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Determination of Phylogenetic and Molecular Characteristics of Three Malaysian Ginger Cultivars (*Zingiber officinale* Roscoe) Using Microsatellite DNA

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Abstrak: Tiga kultivar halia Malaysia (Bukit Tinggi, Tanjung Sepat dan Sabah) telah dikumpul dan diperiksa untuk polimorfisme genetik menggunakan primer DNA mikrosatelit. Primer oligonukleotida mikrosatelit tunggal $(CATA)_5$, $(GATA)_5$ dan $(GAC)_6$ telah digunakan dalam reaksi rantai polimerase (PCR). Reaksi PCR ini telah menghasilkan 7 jalur polimorfik dengan purata 2.334 jalur per primer, dengan kadar purata polimorfisme 17.9%. Analisis kluster telah menunjukkan bahawa terdapat 87.50% persamaan antara Bukit Tinggi dan Tanjung Sepat, 64.27% persamaan antara Bukit Tinggi dan Sabah dan 56.25% persamaan antara Tanjung Sepat dan Sabah. Jujukan DNA produk polimorfik PCR menunjukkan ciri-ciri gen baru: satu jujukan promoter teras, satu penggalak dan satu lokasi mula transkripsi. Analisis kluster menggunakan *unweighted pair group method with arithmetic average* (UPGMA) telah digunakan untuk membina pokok filogenetik, yang telah menunjukkan bahawa halia Bukit Tinggi lebih mempunyai hubungan dengan halia Tanjung Sepat berbanding halia Sabah. Berdasarkan dapatan kajian ini, kami telah membuat kesimpulan bahawa terdapat variasi genotipik antara kultivar halia, dan primer mikrosatelit DNA yang telah dihuraikan disini berguna untuk mengesan DNA polimorfik kultivar halia Malaysia. Selain itu, primer mikrosatelit DNA ini boleh digunakan sebagai penanda molekul untuk membezakan kultivar terpilih halia Malaysia.

Kata kunci: Halia (*Zingiber officinale* Roscoe), DNA Mikrosatelit, PCR, DNA Polimorfik

Abstract: Three Malaysian ginger cultivars (Bukit Tinggi, Tanjung Sepat and Sabah) were collected and examined for genetic polymorphisms using microsatellite DNA primers. The single microsatellite oligonucleotide primers $(CATA)_5$, $(GATA)_5$ and $(GAC)_6$ were used in polymerase chain reactions (PCRs). These PCR reactions produced 7 polymorphic bands with an average of 2.334 polymorphic bands per primer, leading to an average polymorphism rate of 17.9%. Cluster analysis revealed 87.50% similarity between Bukit Tinggi and Tanjung Sepat, 64.27% similarity between Bukit Tinggi and Sabah and 56.25% similarity between Tanjung Sepat and Sabah. DNA sequencing of the polymorphic PCR products of Tanjung Sepat ginger revealed the characteristic features of a putative new gene: a core promoter sequence, an enhancer and a transcription start site. Cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) was used to construct a phylogenetic tree, which indicated that Bukit Tinggi ginger is genetically more closely related to Tanjung Sepat ginger than to Sabah ginger. Based on the results of this study, we concluded that there is genotypic variation among ginger cultivars, and the microsatellite DNA primers described here are useful for detecting polymorphic DNA in Malaysian ginger cultivars. Additionally, these microsatellite DNA

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primers may be used as molecular markers for discriminating among select Malaysian ginger cultivars.

Keywords: Ginger (*Zingiber officinale* Roscoe), Microsatellite DNA, PCR, Polymorphic DNA

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is an important tropical horticultural plant that is valued worldwide as a spice and for its medicinal properties. Members of the family are distributed in the tropics of southern and southeastern Asia, especially in Indo-Malaysia (Simpson 2006; Awang 1992). Chinese records show that ginger was cultivated in the Malacca region of Malaysia in 1416 (Weiss 2002). Currently, ginger is cultivated throughout tropical and sub-tropical regions worldwide, including areas of Australia, Brazil, China, India, Jamaica, West Africa and parts of the United States.

