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GENETIC CHARACTERIZATION OF RHODODENDRON ARBOREUM SM. THROUGH RAPD STUDY

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ABSTRACT

Rhododendron arboreum Sm., commonly known as *Burans*, belongs to family Ericaceae. It is an ethno-medicinal plant used by the locals of Himachal Pradesh in nasal bleeding, cardiac and menstrual disorders. The present study deals with molecular characterization of leaves through RAPD study. Fresh leaves of *Rhododendron arboreum* Sm. were collected from its natural habitat, Jhatingri, Himachal Pradesh during April 2018 and sent for Random Amplified Polymorphic DNA analysis at Food testing laboratory, Junagadh Agriculture University, Gujarat. All the primers gave good band patterns. Total 27 bands are produced from the primers. viz. among 13 very clear bright bands and 14 light bands. Primer 4 shows maximum no of bright bands. Only sample 4 and sample 9 produce bands above 1000 bp. The results obtained from the study help in generating RAPD profile of *Rhododendron arboreum* Sm. that can be used further for authentication purpose.

***Keywords*:** *Burans*, Ericaceae, ethanomedicinal, RAPD.

INTRODUCTION

Medicinal plants have been used for centuries by several indigenous communities to maintain health and to treat diseases. *Rhododendron arboreum* Sm. is an ethnomedicinal plant found in temperate Himalayas, from Kashmir to Bhutan 4000-11000 ft., Khasia Hills 4000-6000 ft., Burma, Nilgiris, Pulneys, Travancore, above 5000 ft[1]. Its flowers are used in nasal bleeding[2] cardiac disorders[3]. Its bark is used in the treatment of jaundice, piles, liver disorders.[4]

Different varieties of *Rhoododendron* genus are available worldwide. About 80 species of *Rhododendron* are reported from India[5]. Most of the varieties shows more or less similarity in morphological, anatomical and phytochemical characters. Appropriate identification and characterization of plant materials is necessary for their particular use. Genetic characterization using molecular markers serve as a valid tool to assess genetic diversity and trace the phylogeny of diverse species of plant. Several methods of genetic characterization are available. Among all of them, RAPD is one of the best method to assess the plant as it is comparably fast, not expensive and have applicability to any organism without prior information on the nucleotide sequence and in the potential detection of DNA damage and mutation[6]. Hence the present study is carried out to establish standards for identification and develop RAPD profile of *Rhododendron arboreum* Sm. using random primers.

MATERIALS AND METHODS

Collection of sample

Fresh leaves of *Rhododendron arboreum* Sm. were collected from its natural habitat, Jhatingri (distt. Mandi), Himachal Pradesh, in month of April 2018. The plant was authenticated from Pharmacognosy Laboratory, IPGT & RA, GAU (specimen no. Ph.M: 6263/ 2018-19) and sent for RAPD study. Plant herbarium was authentified from BSI, Kolkata (Specimen no. AT-01/CHN/Tech.II/2019/42) as *Rhododendron arboreum* Sm. of family Ericaceae.

Fig 1 : Natural habitat of plant

Fig 2 : Flowers along with leaves

Fig 3 : Herbarium

Fig 4 : BSI identification

RAPD analysis

The RAPD study was performed following standard procedures at Food testing laboratory, Junagadh Agriculture University, Gujarat, India.

DNA of the given plant material was extracted by using Doyle and Doyle method with minor modifications[7].

DNA isolation: Fresh were cut into small pieces, washed with distilled water and ethanol, frozen with dry ice and crushed. To that, 2 ml of plant DNA extraction buffer was added. The samples were ground thoroughly, transferred into centrifuge tube and added 10 ml plant DNA extraction buffer. 50 µl of BME added to each tube mixed well. Incubated at 65ºC for 1 hour with intermittent mixing and centrifuged for 15 minutes at 10 K (10000 rpm). Supernatant was transferred carefully into fresh tube and added equal volume of chloroform and mixed well. Centrifuged for 15 minutes at 10 K (10000 rpm). Aqueous layer was carefully pipetted into fresh tube and precipitated with isopropanol. DNA pellet suspended in 300 µl of TE and subjected to column purification. Column purification Silica spin columns and buffers were from Qiagen. The column was placed in collection tube, 400µl of equilibration buffer was added to the column and centrifuged at 10000 rpm for 1min. Collected buffer was discarded. 400 µl of equilibration buffer was added to the DNA samples, mixed and loaded into the column (This step was repeated till the DNA sample was completed). Flow through was collected. 500 µl of wash buffer 1 was added, centrifuged at 10000 rpm for 1minute and buffer was collected. 500 µl of wash buffer 2 was added, centrifuged at 10000 rpm for 1 minute and buffer was collected. The empty column was centrifuged with collection tube to completely remove the wash buffer for 2 minute. 50 µl of elution buffer was added to the column placed in new collection tube. Incubated at room temperature for 2 minutes and centrifuge at 10000 rpm for 1 minute and eluted sample was saved (elution 1). Previous step was repeated (elution 2). DNA quantification was done using a Pico drop spectrophotometer and DNA sample was diluted by using TE buffer up to 50 ng/µl. Quantity of DNA sample 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 primers used in the RAPD study are listed in table 1.

