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ORIGINAL ARTICLE

Analysis of Breadfruit Diversity Based on Random Amplified Polymorphic DNA (RAPD) and Morphology

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ABSTRACT
Artocarpus altilis(Parkinson) Fosberg is a plant that is widely found in the tropics. Efforts to improve the quality of Artocarpus altilis(Parkinson) Fosberg or breadfruit plants are a breeding program to obtain high productivity that is by morphological characterization and analyze the genetic diversity. The purpose of this study is to assess the diversity of breadfruit plants based on morphological and molecular characters. Morphological character was performed with the description of 9 breadfruit plants obtained 33 characters of the phenotype. Molecular characterization were performed with DNA extraction followed by RAPD method to obtain molecular data in the form of DNA fragments and analyzed by using NTSYS software. Based on the results of morphological data analysis showed diversity, from dendrogram there were 9 clusters of breadfruit plants into 3 clusters, namely cluster I consisting of GK1, GK3, GK2, B3, B2, cluster II consisting of S1, S2, S2 and cluster III consisting of B1, and grouped on the coefficient value of 0.79. Based on the results of molecular data analysis showed diversity that 9 breadfruit plants that were screened with 6 primers showed polymorphic fragment of 91.8%. The grouping of 9 breadfruit plants into 3 clusters i.e. cluster I consists of GK3, B1, B2, B3, S1, S2, S3, cluster II GK2 and cluster III GK1, the three clusters are grouped on the coefficient value of 0.95.
Keywords: RAPD, DNA, breadfruit, NTSYS, molecular, polymorphic.

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INTRODUCTION

Breadfruit *Artocarpus altilis*(Parkinson) Fosberg is a plant of the genus Artocarpus within the family of Moraceae which is found in many tropical regions such as Malaysia and Indonesia. Breadfruit is one type of multipurpose plant that is economical because it produces fruit with a high enough nutrient content [1]. The fruit can be used as alternative food because its existence is not in line with conventional food (rice), meaning that the existence of this food can cover the void of conventional food production [2].

Breadfruit has a variety of morphological variations, so that the variation of morphology can be used as one way to determine a type of breadfruit especially the quality is superior or not [2]. The morphological information presented in this study is expected to provide scientific information so as to support efforts to determine the quality of breadfruit. The characteristics of breadfruit plants can be seen based on vegetative characteristics and generative characteristics [3]. Characteristic is very useful to get the description and classification of breadfruit plants so that people can easily determine the cultivars of breadfruit crops. In addition, the uniformity of the specific characteristics possessed among the breadfruit plants can indicate their kinship relationship. The more the same character the closer its kinship [4].

An alternative to assessing of breadfruit diversity is to use molecular markers (protein, isozim, and DNA). The diversity of breadfruit DNA can be analyzed using multiple markers, including RAPD markers (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), SSR or microsatelit [5]. Compared to some of these molecular markers, the advantages of RAPD is easy to use, fast and requires only a little DNA as a mold [6].

In Indonesia, breadfruit plants include plants that have not been widely cultivated. Some breadfruit plants grow in the yard of the house without any special cultivation. People use wood breadfruit plants as the basic ingredients of building the rest of the house fruits are taken for consumption purposes. So with the study of this diversity can provide an alternative for cultivating breadfruit crops.

The study of diversity is essential to provide information on the magnitude of genetic diversity in a population so that genetic conservation efforts, improved varieties and breeding become directed. Exploration should be undertaken in areas with high genetic diversity, where the origin of the plant species (center of origin) or the area where the plant is cultivated intensively (center of diversity) [7]. Annual crops such as breadfruit generally have many components of phenol, polysaccharides, and other secondary metabolites that can degrade the quality of the resulting DNA so it is necessary to assess the diversity of DNA to produce high-quality breadfruit plants [8].

MATERIAL AND METHODS

Research materials in the form genotypes of breadfruit from three different locations, namely Gunung Kidul, Bantul and Sleman (Table 1).

