

# Interspecies Variability between *Wrightiatomentosa* and *W. tinctoria* as Assessed by RAPD Analysis

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**Abstract:** RAPD markers were used for detection of variability between the two species in the genus *Wrightia* i.e. *W. tomentosa* and *W. tinctoria*. A total of 188 amplified bands were scored from 29 random decamer primers out of which 96 (51.06%) were found to be polymorphic. On comparing the amplification products of DNA from both the species, it was observed that 2 primers produced similar amplified products in both the species, whereas 27 primers showed highly reproducible polymorphic banding pattern between these species. Highest percentage of polymorphism was found with the primer OP 26 (85%) whereas no polymorphism was found with primers OP 16 and 27. The band size ranged from 200 bp to 3000 bp.

**Keywords:** *Wrightia* species, Aravallis, Genetic markers, RAPD

## 1. Introduction

The characterization of germplasm is essential for identification of various species, varieties and cultivars and also to determine their genetic relationships. Traditionally, morphological characters were used for establishing the identity of species/cultivar. But these characters are under the influence of environmental changes, epistatic interactions, and pleiotropic effects. Therefore, DNA fingerprinting techniques have been developed for measuring genetic variability and species/cultivar identification. The most common techniques include isozymes and PCR-based assays such as Randomly Amplified Polymorphic DNA [1] Simple Sequence Repeats [2] Amplified Fragment Length Polymorphism [3] and Inter Simple Sequence Repeats [4].

RAPD markers provide a rapid, inexpensive and effective system for studying plant genetic relationships. They are particularly suitable for less well-known species because they can be applied without prior knowledge of DNA sequence information. Since 1990, the RAPD technique has been extensively used in plant systematic studies, especially in the identification of germplasm resources and the measurement of variation to establish evolutionary relationships within or between species, sub-species or populations and genomes. While RAPD markers are considered to be dominant markers, it has been shown that RAPD markers can be efficient in estimating genetic diversity and in analyzing genetic relationships. Moreover, the combined use of different marker systems may provide more reliable information about genetic diversity when compared to the use of only one marker system so that some errors or problems occurring with the use of a certain marker system could be minimized when combined [5], [6]. RAPD technique has been successfully applied for assessment of inter-species/varietal differentiation in different plants such as *Coffea arabica* [7], *Plantago ovata* [8], *Bougainvillea glabra* [9] and *Dioscorea* spp. [10].

*Wrightiatomentosa* (Roxb.) Roemer-Schult and *W. tinctoria* (Family: Apocynaceae), are two important tree species of Aravallis in South-East Rajasthan. Both the species are of immense medicinal and economic value. The plants have

been traditionally exploited by local artisans for their wood in making toys and artifacts. The root bark of *W. tomentosa* has been reported useful in snake bite and scorpion sting [11]. It yields yellow dye which imparts fast colour to cotton fabrics. *W. tinctoria*, a small deciduous tree, provides timber mainly for turnery and carving, making it preferable for toy making by local artisans. Seeds are reported to have aphrodisiac properties. The latex present in the plant contains ca. 28.4% rubber content [12]. A novel isoflavone; wrightidione isolated from *W. tomentosa* has been reported to have cytotoxic activity against murine p388 lymphocytic leukemia cell line [13]. The butanol extract of the plant has shown anti-microbial activity [14]. The ethanolic extract of the bark and leaf of *W. tomentosa* possesses significant anti-allodynic effect [15] and anti-hyperglycemic activity [16]. There is no report in the available literature on molecular evaluation of these species using genetic markers.

In the present study interspecific variability between *W. tomentosa* and *W. tinctoria* has been analysed using RAPD markers

## 2. Materials and Methods

### 2.1 Plant materials

For genomic DNA isolation, leaf samples of *Wrightiatomentosa* and *W. tinctoria* were collected from the mature plus trees growing in Kevre Ki Nal (Udaipur) situated in Aravalli hill region in South-east Rajasthan.

