RAPD Primer

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RAPD-PCR primer selection to analyze genetic diversity of Cinnamon plan

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Abstract. RAPD is one of the DNA markers that utilize the principle of Polymerase Chain Reaction (PCR) machine which can amplify specific DNA sequences in vitro. The primary selection is made to display the DNA band pattern which can be used to obtain polymorphism information. This study aims to determine the RAPD-PCR primer that can be used to differentiate accession of cinnamon bark in Jambi Province, Indonesia. RAPD-PCR primer selection was carried out by using 100 primers which produces a polymorphic DNA band. Ten primers were identified to produce polymorphic DNA bands for cinnamon bark DNA namely: OPE-6, OPE-20, OPH-7, OPH-14, OPH-19, OPM-2, OPM-5, OPM-6, OPM-16 and OPM-19.

1. Introduction

Cinnamon plant (*Cinnamomum burmanii* L.) is a mainstay commodity and potential for Indonesian exports that has a high and economic selling value because cinnamon is the third largest foreign exchange contributor commodity from the plantation sector after pepper and nutmeg. The main results of cinnamon are taken from the bark and branches, while the additional products are from twigs and leaves. Besides the cinnamon skin used as spices, the processed products such as essential oils and oleoresin are widely used in the pharmaceutical, cosmetic, food and beverage industries.

There are about 54 species of Cinnamon, but only four of them have economic value. *Cinnamomum cassia* grows in China known as the name Chinese cinnamon. *Cinnamomum zeylanicum* or *Cinnamomum verum* from Sri Lanka with its products famous by name Ceylon cinnamon. *Cinnamomum burmanii* is a species which grows in Indonesia where the product is called Cassiavera or Indonesia cassia. *C. burmanii* is mostly planted by the people of Indonesia, especially in the Sumatera region, namely in West Sumatera Province, precisely along the Highland of Bukit Barisan and in Jambi Province in the Kerinci Regency, Sungai Penuh City and Merangin Regency.

In West Sumatra and Jambi Province, cinnamon plants are cultivated in areas that have various altitudes ranging from 50-1000 m asl which this situation causes differences in the growth and production of the cinnamon. The results of the study by Lizawati et al. reported that cinnamon plants that grow in various elevations showed a diversity of morphological characteristics, which can be seen from the diversity of canopy shape, shoot colour, base, leaf length, leaf length, leaf width ratio of length of leaf width and length of fruit and thickness of skin. The morphological character is strongly influenced by environmental factors. Therefore, molecular techniques are needed to see the genetic diversity of the cinnamon plant.

The molecular technique is useful and accurate to determine genetic variation in plants. Molecular techniques that are now often used to analyse plant fingerprint patterns to see their genetic diversity in

the form of phylogenetic trees are known as molecular markers. The molecular markers is used due to its relatively simple technique namely the Random Amplified Polymorphism DNA (RAPD). Many studies have been conducted to look at the genetic diversity of plants using RAPD markers, such as in yams, Labeo rohita, Catla catla and their hybrid, Mustard, and canna.

RAPD markers is one of the DNA markers using the working principle of the Polymerase Chain Reaction (PCR) machine which can amplify specific DNA sequences in vitro. The advantages of RAPD techniques are: simple, because they are relatively straightforward in preparation, randomly used primers without the need for gene DNA information or initial genomes, results are obtained faster, and the resulting characters are relatively unlimited. It is very helpful to analyze the genetic variability of plants which the background of the genome is unknown.

The success of genomic DNA amplification using the RAPD technique is largely determined by the order of primary bases used. It is identified that not all RAPD primers can be used to analyse polymorphisms that show different banding patterns between several plants including one species. Therefore, information about the primer type for RAPD analysis in each plant, especially cinnamon plant, needs to be investigated. In addition, limited research on molecular markers in the *Cinnamomum burmanii* species has been conducted.

This study aims to select several types of RAPD-PCR primers that can display the DNA band pattern among plant accessions. It identified polymorphism information so that it can be used to analyze the genetic diversity of cinnamon plant.

2. Materials and methods

2.1 Sampling

Young leaves of 1 year old of cinnamon plant were selected as sample in this research. The leaves come from the Jambi Province (Lempur Mudik Village, Gunung Raya District and Muara Madras Village, Kec. Jangkat) and West Sumatra Province (Puluik-puluik Village, North Bayang District and Guguk Village Gunung Talang District). Table 1 present the location of sample.

