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Full Length Research Paper

Assessment of inter simple sequence repeat (ISSR) technique in mangosteen (*Garcinia mangostana* L.) grown in different Sumatra region

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Inter-simple sequence repeat (ISSR) markers were used to examine the level of genetic diversity in mangosteen. Twenty three accessions of the *Garcinia mangostana* collection from Sumatra region were screened for ISSR markers. Eleven random ISSR primers were chosen to differentiate the investigated accessions. The primers generated 72 bands of which 42 (58%) were polymorphic and 30 bands (42%) monomorphic. From the 11 primers tested, two primers were monomorphic. Seven of the nine polymorphic primers produced fingerprint profiles unique to the RT accession from Tembilahan (Riau Province). Cluster analysis divided the accessions into two major groups with genetic similarity coefficient ranging from 0.44 - 0.96. The first group contained only cultivar RT (elliptical stigma lobe) and the second group consisted of 22 accessions (round stigma lobe) which could be divided clearly into six sub-clusters. One of the sub-clusters contained accessions from the same region of Bangka Island and the others contained combination of accessions derived from different locations. The result shows that mangosteen accessions with different genetic background exist in this region. This confirms the general opinion that mangosteen is uniform in genetic.

Key words: Inter-simple sequence repeat, mangosteen, genetic similarity.

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is a unique plant of tropical fruit. Their origin and distribution extremely abundant across the area in Indonesia and South East Asia. This crop classified in Clusiaceae family (Lindley, 1836; cit. ZippcodeZoo.com, 2009) is an apomictic fruit species, from which the seed develops without fertilization. Most of tropical fruit species belong to facultative apomict, while the mangosteen is an obligate apomict and it is believed that all of its progenies may have the same genotype as their mother plant (Richards,. 1990a; Koltunow et al., 1993). Because of the antiquity, attractiveness and good economical value of mangosteen, researcher have become more interested in them recently.

The rule of genetic diversity in mangosteen, which will facilitate the effective conservation for this crop, is not clear-cut. Current research displays phenotypic and genotypic variability among mangosteen populations. Phenotypic variations found such as canopy shape, fruit shape, inflorescence, pedicel length, stigma lobe, etc (Mansyah et al., 2003a) . Genetic variations occurred among 23 mangosteen accessions from Java and Sumatra by using Random Amplified Polymorphic DNA (RAPD) technique (Mansyah et al., 2003b). Genetic variations were also occurred among the progenies themselves and between the progenies and the mother plant both in polyembyonic and monoembryonic seedlings (Mansyah et al., 2008). Ramage et al. (2004) found genetic diversity among 37 *G. mangostana* accessions, nine different genotypes were identified clustered into three distinct groups.

From these reports there is a need to clarify the extent of genetic variation within *G. Mangostana* and whether these molecular marker-based variations are correlated to accessions or any other traits. Inter-simple sequence repeat (ISSR) are semiarbitrary markers amplified by polymerase chain reaction (PCR) in the presence of one

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Table 1. Mangosteen accessions used in this study.

Accession code	Origin	Primary morphological traits
RT*	Tembilahan, (Riau)	Elliptical fruits, short stalk, large and elliptical stigma lobe, flat base.
RK * BK	Bukittinggi (West Sumatra) Rejang Lebong, Bengkulu)	Ovoid fruits, long stalk, small and round stigma lobe, pointed base.
K5 S6	Bukittinggi (West Sumatra) Payakumbuh (West Sumatra)	Round fruits, medium stalk, medium and round stigma lobe, round base.
SR S4	Alahan Panjang (West Sumatra) Payakumbuh (West Sumatra)	Round fruits, medium stalk, large and round stigma lobe, round base.
B2	Bangka	Elliptical fruits, medium fruit stalk, large and round stigma lobe, flat base
SG, KP L7, Ki6, Ki8 B3 B5, B6, B8. B11, KPS KG*	Painan (West Sumatra); Lahat, (South Sumatra); Bangka Kaligesing (West Java)	Elliptical fruits, medium stalk, medium and round stigma lobe, flat base.
L8 B10,B12	Lahat, (South Sumatra); Bangka	Elliptical fruits, medium fruit stalk, small and round stigma lobe, flat base.

