WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 17, 886-892. Research Article ISSN 2277– 7105

DIFFERENTIATION AND AUTHENTICATION OF MEDICINAL ORCHIDS OF *HABENARIA* SPECIES USING RAPD- A MOLECULAR PROFILE

B. N. Satish[1] and Mallya Suma V.*

[1]Ph.D Scholar and *Associate Professor

Department of Dravyagauna, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, 574118.

Article Received on
08 August 2018,

Revised on 28 August 2018,
Accepted on 18 Sept. 2018

DOI: 10.20959/wjpr201817-13348

***Corresponding Author**
Mallya Suma V.
Associate Professor, Department of Dravyagauna, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, 574118.

ABSTRACT

Introduction: *Habenaria longicorniculata J.Graham* medicinal orchid with multiple therapeutic usages also found adulterated with some *Ashtavarga* drugs. Authentic quality parameters of a therapeutic agent are most essential part in any science. Hence an attempt to generate DNA based molecular marker, RAPD. **Materials and Methods:** *Habenaria longicorniculata J.Graham* along with the other two species ie *Habenaria crinifera* Lindl., and *Habenaria plantaginea* Lindl., were selected for study. Leaf samples of these three orchids were used for RAPD. Genomic DNA isolation was carried out through CTAB method and purified. This was randomly amplified through PCR using different primers. **Result:** Five random primers were used OPD-02, OPB – 08, OPAI -05, OPC-07 and OPZ – 11 and the sequence obtained respectively was GGACCCAACC, GTCCACACGG, GTCGTAGCGG, GTCCCGACGA and CTCAGTCGCA. These can be used to differentiate and authenticate study sample.

KEYWORDS: *Habenaria longicorniculata* J. Graham, *RAPD, DNA, Primer.*

INTRODUCTION

Traditional herbal and herbo-mineral drugs have been used since the dawn of civilization to maintain and alleviate human sufferings from diseases. *Habenaria longicorniculata* J. Graham from family *Orchidaceae*, are tuberous herbs, endemic to peninsular India, which is found commonly at foot hills and slopes amongst grasses.[1] The underground tubers are

widely used by traditional *Vaidyas* in the treatment of malignancy as a rejuvenator.[2] These are also said to be found adulterated for *Riddhi*, one among *Ashtavarga dravya* used as a rejuvenator.[3] Around eight species of *Habenaria* are reported from India among which *Habenaria longicorniculata* J. Graham is one of them.[4] It is specifically terrestrial erect herbs having 1-2 tubers and white flowers.[5] Tubers of which are used in wasting disease, fever, disorders of blood, haemorrhage and fainting and specifically used as overall rejuvenators.[6]

Herbal drug authentication has presented a great challenge for people using them for medicinal purposes. Usage of a wrong herb may be ineffective or it may worsen the condition and may even cause death. Ideally, authentication should be done from the harvesting of the plant material to the final product. Morphological as well as biochemical markers used in the authentication of herbal drugs have many limitations due to the impact of environmental conditions.[7] DNA based molecular markers however, are important tool in quality assurance and preservation of germplasm of medicinal plant species in the plant kingdom.[8] RAPD stands for Random Amplification of Polymorphic DNA. RAPD markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence. By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction.[9]

Hence an attempt to develop DNA based molecular tools for accurate identification of *Habenaria longicorniculata J.Graham* is tired along with *Habenaria crinifera* Lindl., and *Habenaria plantaginea* Lindl., most commonly available medicinal orchid species.

MATERIALS AND METHODS

Test drug *Habenaria longicorniculata* J.Graham, was collected from its natural habitat, during its flowering time, along with other species like *Habenaria plantaginea* Lindl., and *Habenaria crinifera* Lindl., properly identified, authenticated using floras and botanists. Sample cleaned properly with extraneous matter. Leaf samples of these three orchids named and separately packed and preserved for further study.[11]

METHODOLOGY

Genomic DNA isolation through CTAB method Column purification and quantification of DNA

200 mg of plant sample was taken and homogenized using pestle and mortar, washed with 500 µl of 70% ethanol, centrifuged at 10,000 rpm for 5 minutes. To the concentrate 15 ml of CTAB buffer and 20 µl of beta mercaptoethanol was added and mixed. Then it was incubated for 90 minutes at 60^0C in water bath and centrifuged at 10,000 rpm for 15 minutes. Later supernatant was decanted carefully in fresh tubes and added equal volumes of 100% chloroform; mixed well for 10 minutes by inverting tubes, centrifuged at 10,000 rpm for 15 minutes. Next separated aqueous layer transferred in to fresh tube and added equal volumes of isopropyl alcohol and centrifuged at 10,000 rpm for 15 minutes. Again supernatant was discarded and washed DNA pellets with 0.5 ml of 70% ethanol at 10,000 rpm for 5 minutes. Ethanol was discarded and DNA pellet were air dried; DNA Suspended in 500 µl of 1X TE buffer.[12] The DNA was further purified using silica membrane based column and quantitated on agarose gel.(Figure 1).