Table 1. The location of the plantation

No	Access Code	Location	Coordinate	Altitude (m dpl)
1	GR 01 S2	Desa Lempur Mudik Kec. Gunung Raya (Prov.Jambi)	S 02º 15' 56"; E101º 32' 59,0"	1.017
2	MM 01S1	Desa Muara Madras Kec. Jangkat (Prov.Jambi)	S 02º 38'06,2"; E101º 54' 14,5"	1.226
3	BU01	Desa Puluik-puluik Kec. Bayang Utara (Prov. Sumbar)	S 01º 11'14,2"; E100º 35' 50,9"	197
4	BU03	Desa Puluik-puluik Kec. Bayang Utara (Prov. Sumbar)	S 01º 10'58,5"; E100º 36' 04,1"	253
5	GT 01	Desa Guguk Kec. Gunung Talang (Prov. Sumbar)	S 00º 55'02,9"; E100º 37' 59,3"	808

2.2 DNA isolation

DNA isolation for molecular analysis was carried out using a modified CTAB-based isolation protocol. The material analysed was 0.5 g cinnamon plant leaves that was put into the mortar, then added liquid nitrogen and PVPP, then crushed. The results of scouring were inserted into the Eppendorf tube which contained 1 ml of 2% CTAB buffer and five µl mercaptoetanol. The DNA pellet is dissolved with 100 µl TE buffer. DNA purification used the Sambrook method. DNA solution was mixed to one µl RNAse to remove RNA, then it was incubated at room temperature for 2 hours. The pure DNA pellets were dried and then it was added 100 µl buffer TE pH 8. 0.8% agarose gel was made by mixed 0.32 g agarose and 40 ml TAE 1X solution to test the quality of DNA. The mixture of 5 µl DNA extraction and one µl loading dye was inserted into the gel well. The gel is soaked in a solution of ethidium bromide 0.1% and then it rinsed with water. The DNA tape can be seen in the UV transilluminator.

2.3 Primer Selection

Primer selection is carried out to find random primers that produce ribbon markers. It is selected by making several PCR reactions to several different primers under the same conditions and using the same DNA sample, so that the optimum conditions and the level of variation of the tape produced by each primer can be determined. A total of 100 primers have been tried to see the chance of finding a polymorphism between the genotypes tested in each primary tested. The list of the 100 primers used can be looked at in Table 2.

Table 2. The summary of primers used

Primer	Number of Poly-morphic Bands	Primer	Number of Poly-morphic Bands	Primer	Number of Poly-morphic Bands	Primer	Number of Poly-morphic Bands
OPA-1	6	OPB-5	5	OPE-9	10	OPH-16	5
OPA-2	2	OPB-6	4	OPE-10	0	OPH-17	2
OPA-3	10	OPB-7	5	OPE-11	6	OPH-18	3
OPA-4	4	OPB-8	6	OPE-12	1	OPH-19	9
OPA-5	0	OPB-9	4	OPE-13	7	OPH-20	5
OPA-6	4	OPB-10	3	OPE-14	4	OPM-1	6
OPA-7	5	OPB-11	2	OPE-15	0	OPM-2	13
OPA-8	7	OPB-12	3	OPE-16	8	OPM-3	1
OPA-9	1	OPB-13	0	OPE-17	1	OPM-4	0
OPA-10	2	OPB-14	4	OPE-18	3	OPM-5	8
OPA-11	3	OPB-15	0	OPE-19	3	OPM-6	8
OPA-12	0	OPB-16	0	OPE-20	9	OPM-7	2
OPA-13	2	OPB-17	4	OPH-1	6	OPM-8	2
OPA-14	0	OPB-18	5	OPH-3	4	OPM-9	2
OPA-15	2	OPB-19	1	OPH-4	4	OPM-10	3
OPA-16	3	OPB-20	3	OPH-5	4	OPM-12	1
OPA-17	4	OPE-1	2	OPH-6	2	OPM-14	6
OPA-18	1	OPE-2	3	OPH-7	9	OPM-17	2
OPA-19	0	OPE-3	3	OPH-9	5	OPM-18	7
OPA-20	2	OPE-4	2	OPH-10	2	OPM-20	6
OPH-2	6	OPE-5	4	OPH-11	2	OPM-13	6
OPB-1	1	OPE-6	11	OPH-12	2	OPM-15	0
OPB-2	2	OPE-7	0	OPH-13	1	OPM-11	5
OPB-3	6	OPE-7	4	OPH-14	13	OPM-16	13
OPB-4	0	OPE-8	3	OPH-15	7	OPM-19	12

