DAFTAR ISI

Progeny Analysis of the Tasikmalayan Mangosteens (Garcinia mangostana) with E-RAPD Markers
Soaloon Sinaga, Sobir, Roedhy Poerwanto, Hajrial Aswidinnoor & Dedy Duryadi

Genetic Diversity of Pandanus and Freycinetia from Java Based on ISSR Marker
Sri Endarti Rahayu, Alex Hartana, Tatiek Chikmawati & Kuswata Kartawinata

Anatomi Daun dan Taksonomi Syzygium zippelianum Miq., S. javanicum Miq. dan S. racemosum (Bl.) DC
Siti Sunarti

Two Wild Edible Russula (Agaricales: Russulaceae) from East Kalimantan
Atik Retnowati

85–94
95–103
104–108
109–112
Floribunda merupakan organ resmi Penggalang Taksonomi Tumbuhan Indonesia, diterbitkan dua kali setahun dan menerbitkan makalah dalam bahasa Indonesia dan Inggris mengenai pelbagai gatra sistematika keanekaragaman flora Malesia pada umumnya dan Indonesia pada khususnya yang berasal dari hasil penelitian, pengamatan lapangan, pengalaman pribadi, telaahan bergagasan, dan tinjauan kritis.

Sidang Penyunting
Ketua Penyunting
Tutie Djarwaningsih (BO)

Penyunting
Tri Mulyaningsih (UNRAM)
Atik Retnowati (BO)
Novita Kartika Indah (UNESA)
Titien N. Praptosuwiryio (KRI)
Nunik Sri Ariyanti (IPB)

Penyunting Pelaksana
Himmah Rustiani (BO)

Tata Letak
Muhamad Ruslan (BO)

Petunjuk kepada pengarang

Jenis tulisan
Makalah lengkap memuat hasil penelitian floristik, revisi, atau monografi unsur-unsur flora Malesia.

Komunikasi pendek mencakup laporan kemajuan kegiatan penelitian, pengembangan dan rekapitulasi keanekaragaman flora Malesia yang perlu segera dikomunikasikan.

Tulisan lain meliputi obituary tokoh keanekaragaman flora, tinjauan kritis bergagasan, telaahan serta pembahasan persoalan aktal seputar kegiatan penelitian, pengembangan dan rekapitulasi tumbuhan Indonesia, serta timbangannya buku akan dimuat berdasarkan undangan.

Rujukan pembukaan

Gaya penulisan
Penulisan naskah yang akan diajukan supaya disesuaikan dengan gaya penulisan yang terdapat dalam nomor terakhir terbitan Floribunda.

Abstrak informatif supaya diberikan dalam bahasa Indonesia dan Inggris yang masing-masing tidak melebihi 200 kata. Sediaan sekitar 7 kata kunci untuk keperluan pengindeksan dan pemindaian.

Bilamana diperlukan ucapan terima kasih dan bentuk persantunan lain dapat dicantumkan sesudah lubuk teks tetapi sebelum daftar pustaka.


Gambar dan tabel merupakan pendukung teks sehingga perlu disusun secara logis dalam bentuk yang mudah dimengerti. Data supaya disajikan dalam bentuk teks atau tabel atau sebagai gambar, tetapi tidak dalam bentuk ketiganya sekaligus. Siapkan gambar yang lebarnya dua kolom cetak.

Penyumbangan naskah
Naskah dikirimkan dalam bentuk ketikan atau cetakan komputer pada kertas HVS berukuran A4 bersama-sama dengan disket komputer yang diprogram untuk serasi dengan IBM, atau melalui e-mail.

Naskah yang ingin diterbitkan dalam Floribunda akan diperintimbangkan pemuatannya hanya jika pengirimannya disertai pernyataan tertulis dari 2 (dua) orang mitra bestari yang dipilih sendiri oleh penulisnya (akan lebih diuntamakan bila mitra bestari dipilihkan dari luar lingkungan kerja penulis), yang menyatakan bahwa secara ilmiah keakuratan dan makna sumbangan naskah tersebut memang layak diterbitkan.

