measured at the widest part of the leaves. The leaflets number was counted manually from one compound leaf. The leaflets area was calculated by measuring 5 leaflets taken randomly from each 4th compound leaf, scanned and calculated using ImageJ 1.47v software. Observation of the number and the length of roots were carried out by counting the tuberous

roots and by measuring the base of the tuberous root until the longest part of root tip. The tuberous root diameter was measured on the largest part. The observations of the shoot were carried out at the age of 3 months after planting (map), while the observations of the roots were carried out with destructive measurement at the age of 7 maps. The observation of tuberous root color was carried out using the RHS mini color chart.

RESULTS

Molecular analysis of Moringa: PCR analysis using 10 primer pairs of 10 accessions of Moringa plants obtained an average of 8.6 amplified fragments. As presented in Fig. 2, the primer pairs that produced the most amplified fragments were me2F and em1R (Fig. 2a), while the least was me1F and em4R (Fig. 2b). The primer pairs that produced the highest polymorphisms were me1F and em1R (100%) (Fig. 2c), while the lowest was me2F and em2R (57%) (Fig. 2d). Overall, the average polymorphic fragment was 7.0 or 81.40%. The PIC values ranged from 0.26-0.45 with an average of 0.36. The amplified fragment sizes ranged from 150-2900 base pairs (bp) (Table 3).

The AMOVA analysis showed that the percentage of genetic variation among populations was higher than that within population, i.e.: 54 and 46% respectively (Fig. 3). It means that the genetic variation of 10 accessions of Moringa from various islands in Indonesia was much influenced by the