### 2.2 Extraction and quantification of genomic DNA

Total genomic DNA from leaf samples was extracted using modified cetyltrimethyl ammonium bromide (CTAB) procedure [17]. The impurities of RNA were removed by the treatment of RNase A. All the DNA samples were digested with 100 µg/ml RNase A for 30 min at 37°C, extracted once with chloroform : isoamyl alcohol (24:1), precipitated by adding 0.1 volume of sodium acetate (3M) and 0.6 volume of chilled isopropanol, washed with 70% ethanol and resuspended in 100 µl 1 X TE. The quality of genomic DNA was checked by agarose gel electrophoresis on 0.8% agarose gel (w/v), stained with 0.5 µg/ml ethidium bromide. DNA

concentration was estimated spectrophotometrically (UV-Vis Spectrophotometer, Pharmaspec UV-1700, Shimadzu, Japan) by measuring the absorbance at 260 nm. The original DNA samples were then diluted to 5.0 ng/μl for PCR amplification.

### 2.3 Amplification conditions

RAPD profiles were produced through PCR amplification using the protocol described by Williams *et al.* (1990) with minor modifications. PCR reactions were carried out in 0.2 ml polypropylene PCR tubes (Bangalore Genei, India) using Thermal Cycler (Master Cycler Personal, Eppendorf). Each 20 μl reaction mixture contained 1 X Taq buffer (100 mM Tris-Cl (pH 9.0), 500 mM KCl, 15 mM MgCl<sub>2</sub> and 0.1% Gelatin), 0.2 mM dNTPs (Bangalore Genei, India), 20 pmol oligonucleotide primers (Operon Technology, USA), 1.0 U Taq DNA polymerase (Bangalore Genei, India) and 25 ng template DNA. The reactions were subjected to initial denaturation at 94°C for 4 min followed by 40 amplification cycles, each consisting of 1 min at 94°C (denaturation step), 1 min at 37°C (annealing step) and 2 min at 72°C (extension step) with a final extension of 7 min at 72°C. The amplification products were separated on 1.5% agarose (w/v) gel, stained with 0.5 μg/ml ethidium bromide. DNA ladder, 1 Kb (Bangalore Genei, India) and 100 bp DNA ladder (Bangalore Genei, India) were mixed and used as molecular weight marker for comparison of amplified products. Gels were photographed under UV light using a Gel Documentation System (DP 001. FDC, Consort). All reactions were repeated thrice to confirm the results.

### 2.4 Primer Selection

Thirty three arbitrary decamer oligonucleotide primers (Operon Technology, USA) were used for screening and only those primers were selected for the present study, which provided satisfactory and reproducible amplification products under similar conditions.

### 2.5 Data Analysis

All reactions were performed thrice and only consistently reproducible and well resolved bands were considered for the analysis. Amplified fragments were scored as '1' or '0' for presence or absence of bands on the gel. DNA polymorphism was calculated and given as percentage of the total number of bands produced in RAPD profiles.

## 3. Results and Discussion

For genomic DNA isolation modified CTAB method was used as it gave better quality and yield of DNA. The DNA pellet obtained was white, translucent and without any discoloration. Quality of DNA was also confirmed through spectrophotometric analysis, which showed absorbance ratio between 1.75-1.85 when measured at two different wavelengths (A<sub>260</sub>/A<sub>280</sub>), ensuring that the DNA samples were free from protein contamination and were PCR amplifiable.

Genomic DNA samples when subjected to RAPD analysis revealed that both the species (*W. tomentosa* and *W. tinctoria*) differed significantly at molecular level. A total of 33 random decamer primers were used for initial screening, among these 4 primers did not produce any polymorphic band or did not amplify clear products. Therefore, the remaining 29 primers which produced clear, scorable and highly reproducible bands between two species were used for further analysis (Table 1). These 29 primers produced a total of 188 bands, among them 92 fragments were monomorphic and 96 fragments showed polymorphic banding pattern. Amplification products when compared in both the species revealed that 2 primers (OP16 and OP27) produced similar banding pattern whereas 27 primers showed highly polymorphic bands between the two species (Fig. 1-4).

Highest number of bands were obtained in case of primer OP 04 (13) and lowest were recorded in OP 10, OP 15, OP 16 and OP 27 (03). The band size ranged from 200 bp to 3000 bp. Primer OP 26 showed highest percent of polymorphism (85%) while OP16 and 27 showed complete uniformity between both the species. Each primer generated 3-13 RAPD bands. The overall 51.06% polymorphism was obtained in both the species (Table 2).