*Released variety.

primer complementary to a target microsatellite, it does not require genome sequence information, which leads to multilocus, highly polymorphous patterns and produces dominant markers (Zietkiewicz et al., 1994; Mishra et al., 2003). ISSR were more informative than RAPD in wheat, fruit plants (strawberry, apple and Ribes species) and the common bean for the evaluation of genetic diversity. (Korbin et al., 2002; Rakoczy-Trojanowska et al., 2004). This markers are reproducible, and quick for characterization many cultivars like poplar (Gao et al., 2006), bean (Gonzales et al., 2005), cycads (Xiao et al., 2005), study the relationships of red, small and big ginger (Wahyuni et al., 2004), and *Fusarium culmorum* isolates (Mishra et al., 2003).

In this study, we present the results of ISSR variation of several mangosteen accessions mainly grown in Sumatra region including three released varieties. The objective of this study is fingerprinting and identification of selected important mangosteen accessions and determination of the genetic relationships among these accessions.

MATERIALS AND METHODS

DNA was isolated from 23 mangosteen accessions of Indonesian Tropical Fruit Research Institute, Solok–West Sumatra (Table 1), collected from different Sumatera region and one from central Java (Figure 1) . About 0.1 mg fresh leaflets were grind for DNA extraction. Total DNA was extracted according to the modified CTAB protocol (Doyle and Doyle, 1987) by addition 1% of polyvinyl polypyrolidone (PVPP). DNA concentrations were determined with electrophoresis in agarose gel, ethidium bromide staining solution and visualization on ultraviolet (UV) transilluminator DNA was then used by PCR-amplified by using eleven ISSR primers obtained from the PKBT-IPB laboratory (Table 2) in a 96-well Applied Biosystems 2720 thermal cycler.

Reactions were carried out in a total volume of 25 µl consisting of 2 µl (20 ng) of template DNA, 12.5 µl Go Taq Green Master Mix (Promega M7122), 1 µl primer (20 µM), and 9.5 µl free nuclease water. Amplification was performed under the following conditions: 4 min at 94°C for 1 cycle, followed by 0.5 min at 94°C, 0.5 min at annealing temperature (depend on primer used), and 1 min at 72°C for 35 cycles, and 5 min at 72°C for final extension. PCR products were separated on 1.2% agarose gel and 1X TAE buffer solution, stained with ethidium bromide and visualized with UV light and then scored as present (1) or absent (0) for the 23 accessions. Genetic diversity and the relationship between accessions were calculated through Jaccard coefficient and a sequential, agglo- merative, hierarchical, and nested (SAHN) cluster analysis. This analysis performed using the un-weighted pair group method with arithmetic means (UPGMA) algorithm computed by NTSYS-pc (Numerical Taxonomy and Multivariate Analysis) softwares version 2.1 (Rohlf, 2000).

RESULTS

Polymorphism of inter-simple sequence repeat markers

Table 3 describes polymorphism in twenty three mangosteen accessions revealed by ISSR. Eleven ISSR primers, including nine 3'-anchored di-nuceotides, one

Figure 1. Location sites of mangosteen accessions in Sumatra Island.

Table 2. Primers used for ISSR amplification.

Primer	Sequence	Annealing temperature (°C)
PKBT-2	(AC)8TT	51
PKBT-3	(AG)8T	51
PKBT-4	(AG)8AA	51
PKBT-5	(AG)8TA	51
PKBT-7	(GA)9-A	51
PKBT-8	(GA)9-C	54
PKBT-10	(GT)9-A	54
PKBT-11	(GT)9-C	54
PKBT-12	(GT)9-T	54
PKBT-14	CCCGGATCC(GA)9	53
ISSRED -14	(GACA)4	53

5'anchored di-nucleotide and one un-anchored tetra-nucleotide yielded a total of 72 bands, with a maximum of 11 for PKBT-11 $(GT)_9$ C, and a minimum of 2 for PKBT 12 (GT)9-T. The 72 markers generated consist of 42 (52%) polymorphic bands and 30 (42%) monomorphic bands, ranged in size from 250 to 2200 bp. From the 11 primers tested, two primers (PKBT-5 and PKBT-14) were monomorphic and nine others were polymorphic.