Ginger, as a species, is thought to have arisen as a sterile hybrid between two distant species that survived due to successful vegetative propagation (Peter *et al.* 2007). Malaysia and neighbouring countries recognise two divisions: the *Z. officinale* cultivar group *officinale* "ginger" and the *Z. officinale* cultivar group "rubrum" with small, pungent, reddish rhizomes (Weiss 2002).

Traditionally, ginger was used as an acrid bitter to strengthen and stimulate digestion. Modern uses include prophylaxis for nausea and vomiting, dyspepsia, lack of appetite, anorexia, colic, bronchitis and rheumatic complaints. More than 400 chemicals have been identified in ginger rhizomes (Garner-Wizard *et al.* 2006). Geography, age of the rhizome at harvest, extraction methods, storage and drying determine the relative proportions of each chemical.

It is very difficult to distinguish among ginger cultivars (Ibrahim 1999) without depending on chemical and/or molecular markers because the morphological or phenotypic characteristics of the aboveground biomass are not sufficiently unique to adequately discriminate among ginger cultivars. However, some morphological characteristics of the ginger rhizome can be used to discriminate among Malaysian ginger cultivars. For example, the rhizome of Bukit Tinggi ginger is bigger with a dull, whitish colour and it is less fibrous compared with Tanjung Sepat ginger, which has a yellowish, fibrous, slender rhizome [Global Information Hub on Integrated Medicine (Globinmed) 2013]. The rhizome of Sabah ginger tends to be darker in colour with few red-to-brown ribbons of colour. To differentiate among ginger cultivars, a more precise and accurate method was established based on metabolic fingerprinting, in which variations in chemical constituents were detected and some chemical markers for each ginger cultivar were established (Mahdi *et al.* 2010).

Despite the importance of ginger in cuisine and especially in medicine, very little information is available regarding the genome of ginger. Tools such as bacterial artificial chromosome (BAC) libraries or molecular marker-based genetic maps have not been produced, or at least, no such resources have been released or even mentioned in the literature because this plant is not amenable

to the production of genetic maps (Moore *et al.* 2008). Ginger was reported to be diploid (somatic chromosome number: 2n = 22) with a large genome of 23.618 Mb (Wahyuni *et al.* 2003). The total chromosome length ranged from 64.80 μm to 98.12 μm, and the total chromosome volume ranged from 84.35 μm^3 to 1126.36 μm^3. The chromosome conformation capture (4C DNA) varied significantly among different cultivars of ginger (from 16.234 pg to 22.934 pg) (Ravindran & Babu 2004). Because ginger is vegetatively propagated, local cultivars often tend to be uniform and develop specific characteristics.

Microsatellite DNA, also referred to as simple sequence repeat (SSR) DNA, consists of 1 to 6 nucleotides repeated 5–50 times and can be found within coding or non-coding regions in the genome (Hartwell *et al.* 2008; Walker & Rapley 2005; Weising *et al.* 2005). Microsatellites have been predicted to play important roles in maintaining genetic stability. These elements are highly polymorphic (because of their potential for faulty replication), co-dominant (Jayabalan 2006), evenly dispersed through the eukaryotic genome (Bernot 2004; Weising *et al.* 1995) and require little DNA for analysis, all of which make them favourable for use in genetic mapping experiments (Soltis *et al.* 1998). Microsatellite DNA can be used as markers for the detection of allelic variation, genetic mapping studies, germplasm characterisation and gene tagging (Hayden & Sharp 2001). Recently, microsatellite DNA has been largely used by researchers as a molecular marker (Craft *et al.* 2007). The principal reason for the increasing success of microsatellites as molecular markers is that they can detect more polymorphisms than other techniques such as restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) (Weising *et al.* 2005).