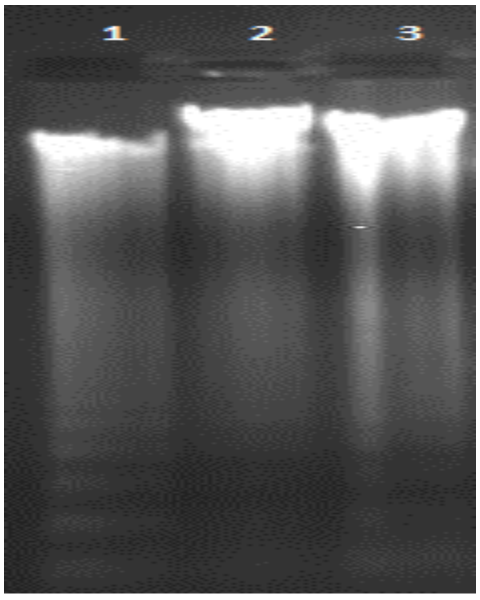

Figure 1: Genomic DNA.

RAPD[13]

PCR was set up using master mix consisting of 100µM each of dNTPs, 100mM KCl, 1.5mM MgCl2, 0.1% each of tween 20 and Nonidet P40, 15p moles of random primer and1u of taq polymerase in 40 µl volume. 38µl of this was aliquoted into 15 different labeled PCR vials and to this 2 µl of different template DNA was added. PCR Cycle conditions were, initial

denaturation at 94˚C for 5 minutes, denaturation for 30 seconds, annealing for 1 minute at 45˚C, extension for 90 seconds at 72˚C and final extension for another 7 minutes. Five different primers used have shown in Table 1.

Table 1: Primers used and sequences obtained.

Sr.no.	Primers	Sequence
1.	OPD-02	GGACCCAACC
2.	OPB-08	GTCCACACGG
3.	OPAI-05	GTCGTAGCGG
4.	OPC-07	GTCCCGACGA
5.	OPZ-11	CTCAGTCGCA

RESULTS

OPB-06,OPAI-05and OPZ-11 primers resulted in RAPD patterns that differentiated the three samples very clearly. These primers can be used to distinguish these three plant species at any given time. (Figure 2). OPB -06 primer gave unique band of ~1.1Kb for sample 1 and unique band of ~400bp for sample 2. OPAI-05 primer gave unique band of~550 and 650 bp for sample 1 and unique band ~1.5kb band for sample 3.OPZ-11 primer gave unique band of ~550 bp for sample 2. These bands can be used for developing SCAR markers which will be easier to identify these species without waiting for the flowering of the plant for identification.

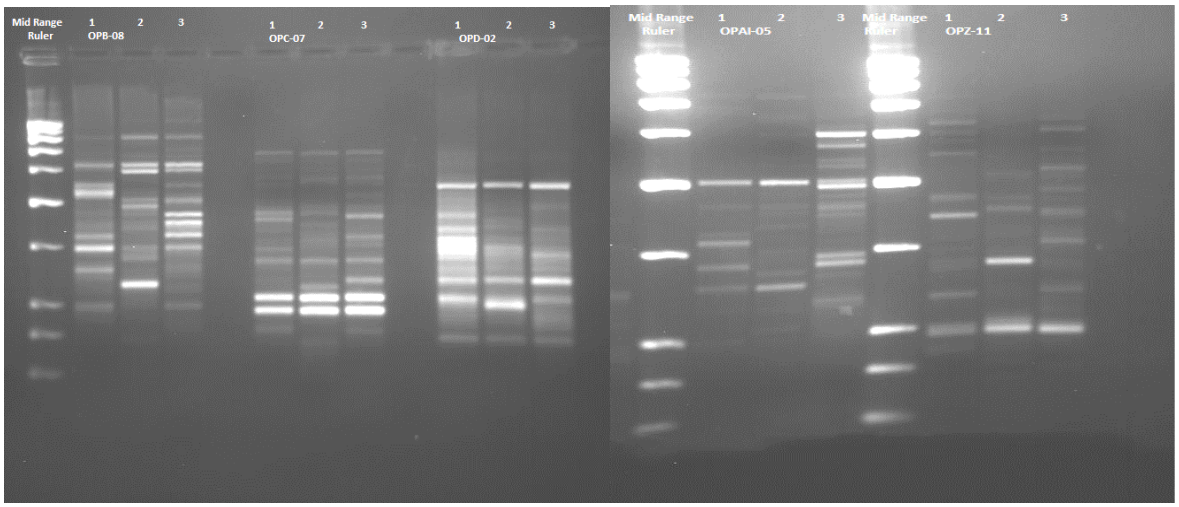

Figure 2: OPB-06, OPAI-05 and OPZ-11 primers resulted in RAPD patterns that differentiated the three plant samples very clearly.

