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Original Article

SCREENING OF RAPD PRIMER FOR TEAK (*Tectona grandis*)

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ABSTRACT

Identification of DNA polymorphisms in teak is important. It is a first step to determine the presence of genetic variation in teak. The information of genetic variation is needed for teak breeding development. RAPD is one of method which can be used for identification of DNA polymorphism. This study aim to get the RAPD primer which can detect the DNA polymorphism in teak. Benefits of this study are provide information about primer which can detect the DNA polymorphism in teak, DNA polymorphism data can be used for genetic variation analysis which needed for teak breeding development. The primers which used in this study shown the DNA polymorphism in teak. The primer are OPF6 (5'-GGGAATTCGG-3 '), OPF8 (5'-GGGATATCGC-3'), and OPF11 (5'-TTGGTACCCC-3 '). The highest DNA polymorphism is shown in DNA which amplified with OPF-8 primer.

Keywords: *RAPD, Primer, Polymorphism, DNA, Tectona grandis*

INTRODUCTION

Teak (*Tectona grandis)* is a species of wood with high demand because it has many benefits, such as used for home appliances (Irwanto, 2006). Teak wood has high economic value in international market. The increasing of teak wood needs cause it becomes the main commodity to be planted by Perhutani and our society. According to this situation, plant breeding is needed. Information about teak genetic variation is needed for further development of teak breeding.Large genetic variation will give good opportunity to obtain the desired characters (Widiastuti et al., 2013). According to Namkoong et al. (1996), large genetic variation is one of indicators which reflect the good forest management. Teak genetic variation can be analyzed using a reliable marker.

The advance biomolecular methods sustain many kind of markers which can help us to detect the genetic variation in species. Random Amplified Polymorphic DNA (RAPD) is method based on PCR (Polymerase Chain Reaction) which can detect the genetic variation by amplify the genomic DNA using random nucleotide sequenceas a primer (Rafalski et al., 1994). Identification of genetic variation using RAPD is widely used because it has some benefits, such as 1) does not require radio active substances; 2) does not require information to the primary target DNA sequence; 3) only requires a little genomic DNA (5-50 ng), also it simple and fast (Black, 1993; Bradeen and Havey, 1995; Hadrys et al., 1992; Hancock et al., 1994). RAPD has been used in various studies, such as rapid detection of genomic polymorphism. This method can be used to reveal the intra-specific variation to suppress the increasing of un favorable alleles in population (Bardakci, 2001). Primer screening is important in polymorphism detection using RAPD method, because the RAPD primers will recognize the DNA by random recognition. We have to determine the exact primer which show the DNA polymorphism before we do the RAPD.

According to Samal et al. (2003) optimization of the RAPD method depens on selection of primers. Although, the RAPD method uses arbitrary primer sequences, many of these primers must be screened in order to select primers that provide useful amplification product. William et al. (1990) and Leal et al. (2010) also reported that the consistency of the amplification product be influenced by primers selection. The primers selection and determine the appropriate annealing temperature needs to be done in order to be optimal PCR conditions (Ahmad and Poerba, 2010). Khan et al. (2015) reported that RAPD primers were evaluated base on intensity of bands, consistency within individual, presence of smearing, and potential for population discrimination. DNA amplification occurs if there is a match between the primer sequences with the template DNA. DNA amplification product does not appear likely caused no match between the primer sequences with the template DNA or other components are not in conformity in the PCR reaction.

Based on recent condition, this study aim to detect the DNA polymorphism of teak. Another expected benefits from this study is provides information about primer which show the teak DNA polymorphism. The information can be used for the teak development by plant breeding.