In this study, three single oligonucleotide microsatellite primers were used: two 20-mers [$(CATA)_5$ and $(GATA)_5$] with 25% GC and one 18-mer [$(GAC)_6$] with 75% GC. The selection of primer sequences was based on the evidence that microsatellite markers with trinucleotide and tetranucleotide repeat motifs amplify more faithfully and are, on average, more polymorphic than dinucleotides (Taylor 1997) in addition to being more abundant (Sharma 1999). Selection of the first two primer sequences [$(CATA)_5$ and $(GATA)_5$] was performed according to Weising *et al.* (1995), who used these two primers to detect DNA polymorphisms in tomato and *Actinidia chinensis*. Our study revealed that these primers could be successfully used in ginger. The third primer, $(GAC)_6$, is a novel primer that has not previously been used for the detection of DNA polymorphisms in plants. To ensure that the polymorphic band(s) were consistently present and could be considered as molecular markers, the procedure was repeated four times for each of the tested ginger cultivars using the selected primers.

This study aimed to detect genetic diversity among three selected Malaysian ginger cultivars and to verify the usefulness of microsatellite DNA as a molecular marker for differentiation between ginger cultivars.

MATERIALS AND METHODS

Genomic DNA Isolation

Isolation of ginger genomic DNA was performed according to Doyle and Doyle (1990) with some modifications. Briefly, ginger leaf tissue was ground to a fine powder under liquid nitrogen using a pre-chilled mortar and pestle. The leaf powder was then transferred into a 2 ml Eppendorf tube containing a smaller volume (750 µl) of pre-heated (60°C) cetyltrimethylammonium bromide (CTAB) extraction buffer.

Polymerase Chain Reaction (PCR) Amplification

PCR with single oligonucleotide primers was performed in a final reaction volume of 50 µl containing 75 ng of template DNA, 100 mM deoxyribonucleotide triphosphates (dNTPs), 60 ng of primer, 1.5 mM $MgCl_2$, 1X Taq buffer + KCl [10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.08% Nonidet P40], 1 U of Taq DNA polymerase and de-ionised water to make up the volume. The PCRs were carried out using the following conditions: a preliminary denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 20 s, annealing at 45°C for 30 s for $(CATA)_5$ and $(GATA)_5$ primers or 55°C for 30 s for the $(GAC)_6$ primer, and an extension step at 72°C for 30 s. A single final extension step was performed at 72°C for 10 min.

Extraction and Amplification of Polymorphic Bands

To determine the existence of polymorphic bands, the amplified DNA was examined by gel electrophoresis using ethidium bromide and a UV spectrophotometer (SmartSpec Plus, BIO-RAD, USA). Polymorphic PCR bands were eluted from the agarose gel using a QIAquick gel extraction kit (Cat. no. 28704; Qiagen, Kuala Lumpur). These eluted DNA fragments were then amplified by PCR using the $(CATA)_5$, $(GATA)_5$ and $(GAC)_5$ primers.

DNA Sequencing of Polymorphic DNA Fragments

DNA sequencing of polymorphic DNA fragments was performed by 1st Base (Seri Kembangan, Selangor, Malaysia) utilising a BigDye® Terminator V 3.1 cycle sequencing kit (Invitrogen, California, USA) (based on Sanger dideoxy sequencing).

RESULTS AND DISCUSSION

Evaluation of Isolated Genomic DNA

The isolated genomic DNA from the 3 ginger cultivars was qualitatively tested using 0.8% (w/v) agarose gels (Fig. 1). Gel electrophoresis showed no fragmentation of the isolated DNA, with only one clear band obtained for each ginger cultivar. Quantitative and qualitative evaluation of the isolated genomic DNA was performed using a UV spectrophotometer to determine its concentration and purity, and the results are presented in Table 1. The results reveal variation in the isolated ginger genomic DNA concentration among the

three samples, which may be due to the use of leaves of different ages. Such leaves may contain different numbers of cells per unit weight and may exhibit variation in cell wall rigidity due to deposition of cellulose, thus causing difficultly in breaking or lysing the cell wall. The isolation of pure and non-fragmented DNA is essential to avoid false positive identification of PCR products after gel electrophoresis.

