

The assessment of genetic variability and taxonomic affinity of local pummelo accessions from Yogyakarta, Indonesia based on RAPD

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Abstract

Pummelo (*Citrus maxima*) is one of three biotypes considered as true species within the genus *Citrus*. A major issue of pummelo taxonomy in Indonesia is the high number of cultivars showing variability in phenotypic characters but of unknown genetic diversity. In this study, the assessment of genetic variability and taxonomic affinity of local accessions of *C. maxima* from Yogyakarta was examined based on RAPD fingerprinting. The availability of universal primers and technical simplicity makes RAPD as a molecular tool of choice for the assessment of genetic variability at various taxonomic levels. In this study, 13 accessions of *C. maxima* collected from Yogyakarta were observed for their genetic variability. An additional three registered cultivars were included for comparative purpose. Two decamer primers used for the amplification of DNA produced 222 bands with 174 of them were polymorphic. The data was subjected to cluster analysis to observe the grouping of accessions and taxonomic affinity. Results indicated high genetic variability among accessions. The dendrogram constructed using UPGMA method based on simple matching coefficient showed two main clusters were which was in line to morphological characters. The grouping of accessions showed clear differentiation between accessions bearing white and those with reddish fruit flesh, and thus demonstrates taxonomic value of this study in recognizing important agronomic character for this tropical fruit crop.

Keywords: *Citrus maxima*, taxonomic affinity, genetic variability, RAPD.

Introduction

Citrus spp. is a group of tropical fruits with high potential as an important agricultural commodity in many countries. The importance of *Citrus* spp. could be observed from its management efforts in some Asian countries under the program of *Global Environment Facilities* (GEF). This program was developed for the documentation and conservation of exotic tropical fruits, including mango (*Mangifera indica*), citrus (*Citrus* spp.), mangosteen (*Garcinia mangostana*), and rambutan (*Nephelium lappaceum*) (GEF, 2006).

Pummelo (*Citrus maxima* (Burm.) Merr.) is one species of citrus originated from

tropical areas of Asia, and known as having high phenotypic variability (Niyomdham, 1992). Pummelo grows well on various soil types at lowland areas to the altitude of 400 m above sea level, and could be harvested twice to four times a year. One of the serious problems of pummelo in Indonesia is the decline of its genetic variability. Arora (2000) suggested that there was an indication on the decline of pummelo variability in Indonesia and Malaysia as a result of genetic erosion. A possible cause of this decline is lack of concern in preserving pummelo which is considered as underutilized citrus fruits due to its relatively low economic value compared to sweet orange and mandarins.

A common problem threatening diversity of tropical fruit species and their uncultivated close relatives exist in natural habitat as landraces also caused by habitat destruction as a result of unsustainable

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development (Trinh *et al.*, 2003; GEF, 2006). Inventory study on pummelo genetic variability is thus worth particular attention to prevent this potential fruit crop from vanishing before its biological potential is fully discovered.

DNA fingerprinting was defined as any multilocus approach of visualizing DNA polymorphisms, either generated from hybridization or PCR-based techniques (Weising *et al.*, 1995). Random amplified polymorphic DNA (RAPD) developed by Williams *et al.* (1990) is a PCR-based fingerprinting technique which uses single primer of arbitrary nucleotide sequence to amplify random DNA segments. The application of RAPD in the assessment of genetic variability studies has been reported at various taxonomic levels, ranging from familial (Prasad, 2014), generic (El-Mouei *et al.*, 2011), to species and cultivar levels (Baig *et al.*, 2009).

Previous research on genetic variability of pummelo in Indonesia is still limited to a small number of cultivars from production center areas such as Magetan, East Java as reported by Karsinah *et al.* (2002), Purwanto *et al.* (2005), Rakhman *et al.* (2005), and Agisismanto & Supriyanto (2007). These studies were conducted only on widely cultivated pummelos, and to this point there was no record on the studies on pummelo landraces occurred as home yard plants. The objective of this study is, therefore, assessing genetic variability of pummelo accessions occurred as home yard plants from Yogyakarta which is assumed to host some local landraces.

