STUDY ON GENETIC DIVERSITY OF *BACOPA MONNIERI* (L.) PENNELL ECOTYPE VARIANTS FROM TAMIL NADU BY THE RAPD MARKERS

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Brahmi is a small prostrate herb that grows wild in marshy and damp places near water logs throughout India. The plant has been used since ancient time in folklore and traditional medicine as nervine, cardio-tonic, and diuretic. The plants were collected from18 different geographical regions of south India. RAPD profiles in order to assess diversity and the similarity matrices were generated from the RAPD data on the basis of Jaccard similarity coefficient estimates of similarity indices and dendrogram were constructed based on UPGMA clustering. OPL-05 having the maximum range of polymorphism was noticed (83.33%) and high level of monomorphism was noticed in the primer OPT-18. The amplification range is between 100 bp to 2900 bp and the total percentage of polymorphism is (50%) was observed. The observed narrow genetic base in *B. monnieri* population in Tamil Nadu may be attributed to the vegetative propagation of the species.

Key words: *Bacopa monnieri*, Ecotypes, Genetic diversity, RAPD.

INTRODUCTION

Plant based drugs and formulations are showing a rising trend globally for the health care due to biosafety attributes they possess over modern medicines. *Bacopa*

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monnieri also referred as water hyssop, and "Brahmi" has been used in the ayurvedic system of medicine for centuries. Recent research focused primarily on Bacopa cognitive enhancing effects, specifically memory learning and concentration, and results support the traditional Ayurvedic claims. The plant has been extensively worked upon for its chemical constituents especially bacosides which are the active principles responsible for memory enhancement. Conventionally, identification of plants was based on morphological, anatomical and chemical analysis but these could be influenced by environmental factors. Identification of DNA markers that can correlate DNA finger printing data with quantity of selected Phytochemical marker associated with that particular class of plant would have extensive application in quality control of raw materials. RAPD technique is used to species or taxon or chemotype identification. It was also used in prediction of phytochemicals in plants (Chen *et al*., 2009). The present study was made to characterize *Bacopa monnieri* ecotypes based on phenology, location and RAPD markers of the wild population of plants.

MATERIALS AND METHODS

Plant material and DNA isolation. *Bacopa monnieri* 8 accessions were collected from the wild populations of the various locations of Tamil Nadu (Table 1). The individual accessions show a wide range of morphological variations including shape of the leaves, colour of the petal, and type of growing. The plants were grown in Department Botanical Garden. Young leaves from the plants were used for DNA isolation by the CTAB method (Saghai-Maroof *et al.*, 1984) with minor modifications.

RAPD analysis. The RAPD analysis was performed following the methodology of Williams *et al*. (1990). Each 15µL PCR reaction mix contained 25ng of DNA, 1X Taq assay buffer B, 15mM $MgCl_2$, 2mM dNTPs, 5nmol Primer, and Hot start Taq polymerase (3U/µL) (GeNei, Bangalore). Amplifications were performed using (EPPENDROF Thermalcycler) for 45 cycles, each cycle consisting of a denaturation at 94º C for 1 min, annealing at 34º C for 1 min and extension at 72º C for 2 min. Reaction mix was heated at 94º C for 5 min before starting the first cycle and the last cycle was followed by 5 min extension at 72º C. The RAPD products were separated on 1.2% agarose gel containing 10 mg/ml ethidium bromide and visualized under UV light and photographed using gel documentation system (GEL DOC SYSTEM GeNei). To measure the level of polymorphism, similarity matrices were generated on the basis of Jaccard similarity coefficient (Jaccard, 1908) to estimate of similarity (shared presence of bands, and ultimately 6 primers pooled data were generated based on UPGMA clustering).

Table 1

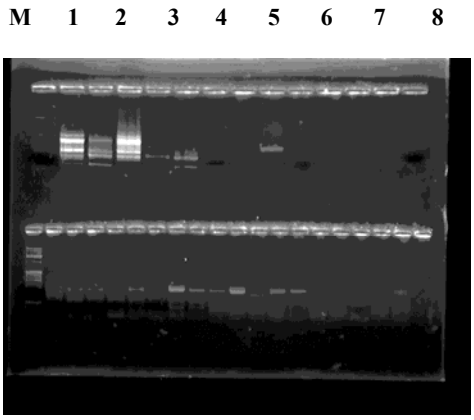
Morphological parameters of *Bacopa monnieri* (L.)
Pennell Tamil Nadu ecotype variants Tamil Nadu