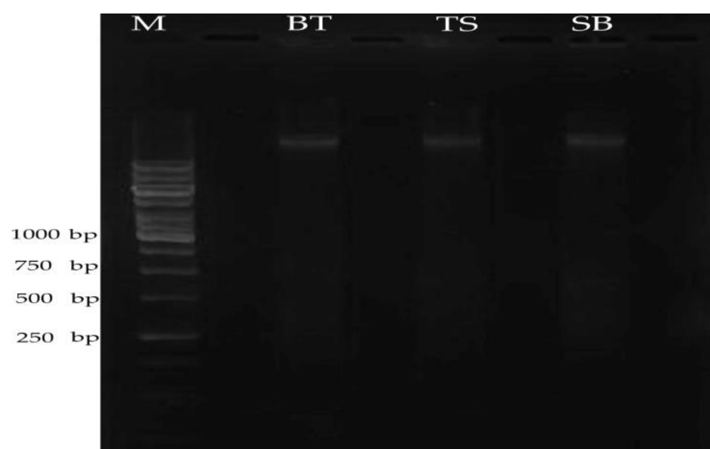

Figure 1: Genomic DNA isolated from the ginger cultivars Bukit Tinggi (BT), Tanjung Sepat (TS) and Sabah (SB).

Table 1: Concentration and purity of the isolated genomic DNA.

Ginger cultivar	DNA concentration (µg/ml)	A260/A280 ratio
Bukit Tinggi	151.6637	1.6624
Tanjung Sepat	30.2700	1.8520
Sabah	150.5050	1.7253

PCR Amplification and Characterisation

Three single oligonucleotide microsatellite primers were used [$(CATA)_5$ and $(GATA)_5$ with 25% GC and $(GAC)_6$ with 75% GC] to amplify specific regions of DNA. Polymorphic bands were visualised and identified in the gel using an electrophoresis imaging documentation system. Several PCR parameters, such as $MgCl_2$ and DNA template concentrations, were examined during the experiment to obtain optimum amplification conditions that yield repeatable and consistent results. The $(GAC)_6$ primer, which is a novel primer that has not been used for the detection of DNA polymorphisms in plants, amplified a specific polymorphic band from Tanjung Sepat ginger (Fig. 2). GAC encodes an aspartate residue in a coding region. The use of the sequence GAC in the $(GAC)_6$ primer therefore enhances the possibility that this primer will bind to a coding region in the genome. The $(CATA)_5$ primer produced strong polymorphic bands 1.25 kb in length (Fig. 3), and the $(GATA)_5$ primer produced a single band in the Sabah cultivar (Fig. 4).

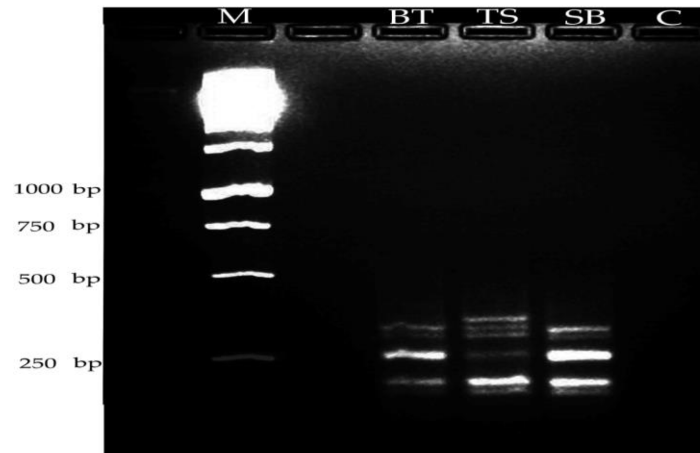

Figure 2: Polymorphic bands were obtained in the Bukit Tinggi (BT) and Tanjung Sepat (TS) cultivars using the $(GAC)_6$ primer.

