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Genetic Variability of *Galoba Durian* (*Amomum* spp) Center Mollucas and North Halmahera Based on RAPD

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Abstract: *Galoba Durian* is one of the endemic flora of Maluku, which is used as a medicinal plant and essential oil with red and green fruit colors. This study aimed to analyze the genetic variation of *Galoba Durian* in Central Maluku and North Halmahera based on the molecular marker of RAPD. This study began with a survey of sampling, isolation, and amplification of DNA using five RAPD primers. The results showed that all DNA bands were polymorphic as many as 67 with genetic similarity ranging from 20% - 31%, which grouped four samples into two groups. The first group is red *Galoba Durian* from North Halmahera (S1), red (S3) and green (S4) from Central Maluku, while the second group is green *Galoba Durian* from North Halmahera (S2).

Keywords: Genetic variation; *Galoba Durian*; RAPD

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Introduction

Zingiberaceae is a family of annual plants classified as plasma germs beneficial as medicinal plants and essential oils (Ewon & Bhagya, 2019). The Zingiberaceae family has several of the largest genera. However, those often used are Curcuma, Kaemferia, Hedychium, Amomum, Zingiber, Alpinia, Elettaria, and Costus (John Kress et al., 2002; Saensouk & Saensouk, 2021). Amomum is one of the largest genera of the Zingiberaceae family, with 150-180 natural plant species used as vegetables (Xia et al., 2004; Ding et al., 2020).

Galoba Durian is widely found in Maluku and North Maluku, spread over various islands with green and red fruit colors. This plant grows a lot in forest areas. The change of forest area status to another designation, uncontrolled logging, and fire accidents cause the decrease of population. It causes the decrease of genetic variation value and reduces the genetic base of the *Galoba Durian* plant. High genetic variation is very useful for genetic conservation and breeding programs or improving plant quality, so it needs to be done.

Currently, genetic variation data generally uses a morphological expression approach based on the phenotypic characteristics to be conserved. It is essential to know the genetic distance and its relationship in plant conservation and breeding. It has a significant impact on plant improvement (Solin et al., 2014). It is necessary to have genetic markers as a selection reference for conservation and breeding activities to determine the level of genetic variation of the population of *Galoba Durian*.

One of the genetic markers that are often used is RAPD (Random Amplified Polymorphic DNA). RAPD can identify DNA polymorphisms in the genome quickly and efficiently in production, evaluate

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variability with good accuracy, and present genetic information in the form of characteristic banding patterns (fingerprints) of complex genomes without prior sequence information. This method can analyze the genetic variation of plants well and present important patterns of kinship. Most of the DNA bands from RAPD are 300-3000 bp in size (Cheema & Pant, 2013). The purpose of this study was to analyze the genetic variation of the *Galoba Durian* plant in Central Maluku and North Halmahera based on the molecular marker RAPD as a recommendation for individual selection for conservation and breeding programs.

Method

The research survey was carried out in July-September 2019 in Porto and Wai Putih-Putih village, Central Maluku district. Sosol village, North Halmahera district. Molecular analysis was carried out at the Sembawa Rubber Research Center, Palembang.

DNA isolation. Isolation of DNA used a method (Orozco-Castillo et al., 1994). Samples of *Galoba Durian* leaves were washed, and dried with a tissue. The sample was cut into small pieces and the leaves were removed to facilitate the refining process. The *Galoba Durian* leaves sample was ground until smooth with a mortar while adding liquid nitrogen, 0.1 gram poly (1-ethenylpyrrolidin-2-one) (PVPP). A total of 5 mL of heated extraction buffer and heated 500 µL β-mercaptoetanol 1% were added, then vortexed and incubated for 30 minutes at 65 ^{0}C. Every 5 minutes, the tube was shaken so that the reaction proceeded quickly. The sample was allowed to cool in a fume hood, then 5 mL of chloroform: isoamyl alcohol (24:1) was added. The sample was centrifuged at 11,000 rpm for 10 minutes at 25 ^{0}C. The supernatant obtained was transferred, added 5 mL of chloroform: isoamyl alcohol (24:1), shaken with a vortex, and centrifuged again at 11,000 rpm for 10 minutes at 25 ^{0}C. The supernatant obtained was transferred and then 1x the volume of cold isopropanol was added. The sample was homogenized by inverting the tube and then stored in the refrigerator (4 ^{0}C) for 30 minutes. Then, it was centrifuged again at 11,000 rpm for 10 minutes at 25 ^{0}C. The supernatant obtained was discarded while the pellet obtained was dried. After drying, the pellet was dissolved with 1 mL of TE buffer, and then it was shaken. A total of 1/10 of the volume of 3M Na-Acetate pH 5.2 and 2.5 mL of absolute ethanol were added and shaken until a collection of white DNA fibers was seen. Samples were stored in a -20 ^{0}C freezer for 30 minutes or overnight. Samples were centrifuged at 12,000 rpm for 10 minutes at 4 ^{0}C. The supernatant obtained was discarded, and the pellet was dried. The pellet was washed with 100 µL of 70% ethanol. The mixture was centrifuged again at 8000 rpm for 5 minutes at 25^{0}C. The supernatant was discarded, and the pellet was dried in an activated laminar airflow cabinet. The dry pellet was added with 30 µL of TE buffer solution and homogenized until the pellet and solution were homogeneous. DNA purity and concentration were measured using a NanoDrop Spectrophotometer.

