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## DAFTAR ISI

Progeny Analysis of the Tasikmalayan Mangosteens (*Garcinia mangostana*) with E-RAPD Markers
Soaloon Sinaga, Sobir, Roedhy Poerwanto, Hajrial Aswidinnoor & Dedy Duryadi ........ 85–94

Genetic Diversity of *Pandanus* and *Freycinetia* from Java Based on ISSR Marker
Sri Endarti Rahayu, Alex Hartana, Tatiek Chikmawati & Kuswata Kartawinata ........ 95–103

Anatomi Daun dan Taksonomi *Syzygium zippelianum* Miq., *S. javanicum* Miq. dan *S. racemosum* (Bl.) DC
Siti Sunarti........ 104–108

Two Wild Edible *Russula* (*Agaricales*: *Russulaceae*) from East Kalimantan
Atik Retnowati........ 109–112

Terakreditasi B berdasarkan Keputusan Dirjen Dikti Depdiknas RI No. 26/DIKTI/Kep./2005

ISSN 0215 – 4706

*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**
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***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 rekayasa keanekaragaman flora Malesia yang perlu segera dikomunikasikan.

Tulisan lain meliputi obituari tokoh keanekaragaman flora, tinjauan kritis bergagasan, telaahan serta pembahasan persoalan aktual seputar kegiatan penelitian, pengembangan dan rekayasa tetumbuhan Indonesia, serta timbangan buku akan dimuat berdasarkan undangan.

**Rujukan pembakuan**

Pemakaian Bahasa Indonesia sepenuhnya mengikuti *Pedoman Umum Ejaan yang Disempurnakan, Pedoman Umum Pembentukan Istilah, Kamus Besar Bahasa Indonesia,* serta kamus-kamus istilah yang dikeluarkan Pusat Bahasa. Bahasa Inggris yang dipakai adalah the Queen English dengan berpedoman pada *The Oxford Dictionary of the English Language.* Ketentuan-ketentuan yang dimuat dalam *Pegangan Gaya Penulisan, Penyuntingan,* dan *Penerbitan Karya Ilmiah Indonesia,* serta *Scientific Style and Format: CBE Manuals for Authors, Editors, and Publishers,* dan buku-buku pegangan pembakuan lain akan sangat diperhatikan. Kepatuhan penuh pada *International Code of Botanical Nomenclature* bersifat mutlak.

**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. Sediakan sekitar 7 kata kunci untuk keperluan pengindeksan dan pemindaian.

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

Pengacuan pada pustaka hendaklah dilakukan dengan sistem nama-tahun. Daftar pustaka supaya disusun berdasarkan alfabet nama pengarang dengan memakai sistem Harvard.

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 dipertimbangkan pemuatannya *hanya* jika pengirimannya disertai pernyataan tertulis dari 2 (dua) orang mitra bestari yang dipilih sendiri oleh penulisnya (akan lebih diutamakan bila mitra bestari dipilihkan dari luar lingkungan kerja penulis), yang menyatakan bahwa secara ilmiah keorisinalan 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) 8765066-67
Fax : (021) 8765059
E-mail : herbogor@indo.net.id

# GENETIC DIVERSITY OF PANDANUS AND FREYCINETIA FROM JAVA BASED ON ISSR MARKER

**Sri Endarti Rahayu**
Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, IPB, Darmaga, Indonesia
(Permanent address: Biology Department, National University)
E-mail: endarti2004@yahoo.com
**Alex Hartana, Tatiek Chikmawati**
Bogor Agricultural University
**Kuswata Kartawinata**
"Herbarium Bogoriense"Bidang Botani, Pusat Penelitian Biologi - LIPI

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 plasma 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 dikoleksi 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 ketidaksamaan 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 pistillate 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 microsatelites (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.

