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Genetic Characterization of Galoba Durian (*Amonum* spp.) in Ambon Island Based on Random Amplified Polymorphic DNA (RAPD)

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Abstract

Galoba durian is one of the endemic floras from Maluku. Galoba durian is a species belonging to the *Amomum* genera *Zingiberaceae* family. It is also used as a medicinal plant for waist and kidney diseases. Based on the color of the fruit, galoba durian is divided into two nmely red galoba durian and green galoba durian. Distribution of this plant in Ambon can found in a few places such as highland and coastal area. Different locations influence phenotypic of plants, but may not show different genetic characteristic. Genetic diversity can detected by molecular markers. Genetic characterization from galoba durian using RAPD markers has not been done before. This study aimed to analyze genetic diversity from galoba durian using molecular markers RAPD. Samples of plants are used red galoba durian from Hatu and green galoba durian from Hatalae. The result of the first study, characterization of the morphology of the galoba durian, showed that both galoba have almost similar characteristics. Further DNA was tested by qualitative and quantitative. Result shows good qualitative and quantitative of DNA genomic. The second study was amplification by PCR-RAPD. DNA amplifications were performed using 3 primers out of 9 screened random primers. The primers selection was based on hight polymorphism. DNA amplification has 36 bands which were 100% polymorphic. The size of each bands from visualization of agarose was determined by linear regression. Number of band amplified was range from 120 to 1612 bp. Polymorphic band of RAPD showed the highest genetic diversity. It can be concluded that the two plants of galoba durian are different species

Keywords: galoba durian, polymorphic, RAPD

A. Introduction

Zingiberaceae is a family of annual herbs classified as germplasm which beneficial as natural herbs, medicinal plants and essential oils (Syamsuardi, Mansyurdin, Nurainas, & Susanti, 2010). *Zingiberaceae* family has several genera, the largest and often exploited genera were *Curcuma, Kaempferia, Hedychium, Amomum, Zingiber, Alpinia, Elettaria and Costus* (Sari, Utami, Wiryani, & Perwati, 2012). Among the dispersed family members, some of them are not widely known and are often regarded as wild plants, as in some species in the Amomum genus (Syamsuardi *et al.* 2010).

Amomum is one of the largest genera of *Zingiberaceae* family with species ranging from 150-180 species often used as herbs, vegetables and natural herbs (Kaewsri, Paisooksantivarana, Veesommai, Eiadthong, & Vajrodaya, 2007). Some species in this genera have not been widely known like galoba durian (*Amomum* spp.). According to Rumphius (1972), galoba durian is an endemic flora in Maluku. Based on the color of galoba durian fruit divided into red galoba and green galoba. One of the characteristics of the galoba durian is the shape of fruit similar to durian fruit with small size.

Generally, the genetic characterization of an organism occured as a result of mutations, recombination and migration of genes which cause changes in nucleotide arrangement in DNA, thus affecting the phenotypic properties of the organism (Sembiring, Putri, & Setiado, 2015). Analyzing of genetic characterization of plant varieties is often conducted of morphological characters observation, but it has limitations since it is easily influenced by the environment as well as uneven genetic characterization depending on the ecosystem in its region. The presence of molecular markers has facilitated accurate genome analysis (Yulita, 2013).

RAPD is an amplification method that can produce millions of copies of DNA segments using a single primer selected at random (Anggraeni, 2008). RAPD markers are often used because they have several advantages over other genomic analysis methods, which are faster and easier to perform and analyze, resulting in large amounts of polymorphisms and the itrequired relatively cheaper in cost compared to other methods (Varesa, syamsuardi, Mansyurdin, Maideliza, & Pratiwi, 2011). Nowadays, RAPD method is widely used in the analysis of the characterization of various plants (Prana & Hartati, 2003) and also to identify plant genotypes (Varesa *et al*., 2011).

Until now there has been no research on genetic characterization of galoba durian (*Amomum* spp.) and it is expected that this genetic characterization information can be used in conservation efforts. The aims of this research was to discover morphological characteristics and genetic characterization of galoba durian (*Amomum* spp.) in Ambon island based on RAPD molecular marker.

B. Methodology

Samples of galoba durian plants were taken from two different locations in Hatalae and Hatu villages. Samples of plants are used red galoba durian from Hatu and green galoba durian from Hatalae.