2.4 The PCR process (Polymerase Chain Reactions)

The isolated DNA was amplified using a random primer. The random primers used are random nucleotides with a length of 10 nucleotides. The selected primers are primers that can produce at least 8 ribbons. The final volume of the reaction was 25 µl with a composition of 16.88 µl MegaMix Blue, 5.62 µl primary and 2.5 µl DNA template. The PCR results were fractionated through gel electrophoresis. The volume of the PCR reaction is 25 µl consisting of 2 µl (20 ng) genomic DNA, 1 µl primer (10 pmole), 12 µl Taq polymerase and 9.5 µl ion-free water. Electrophoresis results were seen using UV trans illuminator. DNA analysis activities were carried out at the Laboratory of the Bogor Agricultural University, West Java, Indonesia.

2.5 Data analysis

The data was analyzed descriptively by looking at the number of bands produced for each primer.

3. Result And Discussion

3.1 DNA isolation

DNA isolation using a modified CTAB-based protocol can produce DNA with good quality. The total DNA produced from 0.5 g of fresh leaves is 5.5 ug. This amount is sufficient to be used as material to analyze the genetic diversity using PCR-RAPD (Figure 1).

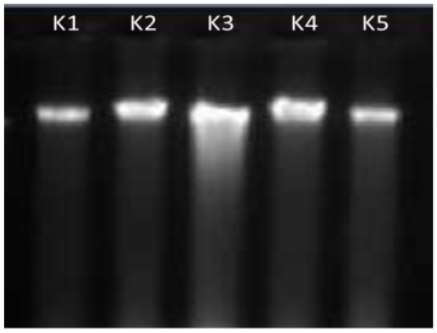

Figure 1. The isolated DNA of the cinnamon leaves using the modified CTAB procedure

Figure 1 shows that the DNA quality is good enough which is characterised by the presence of smear fragments below the main fragment. These smear fragments show the presence of contaminants such as RNA, proteins, polysaccharides and phenolic compounds, due to poor isolation processes. Although each sample showed the indications of DNA smears, the proportion of DNA from the main fragment was still huge, so the DNA was classified as good enough to be used in subsequent analysis.

The DNA bands in agarose gels showed different intensities for each example, and the DNA concentration obtained also varies. K3 and K5 DNA bands show higher band intensity than the intensity of bands K1, K2 and K4. This indicates that the high concentration of genomic DNA will result in high band intensity. It affects the high and low concentration of DNA, namely the content of compounds, DNA extraction procedures, and precipitation methods.

3.2 Primer Selection

100 primers have been selected to see the potential polymorphisms that can be generated by each of these primers. Out of 100 RAPD primers used, each produced some different fragments with a range from 1 to 13 fragments (Table 2). The number of fragments is quite large. As in Garcinia indica plants, 2-11 fragments were produced; in mulberry cultivars, 3 to 10 fragments were obtained; and in canna plants 1 to 12 fragments were produced. Out of 100 primers used, only 10 primers were selected which produced the most polymorphic bands (Table 3; Figures 2, 3 and 4).

Table 3. The selected primer which produced the most polymorphic bands

No	Primer	Number of Polymorphic Bands
1	OPE-6	11
2	OPE-20	9
3	OPH-7	9
4	OPH-14	13
5	OPH-19	9
6	OPM-2	13
7	OPM-5	8
8	OPM-6	8
9	OPM-16	13
10	OPM-19	12

Polymorphism indicates the presence of loci or amplicons (bands) that present in different not size. Figures 2, 3 and 4 showed that the DNA band of the amplification size is between 300-1200 bp. The differences in amplified DNA band profiles, especially the number and size of bands, play an important role in determining the level of genetic diversity of plants. Differences in the size of DNA fragments or amplified polymorphisms of DNA fragments are caused by the distribution of nucleotide base locations in the genome which are the primary attachment sites or sites.