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METHODS

Research Material

Materials which used in this study are: liquid nitrogen, PPVP (Polyvinyl Pyrrolidone Poly), extracts buffer (Tris, EDTA, dH_20 sterile, NaCl) β-mercapto ethanol; chloroform, isoamyl alcohol, isopropanol, sodium acetate, absolute ethanol, 70% ethanol, trisbuffer, agarose, ethidium bromide, TE, TAE, genomic DNA, 3 primary with a size of 10 nucleotides (OPF-06, OPF-07, and OPF-11) of the operon, dNTPs, Taq DNA polymerase amplification buffer, polaroid films.The equipments which used are mortal hammer, gas tubes, centrifuge, pipette, micropipette (1000mL), a micropipette (100 mL), micropipette (10µl), small tip, big tip, small eppendorf, large eppendorf, glass beaker, small centrifuge, electrophoresis tools, shakers, refrigerator, freezer, flask, digital analytical balance, vortex, microwave, incubator, goblet, UV312 nm, the camera. Samples are one parental teak tree (P) and five teak generative offsprings (G1, G2, G3, G4, and G5) which obtained from Bibit Klon Puslitbang Perhutani Cepu Garden and the teak tree wild type (W) as the comparison which obtained locals garden. DNA were isolated from the first leaf or second leaf of teak tree. There are 22 kinds of primer was used in PCR reaction with a size of 10 nucleotides. Base on the amplification results which shown polymorphism only 3 primers namely, OPF-06, OPF-07, and OPF-11.

DNA Isolation

DNA was isolated from the first or second teak leaves using modified CTAB buffer (trimethyl ammonium bromide Hexadecyl Cationic) method which developed by Doyle and Doyle (1987). The obtained DNA solution was purified using "Wizard DNA Clean-Up System". Purified DNA solution then dissolved in 50-100 µl of TE buffer and stored at -20 ° C.

DNA Amplification and Electrophoresis DNA Amplification Results

DNA amplification method which used in this study refers to Rafalski et al. (1994). DNA was first denatured at 94 ° C for 2 min then followed by 45 cycles which has 3 steps. These steps are 94° C for 1 min denaturation, 35° C for 1 minannealing, and 72 ° C for 2 minutes extension. Last cycle was followed by incubation at 72°C for 5 minutes. Amplified DNA then separated in agarose gel with ethidium bromide staining. DNA band then observed under 312 nm of UV light, then documented with a Polaroid 667 films (Sambrook et al., 1989).

Data Analysis

Each band which appeared in agarose gel then compared to the marker bands with the exact size in base pairs (bp). There are two kinds of DNA band, the appeared band and not appeared band. The appeared band then scored with 1 and not appeared scored with 0. This scoring will provide the qualitative data for calculate the coefficient of similarity "Simple Matching".

Table 1. Size of DNA band primers

No	Primer	The Sequence of Bases 5'→3'	Bands Size (bp)
1	OPF-06	5'-GGGAATTCGG-3'	2500, 1480, 1310, 1100, 400
2	OPF-08	5'-GGGATATCGC-3'	3410, 1850, 1610, 900, 610, 520, 460, 410, 370, 330
3	OPF-11	5'-TTGGTACCCC-3'	2290, 1680, 1440, 1290, 1230, 850, 770, 660, 510, 460

Table 2. Primer pattern and total band result

No	Primer	The Sequence of Bases 5'→3'	Total Bands	Monomorphic Bands	Polymorphic Bands	Percentage Polymorphic Bands (%)
1	OPF-06	5'-GGGAATTCGG-3'	5	3	2	40
2	OPF-08	5'-GGGATATCGC-3'	10	0	10	100
3	OPF-11	5'-TTGGTACCC-3'	10	2	8	80
		Total	25	5	20	220
		Mean	8,3	1,7	6,7	73,3

Table 3. Coefficient of Similarity "Simple Matching" in teak DNA which amplified with OPF-06

	G1	G2	G3	G4	G5	W	P
G1	1.00						
G2	1.00	1.00					
G3	0.60	0.60	1.00				
G4	0.60	0.60	1.00	1.00			
G5	0.80	0.80	0.80	0.80	1.00		
W	0.80	0.80	0.80	0.80	0.60	1.00	
P	1.00	1.00	0.60	0.60	0.80	0.80	1.00

Table 4. Coefficient of Similarity "Simple Matching" in teak DNA which amplified with OPF-08

	G1	G2	G3	G4	G5	W	P
G1	1.00						
G2	0.70	1.00					
G3	0.50	0.40	1.00				
G4	0.70	0.80	0.60	1.00			
G5	0.60	0.70	0.50	0.70	1.00		
W	0.40	0.70	0.50	0.70	0.40	1.00	
P	0.40	0.70	0.50	0.50	0.40	0.80	1.00

