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Comparative molecular characterization of *Desmodium gangeticum* DC. and *Desmodium laxiflorum* DC. through random amplified polymorphic DNA (RAPD) analysis

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ABSTRACT

Desmodium gangeticum DC. which belongs to family Fabaceae, known as Shalaparniisa widely used drug in Ayurveda. Shalaparni is one of the contents of Laghupan chamoola as well as that of Dashamoola. In some areas of Gujarat, local Vaidyas use Desmodium laxiflorum DC. (Fabaceae) instead of D.gangeticum, which suggest that they may possess almost similar properties. In the market sample of Shalaparni, adulteration is a common practice now a days. The use of highly discriminatory methods for the identification and characterization of genotypes is essential for plant protection, authentication and appropriate use. Present study utilized the RAPD method for the genetic fingerprinting of 2 species of Desmodium genus i.e. Desmodium gangeticum and Desmodium laxiflorum. The 10 primers were used to amplify the DNA from the plant species. The binary scoring of these primers showed 60-65% of similar characters between species of the same genus. This study is helpful in developing DNA markers of both the species for their authentication as well as for the categorization of Desmodium genus.

Key words: Adulteration, finger prints, Primers, Shalaparni, Substitute.

INTRODUCTION

Herbal medicine has been enjoying renaissance among customers throughout the world. The World Health Organization estimates that 80% of the world's population utilizes traditional medicines for healing and curing diseases.[1]The natural medicines are much safer than synthetic drugs, have gained popularity in recent years, leading to a tremendous growth of phyto-pharmaceutical usage. Pharmaceutical companies are procuring materials from traders, who are getting these materials from untrained persons from rural and/or forest areas. This has given rise to wide-spread adulteration/substitution, leading to poor quality of herbal formulations. Misidentification of herbs can be non-intentional (processed plant parts are inherently difficult to distinguish) or intentional (profit-driven merchants sometimes substitute expensive herbs with less-expensive look-alike ones).[2] Therefore, authentication at various stages, from the harvesting of the plant material to the final product, is the need of the hour. The general approaches to herb identification are dependent on morphological[3], anatomical[4-5], chemical[6-7] and molecular[8]techniques. However, traditional taxonomic studies require expertise of experienced professional taxonomists. In cases where diagnostic morphological traits of the given specimen are lacking, it becomes difficult even for specialists to recognize a species correctly. Genetic analysis has a promising role in resolving disputes of taxonomic identities, relations and authentication of the species in question, as the genetic composition is unique for each species and is not affected by age, physiological conditions and environmental factors. It also helps in the

identification of useful genotypes which are likely to improve efficacy of standard drug formulations; even the plant extract used in the herbal-drug formulations can be authenticated by DNA-based methods.[9] Therefore, DNA-based methods have gained wide acceptance in quality control to authenticate crude materials.

Random amplified polymorphic DNA (RAPD) is a simple and cost effective PCR based method as compared to other DNA based markers. Because of its high utility, it has been widely used for the differentiation of a large number of medicinal species from their close relatives or adulterants, including Echinacea species [10], Turmeric [11], *Astragali radix* [12], *Dendrobium officinale* [13], Typhonium species [14], Dendrobium species and its products [15], *Tinospora cordifolia* [16], *Mimosae tenuiflorae* cortex [17], *Rahmannia glutinosa* cultivars and varieties [18], Desmodium species [19], *Glycyrrhiza glabra* and its adulterant [20], *Piper nigrum* [21], *Cuscuta reflexa* and *Cuscuta chinensis* [22], and *Ruta graveolens* and its adulterant [23]. In the present study two species of Desmodium genus were selected for the DNA analysis i.e. *Desmodium gangeticum* and *Desmodium laxiflorum*. *Desmodium gangeticum* belongs to family Fabaceae is an established source of *Shalaparni*, a very well-known and immensely used drug in Ayurvedic prescription. *Desmodium laxiflorum* which belongs to the same family is being used by local Vaidyas of Gujarat instead of *D.gangeticum* in the formulations. These practices suggest that both these drugs possess almost similar properties and can be substituted each other. So, their authentication and molecular characterization is necessary to maintain the efficacy and quality of herbal formulations.

