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BRIEF COMMUNICATION

A simple and efficient method of DNA isolation from orchid species and hybrids

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Abstract

A simple and reliable method for extracting DNA has been developed for orchid species and hybrids. The high quality of DNA obtained is suitable for amplification via the polymerase chain reaction (PCR) for producing random amplified polymorphic DNA (RAPD) markers.

Additional key words: DNA extraction, PCR, RAPD markers.

Orchids are important ornamental plants. The orchid cut flower industry is increasing in value and contributes significantly to the economy of countries like Thailand, Singapore and Malaysia. Many new hybrids are bred every year for both hobbyists and the cut flower industry. It is therefore important to distinguish between cultivars of commercial value. A very quick way of determining identity and parentage of these orchid hybrids is to study its random amplified polymorphic DNA (RAPD) markers. One prerequisite of amplification via PCR is contamination-free DNA. Unfortunately, orchid leaves and petals have high amounts of polyphenols and polysaccharides and RAPD analysis is often hindered by the presence of contaminating RNA. To overcome this problem, different DNA extraction methods were attempted, one of which proved successful. We report here a quick and efficient method of DNA extraction, a modification of the method by Honda and Hirai (1990) to include RNase A and phenol-chloroform treatment. High yields of pure orchid DNA amplifiable via PCR were obtained for use in the production of RAPD markers.

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Four species and sixteen hybrids, including interspecific and intergeneric hybrids were studied (Table 1). Tissue samples were collected and surface sterilized with 10 % (v/v) sodium hypochlorite for 10 min. They were then rinsed with distilled water 5 times. Approximately 0.1 g of fresh plant tissue was used. First, the tissue was ground to a fine powder using mortar and pestle in the presence of liquid nitrogen. Then, 0.06 cm^3 of polyvinylpolypyrrolidone (100 mg cm^{-3}) was added to the homogenate. 0.6 cm^3 of extraction buffer (100 mM Tris-HCl, pH 8.0, 50 mM EDTA, pH 8.0, 500 mM NaCl and 100 mM mercaptoethanol) was then added followed by 0.04 cm^3 of 20 % sodium dodecyl sulphate. The tubes were inverted several times to mix its contents and the sample was incubated at 65 °C for 10 min. Following this, one tenth volume of 5 M potassium acetate (pH 5.2) was added and the mixture was incubated on ice for 20 min. The samples were then centrifuged at 10 000 *g* for 20 min at 4 °C. The supernatant was decanted and 0.4 cm^3 of isopropanol was added to precipitate the DNA. This was left to incubate at -20 °C for 1 h or -80 °C for 15 min. The mixture was then centrifuged at 10 000 *g* for 15 min at 4 °C to pellet the DNA. Following this, it was then resuspended in 0.2 cm^3 Tris EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) and 1 mm^3 of RNase A (10 mg cm^3) was added. This was left to incubate at 37 °C for 30 min. Then, 0.2 cm^3 of phenol:chloroform (1:1) was added, the mixture vortexed and then centrifuged at

Table 1. DNA yield [μg g^{-1}(f.m.)] from selected orchids (HYP - How Tee Peng orchid nursery, NUS - Department of Biological Sciences, National University of Singapore, SBG - Singapore Botanical Gardens, SMG - Mandai Orchid Gardens of the Singapore Pte Ltd.)