Materials and Methods

Leaves samples were collected from healthy pummelo trees from Gunungkidul, Yogyakarta. Fresh leaves were washed to remove dirt followed by surface sterilization using 70% ethanol. DNA extraction from 0.2 g of fresh leaf sample was done using DNAMITE® Plant Kit Extraction (Microzone, UK) according to manufacturer's protocol.

RAPD reaction was performed in 25 µL tube containing 20 µL volume of reaction mixture with 0.2 µM of primer and 15 ng of genomic DNA. The amplification reaction were carried out using a Biorad™ thermal cycler with the following cycling program: 5 min denaturation step at 95°C; 45 cycles of 30 s at 94°C, 45 s at 36°C, 1 min at 72°C; and 5 min at 72°C for the final elongation step. Six RAPD primers were screened for their suitability in amplifying *C.maxima* DNA. These six primers were OPA-02, OPE-04, OPK-14, OPN-14, OPN-16, and OPW-19. The criteria for determining primer suitability were the number of amplified DNA fragments, their reproducibility, and their quality, in terms of producing clear and unambiguous DNA banding patterns. Two decamer primers were finally chosen based on these criteria: OPA-02 ('TGCCGAGCTG') and OPN-16 ('AAGCCGACCTG'). PCR products are visualized on 2% agarose in 1x TBE buffer, using *Good View* (Microzone, UK) as nucleic acid stain. The gel was run on electrophoresis apparatus on 110V for 45 min. Gel picture was observed under UV illuminator and documented using digital camera. The banding pattern of DNA fragment was coded numerically to produce binary data representing absence (0) or presence (1) of a band at particular molecular weight. The data was then subjected to cluster analysis using MVSP 3.1 (Kovach, 2007).

Results and Discussion

Two primers used in this study were successfully amplified DNA samples from 13 accessions of pummelo from Yogyakarta, and additional three registered pummelo cultivars ("Pameloma Magetan", "Pangkajene Merah", and "Pangkajene Putih"). A total of 222 scorable bands were produced, with 174 being polymorphic (Figure 1). The reproducibility of RAPD was assured from identical results of banding patterns from two consecutive amplification reactions.