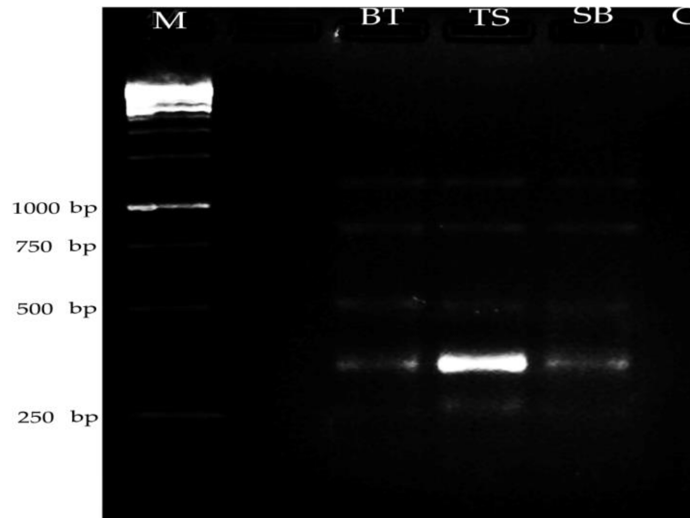

Figure 3: Polymorphic bands were obtained in the Tanjung Sepat (TS) cultivars using the $(CATA)_5$ prime.

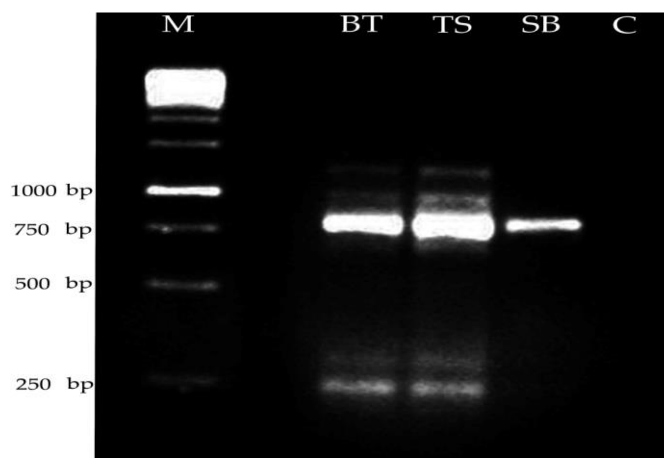

Figure 4: Polymorphic bands were obtained in the Bukit Tinggi (BT) and Tanjung Sepat (TS) cultivars using the $(GATA)_5$ primer.

A band was considered polymorphic if it was present in at least one ginger cultivar and absent in the others. The bands were scored as (1) for present or (0) for absent. A total of 39 bands were amplified, ranging in size from 185.0 bp to 1.3 kb. Band sizes were calculated with reference to a 1 kb gene ladder using a UV spectrophotometer and gel documentation system (Fluorchem 8900 imaging system, Protiensimple, California, USA). Seven polymorphic bands were scored with a polymorphism rate of 17.9%. This polymorphism rate is low compared with that observed in other studies, which may be due to the small sample size. However, the low polymorphism rate may be expected because ginger is known to exhibit extremely narrow genetic diversity compared with other plants (Keshavachandran *et al.* 2007). Furthermore, low genetic variation is typical in an asexually reproducing species (Hangelbroek *et al.* 2002).

Cluster analysis revealed 87.50% similarity between Bukit Tinggi and Tanjung Sepat, 64.27% similarity between Bukit Tinggi and Sabah and 56.25% similarity between Tanjung Sepat and Sabah. A similarity matrix was generated according to the coefficient of Jaccard (Sneath & Sokal 1973).

$$Sij = M_{11} / (M_{01} + M_{10} + M_{11})$$

where Sij is the Jaccard similarity coefficient, M_{11} is the number of 1-1 matches, M_{10} is the number of 1-0 matches and M_{01} is the number of 0-1 matches (Table 2).