Random amplified polymorphic DNA (RAPD) markers have proved to be a reliable marker system for genetic fingerprinting and also for determining the genetic relationships among germplasm collections. RAPD markers have the advantages of simplicity and the ability to detect relatively small amounts of genetic variation and also need no prior information on the genome [18],[19]. Therefore, we employed RAPD markers for the identification of variability between two *Wrightia* species, which showed 51.06% polymorphism. Using RAPD markers a large number of species/varieties/cultivars have been analysed. Our results are in agreement with the results obtained by Kant *et al.* [20] who reported 94% polymorphism in *Pinus gerardiana* genotypes. Similar results were obtained in *Prunus* genotypes where 65% polymorphism was reported by Erturket *et al.* [21]. Mani *et al.* [22] have reported intra-species disparities among rice varieties using these markers. In *Juglans* cultivars, genetic variability was reported by Francesca *et al.* [23]. Roy and Chakraborty [24] observed high partitioning of polymorphism (77.77%) in twenty one cultivars of *Camellia sinensis*. In *Zingiber officinale*, 45.5% of polymorphism was found in eight varieties [25], while in wheat cultivars, 86.80% of polymorphism was reported [26]. Present studies carried out with two species in genus *Wrightia* revealed significant polymorphism.

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**Table 1:** List of Random Decamer Primers used for screening the PCR amplification of total genomic DNA in *W. tomentosa* and *W. tinctoria*

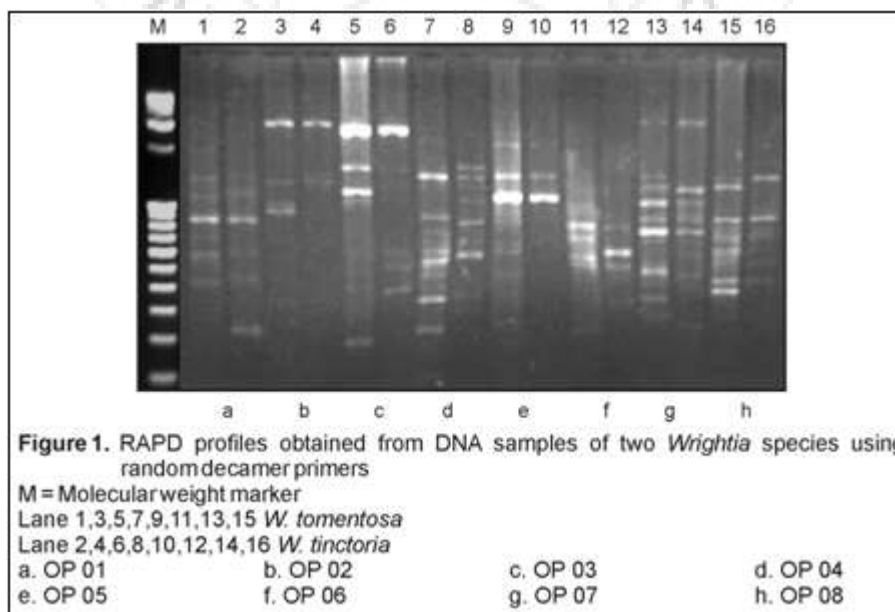
Name	Sequence	Tm	Molecular Weight
OP 01	TGCCGAGCTG	43.6	3044.01
OP 02	TTTGCCCGGA	39.5	3019.01
OP 03	ACCCCCGAAG	43.6	2981.95
OP 04	GGACCCCTTAC	39.5	2987.98
OP 05	TTCGAGCCAG	39.5	3028.01
OP 06	GTGAGGCGTC	43.6	3084.08
OP 07	AAAGCTGCGG	39.5	3077.04
OP 08	CCGATATCCC	39.5	2947.95
OP 09	CTACGGAGGA	39.5	3077.04
OP 10	GGCCTGAGG	43.6	3093.04
OP 11	TCACGTCCAC	39.5	2947.95
OP 12	CTGACGTCAC	39.5	2987.98
OP 13	TGCCCGTCGT	43.6	2994.98
OP 14	CTCTCCGCCA	43.6	2923.92
OP 15	GTCAGGGCAA	39.5	3077.04
OP 16	CATTCGAGCC	39.5	2987.98
OP 17	CCGCCCAAAC	43.6	2941.92
OP 18	AATGCCCCAG	39.5	2996.98
OP 19	CCCGCTACAC	43.6	2932.92
OP 20	GAGCGTCGAA	39.5	3077.04

