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RAPD analysis of *Lycium barbarum* medicine in Taiwan market

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Abstract. In this study, we investigated *Lycium barbarum*, a Chinese medicine sold on the Taiwan market, using RAPD analysis. Sixty oligonucleotide primers were used to screen twenty randomly selected samples in the analysis. Total DNA extracted from the fruit of the medicine material was used as template in the PCR reaction. Four primers: OPD-15, OPG-15, OPT-12 and OPT-17, showed distinct polymorphic patterns, but others exhibited profiles nearly identical to the other samples used in the study. We found that only two RAPD fingerprinting types of these primers were outlined from twenty collected *Lycium* samples. Fifteen samples showed the first type of profiles while only five samples resulted of the second type. A low genetic diversity among the *Lycium barbarum* samples was revealed by RAPD analysis.

Keywords: Chinese medicine; DNA; *Lycium barbarum*; RAPD.

Introduction

The species *Lycium barbarum* L. (Gouqi) of the Solanaceae family is cultivated in Ningsia Province. The fruit of the herb is used for medicinal purposes and is usually found in the herbal markets of Taiwan. It is used to replenish the vital essence of the liver and kidney and to improve visual acuity. Chinese physicians prescribe it to strengthen muscles and bone (Huang, 1993).

The method known as RAPD (random amplified polymorphic DNA), which is simple and faster than other DNA fingerprinting techniques, uses a single oligonucleotide primer in a PCR (polymerase chain reaction) with low stringency. The technique requires no sequence information prior to analysis and only a minute amount of DNA (Welsh and McClelland, 1990; Williams et al., 1990). Therefore, unlimited markers have been created by RAPD for the purpose, for instance, of identifying the component species in Chinese medicine materials (Cheng et al., 1997; Shaw and But, 1995), and differentiating between genuine and counterfeit materials (Cheng et al., 1998). The disadvantage of the technique may be its low fidelity in some circumstances. Questionable DNA quality is considered to be the main factor in this problem (Micheli et al., 1994). The DNA extracted from dried materials, such as root, stem, or fruit, is often contaminated with proteins, polysaccharides, and secondary metabolites, which decrease reproducibility. In our previous studies, Chinese medicines of the *Coptis* species and *Cordyceps* species were identified using RAPD analysis (Cheng et al., 1997; Cheng et al., 1998). In Taiwan, the numerous sources of Chinese medicines in the imported market can lead to great variances in the quality of active compounds. DNA fingerprint patterns could be useful in identifying the species and the sources of various medicine samples and as an aid to quality control. In the present study, twenty Chinese medicine materials of *Lycium barbarum* sold by stores were analyzed using RAPD to understand the genetic variations among them.

Materials and Methods

Materials

Twenty medicine materials of *Lycium barbarum* sold by stores were collected from the Taipei region. The samples used in the study were confirmed by the National Laboratories of the Food and Drug Department of the Health Executive Yuan in Taiwan.

DNA Extraction

Dried rhizome was washed in 70% ethanol for 5 min. and in sterile deionized water for 1 min, using sonication to avoid surface contamination. After being air dried, the sample was cut in pieces and ground into powder with mortar and pestle. DNA was extracted from the sample using a modified CTAB (cetyltrimethylammonium bromide) procedure (Rogers and Bendich, 1985). 0.1 g of the powdered rhizome was added to 1.2 ml of 2X CTAB extraction buffer [2% CTAB; 100 mM Tris-HCl, pH 8; 20

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mM EDTA; 1.4 M NaCl; 0.2% 2-mercaptoethanol]. The suspension was incubated in a water bath at 90°C for 30 min with occasional shaking, then cooled to room temperature and extracted with one vol. aqueous phenol/chloroform/isoamyl alcohol (25:24:1) twice. After centrifugation at 12,000 *g* for 10 min, 0.1 vol. 65°C 10% CTAB buffer [10% CTAB; 0.7 M NaCl] was added to the upper aqueous. The mixture was extracted by phenol/chloroform/isoamyl alcohol extraction once more. The two phases were separated by centrifugation and 1 vol. CTAB precipitate buffer [1% CTAB; 50 mM Tris-HCl, pH 8; 10 mM EDTA, pH 8] was added to the aqueous phase. The mixture was centrifuged at 12,000 *g* for 20 min. The pellet was washed with 70% ethanol twice, then dissolved in 200 µl sterilized 1/10X TE. The solution was used for RAPD reaction.

RAPD Reaction

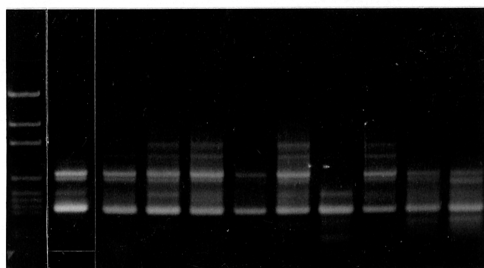
Sixty decamer oligonucleotide primers (kit D, G and T) for RAPD analysis were purchased from Operon Technologies Inc. (Alameda, CA, USA). One µl of template DNA (5 ng) was amplified in 25 µl PCR mixture consisting of 1X PCR buffer (Viogene, USA), 0.2 mM dNTP, 0.8 µM primer and 0.625 U T*aq* polymerase (Viogene). For DNA amplification, a Perkin Elmer Cetus 2400 DNA thermocycler was programmed for 2 cycles of 1 min at 94°C, 2 min at 40°C, and 2 min at 72°C followed by 43 cycles of 1 min at 94°C, 1 min at 45°C, and 2 min at 72°C, then terminating with 7 min at 72°C. The RAPD fragments were separated on 1.5% agarose gel by electrophoresis in 1X TAE buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8) and stained with ethidium bromide.