Table 5. Coefficient of Similarity "Simple Matching" in teak DNA which amplified with OPF-11

	G1	G2	G3	G4	G5	W	P
G1	1.00						
G2	0.60	1.00					
G3	0.80	0.80	1.00				
G4	0.70	0.90	0.90	1.00			
G5	0.70	0.90	0.90	1.00	1.00		
W	0.50	0.90	0.70	0.80	0.80	1.00	
P	0.30	0.70	0.50	0.60	0.60	0.60	1.00

RESULTS

Result 1

The amplification products generated from Teak DNA using OPF-6, OPF-8, OPF-11 primers then separated on agarose gel are shown in Figures below λ symbol for standard DNA with size of band between 0.56-21.2 Kb; G1-G5 symbol for teak generative offsprings, P symbol for parental teak tree, and

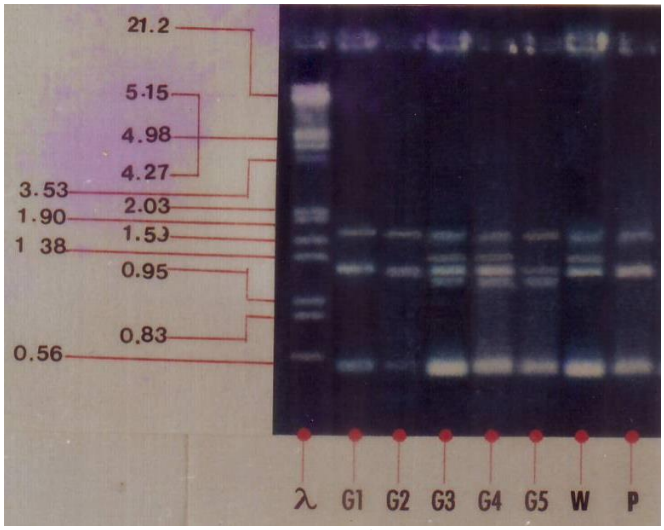

Figure 1. DNA bands which amplified with OPF-6 primer.

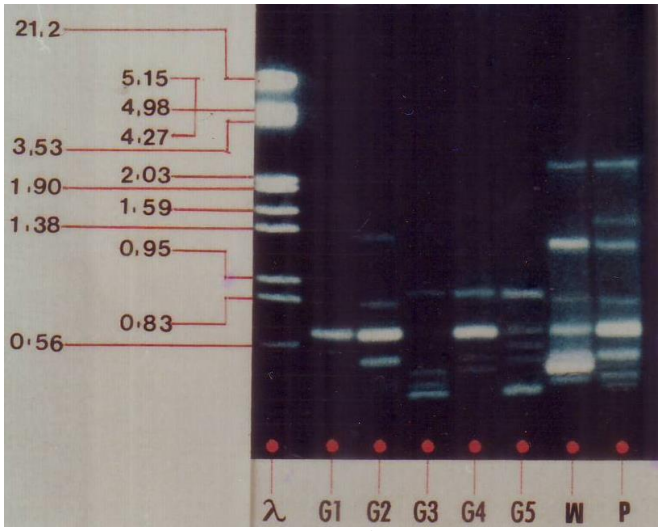

Figure 2. DNA bands which amplified with OPF-8 primer.

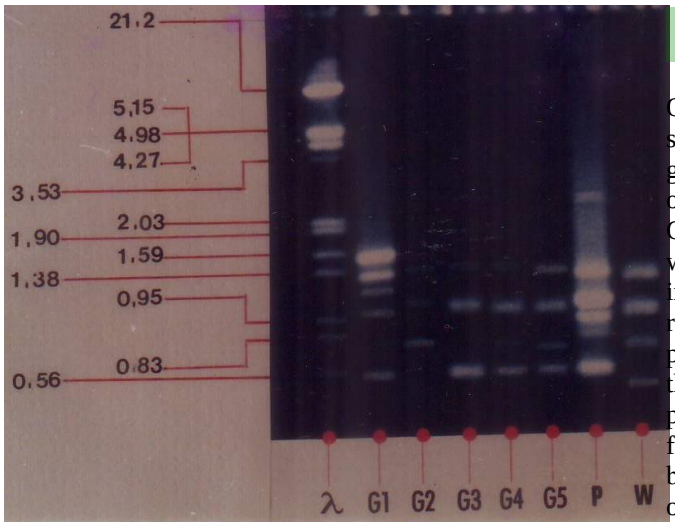

Figure 3. DNA bands which amplified with OPF-11 primer.