Note: Ruler-mid range ruler ranging from 0.1, 0.2, 0.3, 0.6, 1, 1.5, 2.0, 2.5, 3.0 & 3.5kb

DISCUSSION

Medicinal plants constitute an effective source of traditional medicine. Adulterations and substitutions are common in raw material trade of medicinal plants. Development of RAPD markers that can correlate DNA fingerprinting data with quantity of selected phytochemical markers associated with that specific medicinal plant would have extensive applications in quality control of raw materials.[14] These designed markers will act as a qualitative or quantitative diagnostic tool for identification of medicinal herbs.

Habenaria longicorniculata J.Graham was chosen to compare with the other two species ie *Habenaria crinifera* Lindl., and *Habenaria plantaginea* Lindl., using RAPD technique. The RAPD, PCR based assay described here is fast, reliable and easy to conduct in any laboratory. It can be carried out in very short period using unknown genomic DNA from any developmental stages and body parts of herbs.

Five Random Primer used were OPD-02, OPB – 08, OPAI -05, OPC-07 and OPZ – 11 and the sequence obtained respectively was GGACCCAACC, GTCCACACGG, GTCGTAGCGG, GTCCCGACGA and CTCAGTCGCA.

OPB-06, OPAI-05 and OPZ-11 primers resulted in RAPD patterns that differentiated the three samples very clearly. These primers can be used to distinguish these three plant species at any given time. OPB -06 primer gave unique band of ~1.1Kb for sample 1 and unique band of ~400bp for sample 2. OPAI-05 primer gave unique band of~550 and 650 bp for sample 1 and unique band ~1.5kb band for sample 3. OPZ-11 primer gave unique band of ~550 bp for sample 2. These bands can be used for developing SCAR markers which will be easier to identify these species without waiting for the flowering of the plant for identification. OPC-07 and OPD-02 RAPD Patterns did not differentiate the samples- the banding pattern was almost similar.

CONCLUSION

Molecular marking techniques are most reliable newer techniques in herbal drug standardization. *Habenaria longicorniculata J.Graham* a most precious medicinal orchid selected to compare with the other two species ie *Habenaria crinifera* Lindl., and *Habenaria plantaginea* Lindl., using RAPD technique. The results given here will differentiate each sample and these bands can be used for developing SCAR markers.

ACKNOWLEDGEMENT

Authors are grateful to revered President, Dr. D. Veerendra Heggade, SDM Educational Society for constant encouragement. Authors are indebted to Dr. G Shrinivasa Acharya, Principal SDM Udupi for support. Authors are gratified to Dr. Sudha L and Dr. Vasudha, Director, Aristogene Biosceinces Pvt. Ltd, Bangalore.

REFERENCES

1. Dangat et al., Inorganic status of tubers of Habenaria longicorniculata J. GRAHAM; Asian J pharm Clin Res, 2014; 7(2): 172-173.
2. A dhyani et al., Importance of Ashtavarga plants in traditional syatem of medicine in Garhwal, Indian Himalaya; International Journal of Biodiversity Science, Ecosystem Services and Management, 2010; 6(1-2): 13-19.
3. Balakrishna et al., Ashtavarga plants- threatened medicinal herbs of the North-West Himalaya; Int. J. Med. Arom. Plants, Dec. 2012; 2(4): 661-676.
4. JS Gamble, Flora of Presidency of Madras, Volume III, Dehraduna, 1998; 1469.
5. Satish Bn, Mallya Suma V, Prabhu Suchitra; Authentication parameters of Habenaria longicorniculata J. Graham; medicinal orchid used in Ayurveda; Arya vaidyan, Feb-Apr 2018; XXXI(3): 31-40.
6. Kirtikar KR & Basu BD. Indian Mediicnal Plants, Volume III, Dehradun; International Book Distributors, 2008; 2399-415.
7. Mallya et al., Identification of A popularly used Ayurvedic drug Ficus religiosa by DNA SCAR marker; J Ayu Med Sci., 2018 Jan- Mar; 3(1): 289-91.
8. KN Sunilkumar et al., Molecular fingerprinting of Helianthus elastica(Desr.) Danser growing on five different hosts by RAPD; Saudi Journal of Biological Sciences, 2016; 23: 335-40.
9. Devaiah KM, Venkatasubramanian P., Genetic characterization and authentication of Embeliaribes using RAPD-PCR and SCAR marker; Planta Med, 2008; 74: 1306-8.
10. Polashock James J, Development of SCAR markers for DNA fingerprinting and Germplasm analysis of American Cranberry; J. Amer. Soc. Hort. Sci., 2002; 127(4): p677-84.
11. Marym Sarvat et al., A standardized protocol for genomic DNA isolation from *Terminaliaarjuna* for genetic diversity analysis; Electronic Journal of Biotechnology, 2006; 9(1): 87-91.

12. Pirttila AM et al., DNA isolation methods for medicinal and aromatic plants; Plant Molecular Biology Reporter, 2001; 19(3): 273 a-f.
13. Balaraj Suraj Raju et al. Assessment of Genetic diversity of Ficus Species Using Rapd Markers as a Measure of Genomic Polymorphism; IOSR Journal of Pharmacy and Biological Sciences, 2015; 10(1): 22-27.
14. Kalpana Joshi et al., Molecular markers in herbal drug technology; Current Science, 2004; 87(2): 159.