The similarity coefficient value ranged from 0.5625 to 0.8750. The data in the similarity matrix were used to perform a cluster analysis using the unweighted pair group method with arithmetic average (UPGMA). Subsequently, a phylogenetic tree illustrating the calculated relatedness among ginger cultivars was constructed using MINITAB software (release 14, Minitab Inc., USA). Based on these data, the Tanjung Sepat cultivar was genetically more closely related to the Bukit Tinggi cultivar compared with the Sabah cultivar, and the Sabah cultivar

was genetically more closely related to the Tanjung Sepat cultivar (Fig. 5). Palai and Rout (2007) identified eight varieties of ginger using RAPD markers. These authors mentioned that the first major cluster exhibited 43% similarity, whereas the minor cluster exhibited >70% similarity.

Table 2: Similarity coefficients of the three Malaysian ginger cultivars.

Cultivars	M_{11}	M_{01}	M_{10}	Sij
BT-TS	14	2	0	0.8750
BT-SB	9	0	5	0.6427
TS-SB	9	0	7	0.5625

Note: [a] BT: Bukit Tinggi, TS: Tanjung Sepat, SB: Sabah, Sij: Jaccard similarity coefficient

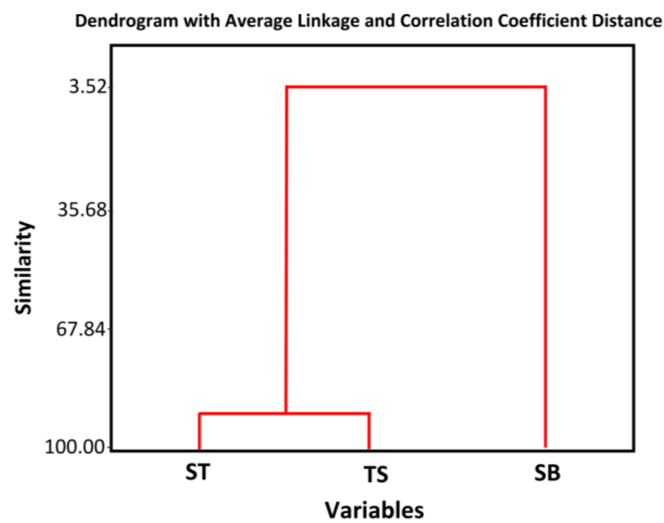

Figure 5: Dendrogram of the three ginger cultivars (Bukit Tinggi, BT; Tanjung Sepat, TS; and Sabah, SB) produced using data obtained with microsatellite DNA markers.

Sequencing of Polymorphic Bands

After separation of the PCR products on a 3% agarose gel, the polymorphic bands of the Tanjung Sepat cultivar were extracted using a gel extraction kit (Qiagen), re-amplified using the same primers and PCR conditions and sequenced. The DNA sequence of the polymorphic bands of Tanjung Sepat contained the characteristic features of a putative gene. According to Toth *et al.* (2000), GC-rich trinucleotide repeat sequences are primarily found within exon regions. The upstream region of the PCR products obtained with the $(GAC)_6$ primer contained a core sequence (CGGCGG), enhancer (CAAT), promoter and TATA box, and a CAAT sequence was identified downstream of the transcription start site. According to Smale and Kadonaga (2003), TATA sequences are recognised by RNA polymerase II for initial transcription. Their locations vary from species to species, although they are predicted to be located 30 to 120 bp upstream of the transcription start site in most eukaryotic genes.

