Genetic Variation of Porang (*Amorphophallus muelleri* Blume) After Mutation Induction Using Colchicine

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Abstract- **Porang (*Amorphophallus muelleri* Blume) is a tuberous plant with the highest glucomannan among *Amorphophallus* genera. However, the low genetic diversity of porang today can affect porang cultivation in the future. Therefore, increasing the genetic diversity of porang by polyploidy induction using colchicine is needed. 30 days after planting porang shoot explants were used for polyploidy induction. After polyploidy induction using colchicine, DNA was extracted using Geneid Genomic DNA Mini Kit. Genetic diversity and clustering analysis was performed to assess the genetic diversity of porang after mutation induction. Mutation induction using colchicine successfully increased the genetic diversity of porang. Polyploidy induction using 0.05% colchicine is recommended to increase genetic variation of porang.**

Keywords: Amorphophallus Muelleri Blume, Genetic Diversity, Issr, Mutation, Polyploidy

I. INTRODUCTION

Porang (*Amorphophallus muelleri* Blume) is a tuberous plant [1] containing the highest glucomannan (up to 65.2%) [2] compared to other *Amorphophallus* species [3] such as *Amorphophallus paeoniifolius* (45.167%) [4], *Amorphophallus variabilis* (44%), and *Amorphophallus oncophyllus* (55%) [5]. The glucomannan is widely used in both food [6] and cosmetic industries [7]. The food industry processes porang flour into gluten-free noodles [8], shirataki [5], and konnyaku [9]. Glucomannan is also used to inhibit acne growth [10] and as a material for facial cleansing sponges [11].

The numerous benefits of glucomannan in porang tubers have made porang an export commodity for Indonesia [12]. However, the cultivation that still used tubers and bulbils [13] makes porang has low genetic diversity [14] threatening porang production in the future. Therefore, efforts to increase genetic diversity through polyploidy induction [15] using colchicine [16] are needed. Colchicine can double the number of chromosomes [17] by inhibiting the anaphase stage [18], where the spindle fibers bind to tubulin [19]. This results in the chromosomes not separating [20], ultimately producing plants with more than one pair of chromosomes [21].

The success of polyploidy induction can be detected using inter simple sequence repeats (ISSR) molecular markers [22] due to their very high polymorphism level [23], producing a large number of fragments [24], and requiring a small amount of DNA template [25]. The ISSR 17899A and ISSR 17899B primers have been proven to identify intra- and inter-species genetic diversity in *Hedysarum chaiyrakanicum* with 100% polymorphic bands in ISSR 17899B and 96.7% in ISSR 17899A [26]. In addition, The ISSR can also characterize *Asparagus acutifolius* with 51.57% polymorphic bands [27]. Therefore, this study aims to analyze the genetic variation of porang after mutated by colchicine using inter simple sequence repeats (ISSR) molecular.

II. MATERIALS AND METHODS

A. Sample preparation

Porang (*Amorphophallus muelleri*) shoot explants (2n=26) were used for polyploidy induction. Explants were subcultured using Murashige and Skoog (MS) media with the addition of 2 mg/l Benzyilidenine (BA). 30 days after planting, porang explants were ready for polyploidy induction.

B. Preparation of Colchicine Solution

The preparation of colchicine solution begins by dissolving colchicine powder in distilled water, then placed in sterile bottles for use as stock or immediate use. A 1% stock solution is prepared by dissolving 0.1 grams of colchicine powder in 10 mL of distilled water. This stock solution is then diluted to various concentrations: 0 (control), 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, and 0.06% using the dilution formula. As per the treatment, the colchicine solution is then sterilized using a 0.22 µm millipore filter.

C. Polyploidy Induction

Polyploidy induction with colchicine is conducted using semi-solid MS medium supplemented with 2 mg/L of benzyladenine (BA), where the colchicine solution is added to culture media that has been sterilized. The sterile medium is placed in a laminar airflow cabinet and allowed to cool to 70°C. Afterward, the sterile colchicine solution (0%, 0.01%, 0.02%, 0.03%, 0.05%, and 0.06%) filtered with a 0.22 µm millipore filter is added to the culture bottles, each containing 10 mL of media. The explants, approximately 0.5 cm in size and yellowish-green in color, are taken out of the bottles, planted in the media containing the colchicine treatments, and stored on culture racks in the incubation room.

D. Explant Maintenance

Explant grown in colchicine media is subsequently transferred to maintenance media using Murashige and Skoog (MS) media with 2 mg/L benzyladenine (BA). The explants are cultured on racks in the incubation room. Daily observations and sterilizations are performed on the explants using alcohol to prevent contamination.

E. DNA Extraction and Isolation

DNA extraction is performed using the Geneaid Genomic DNA Mini Kit according to the protocol provided in the kit. Extracted DNA was then stored in -23°C.

