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Comparative Dna Fingerprinting Analysis of Desmodium Gangeticum Dc. and Flemingia Strobilifera (L). W. Aiton. Through Random Amplified Polymorphic DNA (RAPD) Analysis

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Abstract:
Desmodium gangeticum DC. is the official drug considered for *śālaparṇi*. Previous survey study revealed use of *Flemingia strobilifera*(L). Aiton as *śālaparṇi* in Southern market. Due to the similarities in the leaves, it is difficult to differentiate the two market samples. Hence, the study was considered to know the genotype of both the plants with their similarities and differences. Tender leaves and their buds were utilized in this RAPD method. With minor modification, Doyle and Doyle (1990) method was used to extract the DNA. Verity ABI thermal cycler was used to carry out the RAPD-PCR. Under UV light in Gel document system, the resolved amplification products were visualized by illumination. As comparative analysis of the both species, banding pattern obtained in all the primers were matching at 500, 600 and 700bp range. In this range 9 bright bands from *Desmodium gangeticum* DC. and 7 bright bands from *Flemingia strobilifera*(L). Aiton were matching. These bands are specific for some morphological and some genetically inherited family characters.

Keywords: *śālaparṇi, Flemingia strobilifera, Desmodium gangeticum*

Introduction: *śālaparṇi* is one among *Daśamūla* i.e 10 drugs combination used in Ayurveda. *Daśamūla* is used in many preparations throughout India. Different plants are considered as *śālaparṇi* in different regions of India. Previous survey study revealed use of *Flemingia strobilifera*(L). Aiton as *śālaparṇi* in Southern marke[1]. *Desmodium gangeticum* DC. is the official drug considered for *śālaparṇi*. Due to the similarities in the leaves, it is difficult to differentiate the two market samples. Hence, the study was considered to know the genotype of both the plants with their similarities and differences. Both research drugs, *Desmodium gangeticum* DC. and *Flemingia strobilifera*(L). Aiton belong to the same family i.e Fabaceae.

Materials & Methods
Collection of Sample: Both the research drugs were collected from their natural source. *Desmodium gangeticum* DC. was collected from Sasoi garden, Jamnagar, Gujarat. *Flemingia strobilifera*(L). Aiton collected from Magalore, Karnataka.

Method adopted:
Tender leaves and their buds were utilized in this RAPD method. With minor modification, Doyle and Doyle (1990) method was used to extract the DNA[2]. Picodrop spectrophotometer was utilized to quantify the DNA. TE buffer up to 50ngl pl. was used to dilute the sample of DNA. After several cycles of amplification the DNA is subjected to gel electrophoresis. 0.8% Agarose gel electrophoresis was used to

check the two samples of DNA i.e DNA sample of . and DNA sample of *Flemingia strobilifera* (L). Aiton. Verity ABI thermal cycler was used to carry out the RAPD-PCR. Under UV light in Gel document system, the resolved amplification products were visualized by illumination.

Place of work:

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

Results:

The fingerprinting patterns of *Desmodium gangeticum* DC. and *Flemingia strobilifera*(L). Aiton samples were seen as vertical columns with horizontal light bands on a light background which is depicted in the figures 1 and 2. For the analysis of DNA samples of both the research plants, l0 primers were used. For both samples, primers mentioned in Table 1 were used. Primers have been loaded from left to right. All primers showed amplification in the sample, except for primer 2,3 and 9 primers which showed very less number of bands. In DNA sample, range of band size was observed from ~250 to ~1000bp(Table 2 and 3).

Among 27 total no of bands obtained from the *Desmodium gangeticum* DC. sample, 9 were bright bands and 18 were light bands. Large no of bands obtained from the primer 4, 5, and 8 with four bright bands. Very less number of bands observed in primer 2 and 3.

In primer 1 clear band size was observed from 300 to 900bp; in primer 2 single light band observed at 900bp, in primer 3 the single band size was observed at 400bp; in primer 4, 2 bright bands and 2 light bands are observed at 500bp-600bp and at 1000bp; in primer 5 range of band size was observed at 200bp to 1000bp; in primer 6 range of band size was observed from 400 to 1000bp; in primer 7, band size was observed from 500 to 800bp; in primer 8 band size was observed from 300bp to 700bp; in primer 9 range of bright band size was observed from 700bp; in primer 10, 3 bright bands were observed from 300bp to 700bp;

Among 36 total no of bands obtained from the *Flemingia strobilifera*(L). Aiton sample, 14 were bright bands and 22 were light bands. Large no of bands obtained from the primer 1, 5, 6, 7, 8, 9 and 10 with four bright bands. Very less number of bands observed in primer 3.

In primer 1 clear band size was observed from 300 to 900bp; in primer 2 single light band observed at 1500bp, in primer 3 the single bright band size was observed at 600bp; in primer 4, 2 bright bands and 2 light bands are observed at 400bp and at 1000bp above; in primer 5 range of band size was observed at 350bp to 1000bp above; in primer 6 range of band size was observed from 400 to 1000bp; in primer 7 band size was observed from 400 to 1000bp above; in primer 8 band size was observed from 200bp to 1000bp; in primer 9 range of bright band size was observed from 400bp; in primer 10, 3 bright band size was observed from 600bp to 700bp;

Discussion and Conclusion

Total no of bands obtained from both samples i.e. *Desmodium gangeticum* DC. and *Flemingia strobilifera*(L). Aiton was 63. Among them, 9 bright bands were from *Desmodium gangeticum* DC. and 14 bright bands were from *Flemingia strobilifera*(L). Aiton. 18 light bands produced from *Desmodium gangeticum* DC. and 22 light bands from *Flemingia strobilifera*(L). Aiton. Large no of bands obtained from the primer 1, 5, 6, 7, 8, 9 and 10 with 4 bright bands. Primer 5, 8 and 10 showed excellent matching bands as compared to the other primers. Both the plant species showed the similar banding in the common primers. Especially poor bands observed in primer 2, 3 and 6 in *Desmodium gangeticum* DC. whereas primer 1 and 8 showed poor banding pattern in *Flemingia strobilifera*(L). Aiton. As comparative analysis of the both species, banding pattern obtained in all the primers were matching at 500, 600 and 700bp range. In this range 9 bright bands from *Desmodium gangeticum* DC. and 7 bright bands from *Flemingia strobilifera*(L). Aiton were matching. These bands are specific for some morphological and some genetically inherited family characters.

