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Original Research Article

RAPD Markers for Genetic Diversity Assessment of Critically Endangered Medicinal Plant *Commiphora wightii* (Arn.) Bhandari

G. Krishnamurthy, S. K. Tiwari*, Amit Pandey and S.S. Yadav

Forest Genetics, Plant Propagation and Biotechnology Division, State Forest Research Institute, Jabalpur-482 021, Madhya Pradesh, India

**Corresponding author.*

Abstract

Twenty Random Amplified Polymorphic DNA (RAPD) primers were used to analyze genetic diversity of different populations of *Commiphora wightii* (Arn.) Bhandari, an endangered medicinal plant. These populations were collected from different localities of Rajasthan, Gujarat and north eastern parts of Madhya Pradesh. Upon PCR amplification total of 77 reproducible bands were detected as amplified products, out of which 42 bands (54.54%) were polymorphic. Based on RAPD data similarity index ranged from 0.64 to 0.93 among different populations. Based on similarity value the UPGMA dendrogram showed clustering of different populations into two subgroups.

Keywords

Commiphora wightii

Genetic diversity

Medicinal plant

RAPD

Introduction

Guggul *Commiphora wightii* (Arn.) Bhandari, belongs to the family Burseraceae, is a well known drug plant. Its exudates, oleo-gum-resin which is known to be highly effective in the treatment of rheumatoid arthritis, obesity and peptic ulcer (Atal et al., 1975). *Commiphora wightii* is an important medicinal plant of herbal heritage of India. In Indian languages, it is known by various names like guggul in Hindi, gukkulu and maishakshi in Tamil, guggulu in Sanskrit and Indian bdellium in English. This plant is distributed in arid areas of India, Bangladesh and Pakistan. In India it is found in arid, rocky tracts of Rajasthan Gujarat and North eastern parts of Madhya Pradesh. Collection and conservation of germplasm is gaining importance all over the world with an aim to utilize these genetic resources in the ongoing as well as future plant improvement programmes. A number of techniques are now available for germplasm characterization, depending on the need, type and nature of the species. A number of molecular marker systems are now available for germplasm evaluation and classification.

Genetic diversity stands for all living things on earth. It refers to the range of variations among a set of entities and is commonly used to describe variety and variability of plant in terms of genetic diversity, biodiversity, species diversity and ecological diversity. In simple terms, Genetic diversity is the vast variety of natural plant existing in any region. Molecular markers work by highlighting differences (polymorphism) within a nucleic sequence between different individuals. These differences include

insertion, deletion, translocations, duplications and point mutation. Random Amplified Polymorphic DNA marker (RAPD) was the first PCR-based molecular marker to employ in genetic variation analyses. In this studies Random amplified polymorphic DNA marker was used because these marker shows high polymorphism. Use of DNA markers in germplasm characterization has now been well established and these markers are commonly used in a wide variety of species which can be used for assessing the genetic diversity within and between the populations (Williams et al., 1990). PCR (Polymerase Chain Reaction) technique usually refers to amplification of specific regions of genomic DNA. Utility of RAPD (Randomly amplified polymorphic DNA) markers for genetic mapping has been clearly shown in a number of plant species (Carlson et al., 1991). After scoring of the DNA bands the population/s/genotype/s which shows greater degree of polymorphism can be used for further genetic improvement programme.

Materials and methods

Germplasm exploration and collection

Area surveyed under the study: As per the available literature regarding its natural occurrence from three states viz. Rajasthan, Gujarat and Madhya Pradesh were surveyed for the identification of potential pockets and collection of wild germplasm from these states. For species potentiality in these states the information was collected from the forest officials and with the help of local people. From each site 10 individual plants/genotypes were selected randomly and 25 numbers of vegetative cuttings were prepared from each genotype. *Commiphora wightii* was collected from six populations from Rajasthan (Sajjangadh wild life sanctuary, Thurmagra, Chirvaghat Haldighati, Kiradu (historical guggle in India) Akoolwood Fossil Park) (Table 1) five populations from Gujrat [Daselpur Round (Badhai village), Nakhatrana Round, Mathal nursery, Dwarka Range, Goringa, Poshitra] (Table 2) and Two populations from Madhya Pradesh (Kemera, Bhind) (Table 3). Overall 13 populations were collected from different localities of three states.

Maintenance of germplasm

The population wise stem branch cuttings were placed in mist chamber for obtaining sprouts and young leaves for DNA extraction.

Table 1. Collection of *C. wightii* from State of Rajasthan.