MATERIALS AND METHODS

Collection and preservation of the sample

Desmodium gangeticum and *Desmodium laxiflorum* were collected from Botanical garden of Gujarat Ayurveda University, Jamnagar and farms of Sassoi respectively. The herbarium of respective drugs were prepared and stored in the Pharmacognosy laboratory for further documentation.

Molecular characterization (DNA fingerprints):

Fresh leaves and their buds were used in molecular characterization. For DNA fingerprinting through RAPD markers DNA was extracted by using Doyle and Doyle (1990) method with minor modifications. DNA quantification was done using a Picodrop spectrophotometer and DNA sample was diluted using TE buffer up to 50 ngl pl. Quality of 2 sample of Desmodium DNA was checked by 0.8% Agarose gel electrophoresis. RAPD-PCR was carried out in Verity ABI thermal cycler. The resolved amplification products were visualized by illumination under UV light in Gel document system.

The RAPD reaction was performed following standard procedures at sophisticated instrumentation centre for applied research and testing (SICART), Anand, Gujarat.

RESULTS

Table 1: List of RAPD primers used for the analysis of two plants DNA sample

Sr. no.	Primer Condition	Primer Sequence	5' - 3' Tm (uC)
01	OPC-02	GTGAGGCGTC	25.0
02	OPC-03	GGGGGTCTTI	27.0
03	OPG-01	CTACGGAGGA	25.0
04	OPR-07	CAGCGACAAG	25.0
05	OPI-10	ACAACGCGAG	25.0
06	OPJ-01	CCCGGCATAA	25.0
07	OPJ-06	TCGTTCCGCA	25.0
08	OPJ-08	CAGCACTGAC	25.0
09	OPJ-10	AAGCCCGAGG	27.0
10	OPL-03	GGCATGACCT	25.0

The fingerprinting patterns of *Desmodium gangeticum* DC. And *Desmodium laxiflorum* DC. sample seen as vertical columns with horizontal light bands on a light background, have been depicted in the figure 1 and 2 respectively.

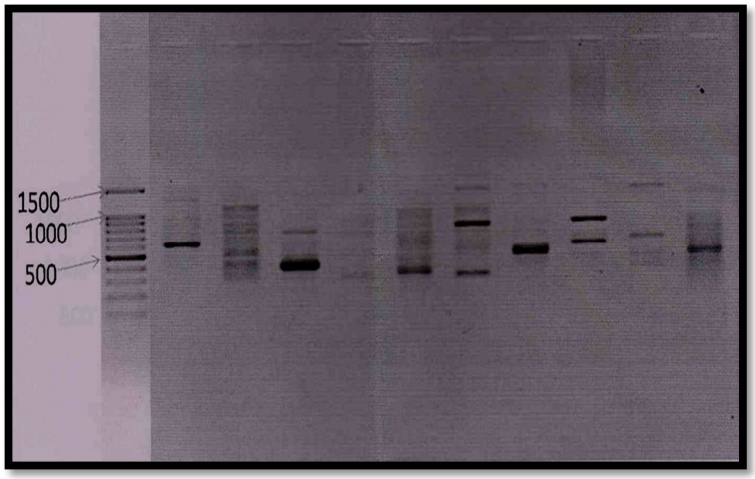

Figure 1: Light and dark bands of DNA fingerprints of *Desmodium gangeticum* DC

For the analysis of DNA sample of *Desmodium gangeticum* DC.l0 primers were used (1 to 10 RAPD Primers mentioned in Table 1). Primers have been loaded from left to right. Primer 1 is on the left most side and primer l0 is on the right side (Figure l). All primers showed amplification. In primer I clear band size was observed at 650bp; 800bp; 900bp and 1200bp; in primer 2 band size was observed at 400bp to 1100bp; in primer 3 the range of band size was observed from 400bp to l600bp; in primer 4 the range of band size was observed from 350bp to900bp; in primer 5 range of band size was observed at 350bp to l000bpt in primer 6 range of band size was observed at 350bp; 600bp; 800bp; 1000bp; l500bp and 1600bp; in primer 7 range of band size was observed from 500bp to l500bpl in primer 8 band size was observed at 600bp to 1600bp; in primer 9 range of band size was observed at 400bp and above the 1500bp; in primer 10 band sizes were observed 500bp to l400bp.