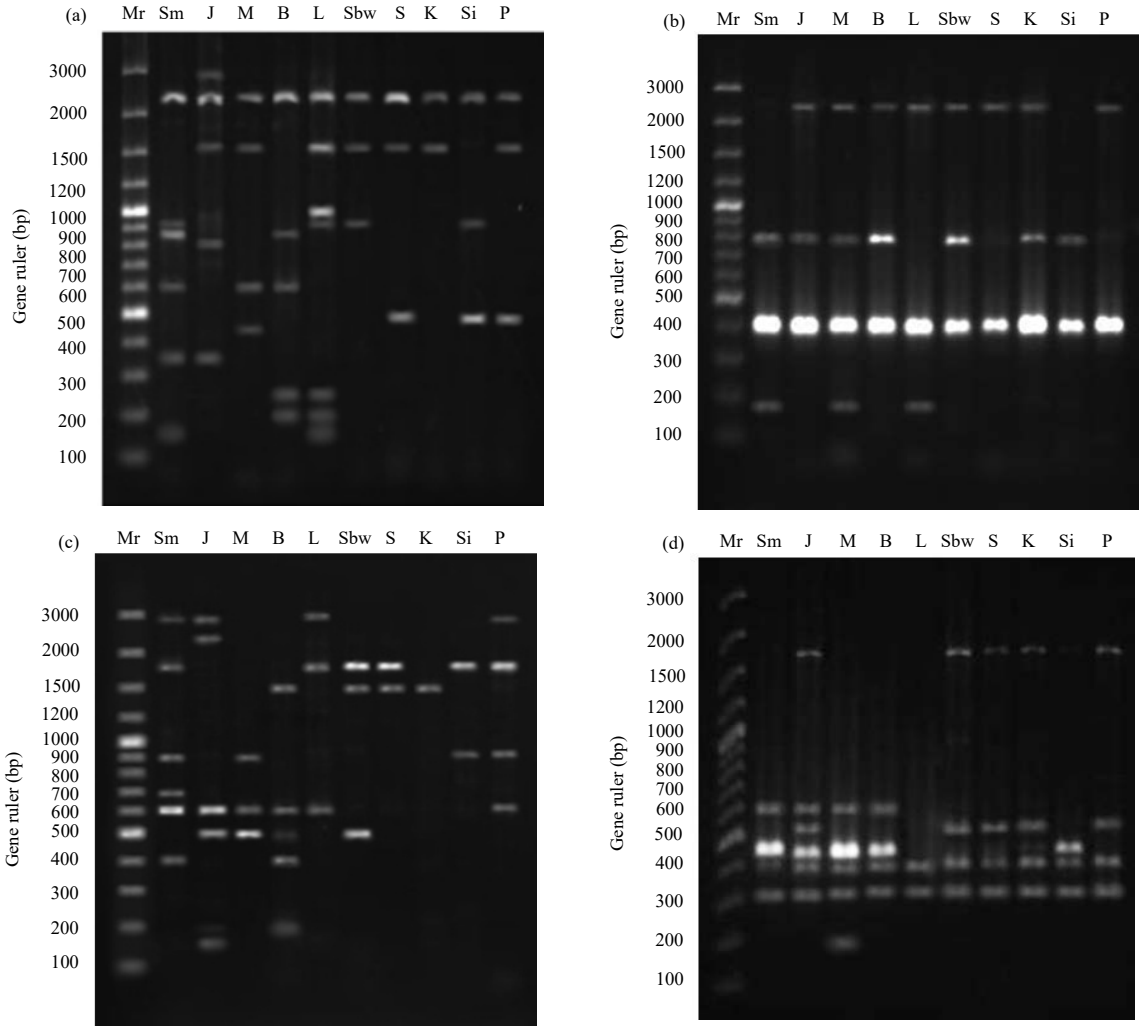

Fig. 2(a-d): SRAP profile of 10 accessions of *Moringa oleifera* using (a) me2F and em1R, (b) me1F and em4R, (c) me1F and em1R and (d) me2F and em2R primer pairs combinations

First lane from the left bands was 100 bp DNA ladder, the second to the eleventh lane were the accession of Sumatera, Java, Madura, Bali, Lombok, Sumbawa, Sumba, Borneo, Celebes and Papua

Table 3: List of primer pairs, number of amplified fragments, number of polymorphic fragments, polymorphic information content (PIC) and fragment size range of 10 *Moringa oleifera* accessions

Primer pairs	Numbers of amplified fragments	Numbers of polymorphic fragments	PIC	Size range (bp)
me 1F and em 1R	11	11	0.34	150-2800
me 1F and em 4R	4	3	0.34	180-2500
me 1F and em 5R	8	6	0.38	200-2500
me 2F and em 1R	15	14	0.26	150-2900
me 2F and em 2R	7	4	0.40	190-2500
me 2F and em 4R	8	5	0.45	200-2500
me 3F and em 1R	7	6	0.41	300-1200
me 3F and em 2R	12	11	0.24	150-2000
me 3F and em 3R	6	5	0.42	200-1000
me 3F and em 4R	8	5	0.35	400-2900
Total	86	70		

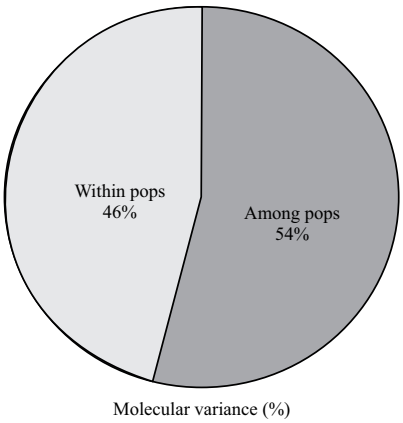

Fig. 3: AMOVA based on SRAP data for 30 genotypes from 10 accessions of *Moringa oleifera*

origin of the accessions/populations, suggesting that Moringa plants from various islands in Indonesia have high genetic differences.

The UPGMA dendrogram showed that the 10 accessions of Moringa were divided into 2 groups. The Java accessions (J) were grouped in a sole group, while the other 9 accessions were grouped into the other (Fig. 4a). The second group was divided into 3 subgroups. The first subgroup was Madura (M) and it was the nearest to Java accession (J). In the second subgroup, Sumatera accessions (Sm) were grouped together with Bali (B) and separated from the third which contained 6 accessions. In the third subgroup, Lombok (L) was grouped alone, while Papua (P) was grouped with Celebes (Si), Sumbawa (Sbw) grouped with Sumba (S) and Borneo (K). The clustering analysis using Principle Component Analysis (PCA) showed the same pattern as well, where Java accessions (J) were grouped in sole group (the first group) and other accessions belong to other groups (the second group). The second group was divided into 3 subgroups. The first subgroup includes Sumbawa (Sbw) and Lombok (L), the second subgroup includes Sumatera (Sm) and Bali (B) and the third subgroup includes Madura (M), Sumba (S), Borneo (K), Celebes (Si) and Papua (P) (Fig. 4b).