Seven of the nine polymorphic primers produced fingerprint profiles unique to the RT accession, which is grown in swamp area of Riau Province. These unique fingerprint profiles indicated by loss and addition of a certain bands. There were specific additional bands, approximately at 875, 815 and 700 bp by primer PKBT-7 (Figure 3d), and 450 bp by primer PKBT -2 (Figure 3a) found in RT accession. In addition RT is the only one accession that showed loss of two bands, 1200 and 750 bp by primer PKBT-4 (Figure 3c). In relation to other accessions, RT showed loss of 2 bands 875 and 600 bp for PKBT-10 (Figure 2b), and one band 950 bp for PKBT-11 (Figure 2c) together with Ki6 accession.

The other accessions also showed specific profiles for certain primers. For example S6 accessions loss one band 1200 bp in size for primer PKBT-7 (Figure 3d). BK, KP, B10 and SR accessions loss 800 bp band for PKBT-12 (Figure 3e). KG, L7, and B12 accessions showed loss in 1250 bp fragment, while B3, B10 and SR loss in 600 bp for by PKBT-2 primer (Figure 3a). Most variations

Table 3. The amplification products by eleven ISSR primers in 23 mangosteen accessions.

Primers	Sequence	Total scorable bands	Polymorphic bands	Monomorphic bands
PKBT-2	(AC)8TT	9	8	1
PKBT-3	(AG)8T	5	5	0
PKBT-4	(AG)8AA	5	2	3
PKBT-5	(AG)8TA	6	0	6
PKBT-7	(GA)9-A	7	5	2
PKBT-8	(GA)9-C	9	5	4
PKBT-10	(GT)9-A	6	3	3
PKBT-11	(GT)9-C	11	10	1
PKBT-12	(GT)9-T	2	1	1
PKBT-14	CCCGGATCC(GA)9	6	0	6
ISSRED -14	(GACA)4	6	3	3
	Total	72	42	30
	Average	6.54	3.82	2.73