1. Morphological Characterization

In both locations, descriptions and measurements of vegetative organs were conducted. The observed characters were the length and width of the leaves, the length of the petiole, the diameter of the stem and the height of the plant. The leaf color, leaf stem and the number of leaves were the other characters observed on one individual plant. Plants used as a reference were the highest and lowest plants and some vegetative characters, because the generative character did not exist yet.

2. DNA Isolation

DNA isolation was performed using the Orozco-Castillo method. Samples of galoba leaf were washed and dried with tissue. After that the dried sample were sliced and the bone leaves were removed to facilitate grinding process. Samples of galoba leaves were crushed to the smallest size with mortar and added with liquid nitrogen. Then put 0.1 gram of poly (1-ethenylpyrrolidin-2-one) (PVPP) into the mortar. Followed by adding the liquid nitrogen slowly as the samples were crushed until powdered and continuously done during the grinding process. The crushed samples were weighted approximately 0.1 grams. The eppendorf tube containing the sample was fed into liquid nitrogen. Added 5 mL of extraction buffer and 500 µL of 1% β-merkaptoetanol that had been heated. Then vortexed and incubated for 30 minutes at 65ºC. Every 5 minutes the tubes were shaken thus reaction reacted faster. The sample was allowed to cool in an acid cabinet, then

added 5 ml of chloroform solution : isoamethanal alcohol (24 : 1). The sample was centrifuged at 11,000 rpm for 10 min at 25ºC. The supernatant was removed, then added 5 mL of chloroform solution : isoamethalcohol (24 : 1), vortexed and centrifuged at 11,000 rpm for 10 min at 25ºC. The supernatant was removed and 1x volume of cold isopropanol was added. The samples were homogenized by moving the tubes back and forth and then stored in refrigerator (4ºC) for 30 minutes. After that centrifuged at 11,000 rpm for 10 minutes at 25ºC. The supernatant was removed while the obtained pellets were dried. After drying, the pellet was dissolved in 1 mL TE buffer and then stirred. Around 1/10 volume of 3 M Na-Acetate pH 5.2 and 2.5 mL of absolute ethanol were added and stirred until a batch of white DNA fibers appeared. Samples were stored in freezer -20ºC for 30 minutes or overnight. The sample was centrifuged at 12,000 rpm for 10 min at 4ºC. The obtained supernatant was removed and the pellet was dried. The pellet was washed using 100 µL of 70% ethanol. The mixture was centrifuged at 8,000 rpm for 5 min at 25ºC. The supernatant was removed and the pellet was dried in a laminar air flow cabinet. About 30 µL of TE buffer solution were added to dry pellets and homogenized. Approximately 1/10 of the DNA volume RNase was added to DNA. A total of 3 µL of RNase was added and incubated at 37 ° C for 30 minutes. DNA Quantity Test using Nanodrop Spectrophotometer. Absorbance (A) was measured at wavelengths of 230, 260 nm and 280 nm.A260: A280 and A260: A230 ratios were used for DNA purity measurements.

3. DNA Amplification

Total DNA genomes were amplified using random primers. Screening was performed using 9 random primers to obtain primer with high repetition rates and consistent results. The DNA amplification process was pre-denatured for 4 min at 94 º C, denatured for 30 s at 94 º C, annealing for 30 s at 36 º C-65 º C, 1 minute elongation at 72 º C and final elongation.

4. Data analysis

DNA ribbon gel photos were analyzed using linear regression to help predict the size of bases, reflecting the genetic characterization between the two species.

C. Result and Discussion

1. Morphological Characterization

Characterization was performed on vegetative characteristics and not performed on generative features (Figure 1). Generative characteristics were not examined because the time of the study had not been in fruit season. Vegetative characteristics were seen in parts of plants which above the soil surface of the leaves and stems.

