

RAPD Profile for Authentication of Medicinal Plant *Glycyrrhiza glabra* Linn.

Salim Khan^{1*} • Khanda Jabeen Mirza² • Md Tayaab² • Malik Zainul Abdin²

¹ Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, 11451, Kingdom of Saudi Arabia

² Centre for Transgenic Plant Development, Department of Biotechnology, Faculty of Science, Jamia Hamdard, Hamdard Nagar, New Delhi- 110062, India

Corresponding author: * salimkhan17@yahoo.com

ABSTRACT

Correct identification and quality assurance is indispensable to ensure reproducible medicinal quality of herbal drugs. Authentication is especially useful in the case of those medicinal herbs that are frequently substituted or adulterated with other species or varieties morphologically and phytochemically indistinguishable. In this study, RAPD (Random Amplified Polymorphic DNA) was employed for authentication of *Glycyrrhiza glabra* L. from its adulterant *Abrus precatorius* L. Fifty two decamer oligonucleotide primers were screened in the RAPD analysis for identification of genuine and adulterant samples using the DNA isolated from the dried root of samples. Sixteen primers gave species-specific reproducible unique amplicons, which could clearly distinguish genuine as well as adulterant samples having similar morphology. RAPD could thus, help to serve as a complementary tool for quality control.

Keywords: *Abrus precatorius*, adulterant, herbal drugs, polymerase chain reaction

INTRODUCTION

Traditional herbal and herbo-mineral drugs have been used since the dawn of civilization to maintain and alleviate human sufferings from diseases. These medicinal herbs have been in use in one form or another, under indigenous systems of medicine like Ayurveda, Sidha and Unani. According to an estimate of the world health organization (WHO; <http://www.who.int/research/en>), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. *Glycyrrhiza glabra* is one of the most frequently employed botanicals in traditional medicine. The root of *G. glabra* is known as licorice and its history as a medicinal plant is very old and has been used in many societies for millennia (Wang 2001). There are many useful compounds in licorice root such as glycyrrhizin, a saponin-like glycoside 50 times sweeter than sugar, and its aglycone, glycyrrhetic acid, which is clinically used for hyperlipidemia (Tamir *et al.* 2001). Licorice flavonoid constituents mainly include flavones, flavonols, isoflavones, chalcones, bihydroflavones and bihydrochalcones. Pharmacological investigations indicate that they have antioxidant, antibacterial and anti-inflammatory activities (Vaya *et al.* 1997). *Abrus precatorius* is a leguminous plant of the Fabacea family that is also called Indian liquorice, Jequirity, Crab eye, *Glycyrrhizin glabra*, among others (Ligha *et al.* 2009). The plant grows widely in fairly dry climates of tropical and subtropical regions, such as India, Sri Lanka, Nigeria and the West Indies. The leaves, roots and seeds of *Abrus precatorius* are used for medicinal purposes, a practice most probably dating back to antiquity (Ivan 2003). Similarly, several herbal drugs on the market still cannot be identified or authenticated based on their morphological or histological characteristics. Use of a wrong herb may be ineffective or it may worsen the condition and may even cause death. In Belgium, the use of a traditional Chinese medicine (TCM) contaminated with plants from the *Aristolochia* species resulted in an epidemic of subacute intestinal nephropathy. Many of the affected patients required kidney transplantation. When 19 kidneys

and urethras removed from 10 patients were examined histologically, neoplasms were detected in 40% (Cosyns *et al.* 1999). *Aristolochia* is now banned in UK but it is still widely available via the internet. A Google search carried out in early April generated no fewer than 86000 hits using the search term “*Aristolochia*” and 60,600 for “snakeroot”, the common name. Ideally, authentication should be done from the harvesting of the plant material to the final product. Morphological as well as biochemical markers used in the authentication of herbal drugs have many limitations due to the impact of environmental conditions. DNA-based molecular markers however, are important tools in quality assurance and preservation of germplasm of medicinal plant species.

Our major objective therefore, was to develop a DNA-based molecular tool for accurate identification of *G. glabra* in a local market to differentiate and authenticate *G. glabra* and *A. precatorius*.