Table 1. The place of breadfruit sampling

No.	Sample code	State/Geographical source	Latitudes/Longitudes
1	GK1	Gunung Kidul	S 07°51'51.0"/E 110°30'30.8"
2	GK2	Gunung Kidul	S 07°52'57.4"/E 110°34'02.8"
3	GK3	Gunung Kidul	S 07°52'29.4"/E 110°34'42.8"
4	B1	Bantul	S 07°55'14,6"/E 110°22'33,7"
5	B2	Bantul	S 07°52'40.9"/E 110°19'06.0"
6	B3	Bantul	S 07°54'14.2"/E 110°19'17.4"
7	S1	Sleman	S 07°41'56.7"/E 110°25'30.6"
8	S2	Sleman	S 07°41'48.1"/E 110°27'25.3"
9	S3	Sleman	S 07°40'43.2"/E 110°28'04.6"

Table 2. Observation variable by morphological characters

No	**Observation Variable**	**Observation Variable**
1.	The shape of the tree canopy	Plant height
2.	Branching patterns	Ring circumference
3.	Direction of growth	Header diameter
4.	Leaf shape	Number of main branches
5.	Leaf bone arrangement	Leaf length
6.	Top surface layer of leaves	Leaf width
7.	Leaf color	The length of the petiole
8.	Leaf tip shape	Male long flower
9.	Leaf base form	Diameter of male flowers
10.	Male flower shape	Male flower width
11.	Female flower shape	The length of the fruit stalk
12.	Color of young male flowers	Fruit length
13.	Color of old male flowers	Fruit diameter
14.	The shape of the fruit	Weight of fruit
15.	The color of the flesh	Number of fruits/trees
16.	The color of the fruit skin	
17.	The shape of the fruit tip	
18.	Form the base of the fruit	

DNA extraction

DNA was extracted from fresh leaves, leaf samples crushed with mortar until soft, then added CTAB buffer solution that had previously been incubated in a water bath at 65° C for 30 minutes [8]. Then the mixture was put into a 2 ml tube, incubated in a water bath at 65° C for 60 minutes and every 10 minutes the mixture was turned back to keep it homogeneous.

After incubation, each sample was added 500 µlCIAA and vortex for 5 minutes then centrifuged for 15 minutes at 12,000 revolutions per minute (rpm). The was taken and added 10% total volume of sodium acetate 1M and cold isopropanol as much as 2/3 total volume (supernatant + sodium acetate). Then mixed by flipping through the tube and allowed to stand at -20° C for 1-24 hours. After that, the mixture was centrifuged for 10 minutes at a speed of 12,000 rpm. The supernatant was removed and the precipitated DNA was washed with 500 µl of ethanol 70%, and then centrifuged for 5 minutes at 12,000

rpm. The supernatant was discarded and the precipitated DNA was dried, after a dry precipitate of DNA, 50 µl ddH_2O was added and then stored in the refrigerator at 4^0C.

DNA Amplification

Amplification of DNA fragments is done by polymerase chain reaction (PCR) which aims to multiply DNA sequences based on the primary was used [9, 10, 11, 12, 13]. The selection was performed by taking four DNA samples as randomized templates that were amplified with a number of primers. Primers that demonstrate polymorphism are used in the *genotyping* stage by using PCR (Bio-Rad T100-Thermal Cycler). The PCR reaction was performed at a total volume of 10 µl for each PCR tube. The DNA of PCR then electrophoresis use 1.5 % agarose added florosafe DNA stain as a dye. Result are visualized by UV light.

Band Scoring and Data Analysis

Morphological characters observation data in analysis through scoring [13]. The bands coded based on the existence of the amplified bands/fragments with a value of *"1"* when there is an amplified band, and *"0"* in the absence of the amplified band.

Cluster analysis with NTSYS 2.02 software uses UPGMA (*Unweight Pair-Group Method with Arithmetic Averaging*) method to group populations in distance concepts based on similarity values. The results of this cluster analysis are then presented in the form of dendrogram of proximity relationship or resemblance genetically between populations [14].

RESULT AND DISCUSSIONS

The morphology of breadfruit plants

Breadfruit grows in many tropical regions of Indonesia. Collection of breadfruit plants in the field is done by collecting information from the local community at the time of observation to research in May-October 2017, not all variations of breadfruit is in the period of fruiting, so that the sampling is done on certain areas that the plants are in the period of fruiting and can represent areas in DIY.

