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Evaluation of Molecular Markers and Morphology In Identification of Local Porang Strain in Karawang District

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Abstract: Porang plant tubers have the potential to have high economic value, because they contain glucomannan which is good for health and can be processed into a food source to replace carbohydrates. The aim of the study was to identify Karawang Local Porang strains using molecular markers and morphological characters as an effort to conserve and utilize Karawang's local biological resources. Exploration of Local Porang Karawang was carried out at the foot of Mount Sanggabuana which is the habitat of Porang in Karawang district. Molecular identification was carried out at BBPP Biotechnology and Agricultural Genetic Resources with comparisons of Porang Sidrat., Madiun 1 and SS1 Bulukumba varieties. Morphological identification was carried out at the Basic Laboratory of the Faculty of Agriculture, Winaya Mukti University. Based on the results of the DNA amplification of the Porang samples using four RAPD primers, it was found that the three primers showed differences in banding patterns between the Porang Karawang sample and the three Porang varieties for comparison (Porang Madiun 1 Sidrap and SS1 Bulukumba). This shows that there is genetic variation between Porang from Karawang and three other Porang. The results of genetic relationship analysis using four RAPD primers showed that the Porang Karawang sample was different from the Porang samples from the other two areas. The morphological identification results showed differences in characteristics with the comparison. The Karawang Local Porang variety has high quality as indicated by high glucomannan levels of 79.80%.

Keywords: Identification, Molecular Markers, Morphology, Porang

INTRODUCTION

Porang (Amorphopallus muelleri Blume) is a herbal plant that can grow to a height of about 1.5 meters. This plant is a tuber producer that lives a lot in tropical forests. Physically,

the porang plant grows with a single stalk or stem with green and white stripes. Porang tubers have the potential to have high economic value, because they contain glucomannan which is good for health and can be easily processed into food ingredients to meet daily needs. Glucomannan is a water-soluble polysaccharide and is considered a dietary fiber. Glucomannan is a hemicellulose component in the cell walls of several plant species. Apart from porang plants, glucomannan can also be found in white pine, lily, orchid, hemp and redwood plants (Center for Research and Development of Food Crops. 2021).

Export opportunities and markets for porang products are still wide open due to the increasing public awareness of health and functional food. The problems faced in developing porang include the unavailability of seeds in sufficient quantities and most farmers do not know the benefits and technology.

Exploration and utilization of the local variety of Porang is very important in efforts to provide Porang seeds which vary from the aspect of superior character and the suitability of the location for the development of Porang. Amorphophallus spp which has been identified worldwide based on the International Plant Names Index (2020) contains 235 species. Indonesia has contributed to the diversity of Amorphophallus spp by 17.87% of all species in the world. Meanwhile, Kalimantan ranks first in contributing to the diversity of Amorphophallus spp in Indonesia, with 20 species out of a total of 42 species, followed by Sumatra with 16 species,

Java with 7 species, Sulawesi with 3 species, Papua and Nusa Tenggara with 2 species each.

The development of biotechnology allows breeders to utilize a tool in the form of molecular markers to assist identification. Molecular markers refer to differences in sequences or sequences of Deoxyribo Nucleic Acid (DNA), which are the main constituent elements of genes. This difference is able to distinguish one individual from another specifically. The basic concept of molecular markers is that one individual has a DNA sequence that is unique to other individuals so that these differences can be used as markers. The advantage of DNA markers in conducting selection is that they are stable, not affected by the growth phase or environmental conditions. The gene arrangement of individuals will remain the same at various growth phases and in different environmental conditions. This makes DNA markers a good tool to assist the process of carrying out the identification and analysis of differences. Various DNA markers have been used in the selection process, including Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Inter-SSR (ISSR), Amplified Fragment Length Polymorphism (AFLP), transposon-based markers, to Single Nucleotide Polymorphism (SNPs). These markers have been widely used and have yielded significant results for improving the properties of various crop commodities such as yield, quality, resistance to drought stress, salinity, and pest attack. Currently, molecular markers continue to experience development on various sides. This is inseparable from the development of Next Generation Sequencing (NGS) technology which enables rapid plant genome sequencing, bioinformatics, and easy and inexpensive molecular detection techniques. Based on its advantages, DNA markers are a tool that has the potential to be utilized in increasing the efficiency and effectiveness of the selection process to improve plant properties through plant breeding or identifying new varieties and observing different characters (Agus R.N. 2019).

