Genetic Diversity of Local Accessions of *Dimocarpus longan* Revealed By ISSR Markers

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ABSTRAK

Keragaman Genetik Aksesi Lenggkeng Lokal *Dimocarpus longan* Berdasarkan Penanda ISSR. Lengkeng telah berkembang di Indonesia, namun studi tentang tanaman ini belum banyak dilakukan. Studi untuk mengetahui keragaman genetik lengkeng lokal telah dilakukan di Balai Penelitian Tanaman Jeruk dan Buah Subtropika (Balitjestro) pada bulan Maret-April 2008. Penanda ISSR digunakan untuk mengidentifikasi keragaman genetik antar delapan genotipe lengkeng lokal yang telah dikoleksi oleh Balitjestro. Dendogram yang dibuat berdasarkan UPGMA menggunakan metode SAHN menunjukkan nilai kemiripan sebesar 0,34-0,86. Nilai kemiripan tertinggi diperoleh dari KL I dan KL II (0,86) dan nilai kemiripan terendah diperoleh dari KL I dan KL V/KL VI (0,34). Penanda ISSR dapat digunakan untuk mengidentifikasi lengkeng dan dapat memberikan informasi yang berguna untuk program pemuliaan lengkeng di kemudian hari.

Kata kunci: *Dimocarpus longan*, keragaman genetik, penanda ISSR, nilai kemiripan.

ABSTRACT

There is no clear history of longan development in Indonesia even though it has developed well in Indonesia for many years. The study aimed at revealing genetic of diversity of local longan was done in Indonesian Citrus and Subtropical Research Institute (ICSFRI) from March to April 2008. ISSR markers were employed to identify the genetic variation among eight morphologically more or less alike accessions of longan ssp. Matrix data was counted and dendogram of samples was constructed using UPGMA and SAHN method. The cluster showed similarity value 0.34-0.86. The highest similarity value was observed between KL I and KL II (0.86) while the lowest one was obtained between KL I and KL V/KL VI (0.34). ISSR markers were able to identify the genetic diversity of longan and were helpful to provide information on genetic diversities especially for future breeding programs.

Keywords: *Dimocarpus longan*, genetic diversity, ISSR markers, similarity value.

INTRODUCTION

Longan, literally meaning 'dragon's eye' due to its round and bright black seed, has a documented history of more than 2200 years in China (Huang *et al.*, 2005). Over this period, 300 varieties have been selected (Lin *et al.*, 2005).

In Indonesia, longan has no clear history about its initial cultivation and development. Longan mostly found in medium-high land, for example in Ambarawa, Temanggung, and Malang. It is believed that longan development begin from those regions and then spread to other area. It is considered because of their subtropical climate which is similar to their origin in China. Longans are predominantly subtropical, requiring a definitive change of seasons for flowering, but are planted in the tropics as well (Puri, 2001).

Longan diversity and its distribution in Indonesia have not been well observed. There are many longan cultivars in Indonesia which known by societies by its local name, mostly refer to where it's found or grown. Even though longan has been developed for long time in Indonesia, researches and observations about the plant are still scarce. According to Directorate of Fruit Crops (2005), there are only two local longans that have been released by the authority, which are Batu and Selarong varieties.

The characterizations of longan in Indonesia are difficult because of unclear history of their first arrival and development. Such effort to trace the history is uneasy due to lack of information. Using morphological characters to identify accessions could be very confusing because such morphological traits largely depend on environmental conditions (Yonemoto *et al.*, 2006). Therefore,

molecular marker was used to identify and characterize the germplasm. A molecular marker is a genetic difference that may exist between two plants, visualized by means of biochemical methods and located at a certain chromosomal position (Ruiz *et al.*, 2000).

One of molecular markers that could be used for genetic identification is ISSR. Inter-simple sequence repeat (ISSR) amplification can rapidly differentiate closely related individuals (Zietkiewcz *et al*., 1994). ISSR analysis is easy to adopt since primers can be derived from existing literature on SSRs in plants, as well as from sequence database information. The primers do not need to be locus-specific since they will target any region of the genome that contains a complementary micro-satellites motif (Wu *et al.*, 1994). ISSR markers have been used to investigate many plants, such as chrysanthemum, *Eleusine*, oilseed rape (Fang *et al.*, 1997), apple (Sugiyatno and Agisimanto, 2007) and citrus (Fang and Rose, 1997; Scarano *et al.*, 2002; Tusa *et al.*, 2002).