F. DNA Amplification and Visualization

DNA amplification follows the program as follows: initial denaturation is 94°C for 4 minutes, followed by 35 cycles consisting of denaturation for 30 seconds at 94°C, annealing for 45 seconds at a temperature corresponding to the primer used, and extension for 90 seconds at 72°C, followed by a final extension step for 6 minutes at 72°C [28].

G. Analysis Data

Scoring of amplified DNA bands aims to estimate the level of polymorphism. Scoring is done by assigning a score of '1' if a DNA band appears and '0' if the DNA band does not appear.

Genetic diversity was analyzed using parameters such as the number of observed alleles (Na), the number of effective alleles per locus (Ne), allele frequency, polymorphic loci, genetic distance, and the Shannon index, using POPGENE software version 1.32.

The genetic characteristics of polyploid Porang were assessed through cluster analysis by comparing the control and treatment groups. Cluster analysis was conducted using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm with the Jaccard similarity index in PAST software version 3.0:

III. RESULTS AND DISCUSSION

A. Genetic Variation of Porang Based on ISSR Molecular Markers

Mutation induction using colchicine successfully increased the genetic diversity of porang (Table 1). Genetic variation (He) of porang increased from 0.0487 in the control and 0.2595 in the porang after mutation induction using colchicine (Table 1). The results clearly indicate that mutation induction significantly enhances the genetic diversity of porang, as reflected in the increased values of polymorphic loci, allele numbers, heterozygosity, and Shannon's index (Table 1). This suggests that induced mutations are an effective method for broadening the genetic diversity of porang, which could benefit breeding and conservation efforts.

These findings align with previous research on mutation-induced genetic diversity in plants. For example, studies of medicinal plants have reported similar outcomes, where induced mutations through chemical agents or radiation increased genetic variation by changing the chromosomal groups and the number of genes in a cell [29]. The sharp contrast between the control group (P = 11.76%) and the treatment group (P = 94.12%) in this study is consistent with these reports, highlighting the effectiveness of mutation induction in generating genetic variability.

The observed phenomenon can be explained by the mechanism of mutation induction. Mutagens such as colchicine introduce random alterations in the DNA sequence, gene duplication, functional divergence and chromosomal rearrangements [30]. These changes disrupt the uniformity of genetic loci, resulting in the emergence of new alleles and polymorphic loci, as observed in the treatment group. The substantial increase in the number of alleles (Na = 15) and effective alleles (Ne = 1.4068) further supports the role of induced mutations in diversifying the genetic makeup of porang.

Additionally, the significant rise in heterozygosity (He = 0.2595) and Shannon's information index (I = 0.4097) suggests enhanced genetic variability and adaptability within the treated population. Higher heterozygosity reflects a greater potential for plants to withstand environmental stresses, which is vital for breeding and conservation programs [31]. This outcome may also be due to the accumulation of advantageous mutations, which increase genetic diversity and promote population robustness.

Interestingly, the relatively low diversity in the control group (He = 0.0487, I = 0.0711) highlights the limited genetic base of porang in its natural or untreated state. This is a common characteristic of many domesticated or underutilized crops, where selective

pressures or low population sizes reduce genetic variability.

In comparison to prior studies, the magnitude of increase in genetic diversity observed here is noteworthy. It may be attributed to the specific mutagenic agent or treatment protocol employed, as these factors greatly influence mutation frequency and spectrum. Further studies are recommended to optimize these parameters and explore the long-term stability and utility of the induced diversity in porang breeding programs.

Table 1. Genetic variation of porang based on ISSR molecular markers. P(%) = percentage of polymorphic loci, Na = number of allels, Ne = number of affective allel , He = genetic diversity index, I = Shannon index.

	P (%)	Na	Na	Ne	He	I
Control	11.76	2	1.1176±0.3321	1.0832±0.2348	0.0487±0.1376	0.0711±0.2008
Treatment	94.12	15	1.9412±0.2425	1.4068±0.3022	0.2595±0.1494	0.4097±0.1951

B. Similarity of Porang Based on ISSR Molecular Markers Using the Jaccard Similarity Index

The similarity coefficient of porang samples ranged from 0.08 to 0.75 (Table 2). In this study, the highest diversity is assessed by comparing the lowest similarity coefficient between the control samples and all treatment samples. The lowest similarity coefficient compared to control is K52 (0.05% colchicine treatment), with similarity values of 0.10 and 0.08, respectively. According to Trimanto, 2012 [32], the similarity index is considered low when the value is <0.60, and if it approaches 1, the similarity is high. It can be concluded that the K52 sample has the highest genetic diversity.

