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Molecular marker analysis of genetic diversity in relation to reproductive behaviour of *Commiphora wightii* populations distributed in Gujarat and Rajasthan states of India

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ABSTRACT

Guggul (*Commiphora wightii*) is a critically endangered medicinal plant distributed in arid and semi-arid agro-climatic zones of Northern-west India. Information on genetic diversity of *C. wightii* using DNA markers even though reported is limited to Rajasthan and Haryana populations and that too without considering its reproductive behaviour. In the present study, we are reporting for the first time the genetic diversity *vis-a-vis* its reproductive behaviour by including more number of accessions from Gujarat, which is the only place in India where sexual populations are distributed. We used RAPD and ISSR markers since genome sequence information is lacking in the species. Twenty four RAPD primers and 16 ISSR primers amplified a total of 185 and 128 reproducible DNA fragments, respectively with fragment sizes ranged from 200 to 3000 bp. RAPD analysis showed higher polymorphism (80%) in comparison to ISSR (69%). Jaccard's coefficient of similarity showed that pair-wise genetic similarity coefficients ranged from 46 to 98.3% in RAPD analysis, whereas it ranged from 47.8 to 98.6% in ISSR analysis. The results showed that the trend of genetic variation was not strictly correlated to the geographical locations, but was related to the reproductive behaviour of the populations. The diversity was somewhat low in Rajasthan populations where only apomictic populations are distributed. Populations of maximum genetic diversity along with sexual forms were found distributed in Gujarat populations, especially in Kutchh, Dwarka, Jamnagar and Porbandar populations. Hence the study indicated these areas as the original areas of the species distribution from where it was spread to the other parts of Gujarat and Rajasthan.

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1. Introduction

Guggal (Indian bdellium), *Commiphora wightii* (Arn.) Bhandari (Syn. *Balsamodendron wightii* Arn; *Commiphora mukul* (Hook. ex Stocks) Engl.; *Balsamodendron mukul* Hook. ex Stocks) is a small aromatic tree or a shrub and is a popular species of the family Burseraceae. The plant is mainly distributed in arid and semi-arid agro-climatic zones of North Western India, especially in Gujarat and Rajasthan and adjoining areas of Pakistan (Hooker, 1872). The plant exudates, oleo gum resin which contains major bioactive compounds guggulsterone E&Z, which possess anti-inflammatory, antirheumatic, hypocholesteremic, hypolipidemic, anti-fertility and anti-cancer activities (Satyavati, 1990) and is used in the allopathic, ayurvedic, and unani systems of medicines. The species is not under cultivation and natural populations of the species are exploited for oleo-gum resin extraction. The oleo-gum resin is extracted from the main trunk by a process known as "tapping" by the local tribal people. Extraction of the gum resin causes the assured death of the plant and overexploitation of the plant for gum resin extraction has resulted in "critically endangered" status of the species (Red Data list of International Union for Conservation of Nature). In order to ensure the sensitive species survival in nature, it is compulsory to adopt *ex situ* conservation strategies and bring the species under cultivation. The ICAR-Directorate of Medicinal and Aromatic Plants Research, (ICAR-DMAPR), Anand, Gujarat, India initiated *ex situ* conservation of the species by collecting the germplasm of guggul from different natural habitats and maintained it in the Field Gene Bank. Characterization of the collected germpalsm is very important before adopting any breeding techniques for the development of elite cultivars. DNA markers like Random Amplified Polymorphic DNAs (RAPD) and inter simple sequence repeats (ISSR) markers have proven very useful tools providing rapid assessment of the genetic difference between accessions, especially when genome sequence information is lacking (Williams et al., 1990; Zietkiewicz et al., 1994; Kulhari et al., 2015; Bishoyi et al., 2016; Bishoyi et al., 2017; Cruz-Martínez et al., 2017).