Results and Discussion

Four of sixty decamer primers, OPD-15: 5'CATCCGTGCT3', OPG-15: 5'ACTGGGACTC3', OPT-12: 5'AGGACTGCCA3' and OPT-17: 5'CCAACGTCGT3', showing distinct polymorphic fingerprints, were selected to reveal the genetic variations among the *Lycium barbarum* samples. The fingerprints of the four primers are consistent. Ten representative samples of the fingerprints are shown in Figure 1. The patterns of the samples were divided into two types: the first type contains No. 1, No. 5, No. 7, No. 9, and No. 10. The other fifteen samples are the second type. We supposed that there were two sources from which the stores imported the *Lycium barbarum* material. In addition to *Lycium barbarum*, *Lycium Chinensis* Mill. is also found in the market. For this study, we could not obtain the standard samples of these species materials or the medicines sold in the Taiwan market would be easily investigated. Ideally, samples

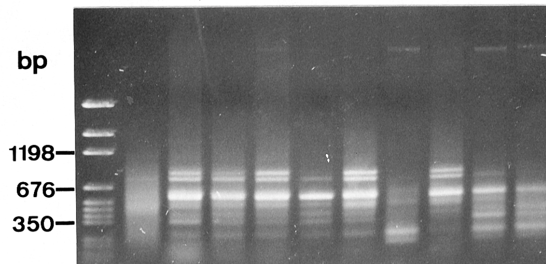
OPD-15

M A B C D E F G H I J



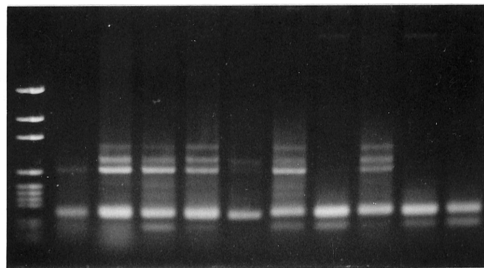
OPG-15

M A B C D E F G H I J



OPT-12

M A B C D E F G H I J



OPT-17

M A B C D E F G H I J

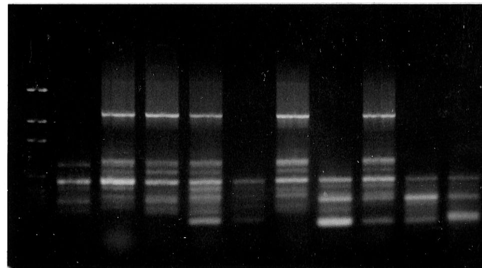


Figure 1. The RAPD fingerprints of sold *Lycium barbarum* medicines generated with OPD-15, OPG-15, OPT-12, and OPT-17 primer, respectively. M: DNA marker; A-J: ten representative samples.

would be collected directly from the fields in China to ensure their purity. The available components content in Chinese herbal medicines varies with the species of the plants, so the work of identification is important to the market.

As for the RAPD analysis of other Chinese medicine materials, such as Rhizoma Coptidis (Huanglian) and Radix Astragali (Huangqi), we found that the Operon primer kit T presents polymorphic patterns among the test samples most easily. The products generated by the primers of the kit will be subjected to further sequencing analysis which should reveal their meanings on the genome.

The DNA is first extracted from the fruit of the Chinese medicine material Fructus Lycii in the RAPD analysis. Unlike rhizome and radix kinds of materials, the DNA extracted from this sample is purer and of a greater quantity. The efficiency of the PCR reaction is dependent on the characteristics of the sample. Low polysaccharides and low polyphenolic content in the sample are thought to make the material suitable for PCR reaction. The relatively small amount of DNA released and the high metabolites existing in the dried rhizomes or radixes might be the limiting factors affecting the reproducibility of RAPD. DNA contamination and wound DNA both will easily introduce variable RAPD patterns (Micheli et al., 1994).

The polymorphic patterns among the samples subjected to RAPD analysis revealed low genetic variations present in the *Lycium barbarum* samples we collected. The RAPD technique provides a sensitive and fast method that facilitates the identification of a large number of herbs. We hope that high quality-linked DNA markers of herbal medicines will be developed in future studies.

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以逢機擴增多型性 DNA 分析市售枸杞藥材之遺傳變異

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本研究以逢機擴增多型性 DNA (RAPD) 技術，分析二十家中藥店市售枸杞藥材之遺傳變異。以六十條含十個逢機排列之核±Ò酸的引子，進行RAPD分析的結果顯示，有四條引子的反應產物，可將樣品呈現不同之電泳圖譜。此四條引子分別為 OPD-15，OPG-15，OPT-12 和 OPT-17。其結果均一致，在二十個市售樣品中，有十五家為第一型，另五家為第二型。該研究顯示，市售枸杞藥材的遺傳變異低，推測這些藥材可能來自兩個不同的來源，或根本為兩個不同的種，若有標準品，即可進一步證實。

關鍵詞：枸杞；逢機擴增多型性DNA。