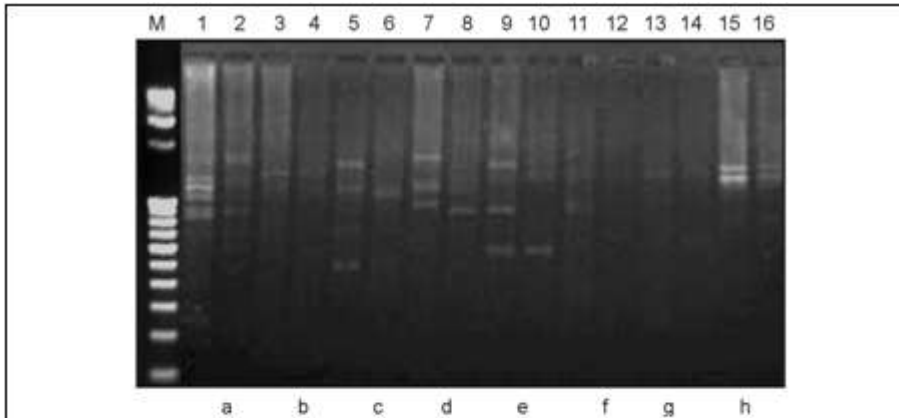
OP 21	CCCAGCTGTG	43.6	3003.98
OP 22	CACAGGCGGA	43.6	3062.01
OP 23	GTGTCGCGAG	43.6	3084.04
OP 24	AGCAGGTGGA	39.5	3117.07
OP 25	CACAGCTGCC	43.6	2972.95
OP 26	TCTGGACGGA	39.5	3068.04
OP 27	GGGTTTGGCA	39.5	3099.07

OP 28	TCGCATCCCT	39.5	2938.95
OP 29	CAAAGCGCTC	39.5	2996.98
OP 30	CAGGGGTGGA	43.6	3133.07
OP 31	ACAGGTGCTG	39.5	3068.04
OP 32	GGCTGCGACA	43.6	3053.01
OP 33	CTCAGTCGCA	39.5	2987.98