District / provenance	Range/village	Accession number
Udaypur	Sajjangadh wild life sanctuary	CW-5
	Thurmagra	CW-10
	Chirvaghat	CW-2
Rajsamand	Haldighati	CW -1
Badmar	Kiradu (historical guggle in India)	CW-4
Jasalmare	Akool Wood Fossil Park	CW-6

Table 2. Collection of *C. wightii* from State of Gujarat.

District / provenance	Range/village	Accession number
Bhuj	Daselpur Round (Badhai village)	CW-11
	Nakhatrana Round	CW-13
	Mathal nursery	CW-8
Jamnagar	Dwarka Range, Goringa	CW-12
	Poshitra	CW-7

Table 3. Collection of *C. wightii* from State of Madhya Pradesh.

District / provenance	Range/village	Accession number
Murena	Kemera	CW-9
Bhind	Bhind	CW-3

DNA isolation

Young leaves (1g wt.) grinded in Pestle and Mortar using liquid nitrogen (LN_2) to convert the leaves into fine powdered form. The powder was transferred in 1.0 ml of CTAB buffer containing 100mM Tris (pH 8.0), 20mM EDTA (pH 8.0), 1.4 M NaCl, 2.5% CTAB (w/v), (Promega). In this solution 1% PVP (Calbiochem) and 10mM B-mercaptoethanol (Merck) added freshly. It was mixed vigorously by vortexer and incubated at 60±5°C for 30 min followed by treatment with equal volume of chloroform: isoamylalcohol (24:1) (Amresco). This mixture was centrifuged (Eppendorf, AG Germany) at 5125X g for 15 min at room temperature. After centrifugation the upper phase (supernatant) was transferred to a fresh autoclaved centrifuge tube and then 1/10 volume of 3M sodium acetate (pH 5.2) and ½ volume of 5M NaCl (Promega) was added to it. DNA was precipitated using 0.6 volume chilled isopropanol (Promega) and pelleted by centrifugation at 5125X g for 10 min at 4°C. The supernatant was decanted and the DNA pellet was washed with 70% ethanol (Merck). The crude DNA pellet was air dried and suspended in 500µl

of 0.5ml high salt TE buffer (10mM Tris pH 8.0, 1mM EDTA, 1M NaCl) (Promega) (Khanuja et al., 1999).

DNA verification

The isolated genomic DNA was verified using 0.8% Agarose gel (Promega) through electrophoresis (Genetix).

Amplification of isolated DNA

The isolated genomic DNA was amplified through PCR reaction which were carried in 0.2ml Polypropylene PCR tubes (Axiva) using thermal cycler EP gradient Master Cycler (Eppendorf, AG Germany). Each 20 µl reaction mixture contain, 1X Taq buffer (100mM Tris-Cl in pH 9, 500mM KCl, 15mM $MgCl_2$ and 0.1% gelatin (Promega), 2.5 mM $MgCl_2$, 0.2 µl dNTPs (Promega), 20 pmols Oligonucleotide primers (IDT Avantor),1U Taq DNA polymerase (Promega) and 20 ng template DNA. This reaction mixture was subjected to the three final PCR steps through (denaturation, annealing and extension) as initial denaturation at 94°C for 5 min followed by 45 amplification cycles, each consisting of 30seconds at 94°C (denaturation step), 1 min at 37°C (annealing step) and 2min at 72°C (extension step) with final extension of 10 min. at 72°C. The amplification products were separated on 1.5% w/v agarose gel (Promega) and stained with 0.7 µg/ml Ethidium bromide solution (Promega). DNA ladders of 1 kbp (Promega) were mixed and used as mol wt. marker for comparison of amplified product. Gels were photographed through Gel Documentation System Geneview 645C, (Genetix). All reactions were repeated thrice to confirm the results. Different random primers (MAP and OPA series consisting of a set of 20 primers each) (Table 6) were used and MAP primers were designed by Khanuja et al. (2000).

Scoring of amplified DNA fragments

The DNA fragments, obtained from different populations using RAPD marker were manually scored for their presence and absence. The data generated subjected to statistical analysis following the method of Jaccard's similarity coefficient. Bands were scored as present (1) or absent (0) for each sample. Weak or spurious bands were not included in the analysis. Similarity estimates were calculated by using Nei and Li coefficients (Nei and Li, 1979). The various populations were utilized for the construction of dendrogram with the help of UPGMA and NT-sys software (Rohlf, 1998).

Results and discussion

The polymorphism in amplified bands might result from changes in either the sequence binding site (e.g. point mutations) or change, which alter the size or prevent successful amplification of target DNA (e.g. insertion, deletion, inversions) (Williams et al., 1993). A dendrogram (Fig. 1) was generated based on RAPD marker analysis among 13 populations collected from three states, which showed genetic diversity within and between the states (Table 4) and amplified gel images (Fig. 2).