| Sample No. | Species | Location |
|---|---|---|
| S1 | *Pandanus bantamensis* Koord. | Jampang Kulon |
| S2 | *Pandanus tectorius* Sol. | Ujung Genteng |
| S3 | *Pandanus bidur* Jungh. | Ujung Kulon |
| S4 | *Pandanus spinistigmaticus* Fagerlind | Bogor Botanical Garden |
| S5 | *Pandanus odoratissimus* L.f | Ujung Kulon |
| S8 | *Pandanus pseudolais* Warb. | Bodogol |
| S13 | *Pandanus multifurcatus* Fagerlind | Bogor Botanical Garden |
| S14 | *Pandanus nitidus* Kurz | Jampang Kulon |
| S21 | *Pandanus amarillyfolius* Roxb | Depok |
| S23 | *Pandanus polycephalus* Lamk | Bogor Botanical Garden |
| S24 | *Pandanus kurzii* Merr. | Bogor Botanical Garden |
| S25 | *Pandanus scabrifolius* Martelli | Cibodas |
| S26 | *Pandanus dubius* Sprengel | Jakarta |

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

| Sample No. | Species | Location |
|---|---|---|
| S6 | *Freycinetia javanica* Blume | Ujung Kulon |
| S7 | *Freycinetia angustifolia* Blume | Halimun |
| S12 | *Freycinetia scandens* Gaud. | Cibodas |
| S19 | *Freycinetia insignis* Blume | Cibodas |
| S20 | *Freycinetia sumatrana* Hemsl. | Jampang Kulon |
| S22 | *Freycinetia imbricata* Blume | Bodogol |

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 MnaCl, 0.2% (v/v) â-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 unambigously visible and polymorphic ISSR bands. A final set of 6 of ISSR primers (Table 2.) which produced unambigously 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 $MgCl_2$, *Taq* polymerase (2.5 units), 0.4 mMdNTPs and 10 µM primer ISSRs 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*

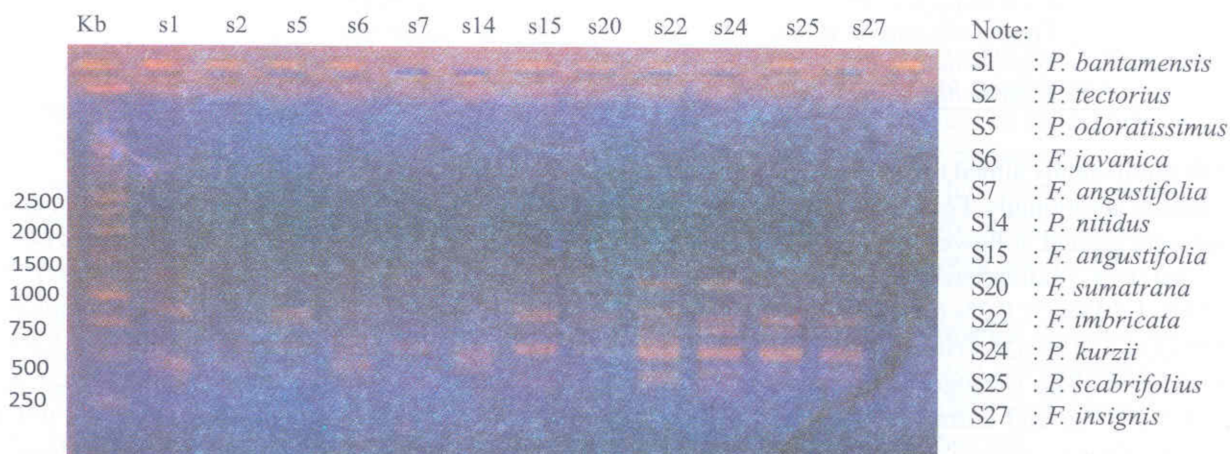


Figure 1. ISSR profile of *Pandanus* spp and *Freycinetia* spp using primer $ISSR_2$.