MATERIALS AND METHODS

A genuine sample of *Glycyrrhiza glabra* (L.) was provided by the Central Council Research for Unani Medicine (CCRUM), Hyderabad. The samples for authentication were purchased from local markets of Khari Baobli, Delhi, India. The material was identified at the National Institute of Science Communication and Information (NISCAIR) by Dr. H. B. Singh and voucher (NISCAIR/RHMD/consult-2007-08/937/121) was deposited in Herbarium.

Reagents and chemicals

Stock solutions used were: 3% (w/v) CTAB (cetyl trimethyl ammonium bromide), 1 M Tris-HCl (pH 8), 0.5 M EDTA (pH 8), 5 M NaCl, absolute ethanol (AR grade), chloroform-IAA (24: 1 [v/v]), polyvinylpyrrolidone (PVP) (40,000 mol wt) (Sigma), β -mercaptoethanol. All the chemicals used in the experiments were of analytical grade. The extraction buffer consisted of 3% (w/v) CTAB, 100 mM Tris-HCl (pH 8), 25 mM EDTA (pH 8), and 2 M NaCl. The PVP and β -mercaptoethanol were added after freshly prepared.

DNA extraction, RAPD reaction and amplification

DNA was isolated from dried root powder using a modified CTAB method (Khan *et al.* 2007). The RAPD reaction was performed according to the method developed by McClelland *et al.* (1995). The reaction was carried out in a 25 µl volume in a tube using sixteen random decanucleotide primers: OPC-1, OPC-2, OPC-3, OPC-4, OPC-5, OPC-6, OPC-7, OPC-8, OPC-13, OPC-14, OPC-15, OPC-16, OPC-17, OPC-18, OPC-19 and OPC-20 (Operon Technologies Inc., USA and Genex PVT, India). Each reaction tube contained 30 ng template DNA, 1.5 mmol/L MgCl₂, 300 µmol/L of dNTPs, 2.5 µl 1×*Taq* DNA polymerase buffer, 30 pmol decanucleotide primer and 1.5 units of *Taq* DNA polymerase (Genex, India). Amplification was performed in a DNA thermal cycler (Techne Thermal cycler, England) using the following conditions: 94°C for 3 min; 36 cycles at 94°C for 1 min, 35.6°C for 30 s and 72°C for 1 min; final extension at 72°C for 2 min. The amplified products were resolved on 1.2% agarose gel in 1xTAE buffer. The DNA was stained with 0.5 mg/ml ethidium bromide, visualized and photographed under a UV transilluminator. A sample without template DNA was included as a negative control in each experiment to check contamination. Electrophoretic profile was visualized under UV radiation and photographed with UV transilluminator, U.K. The sizes of DNA fragments were estimated by comparison with standard DNA ladder of 1 kb and 100 bp (Banglore Genex, India).

RESULTS

G. glabra was chosen to test the reliability of quality control using RAPD. In the local market samples, *A. precatorius* was found as adulterant when later identified at NISCAIR, New Delhi (voucher no, NISCAIR/RHMD/consult/-2007-08/937/121). The root of *A. precatorius* is more or less similar in morphology with those of *G. glabra*. The root of *A. precatorius* is twisted, long, and cylindrical, varying from 1/4 to 1 inch in thickness; reddish-brown externally, the internal or woody portion being yellowish. It is porous and breaks with a short, fibrous fracture (Fig. 1). The bark is thin. The root has little odor, that being somewhat disagreeable at first, but is bitter and acrid to the taste, afterward slightly sweetish, resembling, to a slight extent, that of common liquorice root and easily adulterated with the roots of *G. glabra* in the herbal market. The root of *G. glabra* is round long and straight, tough and fibrous, grayish without and yellowish within, of a sweet taste, and somewhat mucilaginous (Fig. 1). The high quality and purity of genomic DNA free from secondary metabolites was isolated from dried root powder of genuine and adulterant samples by a modified CTAB method for RAPD-PCR (Khan *et al.* 2007). The total number of unique amplicons specific to genuine as well as adulterant samples with different primers is summarized in Figs. 2-5 and Table 1.