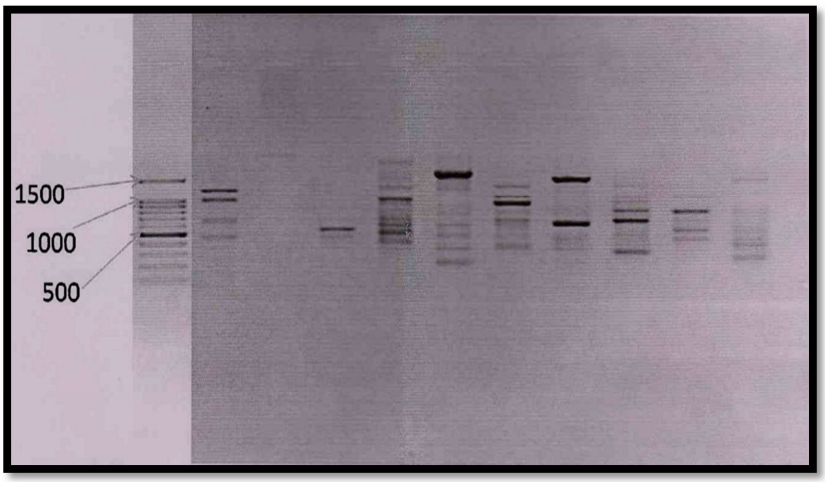

Figure 2.Light and dark bands of DNA fingerprints of *Desmodium laxiflorum* DC

For the analysis of DNA sample of *Desmodium laxiflorum* DC., l0 primers were used (1 to 10 RAPD Primers mentioned in Table 1). Primers have been loaded from left to right. Primer 1 is on the left most side and primer l0 is on the right side (Figure 2). All primers showed amplification. In primer I range of band size was observed at 500bp; 700bp; 1000bp and l200bp; in primer 2 single band size was observed above l500bp; in primer 3 the range of band size was observed from 500bp and 600bp; in primer 4 the range of band size was observed from 400bp to above 1500bp; in primer 5 range of band size was observed at 250bp to l600bpt in primer 6 range of band size was observed at 400bp to around 1500bp; in primer 7 range of band size was observed from 400bp; 700bp and 1500bp;

in primer 8 band size was observed at 300bp to l500bp; in primer 9 range of band size was observed at 450bp and 800bp; in primer l0 band size was observed 300bp to 1500bp.

DISCUSSION

RAPD done with 10 primers showed clear differentiation between samples *Desmodium gangeticum* and *Desmodium laxiflorum*. Each primer showed entirely different pattern for the two samples. The binary scoring of primer OPC-03 and OPJ-08, OPC-02 and OPJ-06, OPR-07 and OPJ-10 and OPG-01 and OPL-03 showed 60-65% of similar characters i.e. Plant nature, chemical constituents, leaves type, flowering nature, type of flowers, type of fruits etc. And 30-35% different characters were found in the two samples i.e. their colour, size etc. (*Desmodium gangeticum* and *Desmodium laxiflorum*). Thus, the DNA fingerprinting result shows almost similar genomic characters that expressed in pharamacognostical study. RAPD analysis has been frequently used method for the detection of genetic variability in plants in present era. The advantages of this method are its rapidity, simplicity and lack of need for any prior genetic information about the plant. RAPD patterns are consistent irrespective of the plant source or age.[24-25]It has been also successfully utilized for the identification of medicinal plants[26-27] and herbal medicinal components.[28] RAPD primers are able to distinguish taxa below the species level[29], because RAPD analysis reflects both coding and non-coding regions of the genome.[30] However, some of the problems with RAPD are related to reproducibility, designing appropriate primers and amplification of RAPD-PCR products. Present work showed that the protocol used worked well for the studied2 plant species. However, the combination of 10 primers showed better resolution. These data corroborate other findings indicating that the combination of primers provides better resolution.[31-32]RAPD analysis is less time-consuming and less expensive[33]as well useful in authentication of medicinal plant species. From the aspects of categorization of Desmodium genus, derived DNA fingerprints of species *gangeticum* and *laxiflorum* can contribute significantly which further need to be elaborated.

CONCLUSION

Present study conclude that the species *Desmodium laxiflorum* possess 60-65% similarity with *D. gangeticum*, which can be a better substitute. Further detail pharmacological, clinical studies are needed to be carried out.

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