The morphological observation of the galoba durian plant from both sites showed some differences in the shape and size of plants (Table 1). Galoba durian in the village of Hatalae has a tapering shape leaf tip, total number of leaves is many, plants growing direction tend to curve down and thick leaf sheets texture. Galoba durian in the village of Hatu has taller plant height than galoba durian from Hatalae, tapered shape leaf tip, the number of leaves is few, the leaf size is longer, the width of the narrow leaf, thicker and slightly stiff leaf texture and the plants growing direction tend to up. Both of these plants had almost the same morphological characteristic. One of the factors causing different characteristics of these two plants might be due to environmental conditions. Environmental conditions in Hatalae village are more humid and shaded, located at an altitude of 230 m above sea level, 25 ° C and has humus soil type. While the environmental conditions in thevillage Hatu are humid, galoba durian growing unshaded, at an altitude of 54 m above sea level, temperature 27 ° C and has laterite soil type which is red soil. Differences topography and environmental conditions at both locations affect the phenotypic of galoba durian. According to Sudomo & Wuri (2013), environmental factors affecting the phenotypic and plant growth conditions such as cardamom can grow well at 630-703 m altitude, 24-28^{0}C temperatures, acid pH 4.87-5.9 with clay soil texture. Furthermore, it also affects the genetic characterization of the galoba durian. Environmental influences on plant growth and phenotypes cause morphologically inefficient identification to examine genetic characterization (Oktavia, Lasminingsih, & Kuswanhadi, 2009).

Figure 1. Galoba Durian

Table 1. Morphological characteristics of Galoba Durian in Hatalae and Hatu villages.

Character	Locations	
	Hatalae	Hatu
Color of leaf sheet	Dark green	Dark green
Tip of leaf sheet	Tapering	Tapered
Base of leaf sheet	Blunt	Blunt
Texture of leaf sheet	Thick	Thick
Length of leaf sheet (cm)	17.9-52.5	3-58.7
Width of leaf sheet (cm)	2.6-10.5	1.7-17.1
Number of leaves	18-49	77-21
Stem color	Light yellow	Light yellow
Length of stem (cm)	0.5-0.7	0.4-0.5
Diameter of stem (cm)	1-2.2	1.3-3
Plants height (cm)	151.5-384	55-340
Plants growing direction	Curve down	Up

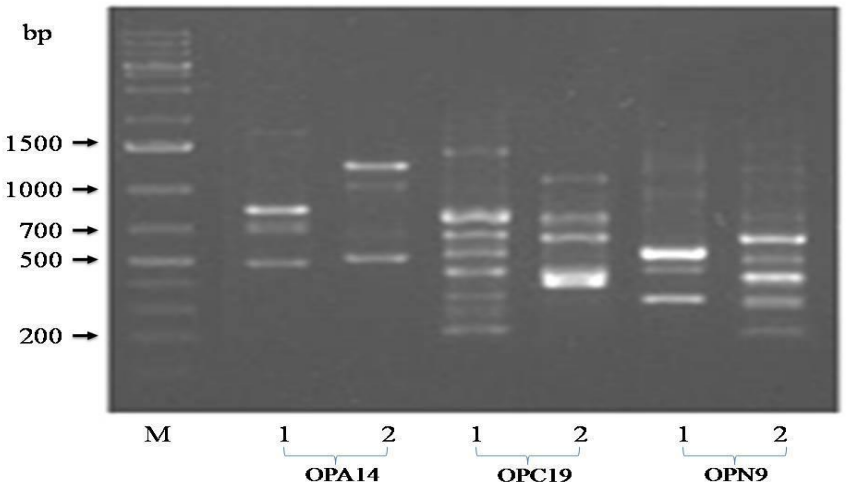

Figure 2. Electroferogram result of DNA amplification using 3 types of primer (M) DNA Lambda Marker sample of green galoba durian in Hatalae (1) and sample of red galoba durian in Hatu (2) which electrophorizedusing 1% Agarose Gel.

From nine primary RAPDs used were selected three primers which had high band polymorphism result. PCR results were electrophorized using 1.5% agarose gel. The visualization results can be seen in Figure 2. The galoba durian DNA bands that appear on the gel were varied on each primer. The band variation occurs due to many sites attach between primary and DNA genome. The primer selection for the analysis affects thelevel of polymorphic band result. The

more random primers used the more varied the pattern and size of the fragments obtained (Palupi, 2010). The highest number of bands weregenerated by OPC19. This means that the OPC19 primer has a complementary base pair with genome DNA thus it has more attachment sites.