Fig. 1 Morphological slides of root for genuine and adulterant samples. (A) *Glycyrrhiza glabra* (L.) (B) *Abrus precatorious* (L.)

DISCUSSION

Medicinal plants constitute an effective source of traditional and modern medicine. Adulterations and substitutions are common in raw material trade of medicinal plants. Adulteration in market samples is one of the greatest drawbacks in promotion of herbal products. Due to this adulteration and

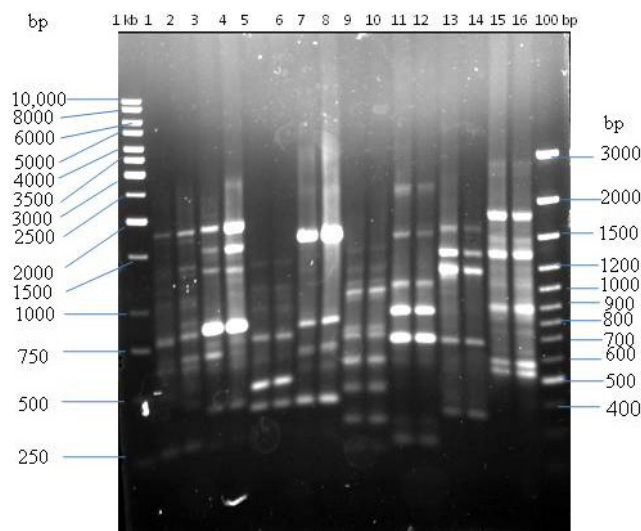


Fig. 2 RAPD analysis carried out with several primers. OPC-1 (lanes; 1-4), OPC-2 (lanes; 5-8), OPC-3 (lanes; 9-12), OPC-4 (lanes; 13-16) on genomic DNA from *G. glabra* (lanes; 1, 2, 5, 6, 9, 10, 13, 14) and *A. precatorius* (lanes; 3, 4, 7, 8, 11, 12, 15, 16). 1 kb and 100 bp are DNA ladder.

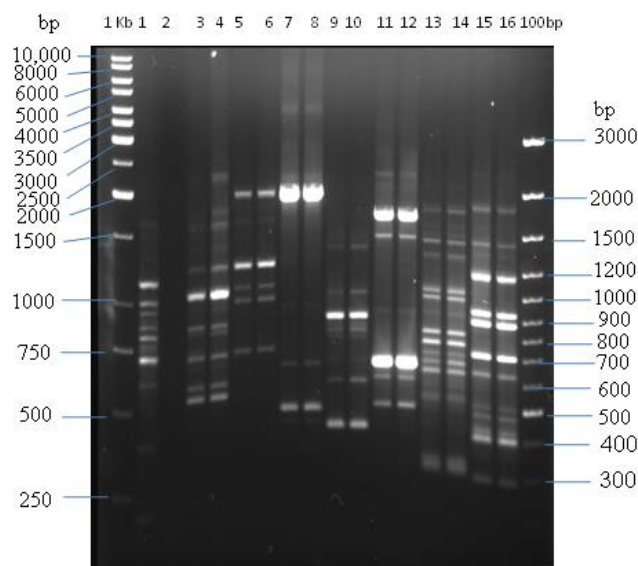


Fig. 3 RAPD analysis carried out with several primers. OPC-5 (lanes; 1-4), OPC-6 (lanes; 5-8), OPC-7 (lanes; 9-12), OPC-8 (lanes; 13-16) on genomic DNA from *G. glabra* (lanes; 1, 2, 5, 6, 9, 10, 13, 14) and *A. precatorius* (lanes; 3, 4, 7, 8, 11, 12, 15, 16). 1 kb and 100 bp are DNA ladder.

altered efficacy, the faith in crude drug promotion has declined (Dubey *et al.* 2004). Development of RAPD markers that can correlate DNA fingerprinting data with quantity of selected phytochemical markers associated with that specific medicinal herb, would have extensive applications in quality control of raw materials. These designed markers would act as a qualitative/quantitative diagnostic tool for identification of medicinal herbs from harvesting to finished product. The RAPD, PCR based assay described here is fast, reliable, and easy to conduct in any laboratory. It can be carried out in very short period using unknown genomic DNA from any developmental stages and body parts of herbs (Kethidi *et al.* 2003).