In guggul, detailed evidences of apomixis, occurrence of obligate sexual reproduction and uneven sex distribution pattern and genetic variations in polyembryony were well studied (Gupta et al., 1996; Geetha et al., 2013; Kawane et al., 2014). Information on genetic diversity of *C. wightii* using DNA markers is very limited. Suthar et al. (2008),

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Haque et al. (2009, 2010), Kulhari et al. (2015), *etc.* studied genetic diversity of *C. wightii* with limited number of either RAPD or ISSR markers and reported that the species retains low genetic diversity. All the reported molecular investigations were carried out in populations of Rajasthan and Haryana and a few from Gujarat and none of the reports described the importance of genetic diversity of Gujarat populations. Genetic variation of populations in relation to gender distribution and reproductive behaviour has not been studied so far. Gujarat populations are especially significant in breeding point of view since sexual populations are available only in Gujarat populations and the other populations consist of only females which are apomictic (Geetha et al., 2013). The earlier reports also reflect limitation of the work in marker/s system as well as number of accessions chosen for the study. Whereas, in this communication we are reporting genetic diversity *vis-a vis* reproductive behaviour of 73 accessions of *C. wightii* collected from Gujarat and Rajasthan (India) by RAPD and ISSR markers.

2. Materials and methods

2.1. Plant materials and DNA isolation

C. wightii populations collected from 73 geographical locations of Gujarat and Rajasthan and conserved in the Field Gene Bank of ICAR-DMAPR, Anand, Gujarat, India were used for the present study. Each population was referred as one accession in the present report. Fifty accessions were from Gujarat and 23 were from Rajasthan. Among these, seven were males (all were from Gujarat), 61 were females (23 from Rajasthan and the rest from Gujarat) and five were hermaphrodites (all from Gujarat). All the Rajasthan populations were apomictic females and populations of Gujarat included apomicts as well as sexuals. Details about the accessions taken for the study are given in Table 1.

Fresh and young leaves were randomly collected from five plants ($n = 5$) of each accession and treated as one sample. A total of 73 samples representing 73 geographical locations were collected and the DNA was isolated from each sample by modified CTAB based protocol (Samantaray et al., 2009). DNA quality and quantity were documented by Molecular Imager Gel Doc XR$^+$ (Biorad, Berkeley, California US), after electrophoresis on 0.8% (w/v) agarose gel at 60 V for 1 h and compared with a known amount of lambda DNA as a standard (MBI, Fermentas). The re-suspended DNA was then diluted in TE buffer to 25 ng/μl and used in polymerase chain reactions (PCR).

2.2. Primer screening

One hundred and forty decamer primers, corresponding to kits A, B, D, J, N, P and T series from Operon Technologies (Alameda, California, USA) and 35 synthesized ISSR primers (M/S Bangalore Genei, Bangalore, India) were initially screened to determine the suitability of each primer for the study. Primers were selected for further analysis based on their ability to detect distinct, clearly resolved and polymorphic amplified products. To ensure reproducibility of the primers, the primers generating no, weak, or complex patterns were discarded.

2.3. RAPD-PCR

RAPD amplification was executed according to Williams et al. (1990) by decamer random primers. Each RAPD-PCR amplification was carried out in 25 μl reaction mixture containing 2 μl of genomic DNA (25 ng/μl), 2 μl (5 pmol/μl) of primer, 0.2 μl (100 mM/μl) of dNTP mix (dATP, dCTP, dGTP and dTTP) (MBI, Fermentas, Maryland, USA), 2.5 μl of 10X assay buffer (10 mM TrisHCl (pH 8.0) with 15 mM $MgCl_2$), 0.2 μl (5 U/μl) of *Taq*DNA polymerase (Bangalore Genei, India) and final volume was made up with sterilized deionized water. PCR reactions were carried out in gradient Mastercycler (Eppendorf AG, Hamburg, Germany), with optimal amplification conditions for the reactions as follows: Initial

Table 1
Details of the studied accessions of *C. wightii*.

