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GENETIC TYPING OF THREE MALAYSIAN DURIAN VARIETIES USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS

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Abstract

There have been few studies on the molecular aspects of durian up to this point. Although morphological classification is practical, simple, and quick, it suffers from phenotypic plasticity due to environmental influences and age. Hence, the genetic characterization of durian varieties needs to be carried out. This study was performed to evaluate the effectiveness of three simple sequence repeat (SSR) markers to identify and discriminate three durian types: MK (Musang King), D24 and D101. Successful amplification of SSR regions were observed in the durian DNA samples. A total of 8 alleles were generated by all primers. Both DZ01 and DZ04 primers showed the highest number of polymorphic fragments. Among the three SSR primers, DZ03 is the least informative with only 2 number of alleles produced. The most important finding of this study was that each primer is unique and specific to one type of durian sample. This preliminary study showed that the SSR loci could be used as genetic markers to assist future durian breeding program.

INTRODUCTION

Production inconsistency is one of the problems encountered by durian fruit due to phenotype variation. Despite being harvested from the same cultivation area, the size of fruits and flesh colour are varied. This situation caused more confusion especially when different types of durian fruit have similar appearance making it difficult to distinguish between them. This is a significant disadvantage to the consumers since they can misjudge the durian types. Several factors contribute to the presence of intra-varietal among the fruits. For example, the phenotypic variation due to adaptation to different natural environment, spontaneously occurrence of genetic variations from their ancestors and people preferences [1]. Hence, the current grading system of durian using a visual morphological method needs to be improved and that will also standardize the durian market price [2].

Many studies were done on the genetic variation of durian types using DNA markers such as inter-simple sequence repeat (ISSR) [2, 3] and random amplified polymorphic DNA (RAPD) [4, 5] from important durian producing countries. The simplicity of these markers is the reason for them to be used for studies on population genetic structure and overall genetic variation [6]. However, they are not suitable for DNA fingerprinting method due to the dominant nature [7]. The feasibility and reliability of these markers are also questioned as the data generated are not as informative compared to the co-dominant markers [8]. They are also known to have a poor reproducibility.

Simple sequence repeat (SSR) or also known as microsatellites DNA are codominant, multi-allelic, highly

reproducible, and stable [9, 10]. This marker is easy to be used as the analysis could be conducted automatically [11]. Successful application of this marker has been reported in plant variety identification and genetic variation studies in a wide range of cultivated plant species such as rice [12], oil camellia [13], jute [14], and oil palm [15, 16]. The ability of this marker to produce accurate and reproducible results are highly significant for the evaluation and subsequent management of genetic resources. Moreover, SSR markers are capable in detecting expected alleles as early as possible which is important for breeding activity [17]. To our knowledge, very few reports have been published with regards to the genetic variation in durian using SSR markers [18, 19]. This study was aimed to determine the potential of the SSR loci as genetic markers to assist durian breeding activity in the future.

MATERIALS AND METHODS

Materials

The durian samples were obtained from the Federal Agricultural Marketing Authority (FAMA). SSR primers were purchased from Apical Scientific company. Detailed information on the chemicals, kit and instruments used was provided in the paragraph.

Sampling and DNA Extraction

The type of durian tissue used in this study was the flesh from four years old durian tree. The durian samples are popular commercial types grown in Tangkak (Johor) and Jerantut (Pahang), in which most have not been tested with the SSR markers for genetic diversity studies. These durians have already been pre-identified and pre-labelled morphologically. Durian Musang King has thick, golden flesh that is sweet, creamy, and sometimes bitter in taste. Durian D24 or Durian Sultan comes with yellow, normally dry and firm, smooth, and sweet flesh. Meanwhile, durian D101 is almost orange in colour and has a strong, fruity flavour. Fresh samples were obtained from the Federal Agricultural Marketing Authority (FAMA) and immediately frozen in liquid nitrogen. Subsequently, using a pre-chilled mortar and pestle the samples were ground into fine powders in liquid nitrogen. The resulting tissue powders were kept at -80ºC before proceeding with the total DNA extraction.

Total DNA was extracted from 100-200 mg of wet weight of all the durian flesh sample using the cetyl trimethylammonium bromide (CTAB) extraction method as described by Doyle & Doyle (1990) [20]. The GF-1 Plant DNA Extraction Kit (Vivantis Technologies Sdn. Bhd., Malaysia) was used to purify the crude DNA extract before further testing. The quality of extracted DNA was then measured using a Nanodrop spectrophotometer (Beckman Coulter, Brea, CA, USA).