Morphological analysis

Qualitative characters: The qualitative character analysis of the 10 Moringa accessions showed differences in stem bark shape, stem bark color and the tuberous root color, while the leaflets shape and color were not different (Table 4). Borneo accession had a striped bark shape, while other accessions had a uniform bark shape, namely warty. The stem bark color of the 10 accessions was divided into 2 groups, brown color for Sumatra (Sm), Madura (M), Lombok (L), Borneo (K), Celebes (Si) and green color for Java (J), Bali (B), Sumbawa (Sbw), Sumba (S) and Papua (P). Tuberous root color was divided into three grades of yellow, i.e., moderate orange yellow 164 B for Sumatra (Sm), Bali (B), Lombok (L), Sumbawa (Sbw) and Sumba (S), moderate orange yellow 164 C for Madura (M) and Borneo (K) and pale yellow 164 D for Java (J), Celebes (Si) and Papua (P).

Quantitative characters: The analysis of ANOVA to morphological characters showed that plant height, stem diameter, canopy diameter and number of taproots were significantly different among the accessions. Accession of Madura (M) was the tallest even though not significantly different from Java (J) and Celebes (Si) accessions. The shortest accessions were Sumba (S) and Borneo (K) (Fig. 5a). The Java accession (J) had the largest trunk diameter, while Lombok (L), Sumbawa (Sbw), Sumba (S) and Borneo (K) had the smallest (Fig. 5b). The biggest diameter of the canopy was found in Java accession (J), while the smallest was in Bali (B) (Fig. 5c). The highest number of tuberous roots was observed in Java (J)

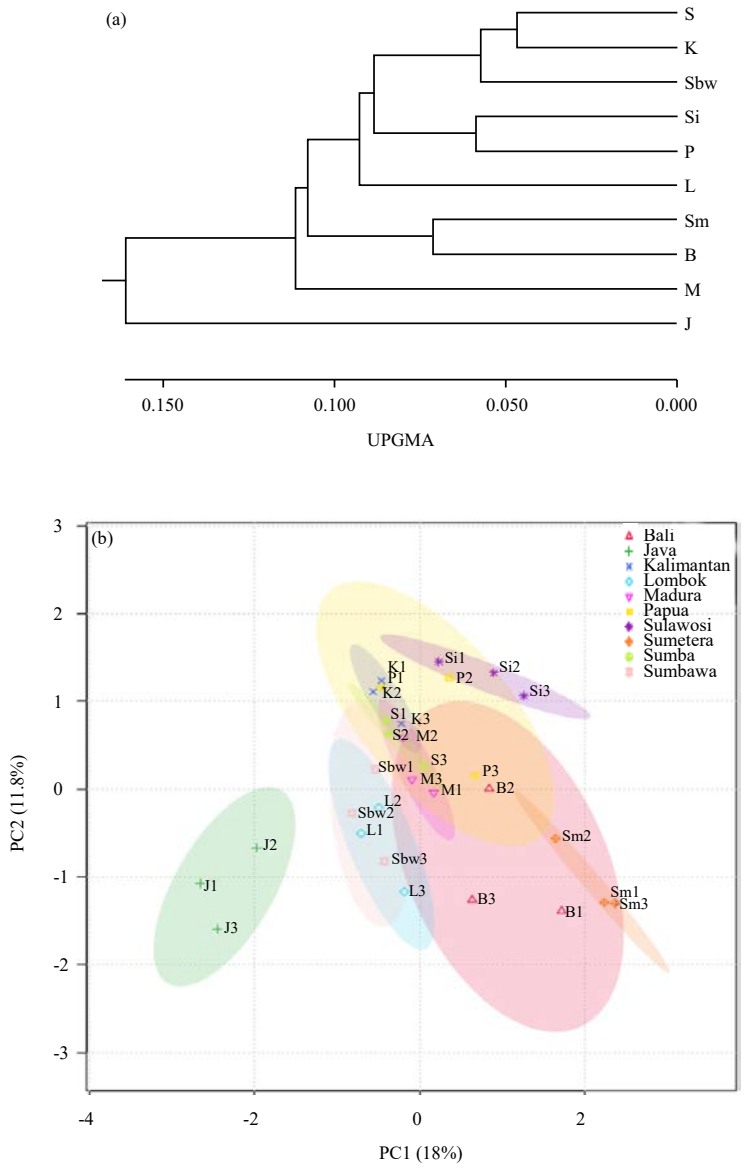

Fig. 4(a-b): (a) UPGMA dendrogram of 10 accessions of *Moringa oleifera* based on Nei's genetic distance and (b) PCA based on SRAP data using Metaboanalyst 4.0