The DNA sequences of the polymorphic bands of Tanjung Sepat were analysed and compared to determine their similarity with accessible published nucleotide sequences using National Centre for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) (retrieved at http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of the polymorphic bands produced with the $(CATA)_5$ primer were most similar to *Brassica napus* subsp. of the family Brassicaceae (96% similarity) and to *Oryza sativa* japonica of the family Poaceae (85% similarity). The sequence of the polymorphic band produced with the $(GAC)_6$ primer was most similar to *Larix lyallii* of the family Pinaceae (94% similarity), *Morella faya* of the family Myricaceae (96% similarity) and *Pinus pinaster* of the family Pinaceae (93% similarity), whereas the sequence of the polymorphic band produced with the $(GATA)_5$ primer was most similar to *Medicago truncatula* of the family Fabaceae (93% similarity) and to *Vitis vinifera* of the family Vitaceae (92% similarity). The putative promoter sequences TATAAAAA, CAAT and CGGCGG, which were observed in the Tanjung Sepat genomic DNA fragment amplified with the $(CATA)_5$ primer, are underlined in DNA sequence below:

Sequence of a polymorphic PCR product amplified from the Tanjung Sepat cultivar using the $(CATA)_5$ primer (774 bases)

1 AGTTCCTACTAGTACGGTGGGATAGAGGTGAGTGATGAATAGTACGTTGA
51 TTGGTGTTAAGTGAAAGACTGCTGCATGACCCCATGAAAAGACTAACTTG
101 CCCGCTTACTGCATCAGGGTACCACTGTGGTCGGGCCCTATAATGAGTCG
151 TGTTTGAATTCACCGAACGTCGTGTTGGGGAAAAACGAGTCCCAAGGAGT
201 AATAGGCACAACGCCAACACCAATCGGCCTTCCCATGGATTGGGGAACGT
251 GCATGGCGAATGGACGCGCCCTGGCCCGGCGCATTGAGCGCGTCGGGTG**C**
301 **GGCGG**ATACGCTCATCTTGACCCTCAACCGTATCTGGGGGTCTATTCATA
351 TGATCCGAAGGGAATTTTGCCGATTTACAGCTCTATTGG**TATAAAAAATG**
401 AGCTTGTTTCAACAATTTTTTTAACGCTGAATTCTTCACAAAAGTTATTC
451 GGGGTAACAATTTTCCCTGGATGCGGTATTTTCCTCCTTAACTCATCAGG
501 GCCGGTAGTTTCACACCCTCGTAAGTGCCCCTCCACAAGACAAATATGCT
551 TCAGAT**GCCGCC**TTAATATAACTCCAACCCCCCCACTTCAGGCCAACTAT
601 CGGTTAACGCGTCCCTCACGGGCTTTTGTCTTGCACACTGTTCTATCGAG
651 CTTCCCACCACAGGGTCTTGGTGGAACGTTCCATCGGGATAGTTCTTGAT
701 GCTGCCCCCTAGAAAAGTTATTATCCCCGCATGTCAATGAAGAGAGAAAA
751 CCCGGGTGAAGAAAACCAAAGAAA (774)

Sequence of a polymorphic PCR product amplified from the Tanjung Sepat cultivar using the $(GATA)_5$ primer (350 bases)

1 GTCGAAGTAAGTGACTCTTTGTGATGTCTTCGATCCTAACTACTCCGGAT
51 GCTTTCGGGCATGGGTTTCTATGTTACATTTAGATGTTTGATCTCTGTGG
101 CTCTCTTTCATTATACTTACTTCCGTCGTTCCTTTCTTATTGTTTGGCGG
151 AACATGGCTATCTTGCGCTTGCGCGAGGTTGAAGGTGGGGGAGCCCCCAA
201 TTTGGGAACCGCCAGCCTGGGGAACTCCCGGA**TTTTTTTTTTT**CCAACCCC
251 TTTATATGAATCCCCCGGAGCTTTGTGGGGAAACACCCGGGATGCCCCAA
301 ATTCCTATCACCAATTAGACAAATCAACCTCCTTCC**AAAAAAAAAAAA**TG (350)

Sequence of a polymorphic PCR product amplified from the Tanjung Sepat cultivar using the $(GAC)_6$ primer (950 bases)