PCR amplification was tested with RAPD primers from kits (Operon Technologies, CA and USA) for authentication of *G. glabra* and *A. precatorius*. Out of 52 primers, only 16 produced reproducible amplicons in RAPD-PCR. The 16 primers were tested three times in PCR amplification to check the reliability of primers and unique amplicons were counted for each primer (Table 1). The random primers

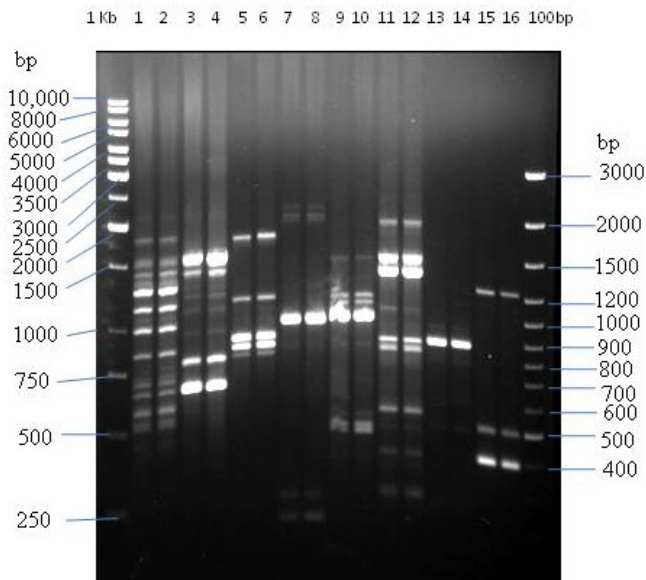


Fig. 4 RAPD analysis carried out with several primers. OPC-13 (lanes; 1-4), OPC-14 (lanes; 5-8), OPC-15 (lanes; 9-12), OPC-16 (lanes; 13-16) on genomic DNA from *G. glabra* (lanes; 1, 2, 5, 6, 9, 10, 13, 14) and *A. precatarius* (lanes; 3, 4, 7, 8, 11, 12, 15, 16). 1 kb and 100 bp are DNA ladder.

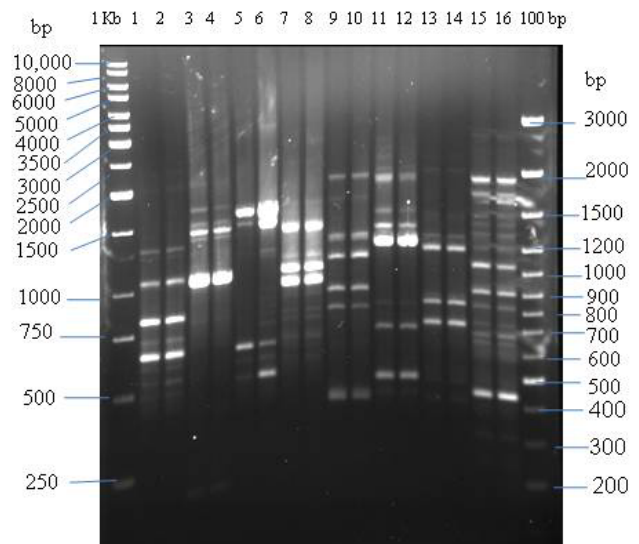


Fig. 5 RAPD analysis carried out with several primers. OPC-17 (lanes; 1-4), OPC-18 (lanes; 5-8), OPC-19 (lanes; 9-12), OPC-20 (lanes; 13-16) on genomic DNA from *G. glabra* (lanes; 1, 2, 5, 6, 9, 10, 13, 14) and *A. precatarius* (lanes; 3, 4, 7, 8, 11, 12, 15, 16). 1 kb and 100 bp are DNA ladder.

Table 1 The unique amplicons specific to *G. glabra* and *A. precatarius* samples with 16 decamer oligonucleotides primers (OPC) obtained in PCR amplification.