Sm: Sumatera, J: Java, M: Madura, B: Bali, L: Lombok, Sbw: Sumbawa, S: Sumba, K: Borneo, Si: Celebes, P: Papua

Table 4: Qualitative characters of 10 *Moringa oleifera* accessions

Accessions	Leaflet shape	Leaflet color	Stem bark shape	Stem bark color	Tuberous root color
Sumatera	Elliptical	Moderate olive green A	Warty	Brown 47 RHS 199C	Moderate orange yellow 164 B
Java	Elliptical	Moderate olive green A	Warty	Green 42 RHS 194A	Pale yellow 164 D
Madura	Elliptical	Moderate olive green A	Warty	Brown 47 RHS 199C	Moderate orange yellow 164 C
Bali	Elliptical	Moderate olive green A	Warty	Green 42 RHS 194A	Moderate orange yellow 164 B
Lombok	Elliptical	Moderate olive green A	Warty	Brown 47 RHS 199C	Moderate orange yellow 164 B
Sumbawa	Elliptical	Moderate olive green A	Warty	Green 42 RHS 194A	Moderate orange yellow 164 B
Sumba	Elliptical	Moderate olive green A	Warty	Green 42 RHS 194A	Moderate orange yellow 164 B
Borneo	Elliptical	Moderate olive green A	Striped	Brown 47 RHS 199C	Moderate orange yellow 164 C
Celebes	Elliptical	Moderate olive green A	Warty	Brown 47 RHS 199C	Pale yellow 164 D
Papua	Elliptical	Moderate olive green A	Warty	Green 42 RHS 194A	Pale yellow 164 D