1 ACGAGATAGCATTCGCCCTGGGTGGGTAGCAGTGCACTCACTGGAGGGCG
51 TTGACTACGATTCGACGGGGAGGTTTTGGCCGTCCCAACTGGAAAAACCC
151 GGGGGACGCCCCCCTTAATCCCCTTGGCGTAGTACCCCATAGGCCCTGCGG
201 GCTCTGGCGTCCCAAGGCCCACGCCGATGCCGGGCCCGGGTAGCAGTTGCG
251 GAAT**GGGAAAGGGAAA**CGCACGGT**GGCGGC**ACCTTAGCGTTAGCGGGCGC
301 GGGGGGGGCGCGCACCCGGACCGTGACCGTTGCACTTGCCAATAGCGCCA
351 GCGCCCACTGCTTTCGTCTCTTCCCCTTAAGTTATAGTAAGGGCGCCGCT
401 TTTGCCTTCGAAACGAGGGGTGGGCACCGGGTACGGATCCGATTTTTGCG
451 TTACCGCGACTCCACAATAGTTGATTTGATTGATGGGAGGATTCACGCAC
501 TTTCCCCTCGATTGAAATACTTTATTTTTGGATTTTGGATCAAACTCTAT
551 TAAAAAAAGAACCCATTTTTGATAATTATTGGAACATTAATCAAATTTAG
601 CTCATTTTATTTTTTTTATAAAAAAAATGAATTTTAATTTTTTCAATTTA
651 AAGCAAAATTAAACCAGATTAAAAAAAAAATTTTTTTCCCAAATTTTTAA
701 AAAAAATAATTAACCTTAAATTTTTCCTTTTCAAAATTTTATTCGGTGTA
751 ATTTTTTTGTCGCGTTTTTTTCTCAACCCCAAATGGTTGAACCCTCATTA
801 AAATCCGGCCCAAAACCCCTAAATTAAAACTAACCCCCTAAAAACCCGCA
851 AAACCCTCTGTATACAAGCACCCGACTGGGGATTCTTTTTCCTCCACATC
901 ATGTTATTTTTATATAAAAATAATATTCTCTATTTATCTTCCAAAAATTTC (950)

The specific DNA sequences of the PCR products obtained with the $(GATA)_5$ primer were found to contain poly deoxy thymine nucleotides (dT) and poly deoxy adenine nucleotides (dA) sequences (underlined), which encode phenylalanine and lysine, respectively. The sequences of the polymorphic bands amplified from the Tanjung Sepat cultivar with the $(CATA)_5$ primer also showed characteristic features of a putative gene; however, more detailed study is required to support this hypothesis.

Ginger is a sterile plant that is only vegetatively propagated by rhizomes, and the phenotypic properties of such plants cannot be improved using traditional breeding methods. Thus, the only available solution is the use of "selective breeding" based on the identification of specific genes that are responsible for the desired phenotype. This process requires isolation and cloning of the gene as well as transformation of plant cells with the gene before a plant with new and improved properties can be genetically engineered. Metabolic profiling of the same three ginger cultivars used in this study, which were micropropagated under the same growth conditions, were determined using gas chromatography-mass spectrometry (GC-MS), and chemical variations were detected among the three cultivars (Mahdi *et al.* 2010). It is possible that these chemical variations may be due to underlying genetic variation. This study is considered to be the first step toward further studies in which transgenic ginger plants with improved yield and increased medically active components may be produced.

CONCLUSION

In the current study, microsatellite primers were successfully used to identify genetic polymorphisms among three ginger cultivars (Bukit Tinggi, Tanjung Sepat

and Sabah). Microsatellite primer PCR amplification can therefore be used successfully to differentiate between different ginger cultivars. A cluster analysis of polymorphic bands using MINITAB software and the Jaccard similarity coefficient revealed that Bukit Tinggi ginger is more closely genetically related to Tanjung Sepat ginger than to Sabah ginger. Additionally, this genetic polymorphism may be responsible for the quantitative and qualitative variations in chemical constituents that were detected in previous studies. Further investigations are required to support this hypothesis.

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