**Table 2:** Interspecies Polymorphisms in Genus *Wrightia* using Random Decamer Primers

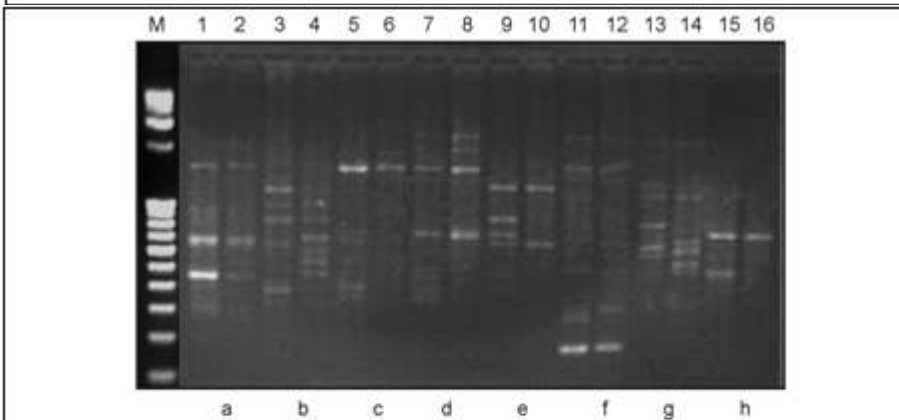
S. No.	Decamer Primers	Total No of Bands	No. of Polymorphic Bands	% Polymorphism	Range of Fragment Size	Jaccards Similarity Coefficient
1.	OP-01	10	03	30	200-2000	0.7
2.	OP-02	05	03	60	700-3000	0.4
3.	OP-03	07	05	71	200-3000	0.28
4.	OP-04	13	10	76	200-2000	0.23
5.	OP-05	05	02	40	300-2000	0.6
6.	OP-06	07	05	71	400-3000	0.28
7.	OP-07	09	03	33	300-3000	0.66
8.	OP-08	07	05	71	200-2000	0.28
9.	OP-09	04	02	50	200-2000	0.5
10.	OP-10	03	01	33	400-2000	0.66
11.	OP-11	04	02	50	400-2000	0.5
12.	OP-12	07	05	71	600-2000	0.33
13.	OP-13	04	02	50	600-2000	0.5
14.	OP-14	04	02	50	600-2000	0.5
15.	OP-15	03	01	33	600-2000	0.66
16.	OP-16	03	00	00	600-2000	1.0
17.	OP-17	07	03	42	200-2000	0.57
18.	OP-18	12	05	41	200-2000	0.58
19.	OP-19	08	05	62	200-2000	0.37
20.	OP-20	07	03	42	200-2000	0.57
21.	OP-21	04	02	50	400-2000	0.5
22.	OP-22	08	03	37	200-3000	0.62
23.	OP-23	10	07	70	200-2000	0.3
24.	OP-24	04	01	25	400-2000	0.75
25.	OP-25	07	03	42	600-2000	0.57
26.	OP-26	07	06	85	400-2000	0.14
27.	OP-27	03	00	00	300-2000	1.0
28.	OP-28	08	01	12	300-2000	0.87
29.	OP-29	08	06	75	300-2000	0.25





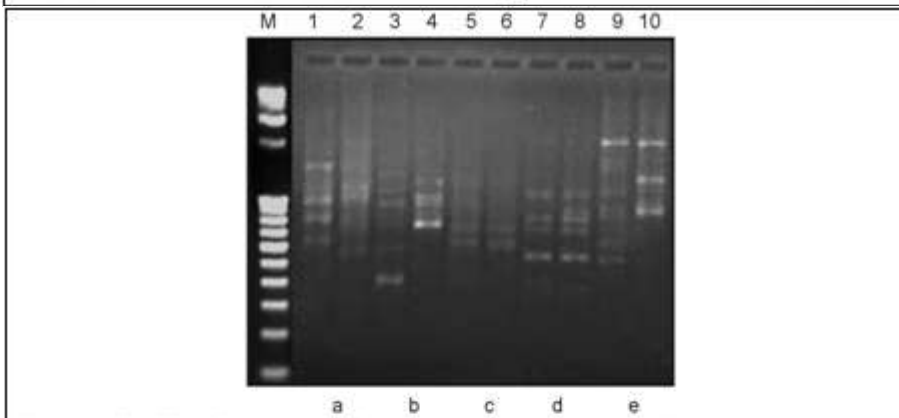
**Figure 2.** RAPD profiles obtained from DNA samples of two *Wrightia* species using random decamer primers

M = Molecular weight marker  
 Lane 1,3,5,7,9,11,13,15 *W. tomentosa*  
 Lane 2,4,6,8,10,12,14,16 *W. tinctoria*  
 a. OP 09                      b. OP 10                      c. OP 11                      d. OP 12  
 e. OP 13                      f. OP 14                      g. OP 15                      h. OP 16



**Figure 3.** RAPD profiles obtained from DNA samples of two *Wrightia* species using random decamer primers

M = Molecular weight marker  
 Lane 1,3,5,7,9,11,13,15 *W. tomentosa*  
 Lane 2,4,6,8,10,12,14,16 *W. tinctoria*  
 a. OP 17                      b. OP 18                      c. OP 19                      d. OP 20  
 e. OP 21                      f. OP 22                      g. OP 23                      h. OP 24



**Figure 4.** RAPD profiles obtained from DNA samples of two *Wrightia* species using random decamer primers

M = Molecular weight marker  
 Lane 1,3,5,7,9 *W. tomentosa*  
 Lane 2,4,6,8,10 *W. tinctoria*  
 a. OP 25                      b. OP 26                      c. OP 27                      d. OP 28  
 e. OP 29