DNA barcoding has been used as a powerful tool and a practical method for species characterization and delimitation. The present work aims to evaluate molecular markers for barcoding three Piper species native to Brazil: P. gaudichaudianum (“jaborandi” or “pariparoba”), P. malacophyllum (“pariparoba-murta”) and P. regnellii (“caapeba” or “pariparoba). The Malva genus contains species that reveal therapeutic properties and are mostly important in medicine and the functional food industry. Its breeding, cultivation, and utilization are based on proper germplasm/plant identification, which is difficult using

morphological features. For this reason, we applied flow cytometry and inter simple sequence repeat polymerase chain reaction (ISSR-PCR) for fast and accurate species identification. (Iwona Jedrzejczyk and Monika Rewers, 2020). The study aimed to get the molecular and morphological identity of patchouli (Pogostemon sp.) from BPP Kabupaten Batang. Amplification of ITS fragment patchouli produced a 670 bp-sized single band. Phylogenetic analysis showed patchouli BPP related to Pogostemon cablin (KR608752.1) with 98% coverage identity (Aeldo Yudifian et al., 2022). Identify the agarwood-producing plant species in North Aceh District, Aceh Province, Indonesia, based on their morphological and molecular characters. The evaluated morphological characteristics include the bark and leaf structures of endemic plants. Molecular characterization was carried out by sequencingof matK, ITS, and trnL-trnF genes (Lukman et al.)

West Java Province is one of the centers of diversity in the world, one of which is in Karawang District. The local Karawang specis of porang need to be explored, identified and developed so as to provide benefits for increasing food security. Exploration is an activity of searching, collecting and researching certain types of local varieties (in certain areas) to protect them for extinction. Exploration activities should be carried out in production centers, traditional production areas, isolated areas, mountain slope agricultural areas, remote islands, indigenous tribal areas, areas with undeveloped/traditional agricultural systems, area where people use these commodities as staple food, areas endemic pests/diseases, as well as old and new transmigration areas. Porang of Karawang local varieties are widely spread at the foot of Mount Sanggabuana and has different characteristics from porang in other areas. The local porang of Karawang need to be explored, identified and described so that their characteristics are known both quantitatively and qualitatively and can be known to be different of the same as the porang that has been identified Germplasm research activities include collection, rejuvenation, characterization and evaluation of local cultivars, wild cultivars or introductions from abroad, past and present superior cultivars (Baihaki, 2004; Sutrisno and Silitonga 2003 in Imelda et al. 2021). The advantages possessed by local varieties (land-races) such as resistance to environmental stress are assets in work so they need to be protected from extinction. According to Sumarno and Zuraida (2004) the more germplasm collections, the greater the chance to get superior varieties. Collection of potential local rice germplasm needs to be continuously carried out because the variability of germplasm which has a large variety is a source of genes that support efforts to form new varities that have high yields, resistance to pests or disease, early maturity and other characteristics (Sitaresmi et al, 2013). Porang varieties that are popular in the community at this time are Sidrap, Madiun 1 and SS1 Bulukumba varieties, so that in this study they were used as a comparison.

MATERIALS AND METHODS

Study area

The local of Karawang species of porang was first explored in 2018 by a Pangkalan resident from the Batu Tumpang area at the foot of Mount Sanggabuana towards Karawang Regency. The exact habitat of the Porang Karawang species is in the Batu Overlapping Curug Cigentis foot of Mount Sanggabuana, Tegalwaru sub-district. Karawang District of West Java Province (Figure 1.)