Plant species	Fig. 2				Fig. 3				Fig. 4				Fig. 5			
	Size of unique amplicons (bp)				Size of unique amplicons (bp)				Size of unique amplicons (bp)				Size of unique amplicons (bp)			
	OPC-1	OPC-2	OPC-3	OPC-4	OPC-5	OPC-6	OPC-7	OPC-8	OPC-13	OPC-14	OPC-15	OPC-16	OPC-17	OPC-18	OPC-19	OPC-20
<i>G. glabra</i>	ND	700 500	600 700	1200 700	ND	1400 1200 1031 800	1500 1200 950 450	1400 1100 1000 850 800 700	1900 1300 1150 1000 850 600	1900 1250 950 900	1300 1100	950 1100	1100 750 600	1500 650 950	1100 850	1200
<i>A. precatarius</i>	1400	1500	1500 900	1800 900 600 500	ND	700 550	1700 1600 700 550	1200 950 900	850 1100	2000 1600 1500 900 600	1300 500 400	1300	1100 1000	1300 750 550 900 450	1900 1600 1100 6	1900 1600 1100 6
Total unique amplicons/primer	1	3	3	6	0	6	8	9	7	5	7	4	4	4	6	6

Note: ND represents the unique band which was not detected

OPC-7, OPC-8, OPC-13 and OPC-15 produced more unique amplicons than the other primers used in the study. Each fragment in RAPD is derived from a region of the genome that contains two short segments in inverted orientation on opposite strands that are complementary to the primer and sufficiently close together for the amplification to work (Hon *et al.* 2003). Each unique amplicon produced in RAPD-PCR differentiated *G. glabra* and *A. precatarius* samples which have similar root morphology. RAPD was used to determine the components in an Ayurvedic herbal prescription, Rasayana Churna to identify three Ayurvedic medicines, dried stem of *Tinospora cordifolia*, dried fruit of *Embllica officinalis* and dried fruit of *Tribulus terrestris* (Shinde *et al.* 2007), which simultaneously generated three distinct amplicons, each specific to one component. A 600 bp marker was specific to *T. cordifolia*, a 500 bp marker was specific to *E. officinalis* and the remaining marker (>1000 bp) was specific to *T. terrestris*. The medicinal species of *Selaginella* and variation of the same species from different habitats was authenticated by RAPD analysis (Li *et al.* 2007). RAPD analysis has been widely used for differentiation of a large number of medicinal species from their close relatives or adulterants, including *Panax* species (Shaw and But 1995), *Coptis* species (Cheng *et al.* 1997),

Astragalus species (Cheng *et al.* 2000), *Lycium barbarum* (Cheng *et al.* 2000), *Panax ginseng* species (Um *et al.* 2001), *Echinacea* species (Nieri *et al.* 2003), turmeric (Sasi-kumar *et al.* 2004), *Astragali radix* (Na *et al.* 2004), *Dendrobium officinale* (Ding *et al.* 2005), *Typhonium* species (Acharya *et al.* 2005), *Dendrobium* species and its products (Zhang *et al.* 2005), *Tinospora cordifolia* (Wild.) Miers ex Hook & Thomas (Rout 2006), *Mimosae tenuiflorae cortex* (Rivera-Arce *et al.* 2007), *Rahmannia glutinosa* cultivars and varieties (Qi *et al.* 2008) and *Desmodium* species (Irshad *et al.* 2009). The advantages of RAPD include its simplicity, rapidity, the low amount of genomic DNA required and the fact that isotopes and prior genetic information are not required (Micheli *et al.* 1994). The RAPD fingerprint patterns obtained are consistent irrespective of the plant source or age (Welsh and McClelland 1990; Kethidi *et al.* 2004). These characters are especially advantageous for the identification of herbal medicine because little DNA exist in the dried material and also because sequence data are difficult to obtain. The most of the primers in our study gave unique amplicons specific to genuine as well as adulterant samples which were selected for further study. Significance of present work is that single primer can differentiate genuine as well as adulterant samples which are sold in

the local markets. The more reproducible DNA marker, sequenced characterized amplified regions (SCAR) will be developed for *G. glabra* and *A. precatarius* samples in further studies for authentication, which can provide an alternative tool to monitor the quality of herbal medicines.

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