The presence of a locus possessed of the same size in all samples analyzed. Locus has the specific location of a gene along the chromosome. The variety number of loci produced is influenced by the primary sequence used. A primer can amplify printed DNA if there are complementary segments. The more printed DNA segments that are complementary to the primary sequence, the more number of bands amplification results.

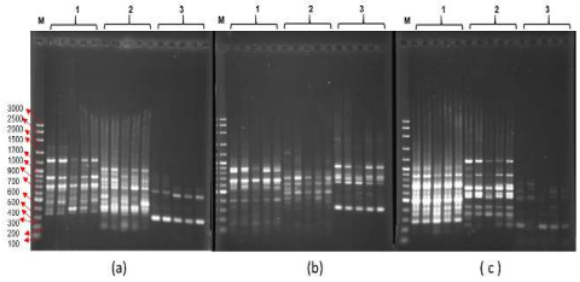

Figure 2. Results of primer selection (**a**). 1 (B5); 2 (H15); 3(B13), (**b**). 1(B10); 2(A11); 3(E8), and (**c**). 1(E7); 2(H11); 3(A6)

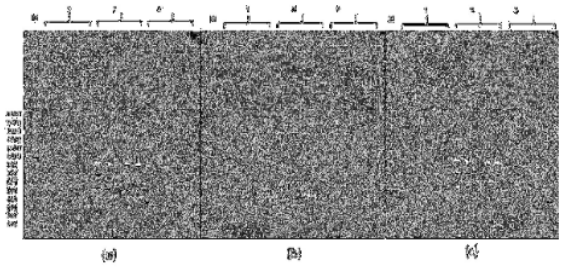

Figure 3. Results of primer selection (**a**). 1(A18);2(A1); 3(H2), (**b**). 1(E12); 2(H18);3(A2), and (**c**) 1(A18); 2(A1); 3(H2)

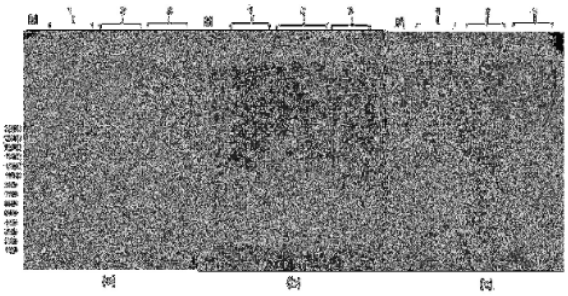

Figure 4. Results of primer selection (**a**). 1(B8); 2(E1); 3(A9), (**b**). 1(E5); 2(M14); 3(H7), and (**c**). 1(A3); 2(A14); 3(B18)

Figures 2, 3 and 4 also indicate that there are differences in the quality of fragments which are assessed from the level of intensity. Single or no fragments produced also vary. Some fragments have higher intensity, and there are also less intensity. Some fragments are very visible, possibly caused by the presence of different fragments, which by chance have the same length of the fragment. In addition to the intensity of the fragments that are differentiated, there are also some primers that still produce smear products. The smear production is caused by the accumulation of several different sizes of fragments that is not too large but they overlap continuously. Thus it is difficult to distinguish the different sizes of each fragment. In such conditions, the primer should not be used further for fingerprinting analysis.

Generally, all primers that successfully amplify DNA showed different amplicon patterns. However, different amplicon patterns are quite high and firmly produced bands compared to others are found in the OPE-6, OPE-20, OPH-7, OPH-14, OPH- 19, OPM-2, OPM-5, OPM-6, OPM-16 and OPM-19 where the primer can produce at least 8 DNA ribbon fragments (Table 3). The primers can be used for further analysis, especially in genetic diversity in several accessions, for example, cinnamon plant. Information on genetic diversity of cinnamon plant can be used as a reference for developing superior seeds, especially for cinnamon skin breeding activities which are beneficial as medicine.