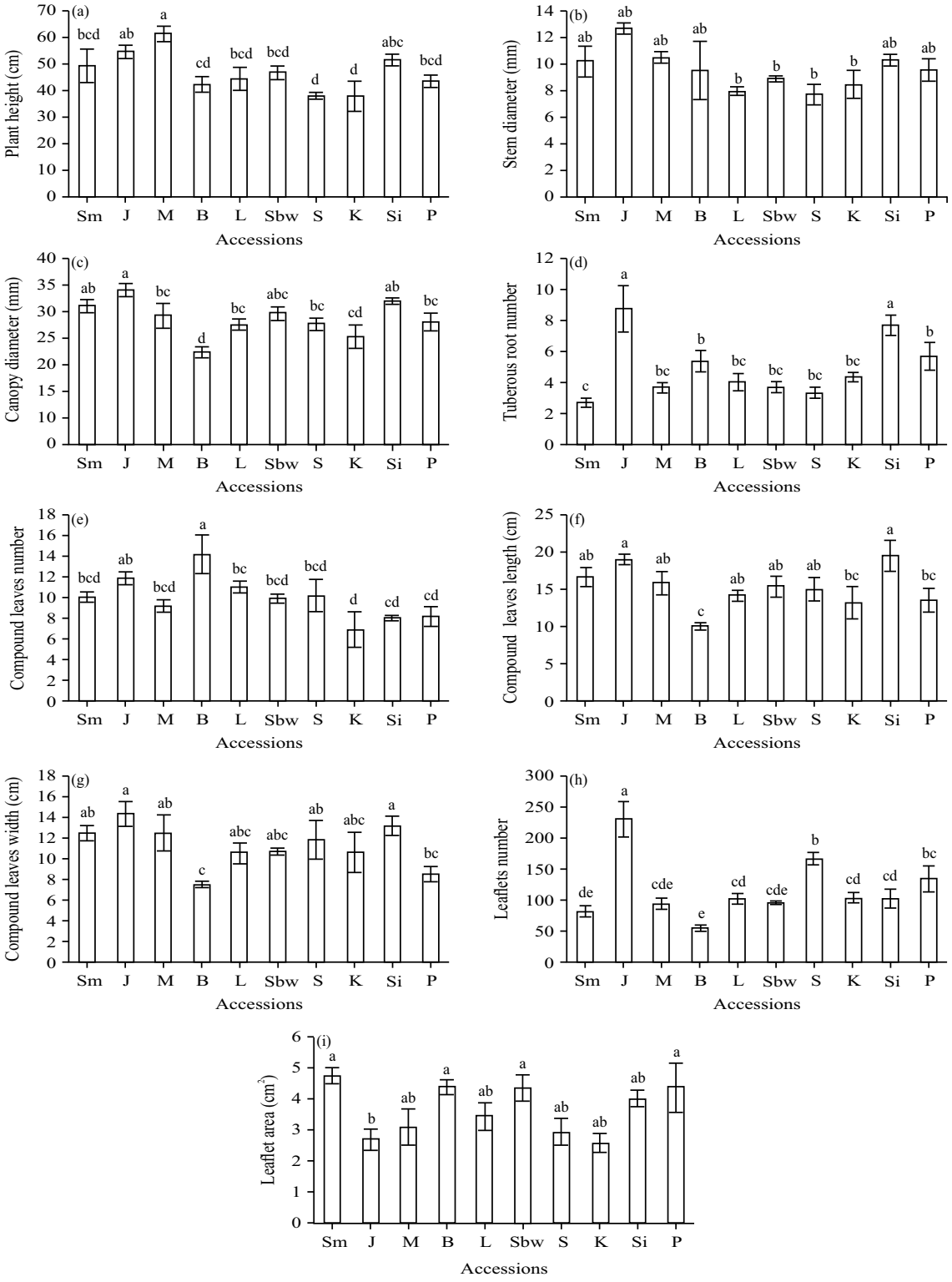

Fig. 5(a-i): *Moringa oleifera* accessions of (a) Plant height, (b) Stem diameter, (c) Canopy diameter, (d) Tuberous root number, (e) Compound leaves number, (f) Compound leaves length, (g) Compound leaves width, (h) Leaflets number and (i) Leaflet area

Sm: Sumatera, J: Java, M: Madura, B: Bali, L: Lombok, Sbw: Sumbawa, S: Sumba, K: Borneo, Si: Celebes, P: Papua. Different letters indicate significant differences at $p \leq 0.05$ DMRT

