Noni (*Morinda citrifolia* L.) relationship analysis based on morphology character and random amplified polymorphic DNA (RAPD)

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Abstract. Noni (*Morinda citrifolia* L.) is a tropical plant with many benefits, one of which is traditional medicine. Moreover, noni can grow in all types of climates. Plant diversity is the main key in plant breeding. The existence of a diverse plant makes it easy for breeders to assemble varieties. This study aims to determine the diversity and relationship of noni germplasm based on morphological characters and RAPD markers. The materials used ten noni accessions at Cimanggu Research Station, Indonesian Spice and Medicinal Crops Research Institute (ISMCRI), Bogor, West Java. The morphological characters observed were leaf length, leaf width, number of pods, fruit diameter, fruit weight, and number of seeds. There were 20 primers used for RAPD. The results showed that morphological characters of leaf shape, fruit shape, and the number of seeds could distinguish noni. Molecular analysis showed that three primers (OPA 9, OPA 17, and OPB 18) could produce polymorphic DNA bands. Based on differences in DNA band patterns, 10 noni accessions were divided into two groups. There is no specific DNA band pattern that distinguishes a total of seeds noni.

1. Introduction

Noni (*Morinda citrifolia*) is a medicinal plant that is believed to have originated from Indonesia. This plant is a small tropical evergreen tree native to Southeast Asia (especially Indonesia), Papua New Guinea, and northern Australia [1]. Noni belongs to the Rubiaceae family, where the coffee plant is also the same in the Rubiaceae family. Noni can grow in an extensive range of environments. It can grow in infertile, acidic, and alkaline soils and is at home in very dry to very wet areas; also, it can grow naturally in relatively dry to mesic sites or lowland areas close to shorelines, or as an important forest understory species in low-elevation Pacific island forests and rain forests [2]. This plant has a characteristic when ripe fruit will smell unpleasant.

The noni plant has been used as traditional medicine by the Polynesians. They believe that every part of the plant can be used as medicine. Flowers, leaves, bark, stems, roots, and fruits of noni can be used [3]. The fruits were used as food treatment for and intestinal problems, while the leaves served to treat wound infections, arthritis, swellings, and similar conditions[4]. Besides that, noni is reputed to have antibacterial, antiviral, antifungal, antitumor, antitubercular effect, analgesic activity, immunological activity, mental health and improve high frequency, anti-helminthic, analgesic, hypotensive, anti-inflammatory, and immune-enhancing [5]. The major components have been

identified in the Noni plant such as scopoletin, octanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin-1-methyl ether, anthraquinone glycoside), b-sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, Alizarin, amino acids, acubin, L-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin, and a putative proxeronine [6].

Nowadays, noni has two recognized varieties of *M. citrifolia* (*M. citrifolia* var. *citrifolia* and *M. citrifolia* var. *bracteata*) and one cultivar (*M. citrifolia* cultivar *Potteri*) where the most commonly found variety is *M. citrifolia* var. *citrifolia*, with the greatest health and economic importance [7]. Indonesian Spices and Medicinal Crops Research Institute is a research institute under the Ministry of Agriculture of Indonesia that collects noni conserved in research installation. This collection is one of the activities in preserving these plants.

Knowing the genetic relationship between plants is one of the breeding activities for creating new varieties. Knowledge about genetic relationships will be useful to avoid the chance of using genetically similar genotypes/landraces. It will also be supportive in future breeding programs to select genetically diverse parents [8]. Plant genetic kinship relationships can be evaluated through pedigree, simultaneous analysis of quantitative traits, and molecular markers analysis [9]. Plants' morphological properties can be observed by looking at the outside appearance, but the environment can influence these morphological characteristics. Meanwhile, the kinship analysis's molecular marker is often used is the *Random Amplified Polymorphic DNA* (RAPD). The marker of RAPD inheritance is dominant so that the homozygous phenotype is indistinguishable from the heterozygous phenotype and is less sensitive. However, this drawback can be overcome by using more primers [10]. This study aims to determine the relationship of noni based on morphological characters and molecular markers. Also, this research can be used in future breeding.

2. Material and method

2.1. Plant material

The material used in this study is 10 noni accessions at Cimanggu Research Station, Indonesian Spice and Medicinal Crops Research Institute (ISMCRI), Bogor, West Java. The research was conducted from January to August 2020 at the Cimanggu Research Station, Indonesian Spice, and Medicinal Research Institute, Bogor, West Java, Indonesia.