Pengolahan naskah
Sidang penyunting bersama sekelompok mitra bestari akan mengaji ulang kesesuaian isi dan keselarasan format setiap naskah dengan Floribunda. Perubahan yang dilakukan akan dikomunikasikan kepada penulis dalam bentuk contoh cetak akhir sebelum diterbitkan.

Cetak lepas
Penulis menerima 5 cetak lepas dari tulisannya secara cuma-cuma.

Kantor penyunting
Sidang Penyunting Floribunda
Herbarium Bogoriense, Cibinong Science Center
Jalan Raya Bogor KM 46 Cibinong 16911
Telepon : (021) 8765006-67
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GENETIC DIVERSITY OF PANDANUS AND FREYCINETIA FROM JAVA BASED ON ISSR MARKER

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Sri Endarti Rahayu, Alex Hartana, Tatiek Chikmawati & Kuswata Kartawinata. 2007. Keragaman Genetik Pandanus dan Freycinetia dari Jawa Berdasarkan Penanda ISSR. Floribunda 3(4): 95–103. — Informasi tentang keragaman genetik plamsa nutfah sangat diperlukan untuk mendukung program pemuliaan dan upaya konservasi. Penelitian ini bertujuan untuk mengetahui keragaman genetik di dalam 13 jenis Pandanus dan 6 jenis Freycinetia yang dikenal dari berbagai lokasi di Jawa dengan menggunakan penanda Inter Simple Sequence Repeat (ISSR). Hasil penelitian menunjukkan bahwa dari 13 jenis Pandanus yang dianalisis dengan menggunakan 6 primer terseleksi diperoleh 50 penanda ISSR, sedangkan dari 6 jenis Freycinetia dihasilkan 32 penanda ISSR, dimana 87.5% dari penanda tersebut adalah polimorfik. Analisis pengelompokan dilakukan berdasarkan profil pita ISSR dengan menggunakan metode UPGMA. Nilai ketidaksamaaan genetik untuk 13 jenis Pandanus berkisar antara 0.250–0.889 dan untuk 6 jenis Freycinetia berkisar antara 0.296–0.923. Nilai ketidaksamaan genetik yang tinggi ini menunjukkan bahwa 13 jenis Pandanus dan 6 jenis Freycinetia yang berasal dari Jawa memiliki keragaman genetik yang tinggi.

Kata kunci: Freycinetia, ISSR, Jawa, keragaman genetik, Pandanus.

Sri Endarti Rahayu, Alex Hartana, Tatiek Chikmawati & Kuswata Kartawinata. 2007. Genetic Diversity of Pandanus and Freycinetia from Java Based on ISSR Marker. Floribunda 3(4): 95–103. — Information on genetic diversity of Pandanus and Freycinetia germplasm is necessary in order to support breeding and conservation program. The objective of the present study is to assess genetic diversity among 13 species of Pandanus and 6 species of Freycinetia collected from different locations in Java by using Inter Simple Sequence Repeat (ISSR). Six primers generated 50 scorable bands in Pandanus and 32 bands in Freycinetia in which 87.5% of them were polymorphic. Clustering analysis was performed based on ISSR profiles using the UPGMA method. The range of genetic dissimilarity value among species of Pandanus was 0.250–0.889 and 0.296–0.923 among species of Freycinetia. These values showed that 13 species of Pandanus and 6 species of Freycinetia from Java have high genetic diversity.

Keywords: Freycinetia, ISSR, Java, genetic diversity, Pandanus.