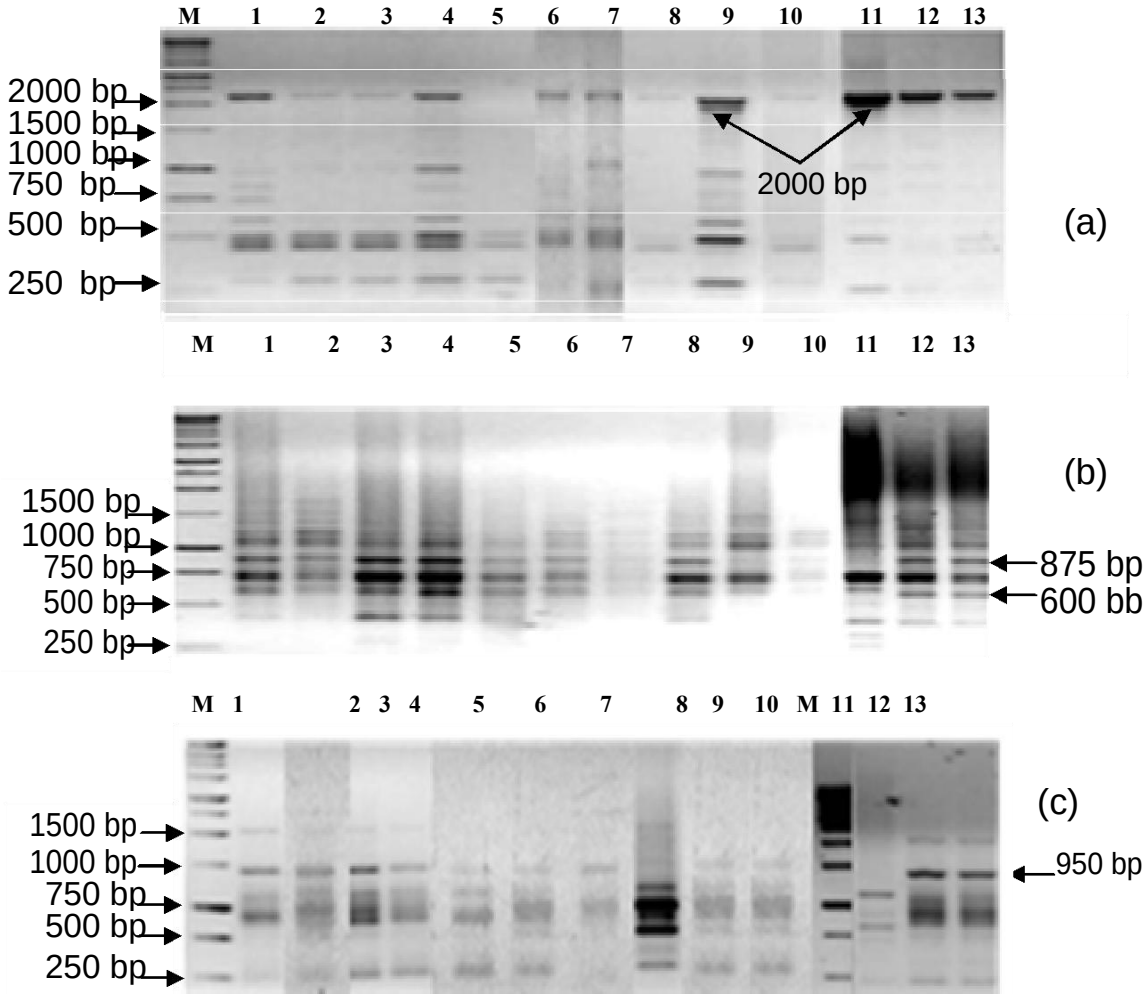

Figure 2. ISSR fingerprint pattern 13 mangosteen accessions generated using three ISSR primers, (a) PKBT- 8, (b) PKBT-10 and (c) PKBT 11, M; Marker, lane 1; BK, lane 2; L7, lane 3; KG, lane 4; ; L8, lane 5; SG, lane 6; S6, lane 7; K5, lane 8; KP, lane 9; Ki6, lane 10; Ki8, lane 11 RT, lane 12; RK, and lane 13; KPS (Arrows indicated addition and loss bands for RT accession).

