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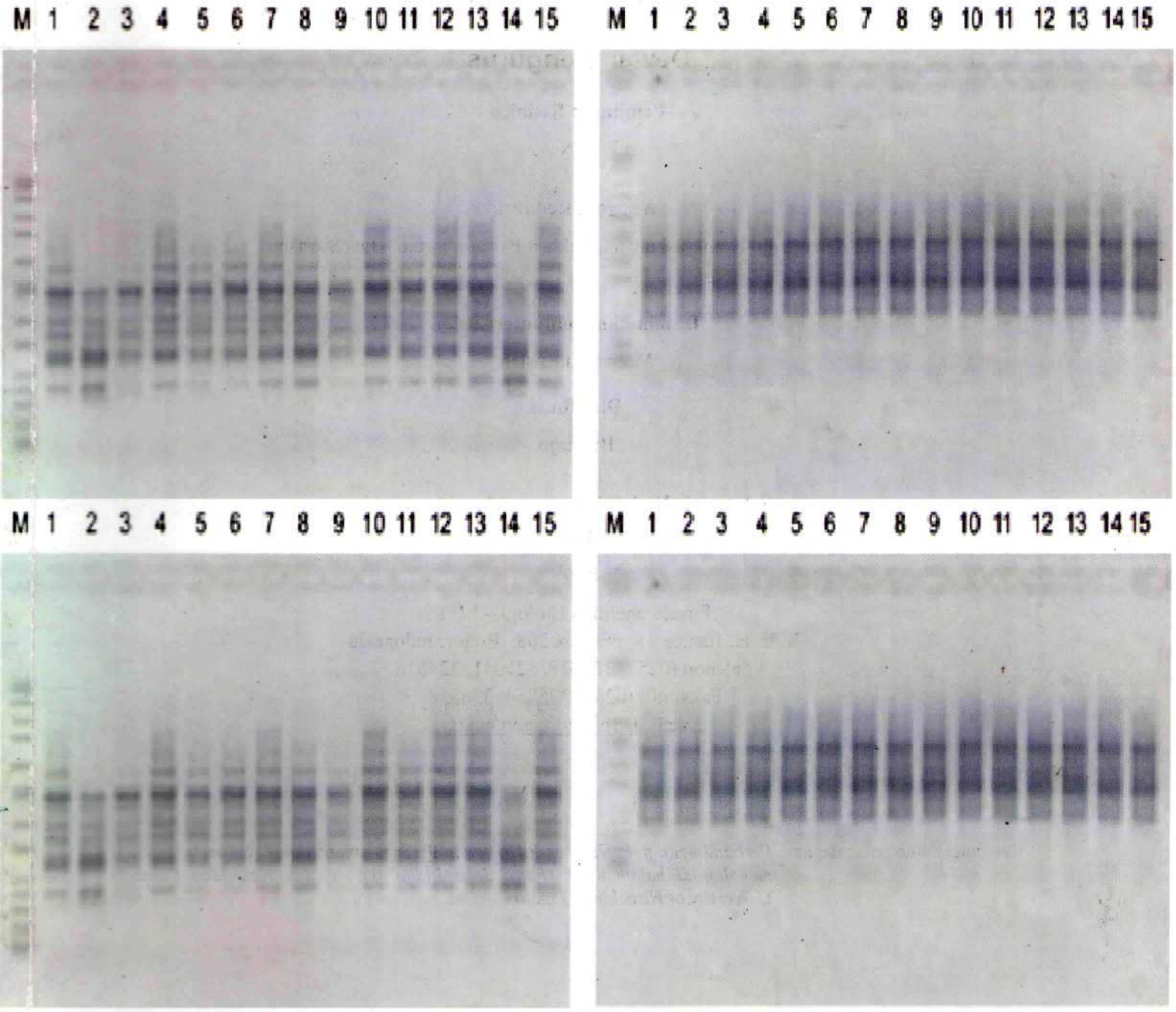

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Keterangan foto cover depan: *Perbandingan pola fragmen RAPD pada* Pinanga javana *dan* P. coronata, *sesuai makalah di halaman 91 (Foto: Joko Ridho Witono dan Katsuhiko Kondo, University of Hiroshima, Japan)*

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KATA PENGANTAR

Jurnal Ilmiah "Berita Biologi" Nomor ini yang tampil sebagai Volume 8 Nomor 2, Agustus 2006, memuat berbagai bahasan terutama dari hasil penelitian maupun tinjauan ulang (review) para peneliti dari berbagai institusi.