The pandan family (Pandanaceae) in Java is represented by two genera, Pandanus and Freycinetia (Stone 1970). Pandanus contains about 500-600 species of trees and shrubs of the old world tropics, distributed from East Africa westward through Indomalaysia to remote island of Polynesia. It extends south to tropical Australia (but not to New Zealand), north to Ryukyus, Bonins, Taiwan, and to Hawaiian island (Stone 1965), while Freycinetia contains about 180-200 species of root climbing lianas or low growing shrubs occur from Sri Lanka throughout South-Eastern Asia to Northern Australia, Polynesia and New Zealand (Cox et al 1995). The habitat of Pandanus is nearly all possible habitats, from sea level to the highest peaks (Stone 1966), whereas Freycinetia are usually not found in open area because they grow on forest (Stone 1982).

Species of Pandanus specialize in wind pollination. This anemophily, coupled with syncarpus dispersal unit (the pistilate phalange) and also the presence of facultative apomixis allows Pandanus species to colonize new areas (Cox 1990). Immediate genetic isolation as well as exposure to new habitats without regard to pollinator availability may account for the extreme richness of species in Pandanus as well as its very wide distribution (Cox et al. 1995). Whereas Freycinetia have been believed to be strictly dioecious (Dahlgren et al. 1985), but recent
fieldwork has indicated that a variety of breeding systems exist in Freycinetia (Cox et al. 1984). Some individuals of Freycinetia imbricata in Sumatra produce staminate and pistillate shoots on the same plant, such divergences from a dioecious breeding system may be important in island colonization (Baker & Cox 1984) particularly since monoecious individuals of Freycinetia scandens in Philippine have been found to be self compatible (Cox 1984). Freycinetia lack facultative apomixis and water dispersal. However, their attractiveness to a wide variety of vertebrate pollination and disperses, as well as infrequent leaky dioecy would assure them a large range and high speciation rate (Cox 1990).

Many species of Pandanus have been used by Indonesian people for daily purposes, i.e as the raw materials for mats, and other handicrafts, such as hat and bag. Not all species of Pandanus are suitable as raw materials for mat. The most suitable species are Pandanus tectorius, Pandanus dubius, and Pandanus furcatus. All along the leaves border of the three species are thorny, but their texture are flexible, unbreakable (Purwanto 2007). Pandanus amaryllifolius is rare in the wild, but it cultivated and widely used as flavouring in cooking (Leam & Yap 2003). Some species are cultivated as ornamentals, i.e Pandanus dubius, Pandanus utilis, Pandanus spurius cv putat, and Pandanus tectorius cv sanderi (Thomson et al. 2006), whereas some species of Freycinetia are important in the pacific as an emergency food and for the construction of fish traps (Brown 1931). In Indonesia, not many people utilize this genus, but looking at its elegant figure, Freycinetia has potential as an ornamental plant (Purwanto 2007).

Learning its importance and considering its availability of the large number of wild species germplasm for Pandanus and Freycinetia in Java, the genetic analysis by using molecular marker is a prerequisite to have a deep insight of the genome organization of the wild species. Therefore it is imperative to establish strategies for the preservation of Pandanus and Freycinetia germplasm. This analysis is a preliminary step that ensured the conservation and the development of genetic resources.

In the past, genetic diversity of species has typically been assessed using morphological, physiological and biochemical traits. Since, morphological and physiological traits are subject to environmental influences, emphasis has shifted to biochemical studies (Moodie et al. 1997). In particular, allozyme analysis has been used to document genetic diversity in a range of different species. However, allozymes may underestimate genetic diversity (Esselman et al. 1999). Recently more sensitive DNA based-techniques have been developed to detect the genetic diversity in different group of plants. Commonly used Polymerase Chain Reaction (PCR)-based DNA marker systems are Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and more recently Simple Sequence Repeat (SSRs) or microsatellites (Staub et al. 1996). The major limitation of these methods are low reproducibility of RAPD, high cost of AFLP and the need to know the flanking sequence to develop species specific primers for SSR polymorphism. ISSR-PCR is a technique that overcome most of these limitations (Zietkiewicz et al. 1994).