DNA amplification. DNA amplification used 5 primers measuring 10 nucleotides (Table 1). The DNA amplification process began with pre-denaturation at 95^{0}C for 3 minutes, denaturation at 95^{0}C for 25 seconds, annealing at 35^{0}C for 15 seconds, extension at 72^{0}C for 15 seconds, and post-extension at 72^{0}C for 7 minutes. The PCR reaction was carried out for 45 cycles. The amplification reaction was carried out at a volume of 25 µL, with the following composition: 12.5 µL of KAPA2G Fast Ready PCR Mix reagent, 1 µL of 10 µM primer, 2 µL of DNA template, and 9.5 µL of nuclease-free water were put into a 0.2 mL tube so that a total reaction 25 µL. DNA electrophoresis. The results of DNA amplification were visualized on 1.5% agarose gel for 90 minutes, then documented using AlphaInnotech doc gel.

Data analysis. DNA bands were screened into binary data and formatted in Microsoft Excel software for genetic distance analysis using GenAlex software (Peakall & Smouse, 2012) and phylogenetic trees were created using MEGA10.0.4 software. All the steps of this procedure shows in figure 1.

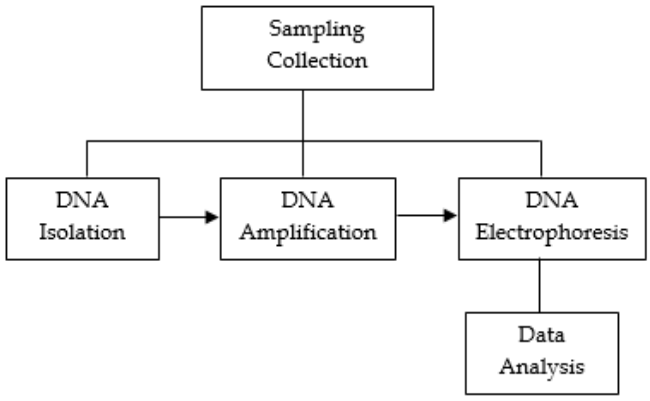

Figure 1. Scheme showing the sampling and DNA analysis of *Galoba Durian*

Result and Discussion

A total of five RAPD primers used in this study produced DNA bands measuring 100 bp to 1400 bp. All DNA bands obtained were polymorphic with variations in the size and number of DNA bands (Figure 2). Primers OPN-19 and OPC-19 produced the highest number of bands, namely 16 and 14 DNA bands. OPN-12 and OPN-9 primers produced 13 DNA

bands, and OPA-14 primers produced 11 polymorphic bands. Motifs of DNA band is an informative figure for representing to form genetic variability between samples (Probojati et al, 2019). RAPD markers show high ability of genetic level due to reveals high polymorphism (Bousba et al, 2020).

The total DNA bands obtained were 67 polymorphic bands. It shows that the five primers used have a high level of polymorphism (Table 1). Polymorphism is a picture of the amplification obtained from the observed differences in DNA fragments and scored as the presence or absence of band differences, indicating variation. The number of bands produced from each primer was determined by how the primer recognizes the complementary DNA sequence on the template DNA (Oktavia et al, 2011).

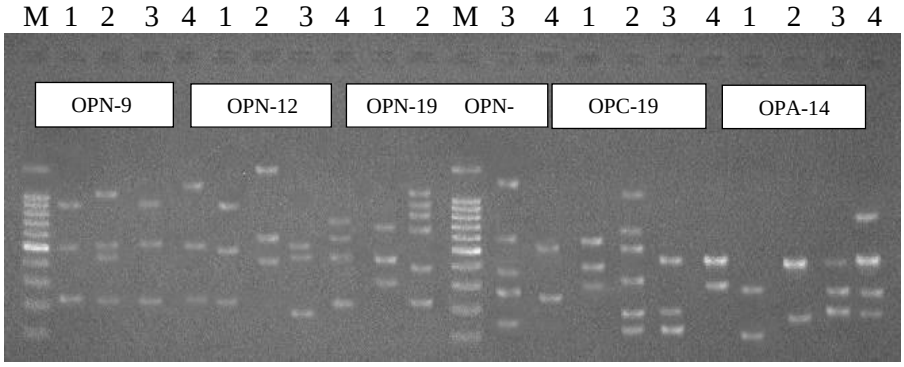

Figure 2. *Galoba Durian* DNA amplification. (M) 100bp DNA marker (1,2) North Halmahera; (3.4) Central Maluku

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Table 1. Data of DNA Bands Polymorphism of Durian Galoba Sample

Primer name	Sequence 5´-3´	Bands Size (bp)	Number of bands		Total bands
			Monomorphic	Polymorphic	
OPN-19	GTCCGTACTG	120-1200	0	16	16
OPC-19	GTTGCCAGCC	110-1200	0	14	14
OPN-9	TGCCGGCTTG	200-1200	0	13	13
OPN-12	CACAGACACC	180-1400	0	13	13
OPA-14	TCTGTGCTGG	100-800	0	11	11
Total number of bands produced					67

The genetic similarity matrix was carried out to determine the genetic relationship of the tested plants. It is necessary to determine the level of genetic diversity in a population (Muriira et al, 2018).