Orasi pengukuhan Ahli Peneliti Utama (APU), kali ini kami pilih dari dunia samudera, yakni karya Dr. Ir. Ngurah Nyoman Wiadnyana yang disampaikan pada tanggal 15 September 2005. Peneliti Senior yang membangun karier penelitiannya di Lembaga Penelitian Oseanografi-LIPI ini mengayakan kita dengan suatu topik yang sangat menarik: plankton dan "red tide" di ekosistem perairan (marine) Indonesia. Pemrasaran secara jelas mengemukakan topik yang belum banyak diteliti di Indonesia. Selain pengayaan pengetahuan tentang plankton, meliputi klasifikasi dan peran ekologis serta manfaat, secara khusus dibahas tentang red tide: fenomena, penyebab dan dampak yang ditimbulkannya. Dr. Wiadnyana mengangkat sebuah tantangan, khususnya bagi para peneliti: akankah Indonesia menjadi lautan red tide?; yang jika tidak dikelola secara bijaksana pertanyaan ini mungkin saja dapat menjadi suatu realita di masa depan, karena permasalahan fenomena red tide, menuratnya tampak semakin meluas di perairan Indonesia. Sementara kita tahu bahwa kebidupan marine adalah juga kehidupan kita masa lalu, sekarang dan masa depan!. Pada salah satu bagian orasinya, ditulis "……harapan saya semoga apa yang saya uraikan ini dapat dijadikan buah pemikiran dalam upaya terus mengembangkan ilmu planktonologi yang pada umumnya kurang mendapat minat dari para ilmuan muda....".

Masih dari Jepang, sebagai kelanjutan studi tentang *Pinanga,* dibahas aspek modifikasi protokol isolasi DNA dari jaringan daun yang dikeringkan dengan silica gel. Hasil penelitian ini merupakan bagian dari program doktor JRW di University of Hiroshima, Jepang. Sementara itu, informasi karakter kimia dari kekayaan keanekaragaman hayati Indonesia tercermin dalam hasil penelitian spesies *Hopea.* Laporan dari dunia hewan ternak tentang imunologi resistensi domba ekor tipis terhadap infeksi cacing hati. Pulai yang dikenal berpotensi sebagai tumbuhan obat dipelajari aspek kultur jaringannya, meliputi penyimpanan dan regenerasi. Selanjutnya masih dalam studi kultur jaringan, dilakukan terhadap jahe sebagai tanaman obat maupun industri, yakni pengaruh perlakuan-perlakuan spesifik terhadap induksi kalusnya. Studi tentang benalu memberikan gambaran ancaman potensial terhadap koleksi Kebun Raya. Suatu tinjauan ulang *{review)* membahas makluk hidup sebagai sumber obat anti-infeksi, dengan penekanan khusus pada aspek diversitas jalur biosintesis senyawa terpena.

Selamat membaca.

Salam Iptek,

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1. Karangan Ilmiah asli, *hasil penelitian* dan belum pemah diterbitkan atau tidak sedang dikirim ke media lain.
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   - Ilmu serumpun dengan biologi: pertanian, kehutanan, peternakan, perikanan dan biologi laut, agrobiologi, agro bioklimatologi, kesehatan, kimia, lingkungan, agroforestri. *Aspek/pendekatan biologi* hams tampak jelas.
4. Deskripsi masalah: hams jelas adanya tantangan ilmiah *(scientific challenge).*
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    a. Jurnal
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    b. Buku
       Kramer PJ, 1983. *Plant Water Relationship.* Academic, New York, 76.
    c. Prosiding atau hasil Simposium/Seminar/Lokakarya dan sebagainya
       Hamzah MS dan Yusuf SA. 1995. Pengamatan Beberapa Aspek Biologi Sotong Buluh *(Sepioteuthis Lessoniana)* di Sekitar Perairan Pantai Wokam Bagian Barat, Kepulauan Am, Maluku Tenggara. *Prosiding Seminar Nasional Biologi XI,* Ujung Pandang 20-21 Juli 1993. M. Hasan, A. Mattimu, JG Nelwan dan M. Littay (Penyunting). Perhimpunan Biologi Indonesia, 769-777.
    d. Makalah sebagai bagian dari buku
       Leegood RC and Walker DA. 1993. Chloroplast and Protoplast. Dalam: *Photosynthesis and Production in a Changing Environment.* DO Hall, JMO Scurlock, HR Bohlar Nordenkampf, RC Leegood and SP Long (Editor). Champman and Hall. London, 268-282.
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DAFTAR ISI