Fig. 1: UPGMA dendrograms generated based on RAPD marker analysis among 9 populations collected from three states showing relationship among *C. wightii.*

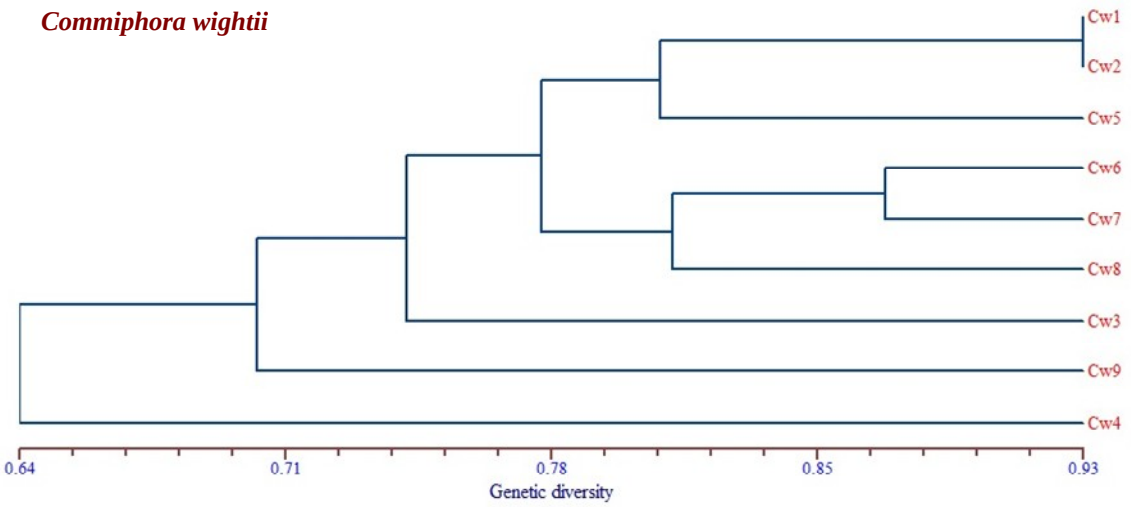
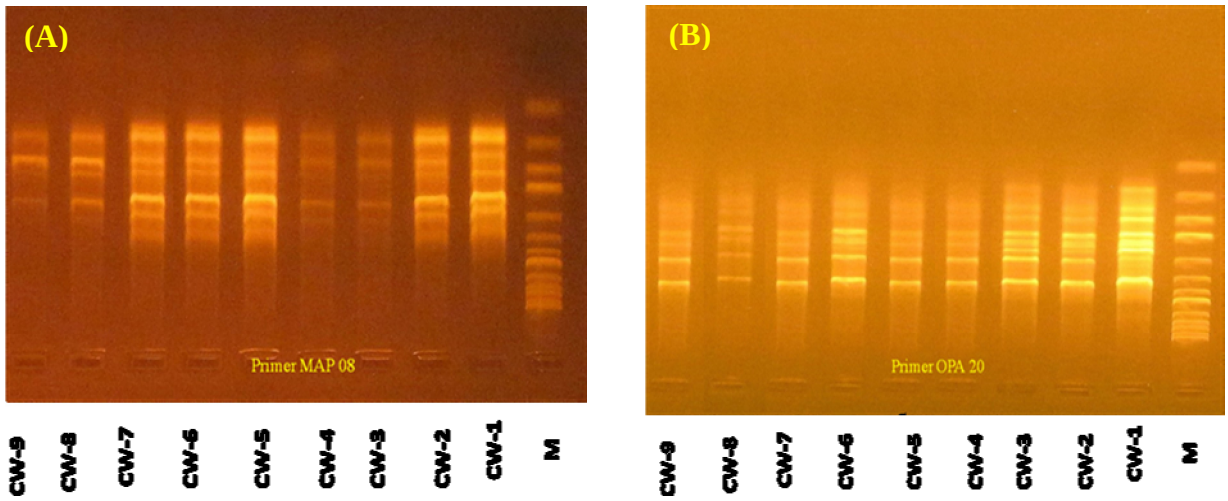


Table 4. Nine populations of *C. wightii* amplified with the applied primers from 13 populations of three states of India.

S. №	Genotype	Accession number	State
1	Sajjangadh wild life sanctuary	CW-5*	State of Rajasthan, India
2	Thurmagra	CW-10	
3	Chirvaghat	CW-2*	
4	Haldighati	CW -1*	
5	Kiradu (historical guggle in India)	CW-4*	
6	Akkool Wood Fossil Park	CW-6*	
7	Daselpur Round (Badhai village)	CW-11	State of Gujarat, India
8	Nakhatrana Round	CW-13	
9	Mathal nursery	CW-8*	
10	Dwarka Range, Goringa	CW-12	
11	Poshitra	CW-7*	
12	Kemera (Murena)	CW-9*	State of Madhya Pradesh, India
13	Bhind (Bhind)	CW-3*	

Note: The populations labeled with star (*) showed response with the applied primers.