PCR Conditions and Detection of PCR Products

Three SSR primers were used in this study. Table 1 shows the detailed primer sequences [21]. Polymerase chain reaction (PCR) was conducted in 20 µl reaction mixtures containing 1x PCR buffer (Banglore Genei), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM each of the forward and reverse primers, 1 U *Taq* DNA polymerase (Banglore Genei) and approximately 20 ng of genomic DNA. The reaction mixtures were placed on a DNA thermal cycler (Bio-Rad). SSR program was performed as one cycle of 95 °C for 3 minutes, followed by 30 cycles of 30 seconds at 95 °C, 30 seconds at 55 °C or 60 °C, and 2 minutes at 72 °C followed by an extension step at 72 °C for 7 minutes. PCR products were analyzed through electrophoresis on 1.5 % (w/v) agarose gels, in 0.5x TBE Buffer at 80 V for one hour and then stained with ethidium bromide (0.5 µg/ml). The Image Lab™ Software Version 6.0 (Bio-Rad) was used to view the amplified products. By comparing sample banding patterns to a 100 bp DNA ladder (Solis BioDyne) loaded in the same gel, the DNA fragment sizes were calculated.

Table 1. SSR primers used in this study

Locus	Primer name	Primer sequence (5' → 3')
DZ01	DZ01_F2	AATTCCACATGACAGACAGG
	DZ01_R	TCATGGATGTTGTATGGCAG
DZ03	DZ03_F	CTCTAAAAAGAATGGGGATATTG
	DZ03_R	ATTCTGGAACAAAAGTTACAAAC
DZ04	DZ04_F2	TGCATGTTTTGAAAAGTACC
	DZ04_R2	ATGGGGAAAAGAAAGTGAAG

RESULTS AND DISCUSSION

High quality and quantity of intact DNA is needed in molecular studies in fruit trees, especially durian. However, due to the presence of large amounts of polysaccharides,

polyphenolics and other secondary metabolites that accumulate during ripening, the isolation and extraction of total DNA can be technically challenging. During the extraction process, these polysaccharides and polyphenolic chemicals frequently co-precipitate and contaminate the DNA, reducing the quality and quantity of extracted DNA [22, 23]. In this study, the incubation period of durian flesh samples during cell lysis step was prolonged to get a significant amount of DNA. The ratio of successfully collected DNA samples averaged 1.895, which is within the range of 1.8 to 2.0 for pure DNA [21] and 18.711 ng/µl for DNA sample concentration.

A total of three durian types (MK, D24 and D101) from Tangkak and Jerantut were investigated to identify molecular variation between them using three SSR primers. The estimated DNA fragment sizes of each sample at each locus were manually recorded. All primers successfully amplified clear and reproducible bands in all three durian types (Figure 1). There were two types of bands found among the amplified DNA products: monomorphic and polymorphic. Monomorphic is the presence of similar size bands in all individuals. Meanwhile, polymorphic bands are those that are present in one or more individuals.