MODIFICATION OF DNA ISOLATION PROTOCOL FROM SILICA GEL DRIED-LEAF TISSUES OF *Pinanga* (PALMAE)

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ABSTRACT

DNA isolation is an important issue in molecular fields especially for specific plant group such as *Pinanga* (Palmae). Typically, leaflets structure of *Pinanga* consists of shiny leaflets as common species of palms and mottled leaflets. *Pinanga javana* and *P. coronata* form commonly the two types of leaflets. The high quality of DNA of *Pinanga* with the shiny leaflet is readily purified and that with the mottled leaflet needed DNA purification due to the presence of high polyphenolic compound. Application of the silica binding method was recommended to purify the template DNA. Modified DNA-isolation protocol is relatively quick, simple, least expensive, minimum equipments and chemicals required, and suitable for PCR and endonucleases digestion reaction.

Key Words: DNA isolation, Java, *Pinanga,* Palmae.

INTRODUCTION

Pinanga consists of 132 species (Govaerts and Dransfield, 2005). The genus is becoming extremely popular in landscape cultivation for ornamental purposes in various countries in tropics and subtropics. It has been utilized to laths, walking sticks, and building materials madeout of stems; matting and thatch made out of leaves and betel substitute made out of fruits (Burkill, 1966).

Pinanga shows great variation in morphological and quantitative characters such as size, form and color of stem, crownshaft, leaf, inflorescence, fruit and seed, but due to unclear distinction patterns of morphological data, a modern monographic account of the genus has not been reported yet. On the other hand, molecular analysis are being forwarded for nearly all groups in plants including in palm family within the last decade.

Molecular techniques require isolation of genomic DNA of suitable purity for PCR *(polymerase chain reaction)* and restriction enzyme digestion. DNA isolated from plant tissue often yields variable results in such applications due to copurification of enzyme inhibitors. These enzyme inhibitors derive either from plant tissue, such as polysaccharides (Murray and Thompson, 1980;Pandey et al., 1996) and polyphenol (Couch and Fritz, 1990; Collins and Symons, 1992) or chemicals used in some DNA isolation protocols, such as CTAB (hexadecyltrimethylammonium bromide), SDS (sodium dodecyl sulphate), phenol, ethanol, isopropanol, sodium acetate, sodium chloride, and EDTA (ethylenediaminetetraacetic acid) (Peist *et al.*, 2001). The growing number of DNA isolation protocols for specific taxa suggested that extraction of DNA is not always simple and published protocols are not necessarily reproducible for all species. DNA extraction is an important issue in molecular field especially for specific taxa which has not been established for that particular crop.

Regardless of the distribution of the taxa under study, the DNA samples examined were isolated from fresh or perhaps freshly lyophilized tissue. Frozen materials at -20°C for short periods or -80°C for longer periods is usually used, but when plant materials obtained from natural population it is not practical. Dry plant materials may be used allowing extraction of DNA from silica gel dried leaf-tissues or herbarium. This method will continue to the method of choice for most systematic studies, especially for tropical angiosperms which are not well represented in botanical gardens, are not available as seeds or generally are not amenable to cultivation (Chase and Hills, 1991).

On *Pinanga* species, the leaves range from being undivided (entire) to pinnate resemble a feather or the backbone and ribs of a fish, with the leaflets

arranged either regularly or irregularly on the rachis. The leaflets *of Pinanga* are not remarkably different from those of other palm genera having the same basic structure and typically it consists of two types of leaflets, regular leaflets with green colour as common species of palms and mottled leaflets (Jones, 199S). The phenomenon of mottled leaflets in some species of *Pinanga* has attracted a great deal attention. Frequently, the mottling is spectacular on the developing new leaves which may have silvery, whitish, or reddish tonings and mature leaves may retain some mottling, such as *Pinanga disticha, P. aristata, P. bicolana* and *P. veitchii,* or becoming green color, such as *Pinanga coronata* and *P. densiflora.*

Here, the DNA isolation protocols described by previous authors were tested and the protocol described by Ban (199S) was modified in order to obtain high quality and consistent results of template DNA of *Pinanga* (Palmae). In the present study, two species *of Pinanga* from Java were used, *P.javana* as the representative for the shiny leaflets with green color and *P. coronata* as the representative for the mottled leaflets.