Taxonomic affinity of pummelo accessions was examined based on result of

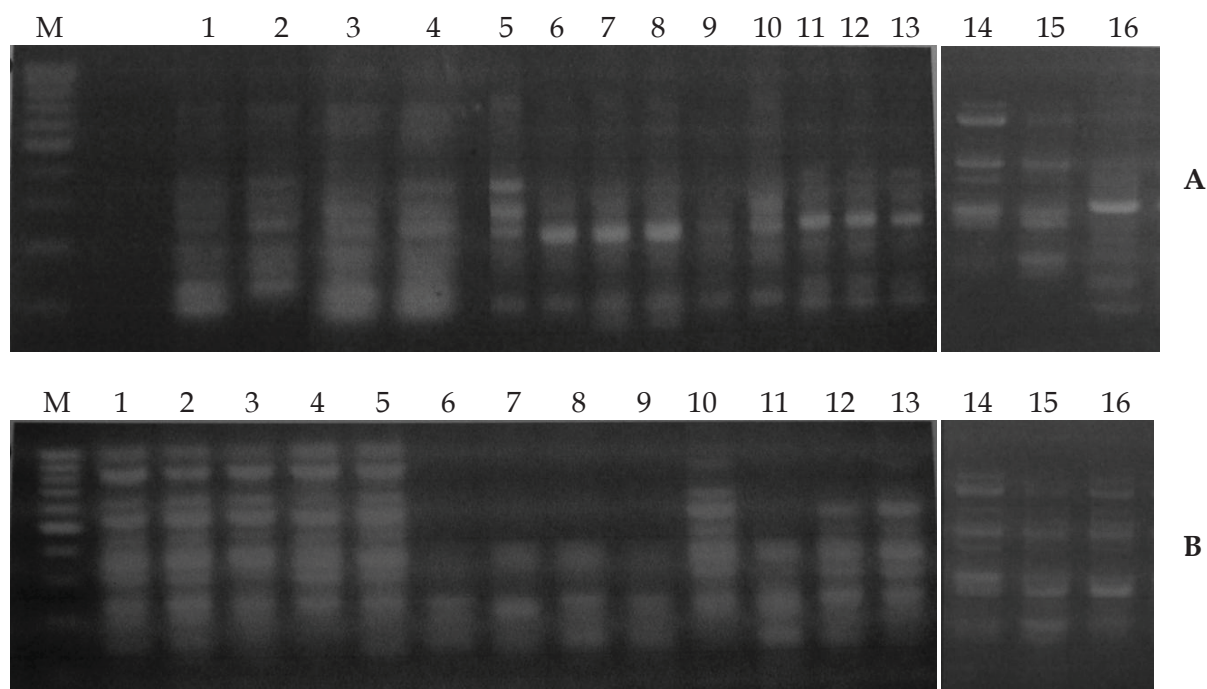


Figure 1. RAPD profile of 13 accessions pummelo from Yogyakarta and three cultivars using primer OPA-02 (A) and OPN-16 (B).

- | | |
|---|---|
| M : molecular weight marker (100-bp DNA ladder) | 9 : TJS-09, tradionally named as "Gulung", pink flesh |
| 1 : TJS-01, unnamed, pink flesh | 10 : TJS-10, tradionally named as "Bali", white flesh |
| 2 : TJS-02, unnamed, pink flesh | 11 : TJS-11, named as "Adas Nambangan", pink flesh |
| 3 : TJS-03, tradionally named as "Bali", white flesh | 12 : TJS-12, unnamed, pink flesh |
| 4 : TJS-04, unnamed, white flesh | 13 : TJS-13, unnamed, pink flesh |
| 5 : TJS-05, tradionally named as "Bali", pink flesh | 14 : "Pamelo Magetan" |
| 6 : TJS-06, tradionally named as "Gulung", pink flesh | 15 : "Pangkajene Merah" |
| 7 : TJS-07, tradionally named as "Gulung", pink flesh | 16 : "Pangkajene Putih" |
| 8 : TJS-08, tradionally named as "Gulung", pink flesh | |

cluster analysis, represented as dendrogram (Figure 2). The dendrogram was constructed based on Simple Matching Coefficient values as a measure of similarity among accessions. The similarity values are indicating the degree of genetic similarity. The overall topology of dendrogram is thus representing the level of genetic variability of pummelo.

Results of RAPD fingerprinting showed a high degree of genetic variability among samples, indicated with 78.4% polymorphism in the amplification products. This result was of comparable extent in defining the degree of genetic variability as reported by Sheidai *et al.* (2010) in the analysis of genetic diversity of brown olive (*Olea cuspidata*). The

polymorphism of RAPD fingerprinting, as represented by the presence or absence of amplification products, functions as genetic markers (Williams *et al.* 1990; Newbury & Ford-Lloyd, 1993), and provide highly informative characters for the evaluation of genetic similarity (Weising *et al.*, 1995).

The grouping pattern of accessions in the dendrogram showed the existence of two main cluster or groups. The first cluster (group I) consisted of four accessions traditionally named as "Jeruk Gulung" with pink flesh. This group of accessions is less-preferred pummelo due to its sweet-sour taste, and even with a reasonable degree of bitterness. The second cluster (group II)