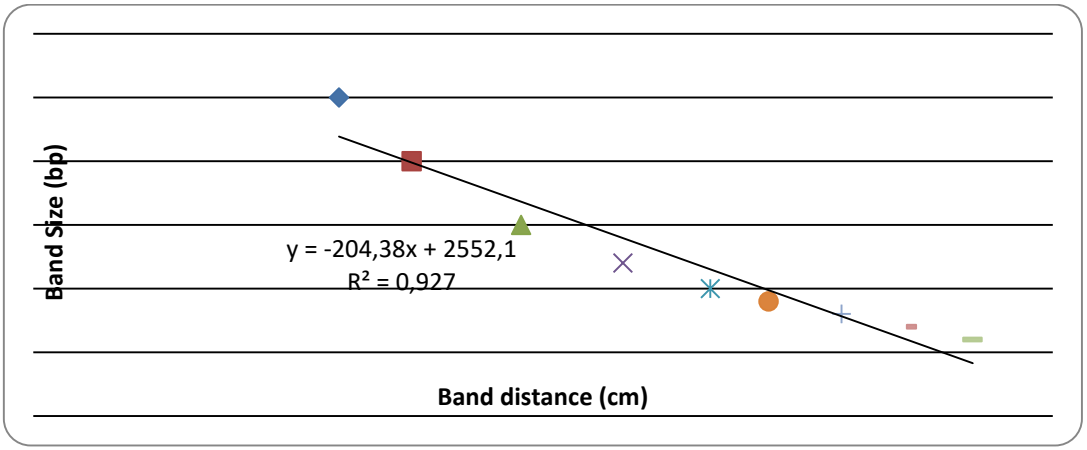

Figure 3. Linear Regression Equation

Table 2. Polymorphic DNA bands of green and red galoba durian samples on each primer

Primer type	Sequence 5-3'	Band size (bp)	Number of bands		Number of resulting bands
			Monomorphic	Polymorphic	
OPA14	TCTGTGCTGG	651-1612	0	8	8
OPC 19	GTTGCCAGCC	161-1469	0	13	13
OPN 9	TGCCGGCTTG	120-1489	0	15	15
Total number of bands					36

To help analyze the resulting band pattern, linear regression analysis was used. Linear regression is one of the methods used to estimate the relationship between independent and dependent variables. The linear regression model parameters consist of simple and multiple properties forming curves with straight lines (Yusnandar, 2004). Using linear regression (Figure 3) yielded 36 polymorphic bands, with band sizes ranging from 120 to 1612 bp (Table 2). The number of bands generated from OPN9 primers is higher than that of OPA14 primers and OPC19 primers. OPN9 produces 15 bands ranging from 120-1489 bp, while OPA14 produces 8 bands and OPC19 produces 13 bands.

The amplification results of both types of galoba durian indicated that all bands were produced 100% polymorphic, there was no monomorphic bands of the three primers were presenced. The high percentage of polymorphic obtained suggested high levels of genetic characterization. Genetic characterization may occur since there are genetic variations of genetic arrangement and genotype interaction with the environment (Anggraeni *et al.*, 2008). Geographical conditions affect the phenotype of plants. Samples of galoba durian leaves were taken from two different locations, namely in the highland (Hatalae village) and coastal area (Hatu village). Several studies had succeeded in showing the relationship of six curcuma species using two RAPD primers which resulted in 86% polymorphic presentation on OPC4 primers and 48% on OPC7 (Donipati & Sreeramulu, 2015). Ikhwani (2014) also said that the number of primers that can amplify the DNA of maniram and local cardamom is 15 of 20. The fifteenth primers had succeeded in amplifying DNA and both plants indicating the presence of polymorphism. The number of DNA bands was produced 182 with the number of polymorphic loci was 164 and the number of monomorphic locus was 18.

The result of DNA bands profile analysis of green and red galoba durian leaves from two locations indicated different species thus it could be concluded that there was a high genetic diversity among the galoba durian plants.

2. Morphological Characterization and Amplification Results

The results of morphological characterization and amplification analysis indicateddifferent results. Both galobadurian plants had similar phenotypical appearance and showed few different traits. DNA amplification produced polymorphic bands. The polymorphic level has showed the

high genetic characteristic between green and red galoba durian. The results show that the phenotypic appearance of both galoba durian plants were not different but genetically they were different hence there was high genetic characterization. Genetic characterization among species is an important aspect for the conservation and utilization of germplasm (Matondang, 2000). High genetic characterization can also be used as breeding materials (Kristamtini, Taryono, Basunanda, & Murti, 2014). This study indicates that both species of galoba durian are important for conservation and can be used as prospective selected resources in the plant crosses breeding process.

D. Conclusion

The morphological characterization of both galoba durian plants had similar appearance. The amplification of galoba durian DNA using three RAPD primers produced36 polymorphic bands. This indicates that both galoba durian plants were genetically different. Similar morphological but has differed genetically, so both of galoba durian is a difference species.

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