Accession No.	Place of collection	Distinguishing Phenotypic characters					
		Colour of stem	Colour of flower	Shape of leaves	Plant type	Latitude	Longitude
1	Palani	Pinkish green	Violet	Spatulate	Semi erect	10º 45 N	77º 50 E
2	Nagar koil	Pinkish green	Violet	Spatulate	Semi erect	08º 13 N	77º 43 E
3	Chidambaram	Pinkish green	Violet	Spatulate	Semi erect	11º 47 N	79º 56 E
4	Kovai Kutralam	Green	Violet	Obovate	Semi erect	11º 32 N	76º 74 E
5	Urachikottai	Pinkish green	Violet	Spatulate	Semi erect	11º 28 N	76º 88 E
6	Aliyar dam	Pinkish green	violet	Spatulate	Spreading	10 º 32 N	76º 94 E
7	Vattakottai	Green	Violet	Spatulate	Spreading	08 º 32 N	77º 54 E
8	Athani	Pinkish green	Violet	Spatulate	Spreading	11º 57 N	77º 69 E

RESULTS

The present study deals with *Bacopa monnieri* (L.) Pennell germplasm collections were assessed for diversity at DNA level, to define its core diversity and identify individual accessions (Table 1). Random amplified polymorphic DNA (RAPD) analysis has been demonstrated as good tool for this purpose (Virk *et al*., 1995). As the first step, the RAPD analysis was carried out using a set of 20 custom-made decamer random primers (OPK-08, OPL-05, OPL-06, OPL-17, OPM-10, OPM-14, OPN-11, OPN-19, OPR-03, OPR-05, OPT-01, OPT-02, OPT-03, OPT-04, OPT-07, OPT-11, OPT-17, OPT-18, OPT-19 & OPU-10) for DNA amplifications through PCR. This profiling showed that yielded PCR products detectable as distinct bands resolved on agarose gels.

The OPL-5&6, OPT-1, 3&18 and OPU-10 primers results were shown in Table 2. OPL-05 having the maximum range of polymorphism was noticed (83.33%) and high level of monomorphism was noticed in the primer OPT-18 (Figs. 1–6). The amplification range is between 100bp and 2900bp and the total percentage of polymorphism (50%) was observed. The phylogenetic tree generated by using Jaccard similarity coefficient (Jaccard, 1908) was measured as well as a phylogram based on similarity coefficients generated by the UPGMA clustering by Sneath and Sokal, 1973 (Table 3). The dendrogram is indicates that the medium level of polymorphism among the 8 accessions at DNA level (Fig. 7).



M. 10 kb ladder 1. Palani 2. Nagar Koil 3. Chidambaram 4. Kovai kutrallam
5. Urachikottai 6. Aliyar dam 7. Vattakottai 8. Athani

Fig. 1. PCR amplified product of *Bacopa monnieri* ecotypes of Tamil Nadu in the primer OPL 05.

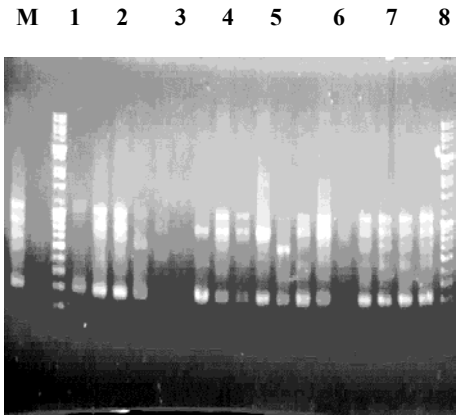


Fig. 2. PCR amplified product of *Bacopa monnieri* ecotypes of Tamil Nadu in the primer OPL 06.

All accessions were grouped into two major clusters. One cluster comprising five accessions is again divided into two branches. One branch contains three accessions (Palani and Nagar koil) and one excluded in this branch (Vattakottai). Aliyar dam and Kovai kutrallam is the other branch of that first cluster. The second cluster is comprised into two branches, the accessions Chidambaram and Urachikottai come under one branch and the accession Athani is divided into another branch.