MATERIALS AND METHODS

Plant materials

Plant materials of *Pinanga* were obtained from natural population in Java, Indonesia. *Pinanga javana* (JW-331) was collected in Mt. Slamet, Central Java and *P. coronata* (JW-335) was collected in Mt. Pangrango, West Java. Total genomic DNA of *Pinanga* species were isolated from 20 mg dried-leaf tissues prepared in silica gel, whereas 100 mg fresh leaf tissues as control in cultivated status.

DNA Isolation Protocols

Chemicals required

. 2% CTAB = 100 mM Tris-HCI (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 2% CTAB (w/v)
. l%CTAB=50mMTris-HCl(pH8.0),10mMEDTA (pH 8.0), 0.7 M NaCl, 1% CTAB (w/v)
• Chloroform buffer = chloroform:isoamyl alcohol=24:l
. lMNaCl
. 99% ethanol
. 70% ethanol
. Sterilized distilled water (sdw).

Modifification of DNA isolation protocol of Ban (1995) as follows:

1. Homogenize the dry leaf lyophilized tissues (20 mg) in a 2.0 ml eppendorf tube with SK Mill to a fine powder
2. Add 25-50 µl of sterilized distilled water (sdw) and 300 µl of preheated 2% CTAB extraction buffer in 65° C, incubate at 65° C for 30 minutes with occasional gentle inversion and cool down at room temperature for 5 minutes
3. Add 400 µl of chloroform buffer, mix gently by inversion for 5 minutes, centrifuge at 12,000 rpm at room temperature for 15 minutes and transfer top aqueous phase into a new tube
4. Repeat step 3
5. Add 1% CTAB buffer 1-1.5 volumes of the supernatant, mix gently by inversion for 5 minutes, incubate at room temperature for 5 minutes, centrifuge at 8,000 rpm at room temperature for 10 minutes and discard the supernatant
6. Add 400 µl of 1 M NaCl to dissolve the pellet, add 800 µl of 99% chilled ethanol, incubate at -20°C for 30 minutes, centrifuge at 14,000 rpm at 4° C for 10 minutes, and discard the supernatant
7. Wash the pellet with 400 µl of 70% chilled ethanol, centrifuge at 14,000 rpm at 4° C for 5 minutes, and discard the ethanol
8. Dry the pellet at room temperature or vaccum dry, dissolve the DNA pellet in 50 µl of sdw and quantify DNA in a spectrophotometer.

Amplification **and** Electrophoresis

Different parameters were detected for optimization of PCR. Examination of the DNA qualities through used of RAPD (random amplified polymorphism DNA) reactions, whereas OPB 8 primer (Operon Technology Inc.) was chosen to amplify those two *Pinanga* species generated by the DNA isolation protocols as described above and other protocols. PCR mixtures were performed in a volume 20 µl containing sterilized distilled water (sdw), 150 µM primer, 250µM dNTP mixtures, l0x Taq buffer, 0.5 U Taq DNA

polymerase (Promega), and 20 ng of template DNA. Amplification was placed in the Gene Amp® PCR System 2700 (Applied Biosystems). The thermal cycling included (1) one cycle of predenaturation at 94°C for 3 minutes; (2) 45 cycles of denaturation at 94°C for 30 seconds, annealing at 36°C for 60 seconds, and extension at 72°C for 2 minutes; (3) one cycle of extension at 72°C for 10 minutes, followed by a soaking at4°C.

The fragments generated by PCR amplification were separated by electrophoresis on 1.5% (w/v) agarose gels submerged in 1 x TAE buffer, and stained with Ethidium Bromide (1.0 µg/ml) for 10 minutes. The fragments pattern was visualized and photographed using a gel documentation system (UVP Inc., UK). RAPD fragments size were estimated by running a DNA MW 0.05-10 kbp marker (Novagen) on the gel as a standard size marker.