Inter simple sequence repeats (ISSR) exhibits a few advantages over other markers, ISSR primer anneal to simple sequence repeats that are abundant through the eukaryotic genome and evolve rapidly, and hence may reveal a high level of polymorphism (Zietkiewicz et al. 1994). In addition, ISSR may produce more reliable and reproducible bands than RAPD because of the higher annealing temperature and longer primer sequences (Qian et al. 2001). Moreover, it has proved their usefulness to detect genetic diversity in Ficus species (Rout & Aparajita 2009) and Morus species (Awasthi et al. 2004).

There is no information regarding the genetic diversity of Pandanus and Freycinetia from Java. Our objective is to obtain information based in genetic diversity among Pandanus and Freycinetia species.

**MATERIALS AND METHODS**

**Plant materials**

Totally 19 samples of species of Pandanus and Freycinetia were collected from various places in Java. They consisted of 13 species of Pandanus and 6 species of Freycinetia. All samples were identified to species based on morphological characters followed the method of Stone (1983) (Table 1) and (Table 2). Two or three leaves were collected from each species and stored in silica gel. In the laboratory, samples were maintained at – 5°C until DNA extraction could be performed.

**DNA extraction**

Genomic DNA of the silica gel dried leaf samples were extracted according to the protocol described by Doyle & Doyle (1987) with minor
Table 1. Thirteen species of *Pandanus* used in this study.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Species</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td><em>Pandanus bantamensis</em> Koord.</td>
<td>Jampang Kulon</td>
</tr>
<tr>
<td>S2</td>
<td><em>Pandanus tectorius</em> Sol.</td>
<td>Ujung Genteng</td>
</tr>
<tr>
<td>S3</td>
<td><em>Pandanus bidur</em> Jungh.</td>
<td>Ujung Kulon</td>
</tr>
<tr>
<td>S4</td>
<td><em>Pandanus spinifistigmaticus</em> Fagerlind</td>
<td>Bogor Botanical Garden</td>
</tr>
<tr>
<td>S5</td>
<td><em>Pandanus odoratissimus</em> L.f.</td>
<td>Ujung Kulon</td>
</tr>
<tr>
<td>S6</td>
<td><em>Pandanus pseudolais</em> Warb.</td>
<td>Bodogol</td>
</tr>
<tr>
<td>S7</td>
<td><em>Pandanus multifloratus</em> Fagerlind</td>
<td>Bogor Botanical Garden</td>
</tr>
<tr>
<td>S8</td>
<td><em>Pandanus nitidus</em> Kurz</td>
<td>Jampang Kulon</td>
</tr>
<tr>
<td>S9</td>
<td><em>Pandanus amarillyfolius</em> Roxb</td>
<td>Depok</td>
</tr>
<tr>
<td>S10</td>
<td><em>Pandanus polycladus</em> Lamk</td>
<td>Bogor Botanical Garden</td>
</tr>
<tr>
<td>S11</td>
<td><em>Pandanus kurzii</em> Merr.</td>
<td>Bogor Botanical Garden</td>
</tr>
<tr>
<td>S12</td>
<td><em>Pandanus scabrifolius</em> Martelli</td>
<td>Cibodas</td>
</tr>
<tr>
<td>S13</td>
<td><em>Pandanus dubius</em> Sprengel</td>
<td>Jakarta</td>
</tr>
</tbody>
</table>

Table 2. Six species of *Freycinetia* used in this study.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Species</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>S6</td>
<td><em>Freycinetia javanica</em> Blume</td>
<td>Ujung Kulon</td>
</tr>
<tr>
<td>S7</td>
<td><em>Freycinetia angustifolia</em> Blume</td>
<td>Halimun</td>
</tr>
<tr>
<td>S12</td>
<td><em>Freycinetia scandens</em> Gaud.</td>
<td>Cibodas</td>
</tr>
<tr>
<td>S19</td>
<td><em>Freycinetia insignis</em> Blume</td>
<td>Cibodas</td>
</tr>
<tr>
<td>S20</td>
<td><em>Freycinetia sumatranca</em> Hems.</td>
<td>Jampang Kulon</td>
</tr>
<tr>
<td>S22</td>
<td><em>Freycinetia imbricata</em> Blume</td>
<td>Bodogol</td>
</tr>
</tbody>
</table>