W symbol for wild type teak tree. In general, primer OPF-8 and OPF-11 generated more DNA bands than on the OPF-7 Primer. Furthermore, We observed that OPF-8 (Figure 2) and OPF-11 (Figure 3) primers yielded more polymorphic bands than OPF-6 (Figure 1) primers.

Result 2

Furthermore of the DNA amplification product, its length is calculated by entering the migration distance of the band into a logarithmic equation, then the value obtained is converted in the form of antilog. Size of DNA band which resulted from the amplification is about 330-3410bp, the resuls are shown in Table 1.

Based on the DNA amplification product , in addition to the size of DNA band also can be detected DNA bands of polymorphic and monomorphic the results are shown in Table 2. DNA amplification product will be analyzed, translated into qualitative data. DNA bands present denoted by 1, while the absence of DNA bands denoted by 0. Then presented in the data matrix. Based on the data matrix then similarity coefficient is calculated by using the similarity coefficient "Simple maching", the results are shown in Table 3, 4, and 5.

DISCUSSION

There are 22 primers which selected have 10 OPERON nucleotides. Samples which used in primer selection are 1 parental teak tree (P), 5 teak tree generative offspring (G1, G2, G3, G4, and G5) which obtained from Bibit Klon Puslitbang Perhutani Cepu Garden and 1 wild type teak tree (W) as the comparison which obtained locals garden. Teak generative offsprings in a Bibit Klon Garden were planted based on complete random design. Teak clones which close kinship were not planted in adjacent. Seed from free pollination results in the Bibit Klon Garden used for teak generative propagation (Rimbawanto, 1995). The teak trees derived from generative propagation have genotype from the both its parent's. Therefore the teak generative offsprings suitable if used for screening of RAPD primer.

There are 3 primer which obtained in this study, 1) amplification which shown polymorphism, 2) all of the DNA samples were successfully amplified, and 3) the amplified DNA band can be measured. Primer screening process is need high accuracy because sometimes it shown the differences in intensity which resulted from the low repetition and primer error (Harris, 1995). The band intensity is resulted from primer annealing variation. High intensity band is resulted when the primer annealed to DNA perfectly, but the low intensity band is resulted when the primer not annealed to DNA perfectly. It is one of problem for researcher in determine DNA band to be analyzed. Hancock et al. (1994) said that only the high intensity band which can be used for analysis. Harris (1995) said that each intensity as a character.Bradeen and Havey (1995) said that only the presence of DNA bands which observed without the intensity.

Based on the amplification of DNA with primers OPF6, OPF 8, and OPF 11 (Figure 1-3) are obtained sized DNA bands were calculated referring to the size standard DNA, namely DNA λ that had been cut with restriction enzymes Eco RI and Hind III . The relationship between the length of the DNA bands (bp) were analyzed with standard DNA migration distance made a logarith-

mic equation. The length of DNA band amplification result is calculated by entering the migration distance of the band into a logarithmic equation, then the value obtained is converted in the form of antilog. Size of DNA band which resulted from the amplification is about 330-3410bp (Table 1).

The combination of DNA template and primer will produce the DNA bands in various amount. Primer OPF6 produces DNA bands less than primer OPF 8 and OPF11. Primer OPF6 generates 5 DNA bands while primer OPF8 and OPF 11are generates 10 DNA bands. Total bands which resulted from 3 primers are 25 bands. Widyatmoko et al. (2013) said that DNA amplification using 25 of primer will bring out 2-7 DNA bands with size about 150-1000 bp. Research of Narayan et al. (2007) showed that RAPD primer bring out more DNA bands than ISSR primer, it is about 133 bands. Nezhad et al. (2010) compared between long and short primers used for RAPD technique in grafe . The results show a short primer to produce DNA more than a long primer. Khaled et al. (2015) reported that RAPD can be detected the genetic polymorphism in sugarcane genotypes with the percentage of polymorphic amplified product ranged from 37,5-72.7%.