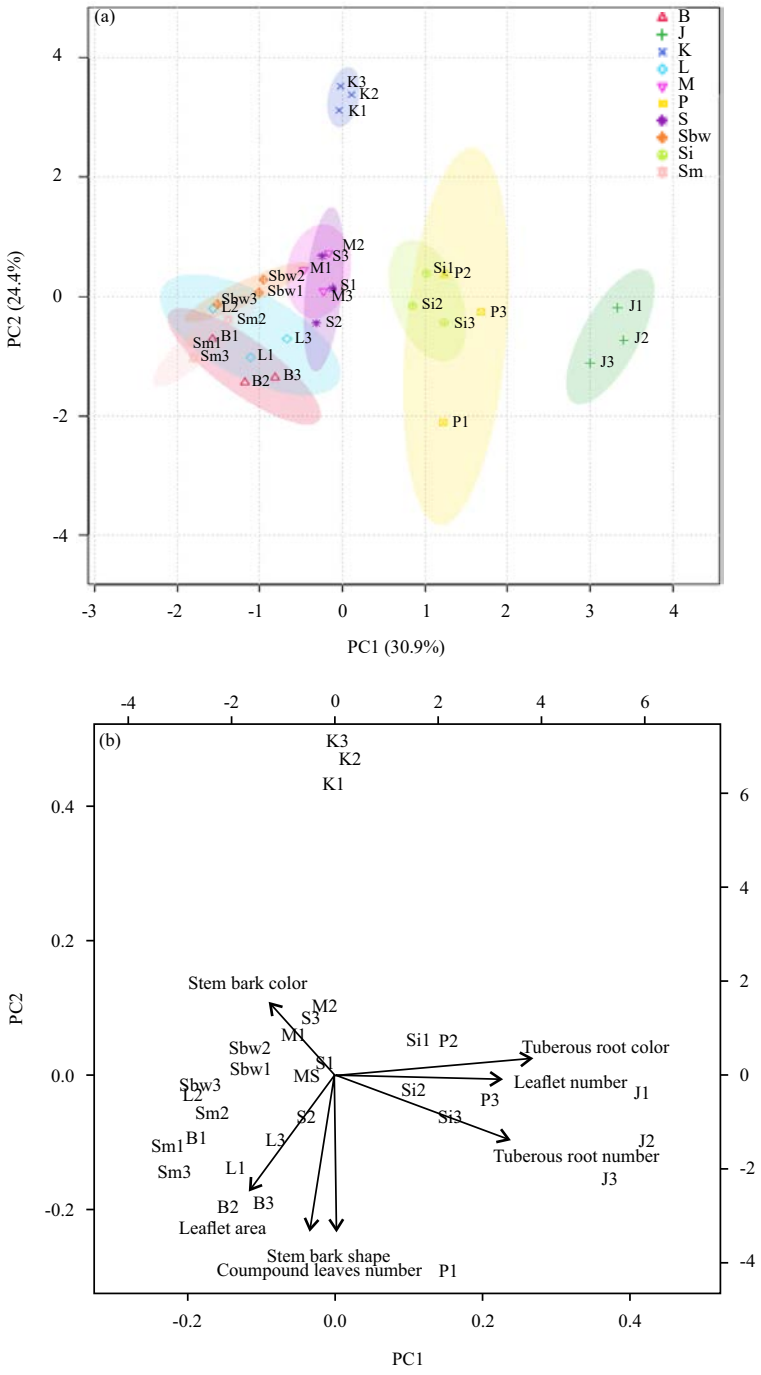

Fig. 6(a-b): The analysis of (a) PCA-2D and (b) PCA-Biplot of 10 *M. oleifera* accessions based on morphological data, using Metaboanalyst 4.0 program

Sm: Sumatera, J: Java, M: Madura, B: Bali, L: Lombok, Sbw: Sumbawa, S: Sumba, K: Borneo, Si: Celebes, P: Papua

and Celebes (Si) accessions, while the lowest was observed in Sumatra accessions (Sm) (Fig. 5d). Based on all those parameters, Java accessions (J) was the most different from others with the specific and distinct characters including relatively higher plant, biggest stem diameter, widest canopy and the most tuberous roots. Based on the quantitative character of the leaves, Bali accession (B) had the largest compound leaves, but not significantly different from Java accession (J), while the accession that have the least compound leaves was Borneo (K) (Fig. 5e). The accessions with the longest and widest compound leaves were Java (J) and Celebes (Si) (Fig. 5f-g). Among all the accessions, Java (J) had

the highest leaflets number (Fig. 5h), even though the size of leaflet was smallest (Fig. 5i).

The PCA analysis carried out using both quantitative and qualitative morphological characters showed that the 10 accessions of Moringa were divided into 4 groups, i.e., Java (J) in the first group, Papua (P) and Celebes (Si) in the second group, Borneo (K) in the third group and the other accession namely Madura (M), Bali (B), Lombok (L), Sumbawa (Sbw) and Sumba (S) gathered in the fourth group (Fig. 6a). Biplot PCA analysis showed that the number of tuberous roots, tuberous root colour and the number of leaflets were the three main variables that caused accession of Java (J) different from other accessions (Fig. 6b).

DISCUSSION

Molecular analysis using SRAP molecular markers effectively identified the genetic diversity of 10 Moringa accessions from various islands in Indonesia (Fig. 2 and Table 3). The percentage of polymorphisms obtained was 81.40% which indicates a fairly high genetic diversity of Moringa. Ravi *et al.*[10] stated that the number of polymorphic loci or the percentage of polymorphisms is a good indicator of Moringa genetic diversity. The percentage of polymorphisms in Moringa genetic diversity reported from several previous studies was lower than this result, such as 66.5% in Moringa from India, Malawi and Kenya using AFLP markers[36], 55.9% in Moringa from Timor Island, Indonesia[11], 48.68% in Moringa from India[19] and 62% in Moringa from the United States[5] using RAPD markers, 48.57% using ISSR markers and 40.00% using cytochrome p450 in Moringa from India[19]. There were also some reports of equivalent or even slightly higher percentage of polymorphisms, such as Ojuederie *et al.*[8] in Moringa from Nigeria using RAPD markers which was 81.50%, Ganesan *et al.*[7] in Moringa from India with SSR markers (82.86%) and Ravi *et al.*[10] in Moringa from India using cytochrome p450 which was much higher (88.25%). The higher percent polymorphism resulted in this experiment indicated the diversity and uniqueness of Moringa collected from different islands separated by seas in the Indonesian archipelago. This was confirmed by the result of AMOVA analysis which shows that the genetic variation among islands was greater than within islands (Fig. 3).