2.2. Observation of morphological traits

The study was conducted with a descriptive method which observed the morphological character of plants. Morphological characters of plants observed are quantitative data. The quantitative plant morphological characters observed were leaf length, leaf width, number of pods, fruit diameter, fruit weight, and number of seeds.

2.3. Analysis of morphological data

The measurement of correlation was based on mean values using R software. The correlation for evaluates the relationships among the different variables in the experiment. The following formula calculates the coefficient of diversity:

$$\boldsymbol{CV} = \frac{\boldsymbol{S}}{\boldsymbol{\bar{x}}}$$

$$S = \frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}$$

Where: S = Standard Deviation

$\bar{x}$ = means of observation value

x_i = observation -i (i = 1, 2, 3, 4, 5)

n = total of sample observation

The value coefficient variance (CV) is used to estimate the level of diversity of the observed accessions characters, namely the CV value of 0–25% indicates low diversity, while the high diversity level is if the CV value is > 25%. The means of each morphological trait were used for cluster analysis. Euclidean distance was used as the similarity coefficient for cluster analysis with the Unweighted Pair Group Arithmetic Means method (UPGMA) using R software. Principal component analysis (PCA) was performed on morphological data to certain that the dendrogram is a good representation of the data

2.4. DNA Extraction and RAPD procedure

Young leaves samples from each noni accession were extracted using the modified CTAB method [10]. DNA concentration was quantified by using the Nano Drop Spectrophotometer 2000 (Thermo scientific, Germany) and qualified using agarose gel electrophoresis (Bio-Rad, USA).

The PCR reaction was carried out with a total volume of 12 µl containing Taq polymerase (6.25 µl), primer (1 µl), DNA (5 µl), and water (0.25). A total of about 20 primers from Operon Technology were used (Table 1). DNA amplification was performed in an RT-PCR rotor gene Q programmed to 1 cycle of 5 min at 95°C followed by 35 cycles 95°C for 1 min, 35°C for 1 min, 75°C for 2 min ending with 1 cycle of 10 min at 72°C (final extension). The PCR amplification results were visualized on 1.5% agarose gel in a TBE buffer (Tris-EDTA) electrophoresis using Mupid Mini Cell for 45 minutes at 80 volts. The results of the electrophoresis were photographed using UVITEC Cambridge. As the standard for the DNA size, a 100 bp DNA ladder (Promega) was used to determine the size of the DNA amplified band

Table 1. RAPD primer that used in the research

No	Primer	No	Primer	No	Primer	No	Primer
1	OPA9	6	OPO 20	11	OPK 12	16	OPC 2
2	OPB 18	7	OPN 10	12	OPO 2	17	OPA 17
3	OPN 13	8	OPB 13	13	OPO 15	18	OPC 4
4	OPN 18	9	OPB 16	14	OPB 8	19	OPA 2
5	OPN 3	10	OPB 7	15	OPA 4	20	OPA 12

2.5. Analysis data of molecular marker

RAPD is the dominant marker, so each RAPD band is considered a single biallelic locus [11]. The results of the visualization of DNA strands were scored based on the DNA fragment bands that were implicated with the classification "1" indicating bands, and "0" showing no bands. The scoring results will be analyzed using R software. Cluster analysis to determine genetic distance by Euclidean distance with Unweighted Pair Group Arithmetic Means method (UPGMA) using R software.

3. 3. Result and Discussion

3.1. Correlation among morphological trait

The analysis results showed a significant level of phenotypic variation between 10 accessions of noni in morphological characters (Table 2). The noni's highest mean value for the number of seeds with value 205, fruit weight with value 102, fruit diameter with value 86.4, and the number of pods with value 86.4. The highest coefficient of variation (CV) is the characteristics of the number of seeds and fruit weight. The value of the coefficient variety of each character is 72.20% and 34.22%. The enormous variance is the number of seeds with a variance value of 21867 and fruit weight s with a variance value of 1217. The CV value is used to estimate the level of observed character diversity if

the CV value of 0–25% indicates low diversity, while if the CV value is > 25% shows high diversity [9]. The high diversity of these characters will provide opportunities for superior accession through selection. A high variance value indicates that the observed data fluctuates.