**Modification mainly aimed to minimize the presence of phenolic compounds.** For each samples, 100 mg of leaf were ground, followed by the addition of 1 ml preheated (65°C) extraction buffer constitute 3% (w/v) CTAB, 1.4 M NaCl, 0.2% (v/v) 6-mercaptoethanol, 20mM EDTA, 100mM Tris-HCl (pH 8.0) and 1% (w/v) PVP-40. The homogenous was incubated at 65°C for 30 min and extracted two times with a phenol chloroform : isoamyl alcohol : phenol (25:24:1) solution, was eliminated by chloroform : isoamyl alcohol (24:1) solution. Then DNA was precipitated in cold isopropanol and treated with Rnase A (37°C) for 60 min. After electrophoresis with a standard DNA on 1% agarose gels, stained with ethidium bromide, DNA concentration was determined by comparison against the standard of DNA with known concentration. The DNA was suspended to a final concentration of 10 ng/µl in 0.5 x TE and stored at 4°C.

**DNA amplification**

Total of 12 ISSR primers (Fermentas GmbH - Germany) were used for screening the amplification of unambiguously visible and polymorphic ISSR bands. A final set of 6 of ISSR primers (Table 2), which produced unambiguously visible and polymorphic bands across the 21 samples was chosen for further analysis.

**PCR conditions**

PCR was performed in a total volume of 25 µl containing 1X reaction buffer, 50 ng genomic DNA, 3.0 mM of MgCl₂, Taq polymerase (2.5 units), 0.4 mM dNTPs and 10 µM primer ISSR were amplified using GeneAmp PCR System 2400 Perkin Elmer. The amplification was programmed for 1 cycle in 2 min at 94°C, and 35 cycles in 1 min at 94°C, 1 min at 53°C, and 1 min at 72°C, followed by a final extension in 10 min at 72°C. PCR products were run at 1% (w/v) agarose gel in 1X TAE buffer. Gels were run for 1 to 2 h at 90 V, with a 100 bp ladder (Promega - USA) as the standard size. Gel was stained with ethidium bromide (10 µg/ml) and visualized under UV light and photograph with a digital camera.

**Data analysis**

The Numerical Taxonomy and Multivariate Analyses System (NTSYS-pc) was used in this study. The presence of band was scored from the photograph. Only clear and reproducible bands were considered. These bands were considered a polymorphic when they were absent in some samples in frequency more than 1% (Jorde 1995). Changing in band intensity was not considered as a polymorphism. Clear bands were scored as present (1) or absent (0) at particular position or distance migrate on the gel. The data matrix of 1's and 0's
has been prepared from the scorable bands and entered into the data analysis package (Amstrong et al. 1994). The indices disimilarity were calculated across all possible pair wise comparisons of species following the method of Nei & Li (1979).

RESULTS

Primer selection

Six primers were applied on 13 species of Pandanus and 6 species of Freycinetia from Java. The results showed that different primers generated various numbers of fragments with different length of amplified products as shown in Table 3 and Table 4. The six primers amplified 50 band positions in Pandanus, and 32 band positions in Freycinetia. The number of amplification bands per primer varied between 6–10 in Pandanus and between 2–8 in Freycinetia.

The repeats (AG)8AA had more bands in Pandanus and (GA)9A primer had more bands in Pandanus and Freycinetia than other dinucleotide repeats primer (Table 3 and Table 4), probably because of its greater abundance in Pandanus and Freycinetia genome. The repeats (GA)n were most abundant in rice (Nagaraju et al. 2002) and date palm (Trifi et al. 2000) genome. This might indicate that dinucleotide-based ISSR-PCR marker could provide potential markers in the Pandanus and Freycinetia genom. A representative amplification pattern obtained by using primer ISSR2 is shown in Fig.1 and Fig. 2.

