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Research Article

The Application Of PCR RAPD Technique In Determining Female Sex Of Kapulasan (Nepheleum Lapaceum Mutabile)

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Abstract

Background and Objective: Kapulasan is one of the horticultural plants derived from the tropical region that has significant economic value. One of the obstacles in cultivating the plant is that kapulasan has the flowering type of androdioceus, i.e. the male and the female flowers are separated. Female flowers produce hermaphroditic flowers that can produce fruits while the male ones will not produce fruits. In the cultivation of the plant, it is very difficult to determine the female flowers if they are propagated by seeds. It takes up to 7 years to determine the nature of the flowering. One of the important steps that can be done is through a system of early detection using molecular marks. RAPD technique as a simple technique can be used to distinguish the characteristics. **Materials and Methods:** Plant materials used are 5 male plants and 5 female plants collected from Guguk Subdistrict 50 Kota Regency and Bonjol Subdistrict Pasaman Regency of West Sumatera. The analysis of bulk segregation is used for primers screening of RAPD related to female sex of 250 decamer primers. **Results:** The results of the research show that of the 250 primers used, the OPM-09 primer produces female sex bands related to the controller of the female kapulasan plants of which the ribbon produced is 512 bp in size.

Keywords: Sex marker-assisted; Kapulasan, RAPD

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Competing Interest: The authors have declared that no competing interest exists.

Introduction

Indonesia has riches (biodiversity) of flora and fauna which other countries in the world don't have. Kapulasan is one of the tropical fruit crops which become one of the riches of flora in Indonesia. Kapulasan is also rare plants. The population and the distribution area of kapulasan plants are almost throughout Indonesia (Uji, 2000). Ediwirman (2007) reported, in West Sumatra, it was found a few distribution areas such as Bonjol, Pasaman regency, and Guguk, 50 Kota Regency. Generally, in the distribution areas, they have a lot of population. However, one of the obstacles in cultivating kapulasan is that there are many kapulasan plants that don't produce fruits and they are classified as male plants.

Kapulasan has the flowering type of androdioecious with male and female flowers separated. Female flowers will produce hermaphroditic flowers that will produce fruits, but the male ones will not produce fruits. The success of kapulasan cultivation is determined by the presence of the female plants that have economic value. According to Prastowo, Roshetko, Maurung, Nugraha, Tukan and Harum (2006), kapulasan and rambutan are very difficult to be propagated vegetatively such as budding. In general, kapulasan is propagated generatively.

Male flowers that grow are economically unprofitable, so they must be avoided. To determine the female sex of kapulasan plants, which are propagated generatively, it can be done when the plants have started flowering or when they are about 7 years old. That is why the usage of spawn derived from seeds requires an effort through a system of early detection for the male and female sex of kapulasan plants.

The development of the female sex marker of kapulasan plants can be done by the usage of PCR (Polymerase Chain Reaction) based technology which was first developed by Karry Mullis in 1983. The PCR technology developed by Karry Mullis became the starting point for the development of genome analysis at the DNA level. This provides an opportunity for the development of a more accurate diagnosis system, especially in the detection of linkage of sex controlling genes. This is the development of an early detection system that can be implemented, and there have been many studies done.

One of the important parts of the early detection system in the plant's sex is the presence of the specific primers as the starting point in the amplification of target fragments derived from genetic material accurately. The primer is a short polynucleotide chain with a new DNA developed from specific fragments such as in the control of sex with fingerprinting analysis based on RAPD.

Some research results done using RAPD technique is able to provide enough information to help in the selection related to sex of Borassus flabellifer L. (George and Karun, 2011); Trichosanthes dioica Roxb (Sing, Kumar, Sing, Ram, and Kalloo (2002); asparagus (Jamsari, et al., 2003), Piper longum (Manoj, et al., 2005). The research aims to get the primer that can produce specific banding pattern related to the control of kapulasan female sex with DNA amplification technique by in vitro using Polymerase Chain Reaction (PCR).

Materials and Methods

Plant Material

Plant materials used are 5 male plants and 5 female plants collected from Guguk Subdistrict 50 Kota Regency and Bonjol Subdistrict Pasaman Regency of West Sumatra. The materials used are young leaves stored in silica gel.

The Isolation and the Extraction of DNA

Genomic DNA is isolated from leaves of males and females kapulasan. The DNA isolation is performed in the laboratory of Biotechnology and Breeding, University of Andalas Padang.