Table 2. Variation in morphological traits of 10 noni accessions

Traits	Mean	Minimum	Maximum	SD	CV (%)	Variance
Leaf Length	26.8	24.4	31	2.17	8.10	4.7
Leaf Width	13.9	12.3	15.6	1.03	7.41	1.07
Fruit Weight	102	56.5	154	34.9	34.22	1217
Fruit Diameter	86.4	59.7	109	14.9	17.25	222
Number of Pods	86.4	59.7	109	14.9	17.25	222
Number of Seeds	205	2.5	371	148	72.20	21876

* CV: Coefficient of Variation

The result of correlation coefficients between morphological traits given in Table 3. The character between fruit morphology has a positive and significant correlation. The morphological characters' leaf length gave a positive correlation to all observed characters except the number of seeds. Likewise, leaf width provides a positive correlation on leaf length, number of pods, and fruit diameter. It was the leaf length and the leaf width, which negatively correlated to the number of seeds and the fruit weight with the leaf width. The positive correlation occurs if the morphological traits interact with each other. The negative correlation is shown no interaction traits with each other. The correlation between characters will make it easier for breeders to determine the plant's important characters [12]. Correlated traits can be used as selection criteria in selecting plants.

Table 3.Correlation coefficients among morphological traits.

	Leaf Length	Leaf Width	Number of Pods	Fruit Diameter	Fruit Weight	Number of Seeds
Leaf Length	—					
Leaf Width	0.378	—				
Number of Pods	0.025	0.113	—			
Fruit Diameter	0.025	0.113	1***	—		
Fruit Weight	0.255	-0.068	0.793**	0.793**	—	
Number of Seeds	-0.028	-0.188	0.466	0.466	0.757*	—

Note. * p < .05, ** p < .01, *** p < .001*

3.2. RAPD fingerprint

Noni accession was analyzed using 20 RAPD primers, out of 8 produced polymorphic and reproducible fragments. These primers produce a total of 28 amplified DNA fragments in all samples, with an average of 3 amplified DNA per accession. The number of amplified DNA ranged from 2-6, with an average of 3 amplified DNA per primer. Almost all the DNA fragments were polymorphic, which 21 bands polymorphic or equivalent with 78% of the total band (Table 4). The number of polymorphic bands varied from 1-5 with an average of 2.6 per primer. The maximum number of polymorphic bands was 5 and amplified with OPB 18. The most informative primer was OPB 18, OPA 9, OPA 17, and OPA 18. Bordallo*et al*. (2017)[13] used 20 RAPD primers on 36 plants of 13 accessions *M.citrifolia* and found OPA4 as the most polymorphic primer with 11 polymorphic bands. The high polymorphism rate (more than 50%) was by other research that used RAPD marker on *Morinda spp* ([14]; [15]; [16]; [17]). The high number of polymorphic bands might be since the samples were obtained from several different places before planted in ISCMRI. Furthermore, although noni has been described as a preferentially self-pollinating plant [18], it also has pistillate florets [19], suggesting the possibility of cross-pollination, which might be associated with the high number of polymorphic bands. There was no significant difference in DNA band pattern found between the

seedling and non-seeding accession (no 3 and 8) in all RAPD primer used. Accession number 3 tends to have a similar band with accession 2,4,5; meanwhile the accession number 8 had a similar band with numbers 7 and 9 (Figure 1). The result confirms that the RAPD technique is considered efficient for determining the genetic variability of noni germplasm. Analyzed 10 germplasm of sweet potato using three DNA markers and found the polymorphic rate of RAPD was the same with AFLP and higher than SAMPL [20].

Table 4. Polymorphism detected with 8 RAPD primer in 10 noni germplasm accessions.

Primer	Sequence (5'-3')	Total no of bands	Polymorphic		Amplicon band size
			No of bands	%	
OPB18	CCACAGCAGT	6	5	83	300-1000
OPA 9	GGGTAACGCC	4	3	75	500-1000
OPA 17	CATTGGGGAG	2	2	100	100-1000
OPN 3	GGTACTCCCC	4	4	100	400-500
OPN 13	AGCGTCACTC	3	3	100	300-500
OPA 18	GGTGAGGTCA	3	2	67	300-500
OPN 10	ACAACTGGGG	2	1	50	100-500
OPN 16	AAGCGACCTG	2	1	50	100-500
	Total	26	21	78	