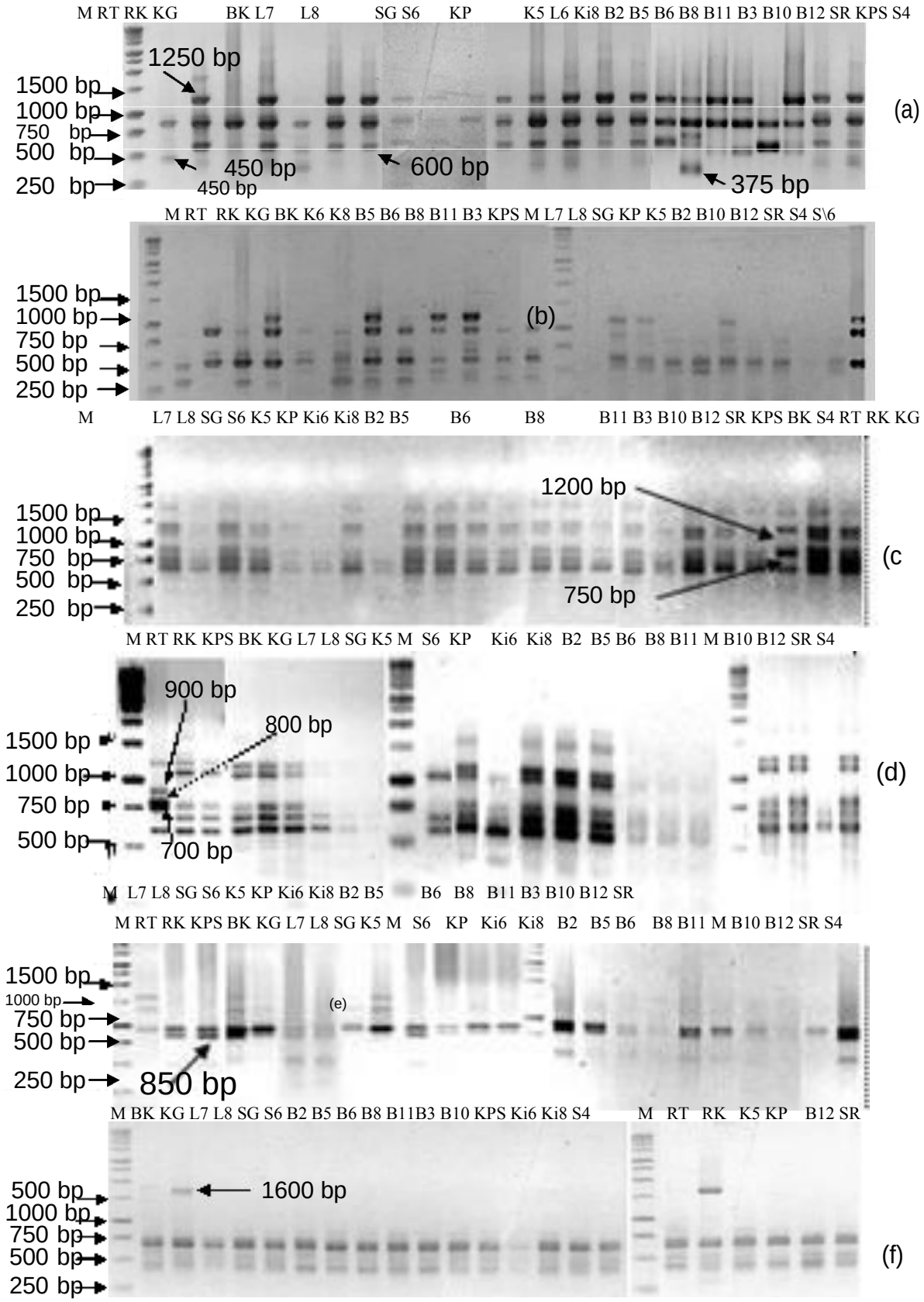

Figure 3. ISSR fingerprint patterns 23 mangosteen accessions generated using primers (a) PKBT-2, (b) PKBT-3, (c) PKBT-4, (d) PKBT-7, (e) PKBT-12 and (f) ISSRED-14. The specific bands are marked by arrows.