Based on the data in Table 2, the genetic similarity of *Galoba Durian* ranges from 20% - 31%. The highest genetic similarity was shown by the pair of green *Galoba Durian* from North Halmahera (S2) with red *Galoba Durian* from North Halmahera (SI) by 31%. The lowest value was found in the green *Galoba Durian* sample from Wai Putih-Putih (S4) with the red *Galoba Durian* from Porto (S3) 20%.

Information on the level of genetic similarity is useful in plant breeding activities. The research of Li, et al. (2020) showed that the increased genetic level indicated the adaptability individuals to divergent habitats as a essential resource for breeding. Plants with a high degree of genetic similarity can be grown separately. It aims to maintain the genetic diversity of the plant population. In addition, it can be used as a reference for crossing plants.

Table 2. *Galoba Durian* Genetic Similarity Matrix based on RAPD markers.

	S1	S2	S3	S4
S1	0.00			
S2	0.31	0.00		
S3	0.27	0.30	0.00	
S4	0.21	0.30	0.20	0.00

Quantitative data obtained from the results of the DNA bands scoring were used for the construction of dendograms showing the distances of genetic differences and similarities between genotypes. Genetic similarity among two organisms or populations may be estimated by various statistical calculate using on the data set (Govindaraj et al, 2015). The clustering used neighbour-joining method of four samples of *Galoba Durian* with 5 RAPD primers resulted in 2 groups (Figure 3), group I consisted of samples of red *Galoba Durian* from North Halmahera (S1), red color from Central Maluku (S3), and green color from Central Maluku (S4) as sub-group while group II only contained samples of green *Galoba Durian* from North Halmahera (S2).

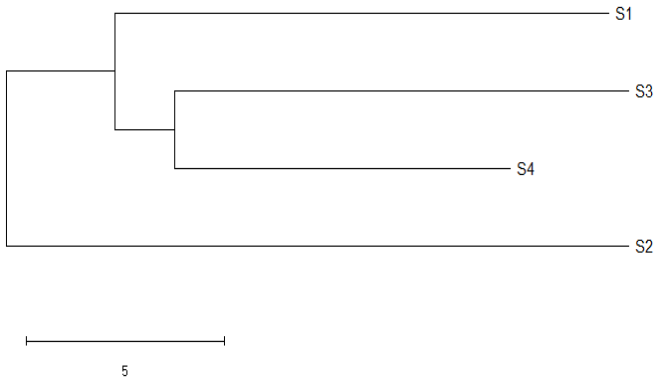

Figure 3. Dendogram of *Galoba Durian* grouping based on genetic distance. (S1;S2) North Halmahera, (S3;S4) Central Maluku

Genetic variation and kinship relationships are essential for genetic conservation and plant breeding. For *ex-situ* conservation, this information is needed to determine the number of populations that need to be collected to maintain its genetic variation. For in-situ conservation, this information is needed to determine the number of locations and individuals to be used (Pamoengkas et al., 2020).

In general, the higher the genetic similarity coefficient will lead to more groupings. A high genetic similarity coefficient value describes the close genetic relationship between individuals in the population. On the other hand, a relatively distant genetic relationship is indicated by a low genetic similarity coefficient. This information is beneficial as a basis for plant crosses for plant breeding (Bolibok-Bragoszewska et al., 2014).

Germplasm diversity can be utilized for genotype improvement if the genetic variation and phenotypic and molecular kinship between genotypes (Abady et al., 2021). The phylogenetic tree shows that the red *Galoba Durian* from North Halmahera and the green *Galoba Durian* from North Halmahera have a high genetic distance. Even though they have different colors and locations, the genetic material is very different. High genetic diversity in a population can be caused by the high potential for genetic diversity it has. Other factors are natural populations that have not been disturbed and random mating, which maintains the stability of genetic diversity (Wu et al., 2020). The red *Galoba Durian* in Porto and the green *Galoba Durian* in Wai Putih-Putih had a low level of genetic variation. The parent's origin influences the genetic variation that appears. Varieties that have genetic closeness are thought to come from closely related parents. On the other hand, varieties with relatively high genetic distance come from parents who are distantly related to other varieties. In addition, the value of genetic diversity is determined by the population size of a species. Smaller population size in an area can be one of the factors causing the low value of population genetic diversity (Huang, 2016).

Conclusion

DNA amplification of *Galoba Durian* in Central Maluku and North Halmahera using 5 primers resulted in 67 polymorphic bands. The highest genetic variation was found in samples of red and green *Galoba Durian* from North Halmahera (S1, S2), while the lowest was in red and green *Galoba Durian* from Central Maluku. (S3, S4).

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