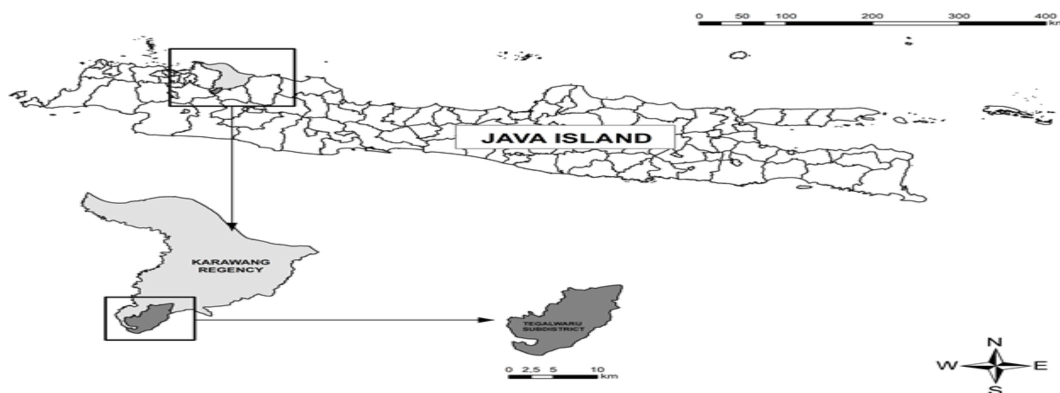


Figure 1. Location of local Porang Karawang

Procedures

Molecular Analysis

The plant genetic material used in this molecular analysis was a Porang plant sample from the Regional Government of Karawang Regency and three (3) control varieties consisting of Madiun 1, Sidrap and SS1 Bulukumba. The stages of Molecular Analysis are as follows:

1. Total Genome DNA Isolation

Isolation of total genomic DNA from porang samples was carried out using the CTAB method on a small scale (miniprep) with reference to the DNA isolation method Doyle and Doyle (1990). The tuber samples were crushed with the help of liquid nitrogen (LN). Until smooth, and put into a 2 mL micro tube followed by the addition of 700 µL of DNA extraction buffer (100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA pH 8.0, 2% (w/v) CTAB (cetyltrimethylammonium bromide), and 2% (w/v) PVP (Polyvinylpyrrolidone)). The mixture was homogenized by carefully inverting the tube and incubated at 65°C for 30 minutes in a water bath. During incubation, the mixture was homogenized again by inverting the microtube every 15 minutes.

In the next step, 1X volume (700 µL) of chisam (chloroform isoamyl alcohol) (24:1 v/v)) was added to the micro-tube containing the mixture. then homogenized using a vortex for a few seconds or using a shaker for 5-10 minutes. This step is followed by the separation of the macromolecules present in the mixture through a centrifugation process (separation of the upper and lower solution phases) at a speed of 12000 g for 10 minutes. The supernatant (upper phase) from each sample was carefully transferred into a new micro tube (1.5 mL eppendorf). The DNA contained in the supernatant was precipitated by adding 700µL of cold iso-propanol solution to the mixture and incubated for 24 hours in a freezer at -20°C. The DNA formed was then separated from isopropanol by centrifuging the microtube containing the mixture at a speed of 12000 g for 5 minutes. The isopropanol solution was then discarded and the DNA pellet formed was then washed twice by adding 700 µL of 70% ethanol. The washed DNA was then air-dried and followed by a resuspension step (dissolving the DNA pellet again) using TE 1X solution (10 mM Tris pH 8.0 and 1 mM EDTA) containing RNAse A (10 mg/ml) to obtain stock DNA solution. The stock DNA solution was then incubated at 370C for 1 hour and then stored at -200C until ready for use in molecular analysis.