RESULTS

Quantification of the DNA can be achieved by using uv spectrophotometer or running the template DNA on 1.5% agarose gel stained with Ethidium Bromide (1.0 µg/ml). Ethidium Bromide is a fluorescent chemical that intercalates between base pairs in a double stranded DNA molecules. Aliquots of template DNA of *Pinanga javana* (Figure la) and *P. coronata* (Figure lb) were loaded 7 µl of DNA mix contains 2 µl of loading dye and 5 *µl* of each DNA samples geneiated by fifteen DNA isolation protocols. High molecular weight DNA will appear as a well-resolved band alongside the lambda DNA bands whilst the smear below the band indicates mechanical or chemical degradator. A smeard band towards the bottom of the gel is an indication of presence of RNA.

In the study, the DNA was quantified using spectrophotometer measurement of uv absorption at wavelength 260 nm, 280 nm, and 320 nm, the DNA contaminants were detected. The average of quantity and quality of DNA generated by some DNA isolation protocols of *Pinanga javana* and *P. coronata* were presented in Table 1.

DISCUSSION

The process of DNA isolation from leaf tissue requires to break cell walls in order to release the cellular constituents. After breaking the cell walls, next step is disrupting the cell membranes to release the DNA into extraction buffer. In general, there are two main detergents are used for extraction buffer, CTAB (Doyle and Doyle, 1988) and SDS (Dellaporta *et al.*, 1983). The other chemicals usually be used to isolate DNA

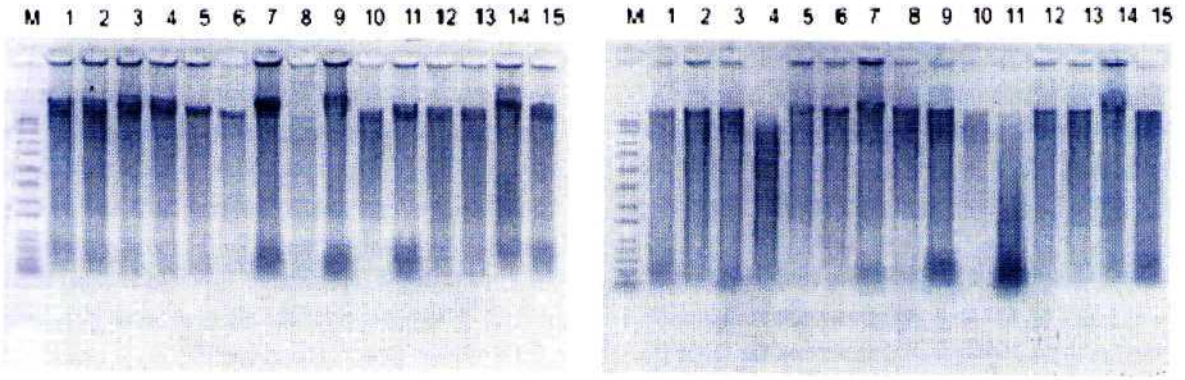

Figure 1. Comparisons of template DNA yields **generated** by **fifteen DNA isolation protocols:** 1. Modification of Ban (1995); 2. Doyle and Doyle (1988); 3. **Saghai-Maroof** *etal.* **(1984); 4. Aras** *et al.* (2003); 5. Lodhi *et al.* (1997);6.Porebskie et al.(1997);7.Cullings(1992);8.Dellaportaet al.(1983);9.Gaweland Jarret(1991); 10. DNeasy Plant Mini Kit (Qiagen); 11. Storchova *et al.* (2000); 12. Wittzel (1999); 13. Ziegenhagen *et al.* (1993); 14. Ban (1995) using fresh **leaf** tissue; 15. **Ban (1995) with addition PVP.** (A) *Pinanga javana* and (B) *P. coronata*

Table 1. The average of quantity and quality of DNA generated by fifteen DNA isolation protocols for *Pinanga javana* and *P. voronata* in a volume 50µl