Genetic diversity which provides high genetic materials is important for breeding program of longan. This preliminary study was to characterize and identify longan germplasm which have been collected by ICSFRI from several regions in Java by molecular marker and also to observe the use of ISSR markers to fingerprint the longan accessions.

MATERIALS AND METHODS

The study was carried out at Plant Breeding Laboratory of ICSFRI Batu-East Java in March-April 2008. Plant materials obtained from Tlekung Experimental Field of ICSFRI consist of eight local longan accessions as listed in Table 1.

Table 1. Origin of accessions.

Accessions	Origin/place of collection
KL I	Temanggung, Central Java
KL II	Poncokusumo, East Java
Selarong	Bantul, Yogyakarta
KL III	Blitar, East Java
Batu	Pringsurat-Temanggung, Central Java
KL IV	Batu, East Java
KL V	Purworejo, Central Java
KL VI	Batu, East Java

DNA Extraction and ISSR-PCR

Total genomic DNA of each accession was extracted from young leaves using the CTAB method proposed by Deng *et al.* (1995) with minor modification. The PCR was carried out in ThermoCycler (Biometra) programmed to run the following temperature profile: a predenaturation for 3 min at 94^{o}C, then 28 cycles each consisting of a denaturation step for 1 min at 95^{o}C, annealing step for 1 min at 45^{o}C, an extension step for 2 min at 72^{o}C. Amplification was terminated by a final extension of 7 min at 72^{o}C. PCR products were electrophoresed in 1,5% agarose gels in 0,5x TBE buffer at 50 V. The gels were stained with ethidium bromide for 50 min, viewed and documented in BioDocAnalyze (Biometra).

Scoring and Data Analysis

The bands observed from photographs were manually scored as 1 for presence and 0 for absence. The scores were entered into a database program (NTedit) and compiled in a binary matrix for statistical analysis. The unweighted pair-group method arithmetic averages (UPGMA) cluster analysis was formed using SAHN method, and relationships among genotypes were visualized as a dendogram using the NTSYS-pc Exeter Software version 2.1.

RESULTS AND DISCUSSION

Table 1 showed the origin of local longan accessions which were used in the present study. Those accessions mostly found at medium-highland about 600-950 m above sea level, except Selarong. Selarong was found at near Cave Selarong Bantul Yogyakarta which is only approximately 50 m above sea level. It is a local low land longan accession of Indonesia.

The local accessions used in this study are morphologically difficult to differentiate except for KL II and KL VI which have dark green and big leaf size. Therefore to identify the cultivars, molecular markers are more reliable.

Some bands observed on the gel were fuzzy and not clear cut. It makes the observation more

difficult. As shown on Figure 1, no band of KL IV appeared at the gel. It is assumed that it was due to the poor quality of DNA. Thus, to avoid bias on the assessment, KL IV was excluded in data scoring.

Number of bands produced by ISSR primers showed in Table 2. Of five primers, only one primer (ISSRa) produced monomorphic bands. It seemed that the primers were able to detect the genetic differences among the local longan accessions. However for accurate result, the polymorphism at all loci must be confirmed by using more markers.

In order to get comprehensive knowledge of the microsatellites presence in longan, other primers with different nucleotides have to be included on next study. Wang *et al.* (1994) reported that the most abundant microsatellites in plant nuclear genomes is $(AT)_n$. Therefore the next study will include the $(AT)_n$ based primers to observe their amplification products.

Many factors influence the PCR amplification results such as amplification condition, DNA quality and quantity, suitability of primers and also the electrophoreses condition. The PCR should be optimized by changing the temperature or increasing the number of cycle, DNA quality and quantity can be improved by using more suitable extraction method for longan (since Deng's method was proposed for citrus) and electrophoreses condition has to be adjusted.

Cluster analysis of the genetic similarity values was performed to generate a dendogram which reflected the genetic relationship among accessions. The dendogram (Figure 2) showed genetic similarities among local longan accessions about 0.34-0.86. The highest similarity value was observed between KL I and KL II (0.86) and the lowest similarity value was obtained between KL I and KL V/KL VI (0.34). Those accessions that showed closed relationship either KL I and KL II or KL V and KL VI were taken from different region which lie separately. It means that there are some probabilities that in the past the seed may be taken or brought from one region to other region that helped the longan spread around Java Island.