Table 1: RAPD primers used for the analysis of DNA samples of *R. arboreum* Sm.

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

OBSERVATION AND RESULTS

The fingerprinting pattern of *Rhododendron arboreum* Sm. sample observed as vertical columns with horizontal light bands on a dark background (Fig. 5). Total 10 primers (Table 1) are used for the study. Primers have been loaded from left to right side. Figure 5 shows the primers numbered 1 to 10. The range of band size is 100 bp to 1000 bp and above.

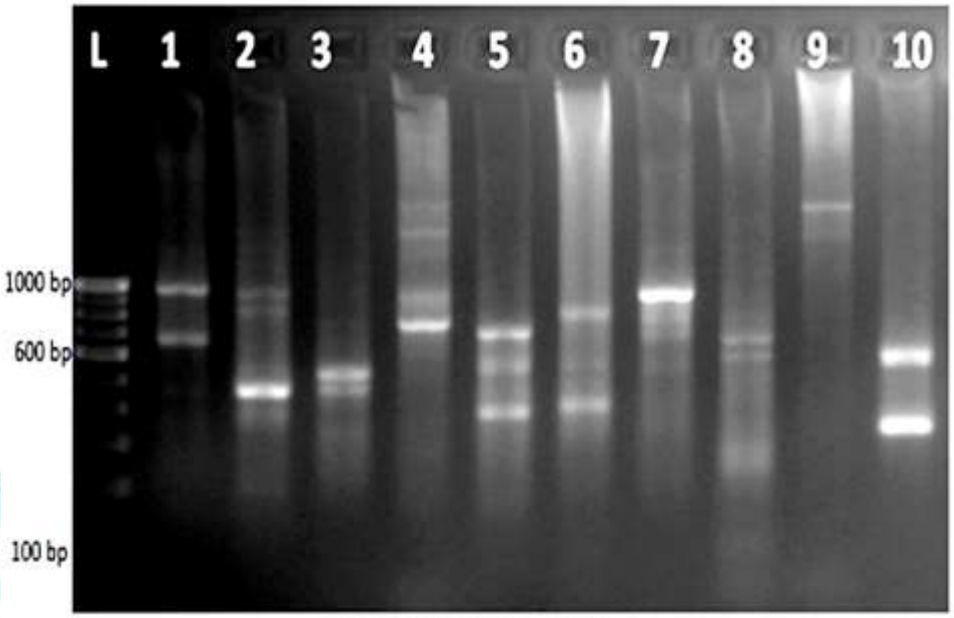

Fig 5 : RAPD fingerprinting

In primer 1 (OPM-07), band size observe from 600 bp to 1000 bp; primer 2 (OPN-03) shows band size from 400 bp to 900 bp; in primer 3 (OPO-01), the range of band size is from 400bp to 600bp; in primer 4 (OPM-09), the band size ranges from 700 bp to above 1000 bp; primer 5 (OPO-06), shows band size from 300 bp to 600 bp; in primer 6 (OPM-06), the range of band size is from 300 bp to 800 bp; in primer 7 (OPM-02), the range of band size is from 600 bp to 800 bp; in primer 8(OPM-05), band size ranges from 400bp to 600bp; in primer 9 (OPM-03)the range of band size is above 1000 bp; in primer 10 (OPM-04), the range of band size is from 300bp to 600bp.

DISCUSSION

In RAPD study of *R. arboreum* Sm. all the primers give result. The range of band size was observed from above 100 to 1000 bp and above. Total 27 bands are produced from the primers. viz. among 13 very clear bright bands and 14 light bands. Primer 3, 5 , 8 and 10 exerts the bright bands in the range of 300 bp to 600 bp. Primers 1, 4 and 7 exerts bright bands in the range of 600 bp to 1000 bp. Primer 2, primer 6 show the bands in the range of 400 to 900 and 300 to 800 bp respectively. Only primer 9 and primer 4 shows band above 1000 bp. Bands produced by Primer 8 and 9 are very poor in nature. Primer 4 shows maximum no of bright bands. Bright band exerted from other 10 primers support the base plant *R. arboreum* Sm. Primer 1, primer 2, primer 4, primer 7 merge with the ladder at 1000bp and primer 8, primer 10 at 600 bp position.

Genetic characterization can be done through various methods. Among all methods, RAPD is one of the best method to assess the plant. The results of the study showed different primers bands are produced by experimental plants, some

bands were coincides at same base pair position of the ladder. They might represent the characters of plant at morphological, anatomical or phytochemical levels.

CONCLUSION

RAPD produces consistent fingerprints irrespective of age or source of the plant. The unique bands obtained in RAPD study serve as standard for determining the genuinity of the plant *R. arboreum* Sm. and help in the identification & differentiation of the plant itself from other varieties.

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