No.	Protocol	Species	OD A260/A280	Quantity (ng/µl)
1	Modification of Ban, 1995	*P. javana*	1.83	485
		P. coronata	**1.79**	269
2	Doyle and Doyle, 1988	*P. javana*	**1.74**	535
		P. coronata	**1.65**	414
3	Saghai-Marooft et al., 1984	*P. javana*	1.85	366
		P. coronata	1.71	533
4	Aras et al., 2003	*P. javana*	1.68	708
		P. coronata	1.61	1018
5	Lodhi et al., 1997	*P. javana*	1.70	289
		P. coronata	1.56	1181
6	Porebski *et al.*, 1997	*P. javana*	1.63	103
		P. coronata	1.53	592
7	Cullings, 1992	*P. javana*	1.86	715
		P. coronata	1.58	1303
8	Dellaporta et al., 1983	*P. javana*	1.75	122
		P. coronata	1.55	1053
9	Gawel and Jarret, 1991	*P. javana*	1.77	862
		P. coronata	1.74	691
10	DNeasy Plant Mini Kit (Qiagen)	*P. javana*	1.84	158
		P. coronata	1.70	51
11	Storchova *et al.*, 2000	*P. javana*	1.98	384
		P. coronata	1.87	852
12	Wittzel, 1999	*P. javana*	1.78	758
		P. coronata	1.57	1199
13	Ziegenhagen *et al.*, 1993	*P. javana*	1.82	310
		P. coronata	1.56	**540**
14	Ban, 1995 (fresh leaf tissue)	*P. javana*	1.84	**1013**
		P. coronata	1.83	686
15	Modification of Ban (1995) by addition PVP	*P. javana*	1.78	619
		P. coronata	1.77	688

from plants are PVP (polyvinylpyrrolidone) or PVPP (polyvinylpolypyrrolidone) to remove polyphenol (Maliyakal, 1992); RNAse A to isolate DNA free from RNA and chloroform and or phenol treatments to remove any excess of protein from the DNA extracts by denaturation and precipitation (Saghai-Maroof *et al.*, 1984); Proteinase-K to digest protein in the **DNA** extracts (Shaw, 1988); EDTA to protect the DNA from endogenous nucleases by chelatting Mg ions as a cofactor for most nucleases; and sodium chloride in the buffer to remove **polysaccharides (Lodhi *et al.*,** 1995).

A pure template DNA should be in a ratio 1.60 to 1.90, whereas lower or higher than those ranges would not be successfully **during PCR.** In our case, even **the DNA ratio ranges from 1.60-1.90, but the template DNA colour was brown indicated that it** contained **high amount of polyphenol and will be** seriously **inhibited during PCR.**

The modification of Ban (1995) protocol described here **was** successfully to obtain high **quality** of DNA on *Pinanga javana* **and other** palm **taxa with** green color as **common** species **of palms** such as *Areca, **Nenga, Hydriastele, Satakentia,* **and *Arenga,* but it could not work on *Pinanga coronata* as other** protocols **tested in the study. However, comparing the** other protocols, the **DNA** contaminants much lower (detailed data were **not** shown). **The** template **DNA**

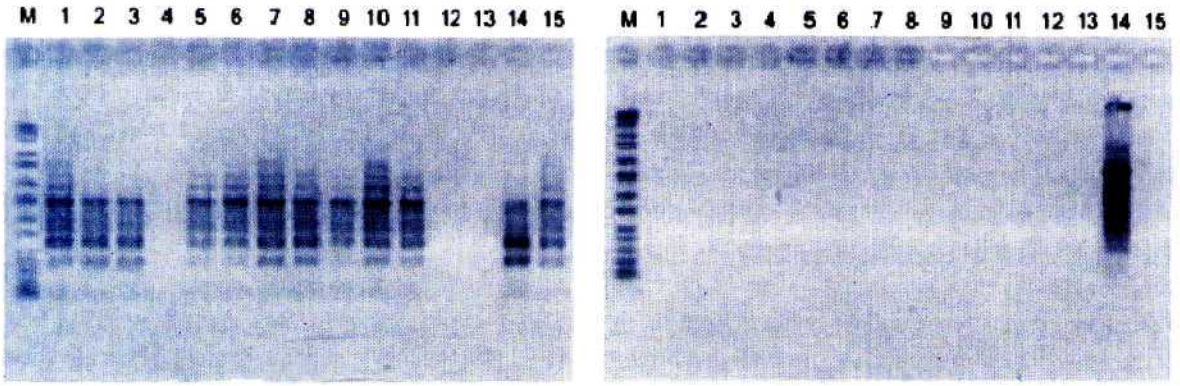

Figure 2. RAPD banding pattern comparisons generated by OPB 8 primer of fifteen DNA isolation protocols described above. (A) *Pinanga javana* and (B) *P. coronata*