Based on field observation in Ambarawa and Malang, it is assumed that longan initially

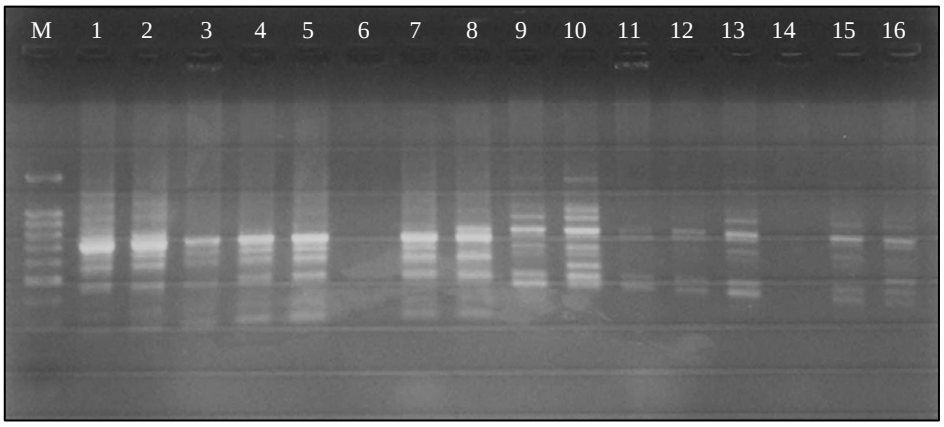

Figure 1. DNA bands pattern of local longan (M is the DNA marker. Lane 1-8/9-16 correspond to ID for accession listed in Table 1. 1-8 amplified by ISSRa while 9-16 amplified by ISSRe).

Table 2. Amplification result of ISSR markers on longan accessions.

Primers	Polymorphic bands	Monomorphic bands
ISSRa {HVH$(CA)_7$}	2	4
ISSRb {$(AC)_8$YA}	4	0
ISSRc {$(AC)_8$YG}	10	0
ISSRd {HVH$(TCC)_5$}	10	0
ISSRe {$(TCC)_5$RY}	9	0
Total	35	4

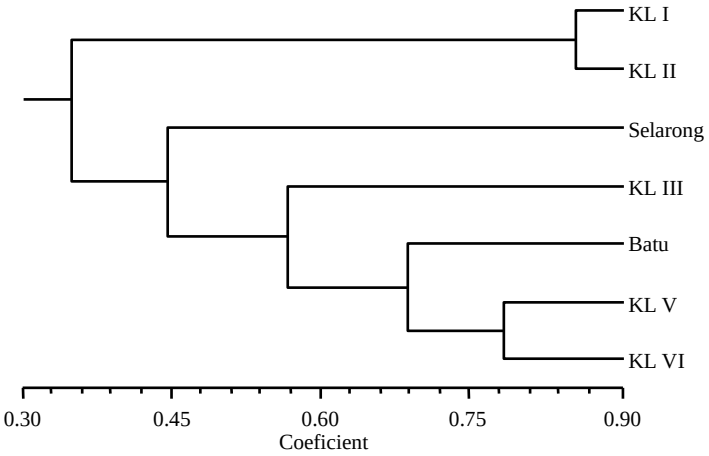

Figure 2. Cluster dendogram of longan accessions based on ISSR markers.

developed at Ambarawa and its surrounding and then developed later in other region (M. Ridwan, personal communication, 24 April 2007). In the beginning, the propagation material used not only vegetative part but also seed. The open pollinated seeds, since longan is cross-pollinated and has three sexually different functions of flowers, were contributed to high variability on longan.

In the present study, Selarong was developed from open pollinated seed of Batu. Batu is an old longan variety that found abundant in highland of Central Java, especially in Pringsurat-Temanggung, where the mother plant is located. Batu has been released as a prime variety in 1997 while Selarong has been released in 1998 (Badan Pengawasan Sertifikasi Benih, 1998). Planted in 1967, the mother plant of Selarong showed its capability to adapt to lowland climate in Yogyakarta that is different from the climate of place where its parents found.

Nowadays, longan propagation is mostly done by vegetative propagation. Therefore, accurate identification is crucial for either nurseries or growers. ISSR markers can realize this accurate identification rapidly. But to gain comprehensive result, further studies with different markers and more accessions are needed to obtain satisfying and comprehensive result of ISSR employment on longan identification.

CONCLUSION

Local longans collected by ICSFRI were genetically related with 0,34-0,86 similarity value. The highest similarity value was observed between KL I and KL II (0,86) while the lowest one was obtained between KL I and KL V/KL VI (0,34). ISSR markers were able to identify the genetic diversity of longan and were helpful to provide information on genetic diversities especially for future breeding programs.

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