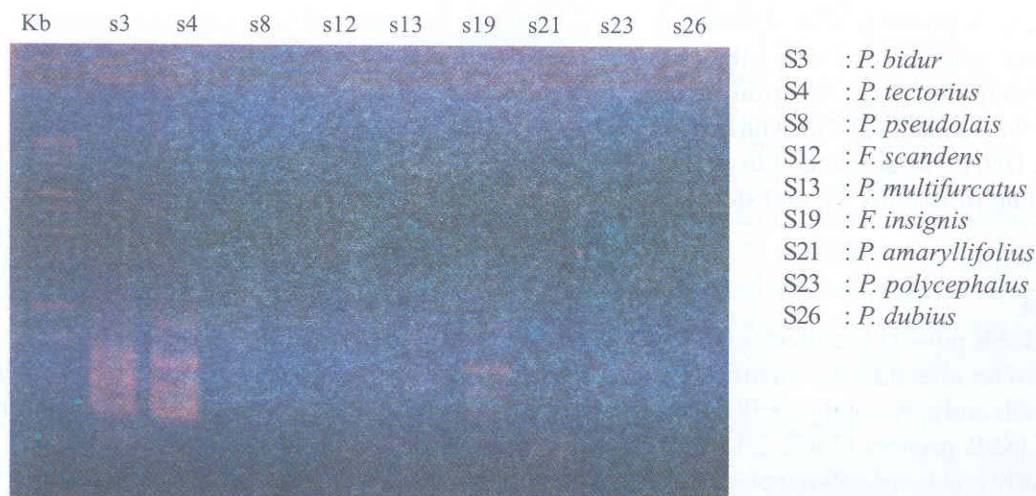


Figure 2. ISSR profile of *Pandanus* spp and *Freycinetia* spp usiong primer $ISSR_2$.

Tabel 3. ISSR primers sequence and amplified results on 13 species of *Pandanus*.

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

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

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

*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 ranged 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*.

| | S1 | S2 | S3 | S4 | S5 | S8 | S13 | S14 | S21 | S23 | S24 | S25 | S26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 1.000 | | | | | | | | | | | | |
| S2 | 0.750 | 1.000 | | | | | | | | | | | |
| S3 | 0.529 | 0.412 | 1.000 | | | | | | | | | | |
| S4 | 0.488 | 0.488 | 0.745 | 1.000 | | | | | | | | | |
| S5 | 0.667 | 0.733 | 0.450 | 0.511 | 1.000 | | | | | | | | |
| S8 | 0.550 | 0.500 | 0.480 | 0.702 | 0.565 | 1.000 | | | | | | | |
| S13 | 0.333 | 0.444 | 0.286 | 0.286 | 0.333 | 0.353 | 1.000 | | | | | | |
| S14 | 0.600 | 0.733 | 0.350 | 0.426 | 0.778 | 0.565 | 0.333 | 1.000 | | | | | |
| S21 | 0.522 | 0.478 | 0.464 | 0.635 | 0.538 | 0.774 | 0.250 | 0.577 | 1.000 | | | | |
| S23 | 0.563 | 0.563 | 0.714 | 0.694 | 0.526 | 0.625 | 0.462 | 0.526 | 0.519 | 1.000 | | | |
| S24 | 0.625 | 0.688 | 0.429 | 0.490 | 0.789 | 0.667 | 0.308 | 0.842 | 0.593 | 0.550 | 1.000 | | |
| S25 | 0.667 | 0.733 | 0.350 | 0.468 | 0.833 | 0.565 | 0.333 | 0.889 | 0.577 | 0.526 | 0.842 | 1.000 | |
| S26 | 0.545 | 0.545 | 0.558 | 0.760 | 0.513 | 0.735 | 0.444 | 0.462 | 0.618 | 0.732 | 0.537 | 0.513 | 1.000 |

Tabel 6. Distance matrix values based on ISSR data between 6 species of *Freycinetia*.

| | S6 | S7 | S12 | S19 | S20 | S22 |
|---|---|---|---|---|---|---|
| S6 | 1.000 | | | | | |
| S7 | 0.833 | 1.000 | | | | |
| S12 | 0.313 | 0.385 | 1.000 | | | |
| S19 | 0.649 | 0.581 | 0.296 | 1.000 | | |
| S20 | 0.872 | 0.848 | 0.345 | 0.647 | 1.000 | |
| S22 | 0.905 | 0.833 | 0.375 | 0.595 | 0.923 | 1.000 |

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. polycephalus*, *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 1 consisted five species, such as *F. javanica*, *F. sumatrana*, *F. imbricata*, *F. angutifolia* 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. multifurcatus* exhibiting the lowest diversity (0.250), whereas in *Freycinetia*, the highest diversity was found between *F. sumatrana* and *F. imbricata* (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. bhengalensis-F. krisnae*) to 0.81 (*F. amottina-F. virens*) (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 posses 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 & Satifah1982) is generally more genetically diverse (Schultz 2009), and anemophily 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 circulate 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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