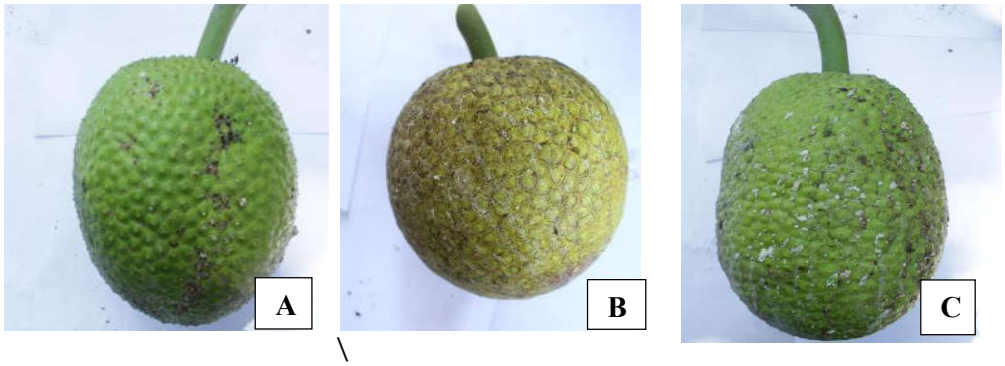

Figure 1. Breadfruit (*Artocarpus altilis*, P.), GK (A), B (B), S (C)

In OTU's research (*Operational Taxonomic Units*) are 9 breadfruit plants and 33 morphological characters used to analyze morphological variations as well as for the preparation of phenetic kinship [16]. Qualitative characters are phenotypes that differ sharply from one another in a qualitative way and each can be grouped in the form of a character category, this character is only slightly influenced by environmental factors because the qualitative character is controlled by a simple gene (one or two genes) , while the quantitative character is generally controlled by many genes (*polygenic*) and is the end result of a process of growth and development is greatly influenced by environmental factors [5, 12].

The results of cluster analysis shown on the dendrogram (Figure 2.) form 3 clusters at the coefficient of 0.65. The morphological combinations of characters (*distinguishing characters*) in each cluster will indicate the number of plants belonging to them. 3 clusters are cluster I, II and III.

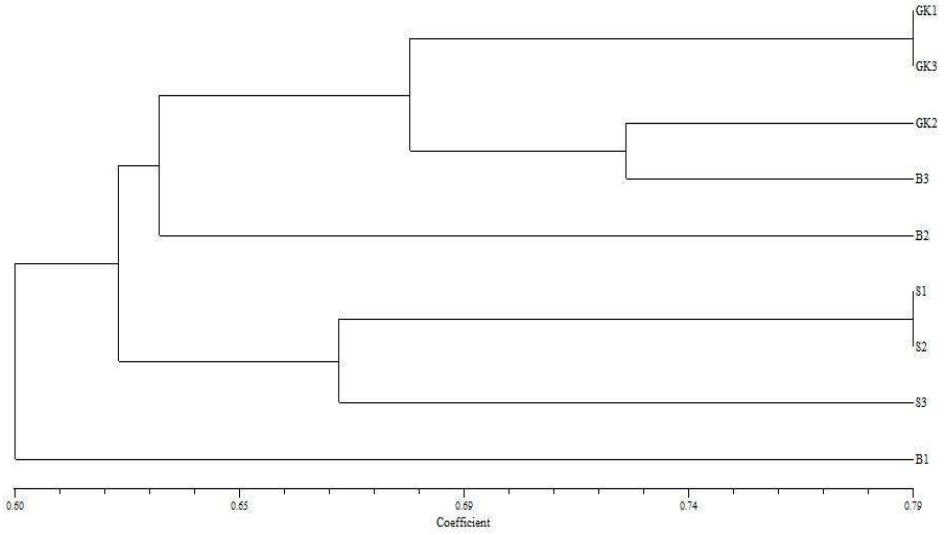

Figure 2. Dendrogram of the phenetic relationship between 9 breadfruit plants in DIY based on morphological characters.

Cluster I consists of 5 plants, namely GK1, GK3, GK2, B3 and B2, from the five plants have similarity coefficient of 0.63, the fifth morphological similarity of the plant is the shape of ovate leaves, the shape of the tip of the sharp leaf (*acuminate*), the shape of the base of the leaf (*cuneate*), jutting male form (*oblong*), shape of female flowers round (*globose*), the color of male flowers and young green flowers, the color of old yellow male flowers, the shape of the longitude (*oblong*), the color of yellow flesh, the color of yellowish green peel, the shape of the tip of convex (*convex*), and the shape of the base of the fruit jutting (*depressed*) .