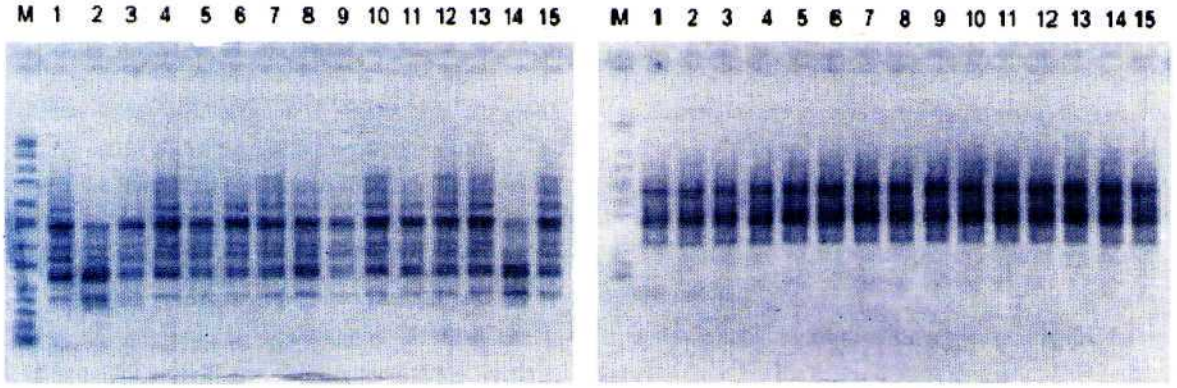

Figure 3. RAPD banding patterns generated by OPB 8 primer after purification using Gene Clen II Kit (Bio. Inc.) of fifteen DNA isolation protocols described above. (A) *Pinangajavana* and (B) *P. coronata*

generated by twelve DNA isolation protocols were successfully amplified during PCR for *Pinangajavana* (Figure 2a) rather than *P. coronata* (Figure 2b) which was only one protocol using fresh leaf tissues as control succeed. Generally, the protocol described by Ban (1995) was successfully to isolate high quality of DNA from fresh leaf tissues without any modifications on palms species. The modification of Ban (1995) protocol was only with addition 25-50 µl of sterilized distilled water (sdw) soon as after homogenization dry leaf lyophilized tissues and it took less than 3 hours to isolate the DNA. In our experiences, there was no or very low of template DNA could be obtained without addition sdw.

In case, the modification of Ban (1995) protocol could not get any satisfactory results, using silica binding method such as Gene Clean II Kit (Bio 101 Inc.) was recommended to purify the template DNA. As a consequence, DNA concentration became lower but it worked succesfully for molecular analysis PCR-based such as **RAPD** and **ISSR** (inter simple sequence repeat) methods, DNA sequencing and also for restriction endonuclease reactions. Figure 3 were shown all samples of template DNA generated by fifteen DNA isolation protocols were successfully amplified during PCR after purification.

No universal protocols is available to isolate the DNA from plant tissues, each protocol has their own

advantage and disadvantage. In our experiences, the best leaf tissues of *Pinanga* for DNA analysis is unexpanded fresh leaf, because the contaminant compounds is very low. Fresh leaf tissues is still the preferred, but silica-gel dried tissues have also proven themselves a nearly equivalent source and a great deal more practical, reliable, and inexpensive. If liquid nitrogen is not available where plant tissues is collected or cultivated, the protocol described here also produced satisfactory result after removing fibrous from leaf tissues.

Regarding to our experience to isolate the DNA from sortie species of *Pinanga,* suggested that modification of Ban protocol (1995) was useful. The protocol modified here is relatively quick, simple, least expensive, minimum equipments and chemicals required, provides clean DNA comparing other DNA isolation protocols and consistently amplifiable in PCR and endonucleases digestion reaction, except particular species which is purification step is necessary due to polyphenolic compound.

ACKNOWLEDGEMENTS

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