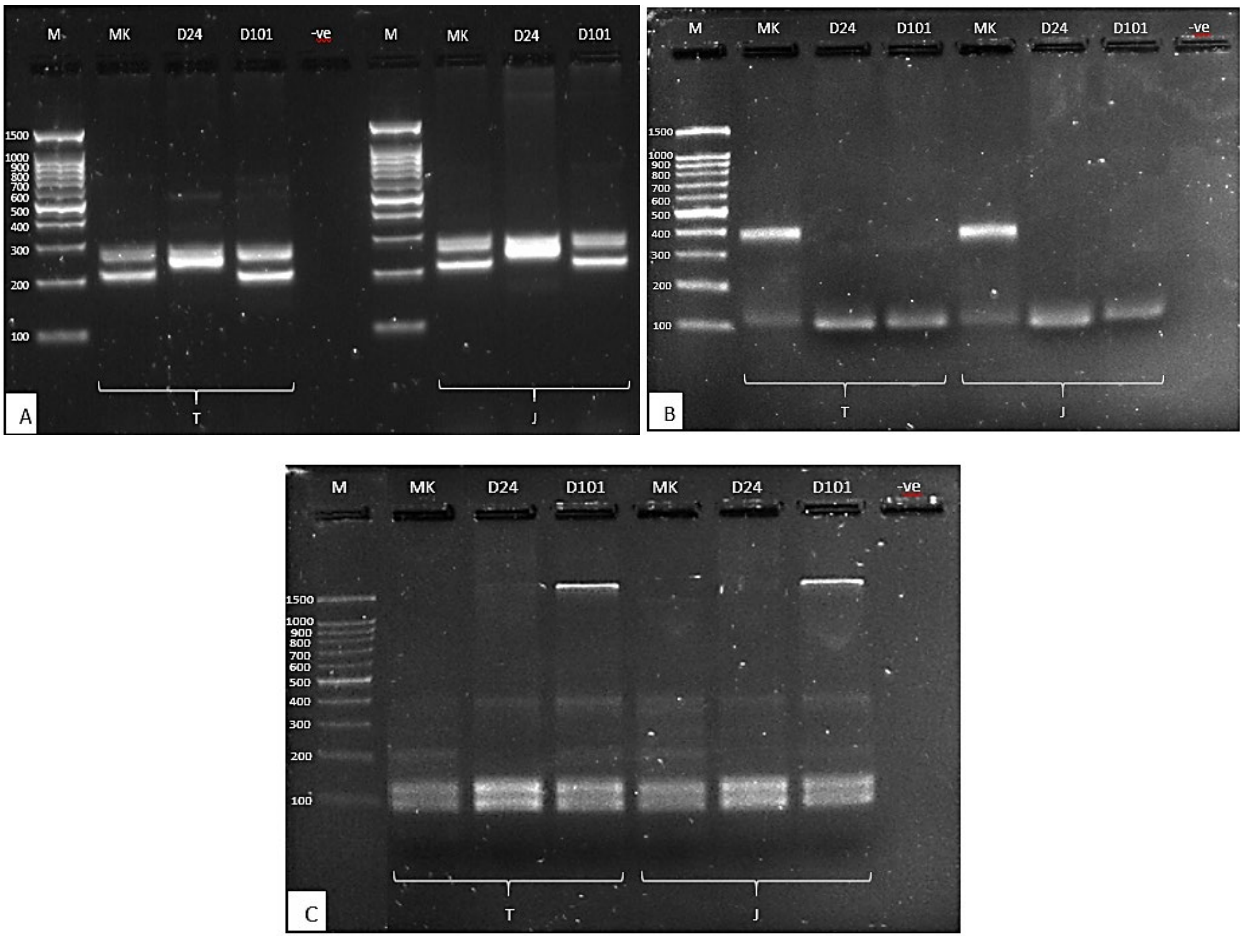

Figure 1. SSR profiles of MK (Musang King), D24 and D101 durian types from Tangkak (T) and Jerantut (J) generated with the DZ01 (A), DZ03 (B) and DZ04 (C). 100 bp of DNA ladder (M) was used to estimate the DNA fragment sizes.

Table 2 shows a total of 8 alleles were scored across three SSR loci with minimum of 2 alleles produced by DZ03 primer. Both DZ01 and DZ04 primers recorded the same number of alleles. Similar banding patterns were observed between the durian samples from Tangkak and Jerantut. Each primer has a unique allele which represented by different durian types. Musang King (MK) is unique to locus DZ03 at 400 bp. D24 durian type produced an allele of 250 bp which is unique to locus DZ01. DZ04 is unique to D101 durian type at 1500 bp. In this study, all durian types tested were successfully evaluated and distinguished with only three SSR loci, which showed the effectiveness of these SSR markers for molecular variation studies of durian types. However, in order to assess the viability of employing SSR markers in the management of registered durian types, thorough studies involving exhaustive sampling of all

registered durian types for a country or region, as well as more markers, are required.

Table 2. Details of alleles produced by each locus

Locus	Number of alleles	Allele
DZ01	3	210
		250
		270
DZ03	2	110
		400
DZ04	3	100
		130
		1500

SSR markers are proven to be beneficial in determining a newly registered variety for Plant Variety Protection (PVP) application [24]. They are being applied as a technique to supplement morphological character assessment [25]. Another advantage of this marker is that it can be used to investigate currently registered plant varieties to see if there are any clones among them, in addition to new plant variety registration [21]. This is especially vital in PVP, because the owner of a novel plant variety has exclusive rights to sell the plant, and others exploiting the plant is prohibited. Successful application of this method reported on several important economic crops including apple cultivars in the Netherlands [25], sugarcanes in Brazil [24] and olive cultivars in Turkey [26]. Therefore, the genetic identification of exported durians is very important to assure that they are genuine breed. Further study is to be taken up in a large population for heterozygosity, and more markers should be tested. As a result, it is capable of providing a high level of discrimination and analysing the phylogenetic relationships among the Malaysian durian population.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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