Sr. no	Genotype	Gender	Place of collection
1	DMAPR CW1	Female	Kutchh, Gujarat
2	DMAPR CW2	Female	Kutchh, Gujarat
3	DMAPR CW3	Female	Kutchh, Gujarat
4	DMAPR CW4	Female	Amreli, Gujarat
5	DMAPR CW5	Female	Kutchh, Gujarat
6	DMAPR CW6	Female	Kheda, Gujarat
7	DMAPR CW7	Hermaphrodite	Kheda, Gujarat
8	DMAPR CW8	Female	Kutchh, Gujarat
9	DMAPR CW9	Female	Rajkot, Gujarat
10	DMAPR W10	Female	Jamnagar, Gujarat
11	DMAPR W11	Female	Kutchh, Gujarat
12	DMAPR W12	Male	Kutchh, Gujarat
13	DMAPR W13	Female	Kutchh, Gujarat
14	DMAPR W14	Hermaphrodite	Kutchh, Gujarat
15	DMAPRCW15	Female	Kutchh, Gujarat
16	DMAPR W16	Female	Kutchh, Gujarat
17	DMAPRCW17	Hermaphrodite	Kheda, Gujarat
18	DMAPR W18	Female	Kutchh, Gujarat
19	DMAPR W19	Male	Kutchh, Gujarat
20	DMAPR W20	Female	Jamnagar, Gujarat
21	DMAPR W21	Male	Jamnagar, Gujarat
22	DMAPR W22	Female	Jamnagar, Gujarat
23	DMAPR W23	Male	Dwarka, Gujarat
24	DMAPR W24	Female	Jamnagar, Gujarat
25	DMAPR W25	Male	Jamnagar, Gujarat
26	DMAPR W26	Female	Kutchh, Gujarat
27	DMAPR W27	Male	Kutchh, Gujarat
28	DMAPR W28	Female	Jamnagar, Gujarat
29	DMAPR W29	Hermaphrodite	Kheda, Gujarat
30	DMAPR W30	Female	Kheda, Gujarat
31	DMAPR W31	Female	Kheda, Gujarat
32	DMAPR W32	Hermaphrodite	Kheda, Gujarat
33	DMAPRCW33	Female	Dwaraka, Gujarat
34	DMAPRCW34	Male	Dwaraka, Gujarat
35	DMAPRCW35	Male	Dwaraka, Gujarat
36	DMAPRCW36	Female	Dwaraka, Gujarat
37	DMAPRCW37	Female	Porbandar, Gujarat
38	DMAPRCW38	Female	Porbandar, Gujarat
39	DMAP CW39	Female	Kutchh, Gujarat
40	DMAPRCW40	Female	Jamnagar, Gujarat
41	DMAPRCW41	Female	Amreli, Gujarat
42	DMAPRCW42	Female	Kutchh, Gujarat
43	DMAPRCW43	Female	Banaskata, Gujarat
44	DMAP CW44	Female	Amreli, Gujarat
45	DMAPRCW45	Female	Surendranagar, Gujarat
46	DMAPRCW46	Female	Banaskata, Gujarat
47	DMAPRCW47	Male	Porbandar, Gujarat
48	DMAPRCW48	Female	Surendranagar, Gujarat
49	DMAPRCW75	Female	Vadodara, Gujarat
50	DMAPRCW76	Female	Banaskatha, Gujarat
51	DMAPRCW49	Female	Badmer, Rajsthan
52	DMAP CW50	Female	Abu road, Rajasthan
53	DMAPRCW51	Female	Balotra, Rajasthan
54	DMAPRCW52	Female	Balotra, Rajasthan
55	DMAPRCW53	Female	Jaiselmer, Rajasthan
56	DMAPRCW54	Female	Balotra, Rajasthan
57	DMAPRCW55	Female	Abu road, Rajasthan
58	DMAPRCW56	Female	Jodhpur, Rajasthan
59	DMAPRCW57	Female	Jodhpur, Rajasthan
60	DMAPRCW58	Female	Balotra, Rajasthan
61	DMAPRCW59	Female	Jodhpur, Rajasthan
62	DMAPRCW60	Female	Jaiselmer, Rajasthan
63	DMAPRCW61	Female	Barmer, Rajasthan
64	DMAPRCW62	Female	Balotra, Rajasthan
65	DMAPRCW63	Female	Jodhpur, Rajasthan
66	DMAPRCW64	Female	Jodhpur, Rajasthan
67	DMAPRCW65	Female	Jaiselmer, Rajasthan
68	DMAP CW66	Female	Jodhpur, Rajasthan
69	DMAPRCW67	Female	Jodhpur, Rajasthan
70	DMAPRCW68	Female	Abu road, Rajasthan
71	DMAPRCW69	Female	Abu road, Rajasthan
72	DMAPRCW70	Female	Balotra, Rajasthan
73	DMAPRCW71	Female	Jodhpur, Rajasthan

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