ISSR survey

A total of 50 ISSR fragments was generated by six primers from 13 species of Pandanus, and 32 ISSR fragments were generated in 6 species of Freycinetia. The highest number of fragments in Pandanus was detected in Pandanus

![Figure 1. ISSR profile of Pandanus spp and Freycinetia spp using primer ISSR2.](image)

![Figure 2. ISSR profile of Pandanus spp and Freycinetia spp usiung primer ISSR2.](image)
Tabel 3. ISSR primers sequence and amplified results on 13 species of Pandanus.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide (5' - 3')</th>
<th>Length of fragment (bp)</th>
<th>The number of bands</th>
<th>The number of polymorphic</th>
<th>polymorphic percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISSR2</td>
<td>(AC)8TT</td>
<td>250 – 1000</td>
<td>7</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>ISSR3</td>
<td>(AG)8T</td>
<td>250 – 1000</td>
<td>9</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>ISSR4</td>
<td>(AG)8AA</td>
<td>200 – 1200</td>
<td>10</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>ISSR5</td>
<td>(AG)8TA</td>
<td>200 – 700</td>
<td>8</td>
<td>7</td>
<td>87.5</td>
</tr>
<tr>
<td>ISSR6</td>
<td>(AG)8TT</td>
<td>250 – 1000</td>
<td>6</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>ISSR7</td>
<td>(GA)9A</td>
<td>200 – 800</td>
<td>10</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>50</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>8.3</td>
<td>87.4</td>
<td></td>
</tr>
</tbody>
</table>

Tabel 4. ISSR primers sequence and amplified results on 6 species of Freycinetia.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide (5' - 3')</th>
<th>Length of fragment (bp)</th>
<th>The number of bands</th>
<th>The number of polymorphic</th>
<th>polymorphic percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISSR2</td>
<td>(AC)8TT</td>
<td>300 – 750</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>ISSR3</td>
<td>(AG)8T</td>
<td>250 – 1000</td>
<td>8</td>
<td>7</td>
<td>87.5</td>
</tr>
<tr>
<td>ISSR4</td>
<td>(AG)8AA</td>
<td>200 – 1000</td>
<td>6</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>ISSR5</td>
<td>(AG)8TA</td>
<td>500 – 700</td>
<td>4</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>ISSR6</td>
<td>(AG)8TT</td>
<td>250 – 500</td>
<td>2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>ISSR7</td>
<td>(GA)9A</td>
<td>200 – 750</td>
<td>7</td>
<td>5</td>
<td>71</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>32</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>5.3</td>
<td>87.5</td>
<td></td>
</tr>
</tbody>
</table>

amaryllifolius (34), Pandanus spinistigmaticus (29) and Pandanus pseudolais (28) respectively, whereas the least number was found in Pandanus multifurcatus containing 6 fragments. In other species the number of fragments varied from 12 in Pandanus bantamensis and Pandanus tectorius to 22 in Pandanus bidur. In Freycinetia, the highest number of fragments was detected in Freycinetia javanica and Freycinetia imbricata containing 21 fragments each, while the least number was found in Freycinetia scandens containing 11 fragments. In other species, the number of fragment ranged from 15 in Freycinetia angustifolia to 18 in Freycinetia sumatrana. The average number of fragments generated by all analyzed primer was 8.3 in Pandanus and 5.3 in Freycinetia. The choice of primers used in amplification is critical to demonstrate high polymorphism. The results show that samples had different banding pattern. Hassel & Gunnarsson (2003) stated that primers had several characteristic that can affect the number and quality of DNA fragment amplified during PCR. Among all the accessions/species subjected in this research, no species specific band was detected.