Fig. 2: Amplified gel images showing different populations (Cw1 (Haldighati), Cw2 (Chirvaghat), Cw3 (Bhind), Cw4 (Kiradu), Cw5 (Sajjangarh), Cw6 (Akkalwood), Cw7 (Poshitra), Cw8 (Matthal) and Cw9 (Murana, Kamera) amplified with primer (A) MAP08 and (B) OPA20.



Genetic diversity assessment within the states

Out of these 13 populations only the 9 populations showed genetic diversity and rest 4 populations could not responded with applied primers (Table 5). According to the dendrogram analysis, all the populations were divided into two major clusters which were further sub divided as from the dendrogram and according to Jaccard's coefficient and cluster analysis it is summarized that as per the similarity index all the populations were placed in the range of 0.64 to 0.94. From this findings the population which were collected from Rajasthan, Cw1 (Haldighati) and Cw2 (Chirvaghat) showed highest genetic similarity index 0.92 and lowest genetic similarity was seen between Cw1 (Haldighati) Cw4 (Kiradu) that means these population are highly diversify with each other. While Cw4 (Kiradu) showed highly genetic diversify population (0.64) among the populations of Cw1 (Haldighati), Cw2 (Chirvaghat), Cw5 (Sajjangarh) and Cw6 (Akkalwood). In case of Gujarat Cw8 (Matthal) population showed more diversify index (0.81) as compared Cw7 (Poshitra) (0.87).The populations which were collected from MP. Cw9 (Murana, Kamera) showed highly diversify index (0.69) as compared to Cw3 (Bhind) (0.80).

Genetic diversity assessment between the states

From the genetic analysis it is concluded that the populations of Cw4 (Kiradu) showed highly genetic diversify (0.64) among the populations of Gujarat and MP. No such populations were found more closes genetic affinity from these three states except Rajasthan. According to Jaccard's coefficient and cluster analysis five RAPD markers were found to be more polymorphic. According to RAPD markers assay, it was found that 42 % bands showed polymorphism while 35% bands showed monomorphism. A polymorphism of 54.54% (Table 5) was found in all the primers used in the study (Table 6). The overall genetic diversity of a taxon has great implications for its long-term survival and evolution. Therefore, knowledge of the levels and patterns of genetic diversity is important for designing conservation strategies for threatened and endangered species Suthar et al. (2008).

Table 5. RAPD markers assay showing the polymorphism and monomorphism.

S. №	Code	Total bands	Monomorphic bands	Polymorphic bands	Percentage of Polymorphism
1	OPA-08	3	1	2	66.60
2	OPC 10	4	2	2	50.00
3	OPA -07	8	3	5	75.00
4	OPC-15	8	2	6	50.00
5	OPA-05	6	3	3	50.00
6	OPA-20	8	5	3	63.00
7	OPA-11	8	4	4	50.00
8	OPA-06	6	3	3	40.00
9	OPA-09	3	2	1	25.00
10	OPA-04	5	1	4	80.00
11	OPN-16	5	3	2	40.00
12	MAP-08	7	2	5	71.00
13	MAP-13	6	4	2	33.00
	Total	77	35	42	54.54

Table 6. List of RAPD primers and their sequences.

S.№	Primer	Sequences 5'-3'	GC content
1	OPA-08	5'-GTGACGTAGG-3'	60
2	OPC 10	5'-GACGGATCAG-3'	60
3	OPA -07	5'-GAAACGGGTG-3'	60
4	OPC-15	5'-GACGGATCAG-3'	60
5	OPA-05	5'-AGGGGTCTTG-3'	60
6	OPA-20	5'-GTTGCGATCC-3'	60
7	OPA-11	5'-CAATCGCCGT-3'	60
8	OPA-06	5'-GGTCCCTGAC-3'	70
9	OPA-09	5'-GGGTAACGCC-3'	70
10	OPA-04	5'-AATCGGGCTG-3'	60
11	OPN-16	5'-AAGCGACCTG-3'	60
12	MAP-08	5'-CTATCGCCGC-3'	70
13	MAP-13	5'-GTGCAATGAG-3'	50

Conclusion

As per the above findings it is concluded that the population which were collected from Kiradu (historical guggle in India) and surrounding areas of Badmer District (Rajasthan) showed highest alkaloid concentration as well as in terms of genetic diversity is concern. This information can be used for its genetic improvement programmes either through breeding or through propagation of elite material for *Commiphora wightii*.

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