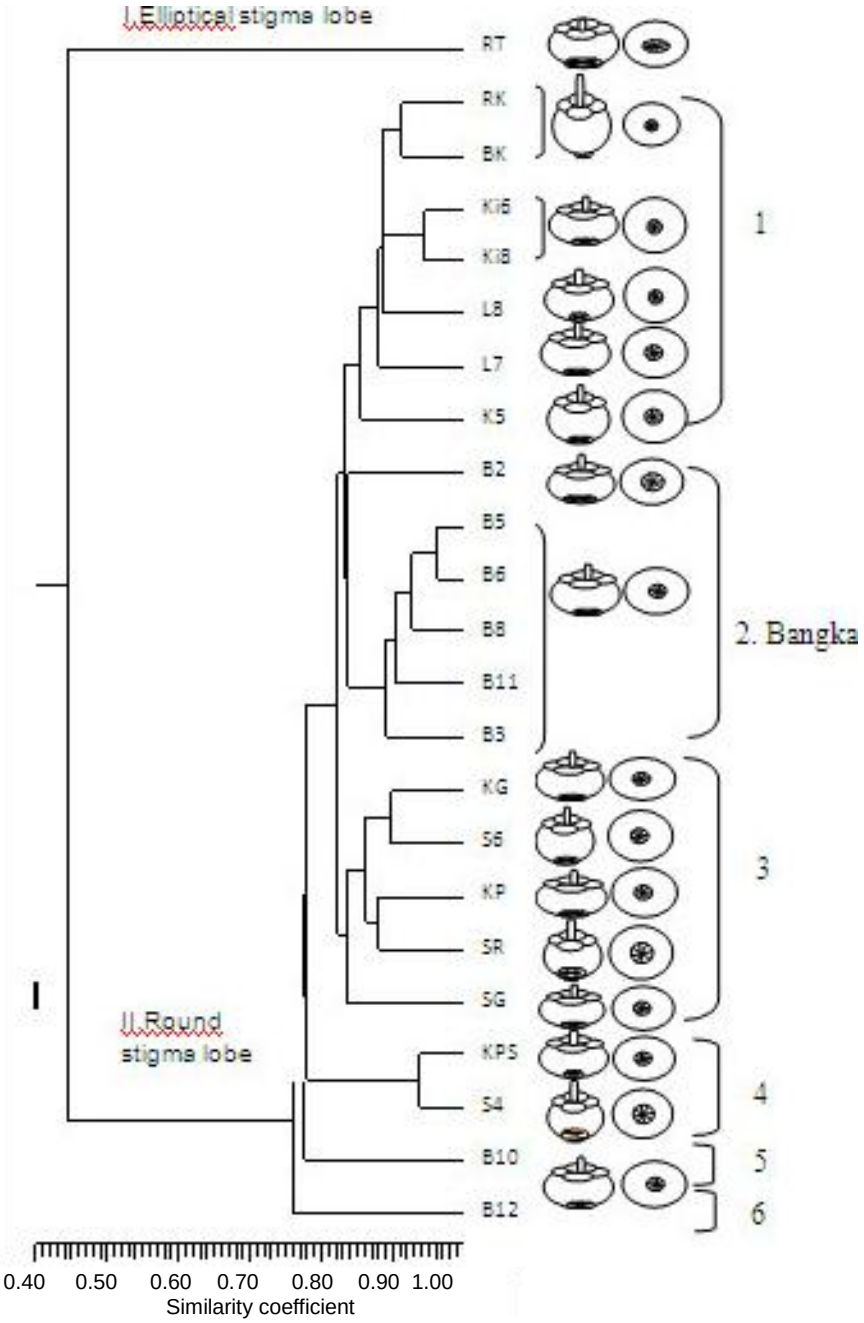

Figure 4. Dendrogram of mangosteen accessions based on ISSR markers.

were exhibited by primer PKBT -3. The primer produced five bands and all of them were polymorphic (Figure 3b). Specific band 1600 bp by primer ISSRED-14 found in KG and RK accessions (Figure 3f).

Genetic relationships among accessions

Genetic similarity among the 23 mangosteen accessions based on ISSR analysis varied from 0.44 - 0.96 (Figure 4) Cluster analysis divided the accessions into two major groups. The first group contained only cultivar RT from Tembilahan (Riau Province) with elliptical fruit and stigma lobe and 44% in genetic similarity from other accessions. The second group consisted of 22 accessions with round stigma lobe and genetic similarity ranged from 0.71 - 0.96. This group is divided clearly into six sub-clusters that is (1). Contained two ovoid fruits accessions and small stigma lobe, BK and RK, 0.91 in genetic similarity, four elliptical fruit accessions from South Sumatra (Ki6, Ki8, L7, L8)

and one round fruits accession from Kamang West Sumatra (K5), (2). Six elliptical fruits accessions from Bangka, B2, B5, B6, B8, B11 and B3, with genetic similarity 0.89 - 0.96, (3). including 5 accessions with elliptical (KG, KP, SG) and round fruits (S6 and SR), 0.82 of similarity, (4) had accession KPS (elliptical and irregular fruits) and S4 (round fruits), 0.93 of similarity. Two accessions from Bangka B10 (elliptical fruits and small stigma lobe) and B12 (elliptical fruit and medium stigma lobe) segregated into different sub-clusters (5) and (6).