DNA isolation uses a modified method of Doyle and Doyle (1987). About 100-150 mg of leaves dried with silica gel is ground and put into Eppendorf (1.5 ml) that is added CTAB buffer (consisting of 1 M of Tris HCl pH 8.5, 5 M of NaCl, 0.5 M of EDTA, 10% of CTAB; b-mercaptoethanol). The extraction is done with vortex and is incubated at 64□ C for 30 minutes. To the extraction solution, it is added 750 µL of Chloroform, Phenol, and Isoamilalkohol (25:24:1) and they are inverted for 10 minutes, then centrifuged at a speed of 14,000 rpm for 10 minutes. The supernatant obtained is then moved to a new Eppendorf and is added 500 mL solution of Chloroform, Isoamyl alcohol (24:1) and is centrifuged with a speed of 14,000 rpm. The supernatant obtained is then moved to a new Eppendorf and is added 500 mL of 96% of cold ethanol and is disposed of, and then added 70% of ethanol. Then, the supernatant is air dried. Pellet (DNA) obtained is dissolved in 1 x TE as much as 100 µL. The quantity of the Genomic DNA is measured using λDNA. The DNA bulk is prepared in the form of the pool with an equal quantity of DNA (1 g/ 1 µ) of 5 female plants individual and 5 male plants individual.

The Analysis of RAPD Marker

The analysis of bulk segregation (Michelmore et al., 1991) is used for primers screening of RAPD related to female sex of 250 decamer primers (Operon Biotechnologies, Germany). The amplification reaction is done using the RTG-PCR kit (GE Healthcare UK Limited) with a reaction volume of 15 µL consisting of 9 µL of ddH2O PCR, 3 µL primers of 20 ρmol, and 3 µL DNA of 20 ng/ µL. The DNA amplification is done with DNA thermocycler respectively: The initiation of 940C for 1 minute, the denaturation of 95°C for 30 seconds, the annealing of 37°C for 30 seconds, 72oC for 1.5 minutes, are done about 45 cycles and then continued with 72oC for 8 minutes. The result of the amplification is moved to 1.5% agarose gel containing 0.5 mg/ml of ethidium bromide. 1 kb ladder (MBI Fermantas) is used as a measurement standard. The gel DNA is documented using documentation gel system (Cybertech, Germany).

Results and Discussion

The isolation of DNA

The results of DNA isolation performed with the method of Doyle and Doyle (1987) produce DNA with enough quantity to be amplified. The DNA produced has 25 to 125 ng/µL sizes. The results of the isolation can be seen in Figure 1.

The isolation done with the method of CTAB without liquid nitrogen produces good enough DNA quality, with the samples used are not in fresh but dry condition.