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

Sr. no.	Primer	Sequence 5' – 3'
1.	OPM-07	CCGTGACTCA
2.	OPN-03	GGTACTCCCC
3.	OPO-01	GGCACGTAAG
4.	OPM-09	GTCTTGCGGA
5.	OPO-06	CCACGGGAAG
6.	OPM-06	CTGGGCAACT
7.	OPM-02	ACAACGCCTC
8.	OPM-05	GGGAACGTGT
9.	OPM-03	GGGGGATGAG
10.	OPM-04	GGCGGTTGTC

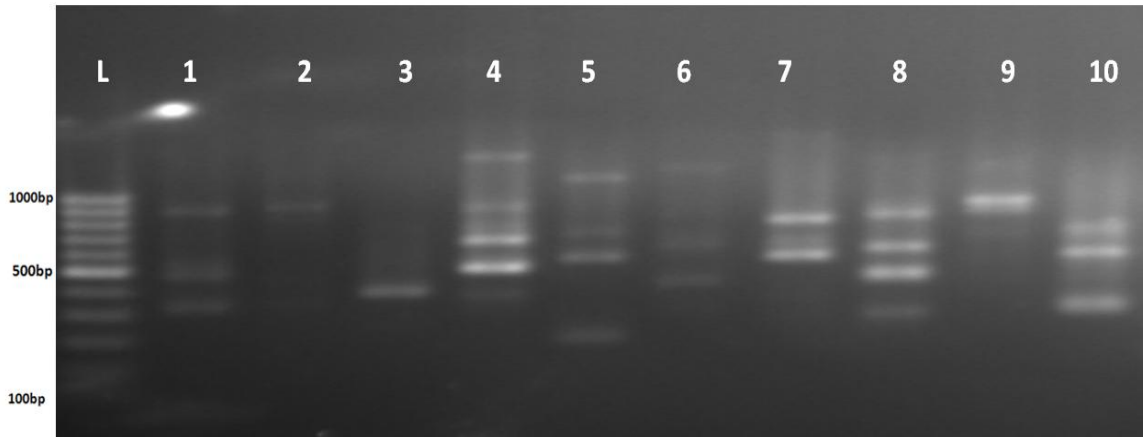

Figure 1: RAPD banding pattern on agarose gel for Sample 1 (*Desmodium gangeticum* DC.)

TABLE: 2 Band observation from figure 1

Primer no:	Band observation range (bp)
1	300bp to 900bp
2	250bp to 900bp
3	400bp to 600bp
4	500bp to above 1000bp
5	200bp to above 1000bp
6	400bp to above 1000bp
7	500bp to 800bp
8	300bp to 700bp
9	700bp to 900bp
10	300bp to 700bp

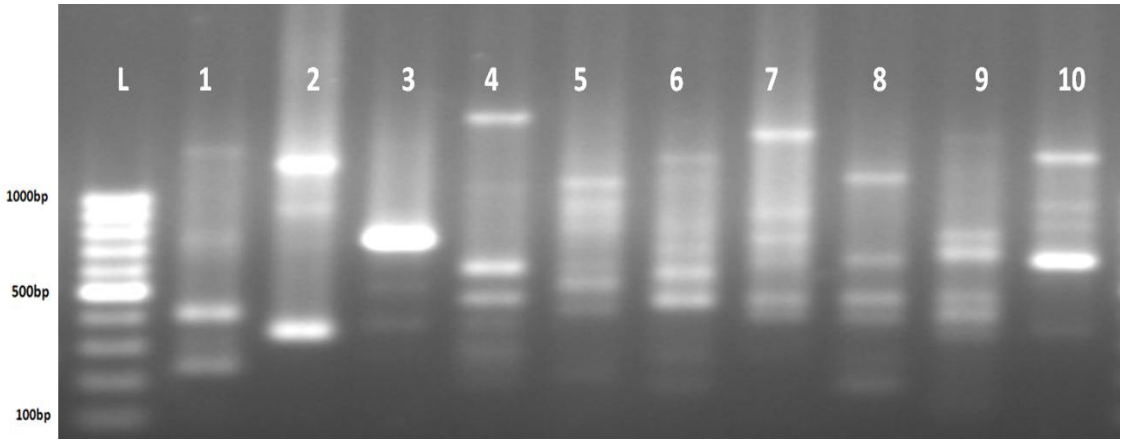

Figure 2: RAPD banding pattern on agarose gel for Sample 2 (*Flemingia strobilifera*(L). Aiton)

TABLE: 3 Band observation from figure 2

Primer no:	Band observation range (bp)
1	250bp to above 1000bp
2	300bp to above 1000bp
3	300bp to 700bp
4	200bp to above 1000bp
5	350bp to above 1000bp
6	400bp to above 1000bp
7	400bp to above 1000bp
8	200bp to above 1000bp
9	400bp to above 1000bp
10	600bp to above 1000bp

Reference

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2. Doyle JJ, Doyle JL. A rapid DNA isolation procedure from small quantities of fresh leaf issue. Phytochem Bull. 1987;19:11–5.