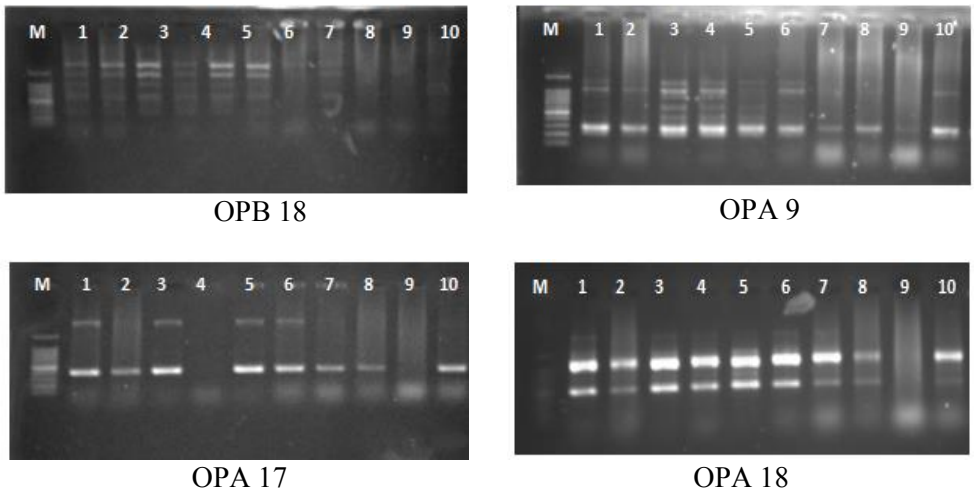

Figure 1. RAPD pattern profile of ten noni accessions described by different RAPD primers M= 100bp DNA marker, 1-10 = noni germplasm.

3.3. Cluster analysis

Cluster analysis was carried out to determine the closeness between plants. The result of cluster analysis is shown in Figure 2. Figure 2a shows a dendrogram made based on the morphological characters of the fruit. This is done because the correlation results show correlates with fruit morphology traits. Meanwhile, Figure 2b of the dendrogram was made based on the RAPD primer. Based on the dendrogram results based on morphology and primer, 10 noni accessions were divided

into 2 major groups: group 1 (1, 3, and 8) and group 2 (2,4,5,6,7,9 10). The small values of the Euclidean distance coefficient between samples show the diversity is low. Based on the dendrogram of accessions 1 with 8, and 9 with 10, morphologically and DNA, it shows a close genetic distance. This proves that the accessions are not different morphologically and DNA. Accessions of noni 1 and 8 have little seeds or seedless. Also, 1 or 8 noni accessions can be selected as parents because it has little seeds or seedless.

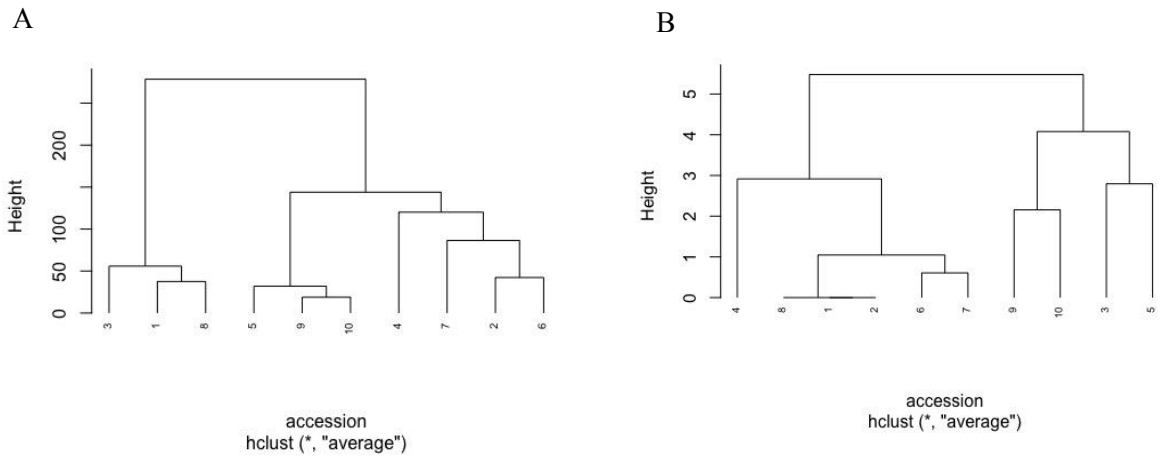

Figure 2. Dendrogram for 10 noni accessions generated by UPGMA clustering using elucidation coefficient of similarity on morphological (a) and RAPD (b) data.

4. Conclusion

The diversity of noni accessions is shown in the characters number of seeds and fruit weight with the coefficient of variation values of more than 25%. Based on the cluster analysis, ten noni accessions were divided into 2 groups: group 1 (1, 3, and 8) and Group 2 (2, 4, 5, 6, 7, 9, and 10). Noni accessions 1 and 8 can be select as parents because they have few fruit seeds or seedless.

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