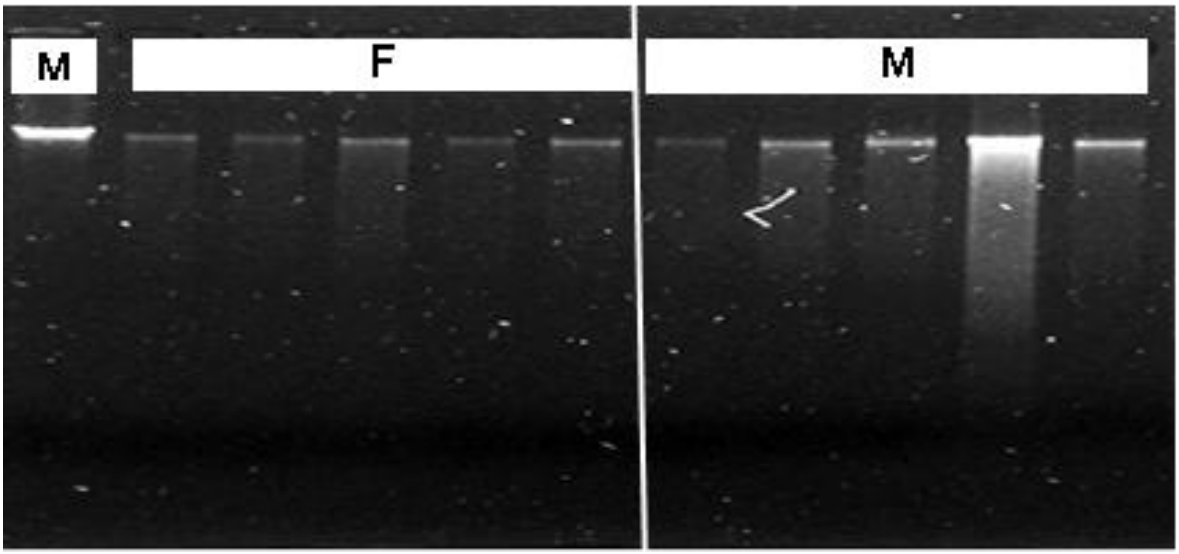

Figure 1. The DNA quantity test resulted from the isolation with the method of Doyle and Doyle (1987). λ DNA Marker 50 ng / mL (M), Row 1-5 of female plants; 6-10 male plants.

The Analysis of Bulk Segregation DNA

Screening is done using 250 primers RAPD, with Bulk DNA from each of the kapulasan samples. Of the 250 RAPD primers, 20 primers have polymorphic bands. The results of the RAPD primary screening using the analysis of bulk DNA is shown in Table 1.

A total of 250 RAPD primers are used to identify the differences in male and female kapulasan with bulk DNA samples, 5 primers of them produce specific bands related to female and male sex. The results of the primers test are shown in Figure 2, indicating that the primers used have a banding pattern of polymorphic DNA.

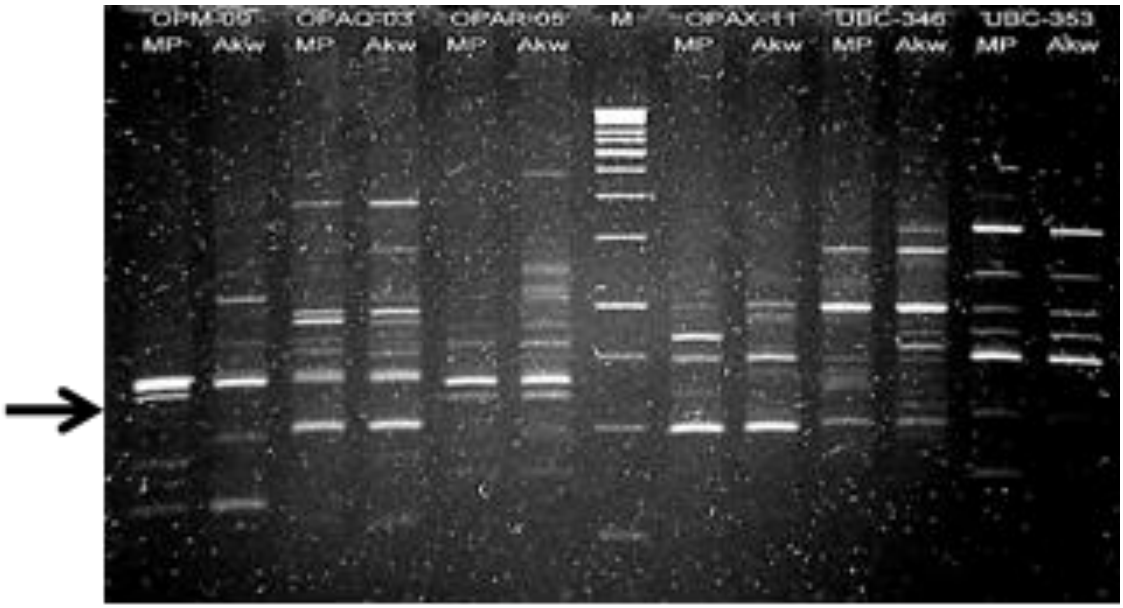

Figure 2. The DNA banding pattern of bulk DNA samples isolated from male (M) and female (F) kapulasan plants. Male and Female of the bulk DNA of 5 female plant genomes and 5 male plant genomes, and M 1 kb ladder.

Testing with more number of individuals is important to estimate the accuracy of specific bands related to female sex on each marker that is used. Hormaza (1994) reported 1,000 RAPD primers used in the determination of P vera sex, it only has one primer that can distinguish male and female sex. The low frequency of band related to sex indicates that the segment of DNA involved in sex determination is small and is influenced by a single gene or a few genes. In order to measure the distance of genetic map related to sex genes and markers, the segregation of population is tested. Plant populations are prepared but still at the juvenile stage. Durand and Durand (1990) reported sex controlled by a single gene presents in some plants (e.g. Asparagus and Vitis), although there is an exception such as on Mercurialis with sex controlling system which is more complex with three genes involved.