Table 2. Jaccard similarity index of 17 porang samples (Amorphophallus muelleri Blume) based on ISSR molecular markers. K01 (Control Replicate 1), K02 (Control Replicate 2), K11 (0.01% Colchicine Replicate 1), K12 (0.01% Colchicine Replicate 2), K13 (0.01% Colchicine Replicate 3), K21 (0.02% Colchicine Replicate 1), K22 (0.02% Colchicine Replicate 2), K23 (0.02% Colchicine Replicate 3), K31 (0.03% Colchicine Replicate 1), K32 (0.03% Colchicine Replicate 2), K33 (0.03% Colchicine Replicate 3), K41 (0.04% Colchicine Replicate 1), K42 (0.04% Colchicine Replicate 2), K51 (0.05% Colchicine Replicate 1), K52 (0.05% Colchicine Replicate 2), K61 (0.06% Colchicine Replicate 1), K62 (0.06% Colchicine Replicate 2).

	K01	K02	K11	K12	K13	K21	K22	K23	K31	K32	K33	K41	K42	K51	K52	K61	K62
K01	1.00																
K02	0.50	1.00															
K11	0.33	0.67	1.00														
K12	0.22	0.30	0.50	1.00													
K13	0.67	0.75	0.50	0.33	1.00												
K21	0.22	0.30	0.36	0.50	0.33	1.00											
K22	0.20	0.40	0.45	0.73	0.30	0.58	1.00										
K23	0.33	0.50	0.33	0.10	0.25	0.10	0.20	1.00									
K31	0.29	0.22	0.30	0.45	0.25	0.45	0.42	0.13	1.00								
K32	0.67	0.40	0.50	0.33	0.50	0.33	0.30	0.25	0.43	1.00							
K33	0.29	0.22	0.30	0.45	0.25	0.45	0.42	0.13	1.00	0.43	1.00						
K41	0.29	0.22	0.30	0.33	0.25	0.45	0.31	0.13	0.75	0.43	0.75	1.00					
K42	0.40	0.29	0.38	0.27	0.33	0.40	0.25	0.17	0.71	0.60	0.71	0.71	1.00				
K51	0.14	0.11	0.20	0.15	0.13	0.25	0.14	0.14	0.44	0.29	0.44	0.63	0.57	1.00			
K52	0.10	0.08	0.15	0.29	0.09	0.20	0.27	0.10	0.45	0.20	0.45	0.45	0.40	0.67	1.00		
K61	0.14	0.11	0.20	0.15	0.13	0.25	0.14	0.14	0.44	0.29	0.44	0.63	0.57	0.71	0.67	1.00	
K62	0.11	0.09	0.17	0.31	0.10	0.21	0.29	0.11	0.50	0.22	0.50	0.50	0.44	0.56	0.89	0.75	1.00

C. Clustering analysis of Porang Based on ISSR Molecular Markers Using the Jaccard Similarity Index

Normal and treatment porang is divided into 4 clusters. The genetic variation of the treatment sample, as seen from the lowest similarity value compared to the control, is K52 which is in cluster 4. The presence of the control sample and sample K52 in different clusters indicates that samples in different clusters exhibit high genetic variation. Sample K52 is on the same branch as K62 in cluster 4. This may be due to the 0.05% and 0.06% colchicine treatment doses producing nearly identical polyploid plants, resulting in a relatively similar number of PCR amplification fragments. The occurrence of polyploidy can be caused by crossing-over events due to the increased number of chromosomes as a result of polyploidy, thereby enhancing physical interactions between homologous chromosomes [33]. Mutations that can arise from crossing-over events include deletions [34], insertions [35], or substitutions [36], which can lead to the formation of identical DNA banding patterns, the addition of new bands, and the loss of DNA bands compared to control plants [37].

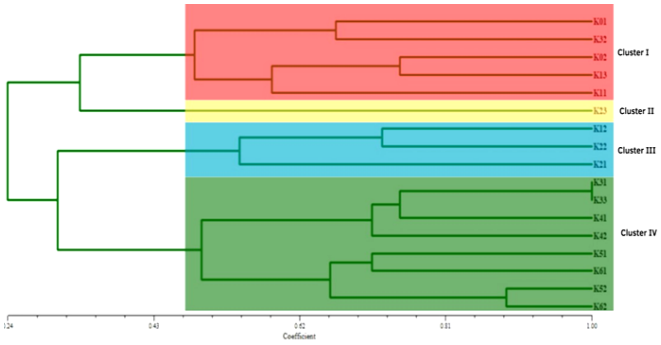

Fig. 1. Dendrogram of 17 Porang Samples Based on ISSR Molecular Markers Using the Jaccard Similarity Index

III. CONCLUSIONS

Mutation induction using colchicine successfully increased the genetic diversity of porang. Polyploidy induction using 0.05% colchicine is recommended to increase genetic variation of porang.

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