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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.

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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