Cluster II consists of 3 plants, namely S1, S2 and S3, from both plants have coefficient similarity 0.66, the similarity of morphology of both plants is in the on the oval leaf shape (*ovate*), sharp leaf tip shape (*acuminate*), the shape of the base of the leaf transverse (*cuneate*), jutting male flower shape (*oblong*), shape of female flowers round (*globose*), a male flower and a light green flower, the old yellow male flowers, shape of longitude (*oblong*), the yellow flesh color, the green fruit skin color yellowish, convex tip shape (*convex*), and the shape of the fruit base protrudes (*depressed*), while cluster II consists only of B1.

Molecular breadfruit plants

Molecular analysis was performed on 9 cultivars of breadfruit cultivars (*Artocarpus altilis*, P.) in Yogyakarta, Indonesia, amplification process of DNA fragment by 6 primer RAPD i.e. OPC5, OPD2, OPD3, OPD8, OPD11, and OPD19 to 9 cultivars of breadfruit plants i.e. GK1, GK2, GK3, B1, B2, B3, S1, S2 and S3, electrophoresis which produce the following electrogram.

Table 3. Baseline RAPD base order, size and number of amplified fragments and number of polymorphic and monomorphic fragments

No.	Primary	Sequence of base 5'-3'	Fragment	Number of fragments DNA	Number of polymorphic fragments	Polymorphism %
1.	OPC5	GATGACCGCC	250-1300	26	26	100%
2.	OPD2	GGACCCAACC	350-1000	28	28	100%
3.	OPD3	GTCGCCGTCA	350-1300	29	29	100%
4.	OPD8	GTGTGCCCCA	400-1600	29	20	68%
5.	OPD11	AGCGCCATTG	150-1100	54	45	83%
6.	OPD19	CTGGGGACTT	300-1300	34	34	100%
Amount				200	193	
Average				33	32	91.8%

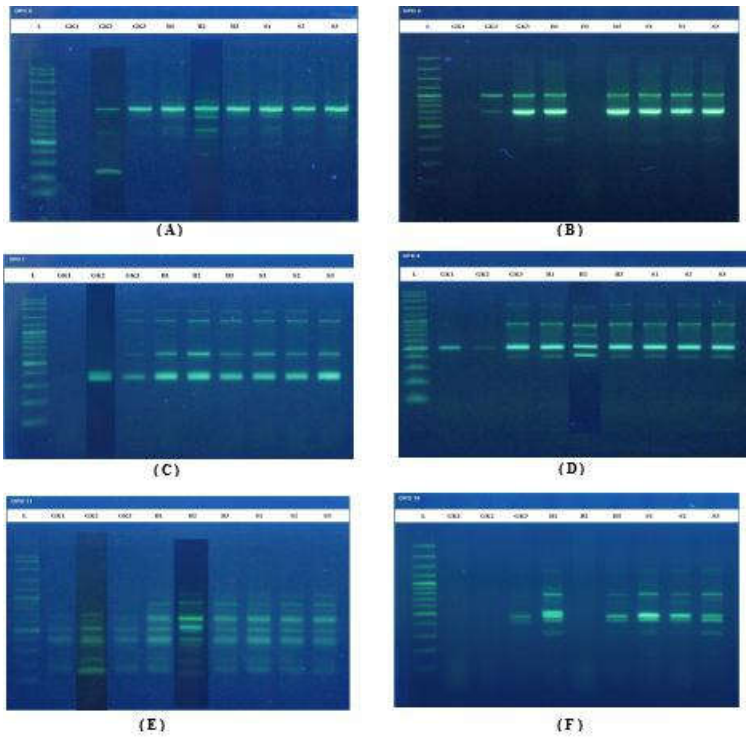

Figure 3. Amplification of electrogram result DNA of PCR-RAPD plant of breadfruit

Molecular analysis showed electrogram result from PCR-RAPD product electrophoresis by using 6 primers. In this study, the use of these 6 primers was based on its ability to detect genetic diversity among samples of breadfruit plants as many as 9 cultivars were analyzed. A total of six primers produced a variable number of DNA fragments, the variation in DNA fragments that appeared to depend heavily on whether or not the homology of the primary nucleotide sequence and DNA nucleotide sequence were printed.

Based on the 6 primers used, it is known that the highest polymorphic percentage is generated by OPD19 primers with a value of 100% and the lowest polymorphic percentage in OPD8 primers is 68%.

However, if averaged to 6 primers produce a percentage of 91.8%, which means that the 6 is either used in the analysis of genetic variation. The more polymorphic DNA fragments that are formed show a variety of genetic variations so that the genotype of an individual can be distinguished from that of the other. The resulting monomorphic fragments represent a locus of a certain size possessed by all individuals in 1 species.