In the next step, quantification of the DNA obtained was carried out using the NanoDrop Spectrophotometer 2000. DNA concentration was measured at a wavelength of 260 nm, while the purity of DNA was determined based on the absorbance ratio of A260/280. Determination of the purity of the extracted DNA is based on the absorbance value of A260/280 where the absorbance value of the DNA sample is in the range of 1.8 to 2.0 indicating the purity of the DNA sample (Sambrook and Russel, 2001).

The stock solution from each DNA sample was then equalized to 10 ng/µL using the solution dilution formula (V1 × M1 = V2 × M2), where V1 is the initial volume of the solution, M1 is the initial concentration/stock solution, V2 is the final solution volume and M2 is the final concentration of the solution. The DNA of each sample with a concentration that has been equalized to one another is hereinafter referred to as the working stock solution (DNA working solution) which will be used as template DNA in PCR analysis.

2. PCR Analysis

A total of 4 RAPD primers, namely OPD-20, OPE-03, OPA-02, and OPC-11 were used in the PCR analysis to obtain information on genetic relationships between Porang samples. The PCR (Polymerase Chain Reaction) reaction was carried out in a total reaction volume of 25 µL. The PCR reaction composition consisted of 10 ng/µl shallot DNA, ddH_2O, 1X PCR buffer, 0.125 mM dNTPs, 0.5 µM primer, and 1 unit of Taq polymerase enzyme. The PCR profile used in this PCR analysis includes the following steps, namely initial denaturation at 94oC for 7 minutes, followed by 45 cycles of denaturation at 94°C for 30 seconds, primer attachment at 37°C for 1 minute, and polymerization at 72°C for 1 minute. minute. PCR analysis ended with a final elongation step at 72°C for 7 minutes.

3. Electrophoresis

Separation of PCR products or DNA amplification results from each test sample was carried out on 1.2% agarose gel using electrophoresis techniques. 3µl of PCR product mixed with 3X loading dye was injected into the wells of the gel which had been placed in an electrophoresis tank containing 0.5X TBE buffer solution. The electrophoretic conditions were set (conditioned) at an electric voltage of 90 Volts for 60 minutes. Next, the gel was soaked in ethidium bromide dye solution for 5-10 minutes, rinsed with water, and irradiated with UV which is available in the chemidoc documentation tool (Biorad). Variations in the test samples that were amplified with primers were indicated by the differences in the band patterns produced at each locus

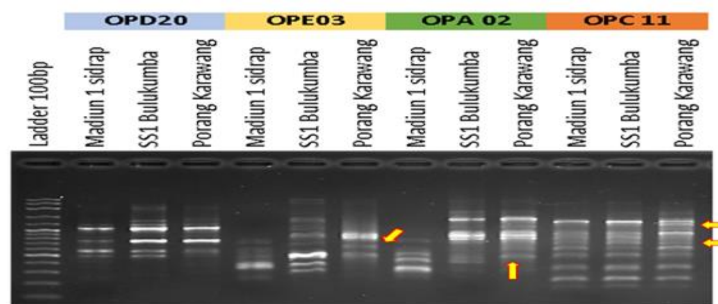
Exploration & Identification

To identify the characteristics of the local porang Karawang, an exploratory survey method was carried out to their habitat at the distribution site, namely at the foot of Mount Sanggabuana, in Karawang Regency. Exploration using Purpose Sampling Method. The tools used are Cameras, Altimeters, thermometer & hygrometers, luxmeter, Digital Calipers, Macro Lenses + Kits, and stationery. Materials used: color chart, local variety of porang, sample plastic bag, and cloth backdrop. The description of the porang Karawang variety was carried out on several observation variables, including: leaf shape, leaf apex, base dun, leaf margin, leaf vein, leaf surface, stem shape, stem length, stem diameter, stem color, root length, root diameter, tuber shape, tuber number, tuber shape, tuber size, carbohydrate content, glucomannan content using standards from IPNI (2020).