The UPGMA clustering analysis based on the genetic distance matrix among populations and PCA based on the SRAP data showed that the accession of Java (J) was the most different accession compared to other accessions (Fig. 4a). The data of PCA analysis generated from morphological characters also separated Java accession (J) from other accessions (Fig. 6a). Even though in this analysis, the 10 accessions were divided into 4 groups, but Java (J) accession was still separated into different group far from others. Java accession (J) had more superior morphological characters such as stem diameter, canopy diameter, number of compound leaves and leaflets, number of tuberous roots, even though it had smallest size of leaflets. This accession also had brightest color of tuberous roots. These characters made Java accession (J) to be relatively far apart from other accessions in the PCA analysis. Among those characters, leaflet number, tuberous root number and color were among the most variables that influenced the distinction of Moringa accession of Java (J) (Fig. 6b). The distinctive of Java accession (J) compared to other accessions indicates a narrow distribution of this accession, while 9 others which had relatively higher similarity which indicated a wider distribution. Chen *et al.*[37] stated that the distribution of plants is influenced by internal factors which refer to the characters of the plant itself and external factors including the role of human intervention. Shahzad *et al.*[6] reported that *M. oleifera* accessions from 9 different countries (India, Senegal, Tanzania, Mozambique, Zimbabwe, Florida, Belize, Mexico and Haiti) had low genetic diversity and had high similarities to one accession from Pakistan (Punjab accession). Based on this, they assumed that the accessions from 9 different countries originated from Punjab and then spread to these 9 countries due to human intervention through introductions by traders, expatriate, immigrants and even by colonialists in the past. Human's intervention also influences the distribution of plants through plant selection activities for breeding and cultivation which is focused on their superior characteristics. Plants with characters considered less interesting tend to be abandoned and usually obtain lower human intervention. Consequently, the distribution of these plants occurs naturally and relatively narrow.

The smaller leaflet size of Java accession (J) may be one reason that caused this accession has less attention by breeders and cultivators, so it tends to be neglected since the leaves are the most widely used organ harvested from this plant[2,38,39]. Morphological characters of a plant are commonly used as the basis for plant selection efforts in breeding and cultivation programs. Some common morphological characters that have been used for breeding selection in many cultivated species include the size, shape, leaf colour, fruit size and weight in almond plants[40], the number, size and weight of pods and seeds in cowpeas[41,42], the size and weight of fruit in chili[43], the size and head yield in cabbage[44], size and color of leaves in *Cleome gynandra* L.[45] and the leaf size and weight in amaranth vegetables[46]. The local community traditions

probably also have determined the narrow distribution of Java accession (J). The people of West Java where the accession was collected generally use the Moringa plant only as an ingredient for bathing corpses and not for food and therefore this accession is not intensively cultivated in this area. This is probably the reason that the introductions of Moringa genotypes from outside islands are rarely found.

CONCLUSION

It can be concluded that SRAP molecular markers can be effectively used to detect the genetic diversity of *M. oleifera* plants by obtaining 81.40% polymorphic bands which indicated higher genetic diversity of Indonesian Moringa. The accession of Java was the most distinct accession than others indicated the narrow distribution of this accession due to probably lower human intervention. The leaflets number, the number of tuberous root and the color of tuberous root were among the most influential variables which caused distinction of the accession.

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SIGNIFICANCE STATEMENT

This study discovered that there were a considerable variation of Drumstick (*Moringa oleifera* Lam.) accessions collected from 10 islands in Indonesia based on morphological and molecular analysis that has never been reported before, which can be beneficial for researchers and breeders in utilizing the accession for further development. This study will help the researchers to uncover the critical areas especially the differences of secondary metabolic content among the accessions that many researchers were not able to explore. Thus a new theory on the diversity of drumstick tree based on bioactive compounds may be arrived at the near future.

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