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Genome Note

Random amplified polymorphic DNA analysis to distinguish *Brugmansia suaveolens, B. candida* and *B. versicolor*

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Abstract DNA isolation followed by random amplified polymorphic DNA (RAPD) analysis was assessed as a possible method to distinguish three Indonesia species of *Brugmansia* because these species cannot be distinguished by metabolite analysis or morphological observation when they are not flowering. The DNA fragments were obtained by PCR, using random primers of isolated DNA, and the RAPD analysis showed that each species had different DNA fragment patterns, thus allowing each species to be individually distinguishable. The DNA patterns of each species remained the same, regardless of the samples region in Indonesia.

Key words: *Brugmansia*, random amplified polymorphic DNA.

Brugmansia leaf (Solanaceae) is an alternative source of tropane alkaloids such as hyocyamine, scopolamine and atropine. Three species of *Brugmansia* can be found in Indonesia, namely *B. suaveolens* B. et Pr., *B. candida* Pers and *B. versicolor*. Both *B. suaveolens* and *B. candida* have white corolla but the former has two layers while the latter has one layer only. *B. versicolor* can be distinguished from the other two *Brugmansia* by a yellowish single corolla layer. The thin layer chromatography (TLC) of the alkaloid from the leaves of *B. suaveolens* and *B. candida* is indistinguishable, although the total alkaloid content of the latter is higher than the former (Meijer 1957; Serrano et al. 1977). However, it is almost impossible to distinguish between these species by metabolite analysis (TLC) or morphological observation when they are not flowering. Recently, random amplified polymorphic DNA (RAPD) analysis has been used to help classify natural medicines (Kohjyouma et al. 1998) and the DNA extraction method has been improved (Yamazaki et al. 1995). In our study, DNA isolation followed by RAPD analysis was conducted to assess the potential for this method to distinguish the three species of *Brugmansia*. Leaves from several regions in West Java in Indonesia were analysed to determine if these was any regional variation in DNA, either between species, or within one species.

Leaves were collected from Lembang, Pangalengan and Bandung (Dago) in District of West Java. Several mg of freeze-dried leaf samples were ground to a powder in a mortar using a pestle with liquid nitrogen. Total DNA was extracted from the leaf powder (ca. 50 mg) of each *Brugmansia* spp., from each location, by the method described in the protocol manual of the Isoplant DNA extraction kit (Wako Chemicals Co. Japan). The reaction mixture (25 μl) for PCR was composed of 2.5 μl of the buffer for Ex-Taq^{TM} polymerase, 0.125 μl of Ex-Taq^{TM} polymerase (0.625 unit, Takara Co), 0.5 μl of dNTP (25 mM each, Takara Co.), 1 μl of primer solution (1 pmole, Operon, U.S.A.), 25 ng of template and added ddH_2O to adjust to 25 μl (pH 7–8). After denaturation at 94°C for 1 min, thermal cycling was carried out for 45 cycles of 1 min at 94°C, 2 min at 41°C and 3 min at 72°C, followed by one cycle of 10 min of 72°C. Four types of commercial random primers (OPF-01; ACGGATCCTG, OPF-14; TGCTGCAGGT, OPF-15; CCAGTACTCC, OPF-16; GGAGTACTGG) were used for PCR amplification. The amplification products were subjected to electrophoresis at 50 V in 2.0% agarose gel with TAE (Tris-Acetate 40 mM and EDTA 1 mM) buffer (pH 8.0). After staining with ethidium bromide, the gel was exposed to UV light.

All four primers used in the experiment generated clear fragment patterns. Interspecific variation was found between *B. candida*, *B. suaveolens* and *B. versicolor* (Figure 1). The three species were distinguishable by their DNA fragment pattern, but there was no difference between the DNA samples of the leaves of any individual species collected from the different locations. Figure 1

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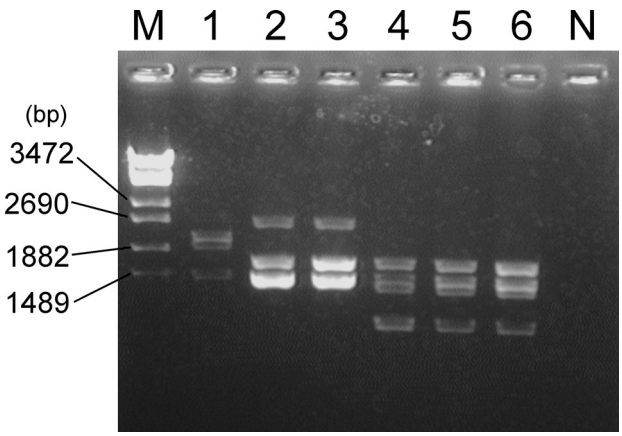

Figure 1. Agarose gel electrophoresis of PCR products obtained from the total DNA of freeze-dried leaves of *Brugmansia candida*, *B. suaveolens* and *B. versicolor*. A DNA primer of OPF-15 was used for the PCR. Lane 1, *Brugmansia candid*a from Lembang; Lane 2, *B. suaveolens* from Lembang; Lane 3, *B. suaveolens* from Pangalengan; Lane 4, *B. versicolor* from Lembang; Lane 5, *B. versicolor* from Pangalengan; Lane 6, *B. versicolor* from Bandung(Dago); Lane N, no template; Lane M, DNA marker of λ phage DNA/Sty I digest.

shows the distinguishable DNA fragment patterns using OPF-15 as a DNA primer. They were also distinguishable when OPF-1, PPF-14 and OPF-16 were used as DNA primers (data not shown).

The results of RAPD analysis in this study suggest that this could be a useful technique to distinguish *Brugmansia candida*, *B. suaveoloens* and *B. versicolor* as they are not distinguishable by simple morphological observation of the plant when they are not flowering.

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References

Kohjyouma M, Iida O, Yoshida H, Hatakeyama Y, Satake M, Sekita S, Kohda H (1998) Random amplified polymorphic DNA analysis of *Angelica acutiloba* and its varieties. *Natural Medicines* 52: 130–134

Meijer TM (1957) The Alkaloids of *Brugmansia suaveolens*, *Suara Pharmasi* 2: 18–31

Serrano JJ, Godeau, J, Liutkus, M, Saumade, R, Agoes G (1977) Pharmacological Study of Plant Extracts BS 177. *Acta Pharmaceutica* 6: 134–148

Yamazaki M, Sato A, Shimomura K, Inoue K, Ebizuka Y, Murakoshi I, Saito K, (1995) Extraction of DNA and RAPD analysis from dried licorice root. *Natural Medicines* 49: 488–490