RESULTS AND DISCUSSION

DNA Apmplification

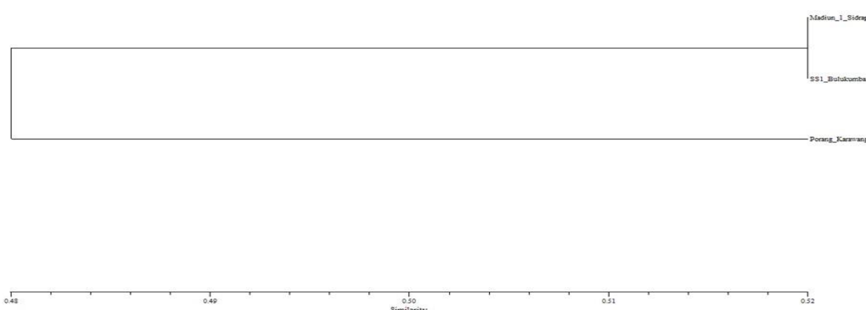
Based on the results of DNA amplification of the porang sample using four RAPD primers, it is known that one primer, namely OPD-20, shows the same DNA banding pattern among the three test samples. On the other hand, the three primers showed different banding patterns between the porang Karawang samples and the three porang varieties (porang Madiun 1 Sidrap and SS1 Bulukumba). This shows that there is genetic variation between porang from Karawang and three other porang. The detailed appearance of the banding pattern of DNA amplification with 4 RAPD primers can be seen in the figure 2:



Figur 2. Results of Porang DNA amplification from Karawang and three comparison samples based on the four RAPD primers

Genetic Relationship Analysis

Based on the results of molecular analysis using 4 RAPD markers, the Porang plant sample from the Karawang Regional Government, West Java, was separated from the other two porang samples at a genetic similarity coefficient of 0.48 (Figure 2). This shows that there is a genetic variation between the porang plant samples from Karawang and the other two porang samples by 52%. Thus, the results of genetic relationship analysis using the four RAPD primers showed that the porang Karawang sample was different from the porang samples from the other two areas.