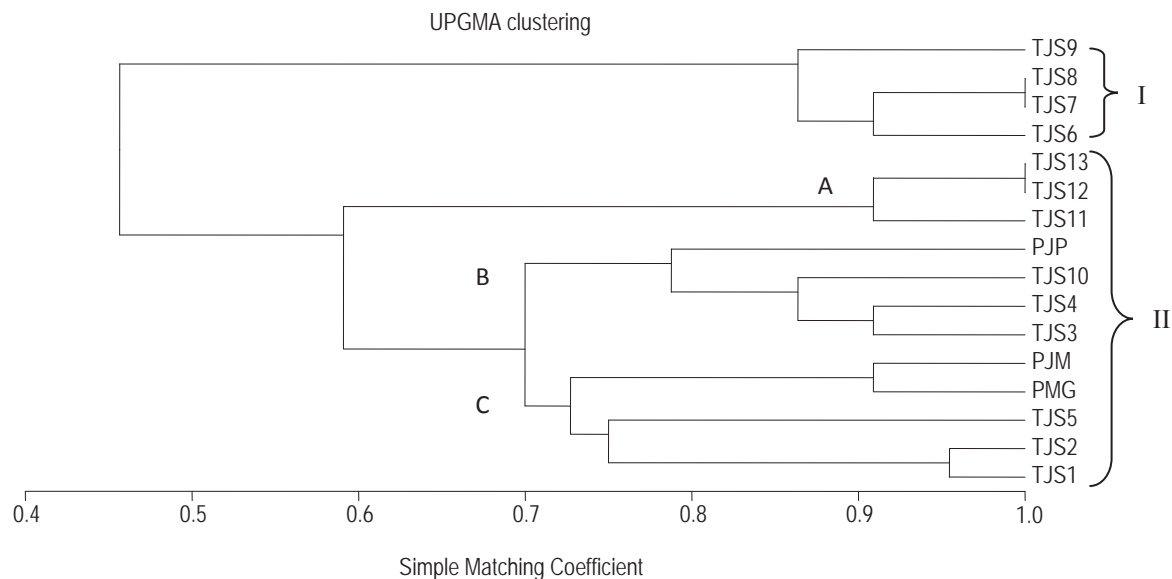


Figure 2. Dendrogram showing genetic variability and taxonomic affinity of pummelo accessions

consisted of 9 local accessions and 3 named cultivars of pummelo. This cluster was clearly divided into three sub-clusters (A, B, and C), with the first sub-cluster being separated at substantial degree from the other two. The three accessions within sub-cluster A could be regarded as a group of “Adas Nambangan”, a typical pummelo with palatable taste of sweet-sour but lack of bitterness in their fruit taste. The sub-cluster B comprised of accessions with white flesh and sweet fruit taste, with “Pangkajene Putih” cultivar was among the members of the group. This sub-cluster was then could be referred to the commonly known as “Bali Putih” pummelo type. Meanwhile, the sub-cluster C with “Pamelo Magetan” and “Pangkajene Merah” cultivars were nested together with two accessions of pummelo with pink flesh and sweet fruit taste could be denoted as those commonly known as “Bali Merah”.

The conformity of clustering result based on RAPD analysis to morphological and agronomical characters observed in this study was in agreement to the finding of Corazza-Nunes *et al.* (2002) in the assessment of genetic variability in *Citrus paradisi* and *Citrus maxima*. Results of this study also

indicated the existence of infraspecific variability within pummelo, and showed the grouping of accessions into recognizable types. This finding was in line with the study of characterization of *Citrus* cultivars and clones using RAPD as reported by Hvarleva *et al.* (2008).

The application of RAPD fingerprinting in infraspecific taxa has been concerned with two major objectives, either for the estimation of genetic variability or cultivar identification. The combination of these two objectives has been reported in various fruit crops, such as in *Cucumis sativus* and *Benincasa hispida* by Saengprajak & Saensouk (2012) and sugar apple (*Annona squamosa*) by Guimarães *et al.* (2013). The ability of RAPD in providing evidence on the occurrence of infraspecific genetic variability within cultivated plants was reported previously by Mahasi *et al.* (2009) in their study on genetic polymorphisms in *Carthamus tinctorius*.

Conclusion

Pummelo (*Citrus maxima*) accessions from Yogyakarta have a high degree of genetic variability as indicated by the high percentage of RAPD fingerprinting polymorphisms. The application of RAPD

fingerprinting technique for the assessment of genetic variability in *C.maxima* was proven to be effective in revealing infraspecific variability and taxonomic affinity. The grouping of pummelo accessions based on RAPD analysis was in agreement to the agromorphological characters.

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