4. Conclusion

Based on the results of this experiment, it can be concluded that:

1) The results of the amplification of 100 primers used obtained 1 to 13 DNA ribbon fragments in 300-1200 bp
2) The primary type that can be used to analyze the genetic diversity of cinnamon plants is the primary that produces a minimum number of DNA ribbon fragments of 8, namely the OPE-6, OPE-20, OPH-7, OPH-14, OPH-19, OPM- 2, OPM-5, OPM-6, OPM-16 and OPM-19

References

[1] Pribadi E R 2016 Perkembangan Produksi dan Ekspor Kayu Manis Indonesia *Warta Penelitian dan Pengembangan Tanaman Industri* **2** pp 10-14
[2] Safithri M, Yasni S, Bintang M and Ranti A S 2012 Toxicity study of antidiabetics functional drink of piper crocatum and cinnamomum burmannii *Hayati Journal of Biosciences* **19** pp 31-36
[3] Nabavi S F, Lorenzo A D, Izadi M, Sobarzo-Sánchez E, Daglia M and Nabavi S M 2015 Antibacterial effects of cinnamon: from farm to food, cosmetic and pharmaceutical industries *Nutrients* **7** pp 7729-7748
[4] Lizawati, Riduan A, Neliyati and Alia Y 2017 Genetic Diversity of Cinnamon Plants (Cinnamomum burmannii BL.) From west Sumatra based on morphological characteristics *Prosiding Seminar Nasional Perhimpunan Agronomi Indonesia (PERAGI) 2017* (Bogor: IPB International Convention Center)
[5] Lizawati, Riduan A, Neliyati, Alia Y and Antony D 2018 Genetic diversity of cinnamon plants (cinnamomum burmannii bl.) at various altitude based on morphological character *Conf. on The 3rd Annual Applied Science and Engineering*
[6] Purnomo, Daryono B S, Rugayah, Sumardi I and Shiwachi H 2016 Genetic variability and classification of indonesian yams (dioscorea spp.) *SABRAO Journal of Breeding and Genetics* **48** pp 377-390
[7] Naveed R, Sultana S, Nawaz M, Ullah I, Al-Ghanim K A, AlThobaiti A and Mahboob S 2017 Genetic Variability in labeo rohita, catla catla and their hybrid (labeo rohita ♂ × catla catla ♀) populations employing randomly amplified polymorphic (rapd)-inter simple sequence repeat (issr) assays *Int. J. Agric. Biol.* **19** pp 643–648
[8] Sharma V, Khandelwal P S S K, Joshi A, Jain D and Rajpurohit D 2018 RAPD analysis of mustard (brassica juncea l.) genotypes differing in response to water stress *J. Pl Sci Res* **34** pp 107-113

[9] Sari N, Suryadiantina, Daryono B S and Purnomo 2018 Variability and intraspecific classification of Indonesian edible canna (canna indica l.) *SABRAO Journal of Breeding and Genetics* **50** pp 156-167
[10] William J G K, Kubelik A R, Livak K J, Rafalski J A and Tingey S V 1990 DNA polymorphism amplified by arbitrary primers are useful as genetic marker *Nucleic Acids Research* **18** pp 6531-6535
[11] Castillo C O, Chalmers K J, Waugh R and Powell W 1994 Detection of genetic diversity and selective gene introgression in coffee using rapd marker *Theor. Appl. Genet.* **87** pp 934-938
[12] Sambrook J F, Frits T and Maniatis 1989 *Molecular Cloning A Laboratory Manual* (New York)
[13] Chen H, Rangasamy M ,Tan S Y, Wang H and Siegfried B D 2010 Evaluation of five methods for total dna extraction from western corn rootworm beetles *Plos ONE* **5** pp 1-6
[14] Sahasrabudhe A and Deodhar M 2010 standardization of dna extraction and optimization of rapd-pcr conditions *garcinia indica International Journal of Botany* **6** pp 293-298
[15] Sheet S Ghosh K Acharya S Kim K and Lee Y S 2018 Estimating genetic conformism of Korean mulberry cultivars using random amplified polymorphic dna and inter-simple sequence repeat profiling *Jour. Plants* **7** pp 1-10
[16] Sari N, Suryadiantina, Daryono B S and Purnomo 2018 Variability and intraspecific classification of Indonesian edible canna (canna indica l.) *SABRAO Journal of Breeding and Genetics* **50** pp 156-167
[17] Berdakci F 2001 Random amplified polymorphic dna (rapd) markers *Turk. J. Biol.* **25** pp 185-196
[18] Semagn K, Bjornstad A and Ndjiondjop M N 2006 An overview of molecular marker methods for plants *Afr. J. Biotechnol* pp 2540-2568
[19] Jamsari, Darusalam, Syahlena R, Syaputra M ,Darnetty R and Putri N E 2007 RAPD primers selection and genetic relationship study of capsicum sp *Jurnal Akta Agrosia* **10** pp 172-181

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