The result show that the genetic similarity of mangosteen from the same region occurred. Based on the cluster analysis, the ISSR primers could be used to identify the mangosteen, but not able to construct good clustering based on morphological characters. Further research is necessary to obtain more detail information concerning the relationships between genetic and morphology variation on mangosteen by using more primers

DISCUSSION

In this study, ISSR markers revealed a few number of polymorphic bands with an average of 3.82 bands per primer compared to 17 bands on the study of sweet potato (He et al., 2009) and 43 for the genus *Populus* L. (Gao et al., 2006). This condition is suggested related to apomictic behavior of the mangosteen. Gonzalez et al. (2005) reported that the level polymorphism of ISSR is dependent on the plant species and the type of simple sequence repeat incorporated in the ISSR primer used. Rakoczy-Trojanowska and Bolibok (2004) reported that ISSR produced highly polymorphic pattern per reaction. Blair et al. (1999) noticed that ISSRs usually amplify 25 to 50 products in one reaction. In rice, a higher percentage of polymorphic bands was produced with the ISSR technique than with amplified fragment length polymorphism (AFLP).

Genetic similarity among the 23 mangosteen accessions based on ISSR analysis varied from 0.44 to 0.96. This result is lower than genetic similarity obtained by RAPD analysis (0.71 - 1.00) (Mansyah et al., 2003b), and similar to AFLP analysis (0.46 - 0.77) (Sobir et al., 2009). The result indicated that ISSR analyzes more effective than RAPD in differentiating genetic diversity of mangosteen. Several characteristics make ISSR noticed as a useful marker are very powerful to detect polymorphism, scan and amplify fragments that are dispersed throughout the genome, inexpensive and easy to generate (Gonzales et al., 2005).

The polymorphism and genetic diversity observed may result from several events. Rhichards (1997) explained that variation in obligate apomixis can occur because one or more of the following mechanisms: (1). accumulation of DNA mutations, (2). accumulation of all the changes to the cytology through disjungsional accident resulting in the deviation polyploids, haploid, polisomic, and oligosomic among derivatives, (3). somatic recombination resulting from chromosomal translocations, and (4). mutations or basic changes in the chromosomal of maternal genome that control apomictic behaviour. Some other information on apomixis mentioned that variations can be caused by the presence of transposable elements. Lines undergoes these events can vary and develop without the intervention of sexual.

The high ploidy levels can also trigger the occurrence of mutations. Tixier (1955) reported that mangosteen is allotetraploid apomictic plants with chromosome number 2n = 96. Rhichards (1990b) suggest that mangosteen has two close related species *Garcinia hombroniana* (2n = 48) and *Garcinia malaccensis* (2n = 42) which are facultative agamospermy. Cytological studies show that mangosteen may be the allotetraploid derivatives of the two species. The relationship between ploidy level and mutation has been widely described. Otto (2007) explained that polyploidization, the addition of a complete set of chromosomes to the genome, represents one of the most dramatic mutations known to occur. Polyploids suffer more from recurrent deleterious mutations than diploid. Adams and Jonathan (2005) stated that polyploidy is an ancient and recurrent process leading to differential gene loss, rapid genomic alterations and has extensive effects on gene expression. Wendel (2000) noticed that a surprising feature of many newly formed polyploids is that their genomes are unstable and undergo rapid reparterning. Mansyah et al. (2008) also found that mangosteen can produce wide variation in its progeny.

In conclusion, the variability of ISSR fingerprinting patterns and relationships observed using the ISSR-based dendrogram may provide information on the morphology and genetic variability of mangosteen grown in Sumatra region. Different genetic similarity among the mangosteen indicated that there are many genetic resources of mangosteen in Indonesia. There was some evidence that the low genetic similarity between ellipse and round stigma lobe accessions and high genetic similarity within ovoid cultivars occurred. Different and numerous mangosteen accessions are important in providing new variety for further improvement. The accessions which have good performance are interest for future breeding work and it is possible for breeders to select the accessions with morphological traits.

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