Table 2

List of RAPD primers used and the level of polymorphism detected

Name of the primer	Sequence 5'→3'	Product size (Kbp)	Total no. of loci	No. of polymorphic loci	No. of monomorphic loci	% Polymorphism
OPL-05	**ACGCAGGCAC**	0.2–1.3	6	5	1	83.00
OPL-06	**GAGGGAAGAG**	0.4–1.3	8	5	3	62.50
OPT-01	**GGGCCACTCA**	1.4–2.9	3	1	2	33.33
OPT-03	**TCCACTCCTG**	0.7–1.9	4	2	2	50.00
OPT-18	**GATGCCAGAC**	0.1–1.5	3	3	0	00.00
OPU-10	**ACCTCGGCAC**	0.2–1.9	6	4	2	33.33
	Total	**0.1-2.9**	**30**	**15**	**15**	**50.00**

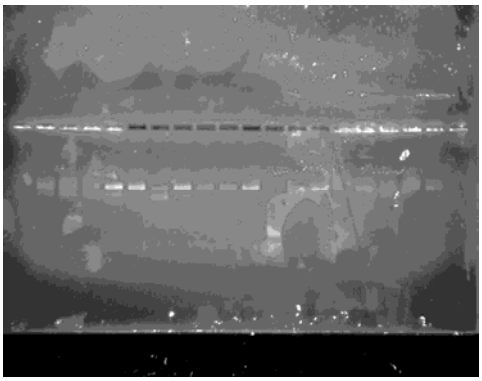
Table 3

Jaccard similarity coefficient matrix
for the Tamil Nadu ecotypes of *Bacopa monnieri*

Pop ID	BM1	BM2	BM3	BM4	BM5	BM6	BM7	BM8
BM1	1	0.957	0.875	0.667	0.72	0.64	0.714	0.75
BM2		1	0.917	0.704	0.76	0.68	0.75	0.786
BM3			1	0.704	0.76	0.68	0.815	0.852
BM4				1	0.833	0.826	0.75	0.724
BM5					1	0.818	0.808	0.778
BM6						1	0.731	0.704
BM7							1	0.963
BM8								1

BM1-Palani BM2-Nagar koil BM3-Chidambaram BM4-Kovai kutrallam
BM5-Urachikottai BM6-Aliyar dam BM7-Vattakottai BM8-Athani

M 1 2 3 4 5 6 7 8



M. 10 kb ladder 1. Palani 2. Nagar Koil 3. Chidambaram 4. Kovai kutrallam
5. Urachikottai 6. Aliyar dam 7. Vattakottai 8. Athani

Fig. 3. PCR amplified product of *Bacopa monnieri* ecotypes of Tamil Nadu in the primer OPT 01.

M 1 2 3 4 5 6 7 8

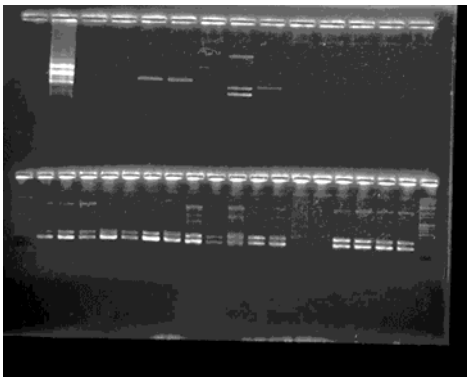
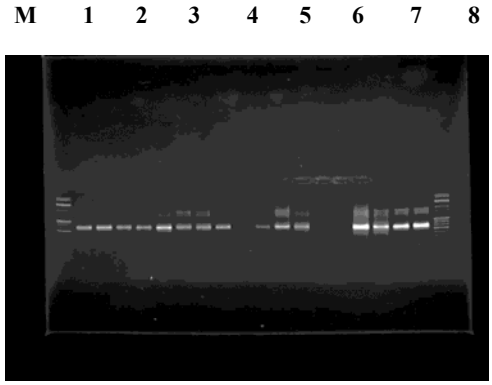


Fig. 4. PCR amplified product of *Bacopa monnieri* ecotypes of Tamil Nadu in the primer OPT 03.



M. 10 kb ladder 1. Palani 2. Nagar Koil 3. Chidambaram 4. Kovai kutrallam
5. Urachikottai 6. Aliyar dam 7. Vattakottai 8. Athani

Fig. 5. PCR amplified product of *Bacopa monnieri* ecotypes of Tamil Nadu in the primer OPT 18.