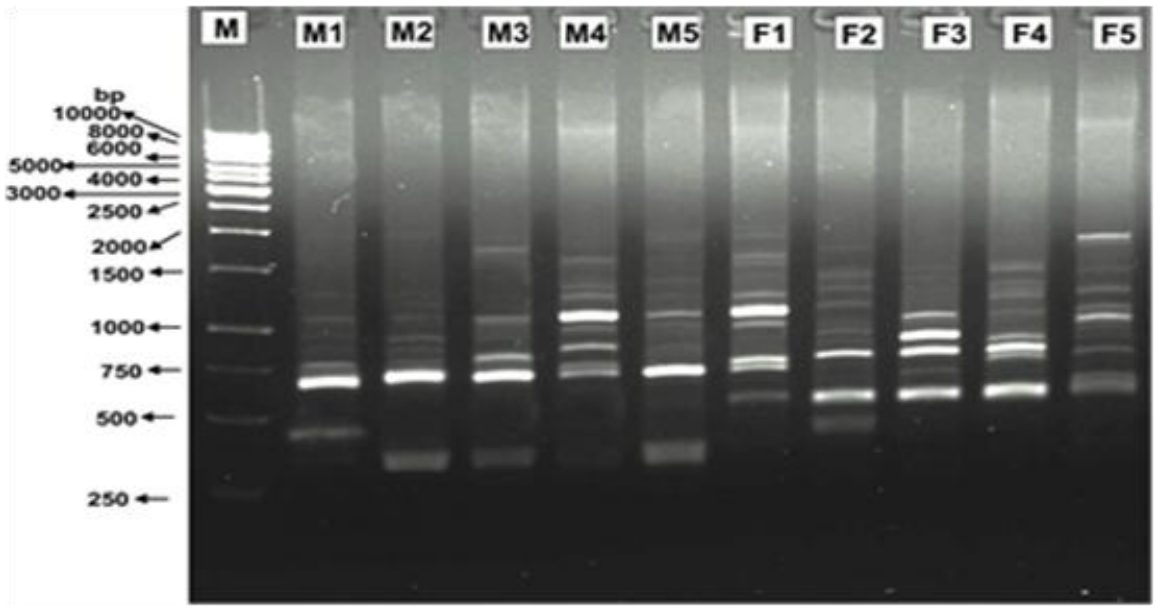

Figure 3. The banding pattern of OPM-09 primer (GTCTTGCGGA) related to female sex controller of the kapulasan plant.

Table 1. The screening of RAPD primers using Bulk Segregation Analysis (BSA) with 20 of the 250 primers tested to determine the specific banding pattern related to controlling genes of the female sex (the data presented here has a high level of band products).

No.	Primers	Screening of Primers			Screening of Individual		Average
		♀♂	♀			♀♂	
1	OPA-01	14	14	0	-	-	14,00
2	OPA-05	19	19	0	-	-	19,00
3	OPA-07	7	7	0	-	-	7,00
4	OPA-08	14	14	0	-	-	14,00
5	OPA-10	17	17	0	-	-	17,00
6	OPA-11	20	23	3	0	3	21,50
7	OPA-11	17	19	2	1	1	18,00
8	OPA-13	17	18	1	0	1	17,50
9	OPA-19	14	15	1	-	1	14,50
10	OPA-19	18	18	2	1	1	18,00
11	OPA-20	18	18	0	-	-	18,00
12	OPAC-12	17	17	0	-	-	17,00
13	OPAE-08	16	16	0	-	-	16,00
14	OPAF-14	14	14	0	-	-	14,00
15	OPAL-12	14	14	0	-	-	14,00
16	OPAV-16	18	16	2	2	1	17,00
17	OPB-01	18	18	0	-	-	18,00
18	OPB-01	14	14	0	-	-	14,00
19	OPR-17	3	4	1	3	1	3,00
20	OPM-09*)	3	3	3	6	7	6,50

Description:
♂ = male sex
♀ = female sex
*) = Candidate primers related to female sex

For kapulasan plants, no information about the number of genes related to the control of sex. If this happens, several genes may be involved in the differentiation of male and female flowers in dioeciously plants, but the sex determination can be controlled by a single locus that acts as a trigger.

Conclusion

The results of the research using the RAPD technique related to female sex controller in kapulasan plants show that the OPM-09 primer produces monomorphic bands related to female sex with 512 bp in size.

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