Based on the amplification results, in addition to the size of DNA band also can be detected DNA bands of polymorphic and monomorphic. Polymorphic bands are DNA bands of a certain size which present in different individuals. Monomorphic bands are DNA bands of a certain size are present in all individuals. Therefore the Polymorphic DNA bands can be detected between the amplification products of different individual. While the monomorphic DNA bands can be detected in all the individual amplification product which compared (Williams et al., 1990). Amplification result of OPF-6 primer shown the lowest DNA polymorphism, while OPF-8 primer shown the highest DNA polymorphism (Table 2).Narayan et al. (2007) also reported that teak polymorphic band which resulted from amplification using RAPD primer is higher than using ISSR primer, it is 12.4 from 15 RAPD primer which used.

Based on the results of the coefficient of similarity "Simple Matching" are shown in the Table 3-5. Coefficient of similarity (Cij) has a value $0.00 \leq Cij \leq 1.00$. If the similarity value is equal to 1.00 indicates that the two individuals being compared are identical or similar, while if the similarity value is equal to 0.00 indicates that the two individuals being compared are not identical or similar (Ayuningrum et al., 2012).Teak which amplified with OPF-6 shown that there are 2 groups which have the coefficient of similarity 1.00 or identic, they are G1, G2, P groups with G3 and G4 groups. While other teak groups shown the coefficient of similarity about 0.60-0.80 which means each other are not identic (Table 3). This mean that among the teak tree GI, G2 and P did not show genetic variation, as well as with the teak tree G3 and G4. Whereas for other teak plants showed genetic variation. Teak which amplified with OPF-8 shown the coefficient of similarity about 0.40-0.80 which means there is no teak which identic (Table 4). This means that all teak tree showed genetic variation. Amplification results using OPF-11 show that teak from G4 and G5 have coefficient of similarity 1.00 or identic, while the other groups are have coefficient of similarity about 0.30-0.90 (Table 5). This mean that among the teak tree G4 and G5 did not show genetic variation. Whereas for other teak plants showed genetic variation.

Therefore OPF-8 primer is the best primer for identification of teak DNA polymorphism. RAPD primer which used are have high polymorphism which shown with more than 70% in DNA band results. This information in needed to reveal the genetic variation of teak. Genetic variation in teak tree can be determined based on the polymorphism of DNA bands and value similarity.High genetic variation is one of the important factors in development of teak seed new variation (Hutami, et al., 2008). A high genetic variation greatly affect the ability of plant species to adapt. High genetic variation will produce resistant properties or resistant to extreme environmental conditions, so that pests and diseases can be avoided (Olivia and Siregar, 2012).

Low genetic variation cause teak will easily infected by pathogens and pest. The inbreeding between two variation of teak which has low genetic variation will increased the homogeneity of recessive genes. These recessive genes probably carry the good or bad character (Aria, 2009). The bad character which carried by genes will make the low quality of offspring life. Therefore primer OPF6, OPF8, and OPF11, can then be used in teak genetic variation analysis. The information of teak genetic variation is the basis of implementation for teak breeding program. These primesr can be used for the analysis of genetic variation other teak, because RAPD primers do not need target DNA sequence information (Black, 1993; Bradeen and Havey, 1995; Hadrys et al., 1992; Hancock et al., 1994).

There are 3 primers which can be used in identification of teak DNA polymorphism. These primers are OPF6 (5'-GGGAATTCGG-3'), OPF8 (5'-GGGATATCGC-3'), and OFF11 (5'-TTGGTACCCC-3 '). The highest DNA polymorphism was found in DNA which amplified with OPF-8 primer with 100% of percentage and coefficient of similarity about 0.40-0.80. There is no teak group which identic each other. The same thing such as Hamid and Zokian (2015) using 5 RAPD primer to analyzed genetic diversity among pomegranates cultivated. The results showed that the genetic polymorphisms value of each RAPD primer was determined and ranged between 31 to 100%.

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