In this study we performed a binary data cluster analysis based on the DNA fragment profile that emerged from the DNA amplification result in each primer used to see the similarity level and phenetic kinship relationship on 9 breadfruit cultivars. The obtained binary data is then created similarity table between OTU's by using *Gower General Coefficient* formula. The results show that the coefficient of similarity between cultivars of breadfruit plants ranged from 0.31 to 0.96. The smaller the coefficient value, the similarity is close to *"0"* then the kinship relationship will be farther and the greater the coefficient value approaching *"1"* then the closer the relationship. The results of the similarity coefficient calculations can be used as an indication to determine the difference between genotypes of breadfruit cultivars analyzed.

The grouping on the dendrogram occurs because of differences in coefficient similarity values between groups of one with other groups based on the emergence of banana bread sample fragments that have different sizes of each primer.

The DNA fragments produced from each RAPD primer vary so that it is used as a character determination of the similarity of each group so that more and more identical fragments the similarity level will be higher. In addition to the primary RAPD molecular method used in this study is also very influential because this method is very sensitive and has weaknesses in reproducibility, RAPD method cannot distinguish homozigotsitas and heterozigositas due to the way of amplification by primers RAPD in the unknown part of the genome, so that the locus of codomain is often has DNA bands of different sizes.

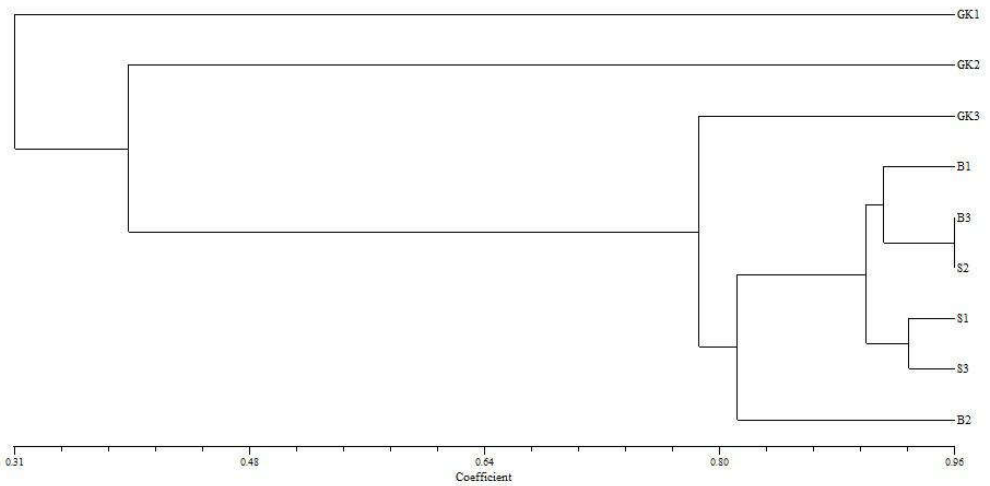

Figure 4. Dendogram of molecular breadfruit plants

Although the results of cluster analysis indicate the grouping but most of the members in each group had a similarity coefficient value above 0.8 (80%), which means that 9 cultivars of breadfruit plants genetically have a close kinship, indicating that the breadfruit plants are likely derived from the same genotype. Genetic diversity that emerges can be caused by environmental factors such as soil types and conditions, rainfall, climate and internal factors such as mutations, in addition the influence of human intervention in the distribution process also plays a very important role and also influence the process of genetic variation, for example in process and cultivation of breadfruit crops. The coefficients of similarity range from 0.31 to 0.96, by forming individual GK1, individual GK2 and large groups (GK3, B1, B3, B2, S1, S2, S3).

CONCLUSION

1. Based on the results of morphological data analysis showed diversity between plants, it is found that the grouping of 9 breadfruit plants into 3 clusters is cluster I consisting of GK1, GK3, GK2, B3, B2, cluster II consisting of S1, S2, S2 and cluster III consisting of B1, the coefficient value of 0.79.
2. Based on the results of molecular data analysis showed that 9 breadfruit plants that were screened with 6 primers showed polymorphic fragment of 91.8%. Dendrogram grouping of 9 breadfruit plants into 3 clusters, cluster I consists of GK3, B1, B2, B3, S1, S2, S3, cluster II GK2 and cluster III GK1, the three clusters are grouped on the coefficient value of 0.95.

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