Genetic diversity

The potential value of ISSR marker has been observed for genetic analysis in Pandanus and Freycinetia. In order to determine genetic diversity among 13 species of Pandanus and 6 species of Freycinetia, it was employed the ISSR technique and found that Pandanus and Freycinetia species could be characterized by ISSR markers. A pairwise genetic distance was calculated to know the distance relationship between the species of Pandanus and between the species of Freycinetia. In Pandanus, the highest genetic distance was observed between Pandanus scabrifolius and Pandanus nitidus (0.889), showing that these two species were most distantly related to each other, while the lowest distance was detected between Pandanus amaryllifolius and Pandanus multifurcatus (0.250) (Table 5). High distance relationship was also observed between Pandanus scabrifolius and Pandanus kurzii (0.842), Pandanus kurzii and Pandanus nitidus (0.842), Pandanus scabrifolius and Pandanus odoratissimus (0.833). The distance in other species varied from 0.333 (Pandanus multifurcatus and Pandanus bantamensis) to 0.789 (Pandanus kurzii and Pandanus odoratissimus) (Table 5). In Freycinetia, the highest genetic distance was observed between Freycinetia sumatrana and Freycinetia imbricata (0.923) showing that these two species were most distantly related to each other,
Tabel 5. Distance matrix values based on ISSR data between 13 species of *Pandanus*.

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
<th>S13</th>
<th>S14</th>
<th>S21</th>
<th>S23</th>
<th>S24</th>
<th>S25</th>
<th>S26</th>
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Tabel 6. Distance matrix values based on ISSR data between 6 species of *Freycinetia*.

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while the lowest distance was detected between *Freycinetia insignis* and *Freycinetia scandens* (0.296). High distance relationship was also observed between *Freycinetia sumatrana* and *Freycinetia javanica* (0.872), *Freycinetia imbricata* and *Freycinetia javanica* (0.905), *Freycinetia sumatrana* and *Freycinetia angustifolia* (0.848), *Freycinetia angustifolia* and *Freycinetia javanica* (0.833) and *Freycinetia imbricata* and *Freycinetia angustifolia* (0.833). The distance in other species varied from 0.313 (*Freycinetia scandens* and *Freycinetia javanica*) to 0.649 (*Freycinetia insignis* and *Freycinetia javanica*) (Table 6).

The wide variation in genetic distance among 13 species of *Pandanus* and 6 species of *Freycinetia* revealed by ISSR technique reflected a high level of polymorphism at the DNA level. *P. multifurcatus* and *F. scandens* as a separate species distinct from the others, which are likely to be independent species. Thus ISSR based molecular markers was able to distinguish difference between species.

Smallest distance value (0.250) is recorded between *P. multifurcatus* and *P. amaryllifolius*, and (0.296) between *F. insignis* and *F. scandens* suggest a relatively high degree of genetic similarities between these species, on the other hand, higher distance values are observed between *P. scabrifolius* and *P. nitidus* (0.889), and between *F. sumatrana* and *F. imbricata* (0.923). It may be assumed that these species represent the maximum of divergence.

**Cluster analysis**

Dendrogram for 13 species of *Pandanus* based on ISSR markers shown as in Fig. 3. In this dendrogram, they could be divided into three groups. Group1 contained 6 species, such as *P. bantamensis*, *P. tectorius*, *P. odoratissimus*, *P. nitidus*, *P. scabrifolius* and *P. kurzii*. Group 2 contained also of six species, such as *P. bidur*, *P. polyccephalus*, *P. spinistigmaticus*, *P. dubius*, *P. pseudolais* and *P. amaryllifolius*. Group 3 only contained one species, i.e *P. multifurcatus*. Dendrogram for 6 species of *Freycinetia* based on ISSR markers shown as in
Fig. 4. In this dendrogram, they could be divided into two groups. Group I consisted five species, such as *F. javanica*, *F. sumatrana*, *F. imbricata*, *F. angustifolia* and *F. insignis*. Group 2 consisted of one species, i.e *F. scandens*.