		Plant part	Source	DNA yield
Species	*Arachnis hookeriana*	petal	SMG	330.1
	Spathoglottis plicata	petal	NUS	81.9
	Vanda deerei	petal	SMG	128.5
	Vanda sanderiana	leaf	SBG	200.2
Hybrids	*Aranda* Christine	petal	SMG	146.1
	Mokara Bangkok Gold	petal	SMG	328.3
	Mokara Bibi	petal	SMG	331.3
	Mokara Chart Kuan	petal	SMG	178.9
	Mokara Esmaco	petal	SMG	106.8
	Mokara Kelvin	petal	SMG	236.1
	Mokara Khaw Phaik Suan	petal	SMG	95.7
	Mokara Luenberger Gold	petal	SMG	127.6
	Mokara Mak Chin On	leaf	SMG	281.2
	Mokara Millie Loon	leaf	SMG	130.7
	Mokara Sumalee	petal	SMG	140.0
	Mokara Walter Oumae 'yellow'	petal	SMG	93.3
	Mokara Willie How	petal	HYP	154.6
	Vanda Josephine van Brero	petal	SMG	151.5
	Vanda Tan Chay Yan	leaf	SMG	173.2
	Vanda T.M.A.	petal	SMG	227.5
	Vanda T.M.A.	leaf	SMG	217.1

10 000 *g* for 5 min at 4 °C. The aqueous phase was removed and the phenol:chloroform extraction was repeated. After this, 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 100 % ethanol was added and the mixture left to incubate at -80 °C for 30 min. The DNA was then pelleted by centrifugation at 10 000 *g* for 10 min at 4 °C. The pellet was washed with 70 % ethanol and dried in a speed vacuum. The pellet was then dissolved in 0.025 cm^3 of Tris EDTA buffer. The isolated genomic DNA can be stored in 4 °C for long periods of time.

This method yielded pure DNA from various orchids. The extracted DNA could also be restricted to completion with *Eco*RI and is thus suitable for Southern analysis (Southern 1975). The DNA is also appropriate for PCR amplification using either specific primers (Erlich 1989) or the RAPD technique (Williams *et al.* 1990) (Fig 1).

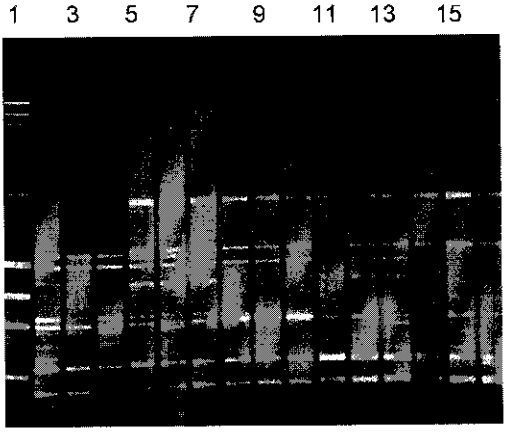

Fig. 1. Amplification profiles of *Arachnis hookeriana* (*lanes 2 - 4*) and resultant hybrids *Mokara* Walter Oumae 'yellow' (*lanes 5 - 7*), *Mokara* Bangkok Gold (*lanes 8 - 10*), *Mokara* Kelvin (*lanes 11 - 13*), and *Mokara* Bibi (*lanes 14 - 16*) obtained from RAPD analysis. *Lane 1* - Lambda *Hind* III and ϕx-174 *Hae* III DNA markers.

DNA yields of 80 to 330 μg g^{-1}(f.m.) were obtained in our laboratory using this method (Table 1). This compares well with other published methods of DNA extraction (Lodhi *et al.* 1994, Li *et al.* 1994). Although the methods of Lodhi *et al.* (1994) and Li *et al* (1994) work well for the recommended plants, it failed to produce a good yield in orchids. In orchids, the extract was found to be contaminated with ribosomal RNA, thus RNase A was included in our protocol. There is also a high content of polysaccharide-like compounds and secondary metabolites in orchid DNA extracts making the DNA pellet more insoluble. Phenol-chloroform extraction was therefore necessary. In our experiments, both leaf and petal samples gave satisfactory yields (Table 1). Many orchids however, have thick, succulent leaves with sclerenchyma fibers which interfere with DNA extraction. Thus, floral parts like petals and sepals are preferred for DNA extraction. When leaves are sampled, it is recommended that DNA be isolated as far as possible from younger leaves which

contain less polysaccharide-like substances. Our method can process approximately 30 samples in a single day and the DNA can be stored for long periods at -20 °C.

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