Figur 3. Porang sample phylogenetic tree based on the four RAPD primers

Exploration & Identification

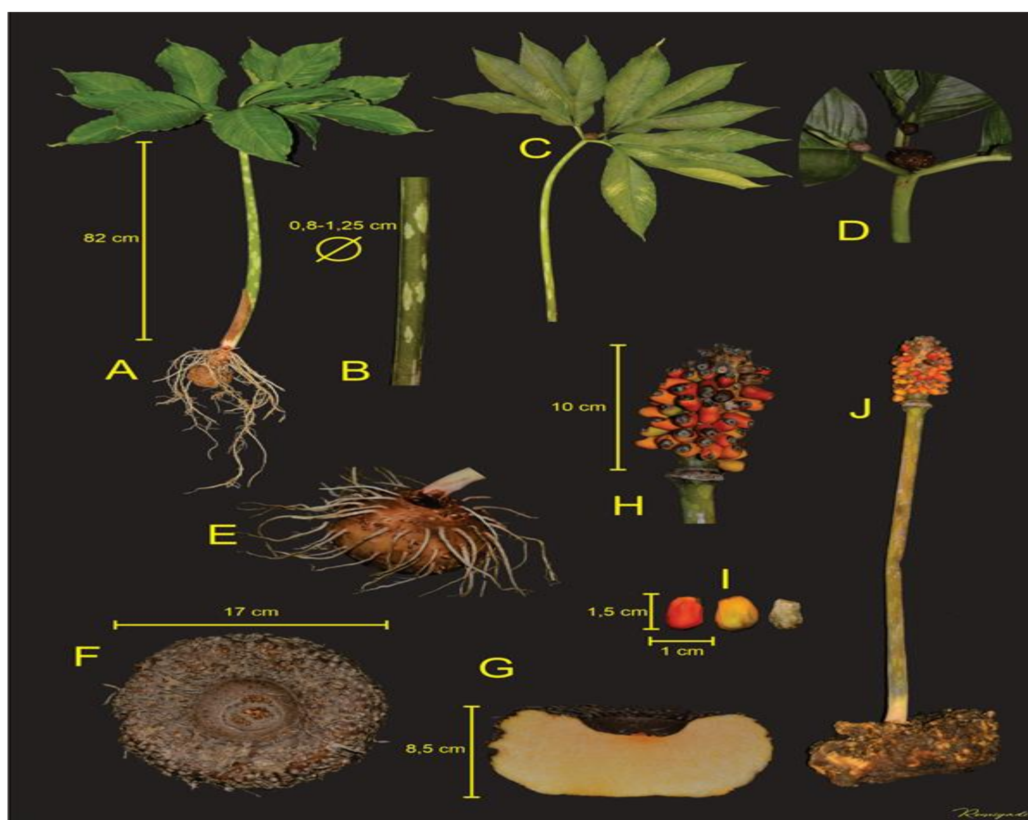
Habitat

Regional specifications are as follows, alttitude 350 - 442 meter above sea level (asl), type of sandy loam soil, light intensity 391 - 1,700 lux (shaded by trees), soil pH 5 - 6, found living side by side with *Colocasia* sp, specifications for the Batu Tumpang region, foot of Mount Sanggabuana Tegalwaru sub-district, Karawang district.

Figure 4. Porang Karawang in its habitat

Table 1. Diagnostic character of Amorphophallus muelleri Bl. Var. Karawang and Madiun 1

Morphological Characters	Karawang Variety	Madiun 1 Variety
Leaf Type	*Digitatopinnatus*	-
Circumsciptio	*Ovalis - oblongus**	Ovalis
Apex Folii	*Acuminatus**	Acutus
Basis Folii	*Acutus*	-
Margo Folii	*Undulatus*	-
Nervatio	*Penninervis*	-
Intervenium	*Herbaceus*	-
Superficies Folii	*Glaber - opacus - undulatus*	Glaber - opacus - undulatus
Leaf Colour	*Light green*, margin of the leaves are pink*	Green, *margin of the leaves are pink when seedling and white when mature*
Caulis	*Teret, stem length 72 cm, 0.75 - 1.35 cm in diameter, color light green from stem top to bottom stem, elongated line motif greenish white (more a little), form spot like a white cloud, smooth grooved surface*	*Teret, green with white spot, smooth*
Root	*Length 30 cm, diameter 0.23 cm, cream white color*	-
Tuber	*Diameter 17 cm, thickness 8.5 cm, surface smooth - wavy*	-
Flos	Green pedicels, cylindrical, spotted motifs such as clouds or white rhombuses. Trumpet shape, reddish purple, overall shape like a spear but not sharp	Green pedicels
Fruit and Seeds	Fruit length 1.5 cm, diameter 1 cm, capsule shape, red when mature, seed length 1 cm, width 0.7 cm	The fruit is red when mature
Carbohydrate	*81.75%*	90.47%
Glucomannan	*79.8%*	45-65%
Resitance	-	Not resistant to *Sclerotium rolfsii*



Figur 5. (a) Young plant, height 82 cm, (b) stem, diameter 0.8 - 1.25 cm, (c) leaf shape, (d) bulbil, (e) tubers and roots, (f) mature tuber, width 17 cm, (g) tuber color, thickness 8.5 cm, (h) mature fruits and seeds, (i) fruit length and fruit width, (j) fruit, pedicel and mature tuber

CONCLUSION

1. Based on the results of the genotype differential test through DNA amplification that had been carried out at the local Porang Karawang, it was stated that it was different from the comparison specimens, namely the Madiun 1, Sidrap and SS1 Bulukumba varieties
2. Based on the identification results, a description of the Karawang Local Porang can be compiled

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