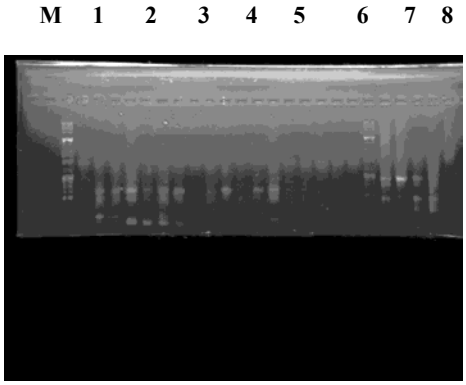


Fig. 6. PCR amplified product of *Bacopa monnieri* ecotypes of Tamil Nadu in the primer OPU-10.

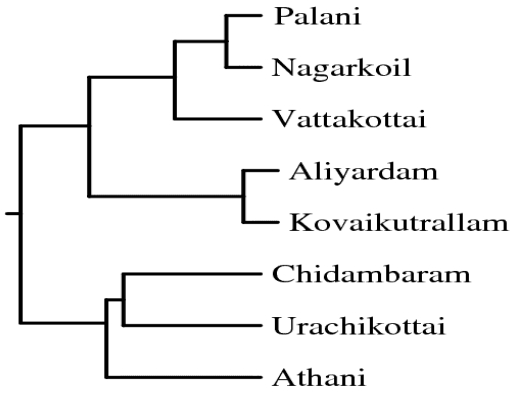


Fig. 7. Dendrogram generated by *Bacopa monnieri* (L.) Pennell ecotypes from Tamil Nadu using UPGMA cluster analysis based on Jaccard similarity coefficient.

DISCUSSION

The present study reveals that similar types of results were observed by Khanuja *et al*. (1997). The previous report was based on the individual accessions

in this germplasm collection which show wide morphological variations including differences in growth rate, leaf size, flower colour and differences in the bacoside – A contents of shoot.

This low level of RAPD variation observed indicates the need for detailed studies on the interplay of sexual and/or vegetative modes of reproduction and natural selection forces operating on the populations of this region. Generally, the observations of this study indicate 8 accessions that there is little variations among the different accessions of *B. monnieri* collected from different geographical regions of South India. It may be noted here that the microenvironments of the habitats of the species are largely similar in geographically distinct locations as the plant is observed to grow near the banks of water bodies. Therefore, the wide heritable phenotypic and chemotypic variation observed in the collection of accessions might be due to qualitative genetic differences. The observed narrow genetic base in *B. monnieri* Sci. population in India could be attributed to the vegetative propagation of the species. The lack of RAPD but that would not be uniform across the different populations throughout the country and in this case the accessions used represent the natural populations from various agro-climatic zones of India. The self-pollinating species like tomato and wheat are also known to show a little polymorphism among various accessions (Joshi and Nguyen 1993; Williams and Clair 1993).

To date, just one report is available on the genetic diversity analysis of *B. monnieri* samples collected from different agro-climatic zones of India using RAPD approach (Darokar *et al*., 2001). In one of the very recent reports, RAPD fingerprinting was successfully applied to analyze the genetic integrity of *in vitro* regenerated *B. monnieri* plants using five random primers (Ceasar *et al*., 2010). Recently Ramesh *et al.* (2011) reported low to moderate RAPD profile variation (21.5%) among the *B. monnieri* plants micropropagated under *in vitro* conditions, synthetic seed derived and hardened plants. RAPD analysis of *B. monnieri* plants collected from different accessions aided in protecting the genetic integrity of the plant the data generated from the present work would go well in conserving the distinct genotypes of this endangered medicinal herb. Karthikeyan *et al.* (2011) noticed the Random amplified polymorphic DNA (RAPD) fingerprinting approach was applied to assess genetic diversity in different accessions of rejuvenating and intellect-promoting ancient ayurvedic medicinal herb *Bacopa monnieri* (L.) Pennell collected from four Southern Indian states, along with *in vitro* micropropagated samples.

The technical simplicity of the RAPD technique has facilitated its use in the analysis of genetic relationship in several genera (Demeke *et al*., 1992; Nair *et al.,* 1999; Wilikie *et al*., 1993). The major concern regarding RAPD-generated phylogenies include homology of bands showing the same rate of migration, causes of variation in fragment mobility and origin of sequence in the genome (Stammers *et al*., 1995). In spite of this limitation, RAPD markers has the greatest advantage

of its capability to scan across all regions of the genome hence highly suited for phylogeny studies at species level (Demeke *et al.*, 1992; Wilikie *et al*., 1993).

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