**DISCUSSION**

Molecular marker techniques, such as RAPD and ISSR, have been used to assess genetic diversity of *Ficus* spp., *Morus* spp and *Pandanus* spp (Rout & Aparajita 2009; Awasthi et al. 2004; Sarile & Manguito 2004; Panda et al. 2009). Based on Nei’s estimates of genetic diversity, the highest diversity was found between *P. scabrifolius* and *P. nitidus* (0.889), with *P. amaryllifolius* and *P. multificatus* exhibiting the lowest diversity (0.250), whereas in *Freycinetia*, the highest diversity was found between *F. sumairana* and *F. imbricate* (0.923) with *F. insignis* and *F. scandens* exhibiting the lowest diversity (0.296). Genetic diversity among these *Pandanus* species and *Freycinetia* species was low when compared with another outcrossing species *Morus* (Awasthi et al. 2004), where ISSR-based genetic diversity between species ranged from 0.419 (*M. rubra-M. bombycis*) to 0.885 (*M. nigra-M. tiloefolia*). However, the genetic diversity estimate among *Pandanus* species and *Freycinetia* species are comparable with another species *Ficus*, where ISSR-band genetic diversity between species ranged from 0.24 (*F. benghalensis-F. krisnae*) to 0.81 (*F. amottina-F. vires*) (Rout et al. 2009). An RAPD-based study reported that in *P. amaryllifolius* and *P. dubius* detected similar (88%) DNA polymorphism (Sarile & Manguito 2004).

The high genetic distance value 0.29–0.889 in *Pandanus*, and 0.296–0.923 in *Freycinetia* species indicated that they possess several different genetic variations, because the genetic difference was obvious among species of *Pandanus*, and among species of *Freycinetia*. Genetic diversity in a species is affected by a number of evolutionary factors including mating system, gene flow, seed dispersal, geographical range as well as natural selection (Hamrick & Godt 1989). Of these factors, breeding system and geographical range are factors that affect the levels of genetic variation in a species (Barret et al 2004).

Maintenance of genetic variability to a maximal extent at the species level will tend to prevent extinction. This is achieved by various devices which enforce or promote outcrossing, such as dioecism, anemophily. Dioecious species, such as *Pandanus* and *Freycinetia*, will harbor a high levels of outcrossing. Outcrossing species which is characterized by production of abundant pollen (Darjanto & Satifiah 1982) is generally more genetically diverse (Schultz 2009), and anemophilous species such as *Pandanus* spp will tend not only to promote outcrossing, but also to promote it over a relatively wide area. The high genetic distance in *Pandanus* is 0.29–0.889, and it is 0.296–0.923 in *Freycinetia*. Both reveal a fairly good value to support the broad range of distribution sites, and the outcrossing devices could potentially facilitate genetic material actively (Carlquist 1966).

High genetic diversity is important, besides as a safeguard against co-evolving biotic factors such as pests and diseases (Namkoong 1986), also allow *Pandanus* and *Freycinetia* species to adjust to the ever-changing environment in Java due to the natural or human factors (Chamberlain & Hubert 2001; Hedrick 2004). An overall loss of genetic variability usually has deleterious effects on species fitness and it may threaten the ability of population to survive and persist via natural regeneration (Reed 2003; Kremer & Reviron 2004).

Assessing the level and distribution of genetic diversity within plant species is crucial for their management and the development of effective conservation strategies (Hedrick 2004).

**CONCLUSION**

The present findings strengthened previous reports that ISSR markers can be used effectively to estimate genetic diversity of the genus *Pandanus* and *Freycinetia* at species level. Six primers generated 50 scorable bands in *Pandanus* and 32 bands in *Freycinetia* which is 87.5% of them were polymorphic. The range of genetic dissimilarity value among species of *Pandanus* was 0.250–0.889 and 0.296–0.923 among species of *Freycinetia*. These values showed that 13 species of *Pandanus* and 6 species of *Freycinetia* from Java have high genetic diversity. Future study by using of other molecular markers, especially AFLP and SSR should also be carried out in order to determine genetic diversity among other species of *Pandanus* and *Freycinetia*.

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