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Analysis of Genetic Variation among Brahmi (*Bacopa monnieri*) Accessions in Andhra Pradesh

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ABSTRACT: The current research entitled "Analysis of genetic variation among brahmi (*Bacopa monnieri*) accessions in Andhra Pradesh" was carried out at Dr. Y.S.R. Horticultural University, Horticultural Research Station, Venkataramannagudem, Andhra Pradesh. Genetic variations were evaluated for 22 accessions of brahmi using 20 ISSR markers.PIC value for each ISSR primer was calculated which ranged from 0.089 to 0.992 with an average of 0.678 which showed 95% polymorphism. Average genetic similarity among twenty two accessions ranged from 0.111 to 1.000. Among the 22 accessions studied APBm-3 was found to have the least genetic distance of 0.111 with APBm-22 and showed maximum genetic divergence. The APBm-9 and APBm-12 accessions had a high genetic similarity of 1.000 and showed minimum genetic divergence. Since, genetic variability is essential for the crop improvement it is important to know genetic variation between the accessions. The findings suggested that ISSR primers can be used to identify the genetic relationships between *B. monnieri* accessions. It is possible to draw the conclusion that knowledge of genetic diversity and genetic similarity among brahmi accessions is essential for their breeding and conservation programmes.

Keywords: *Bacopa monnieri*, Genetic variation, Inter simple sequence repeats (ISSR), Polymorphism.

INTRODUCTION

The Scrophulariaceae family includes small prostrate herb *Bacopa monnieri*, which grows widely in the tropics of Asia, America, and Australia. It is known as "Brahmi" or the "Herb of the Thinker" in India. It has fleshy leaves and light purple blooms (Prasad *et al*., 2008). If enough water is provided, bacopa is a simple plant to grow and has medicinal properties throughout. *B. monnieri* was ranked second among the most valuable Indian medicinal plants in a study by Rajani *et al*. (2004), who considered medical value, commercial importance, and the potential for further research and development. Different types of saponins including bacosides A, B, C and D are found in *B. monnieri*. Bacosides A and B have the most influence on the active ingredients in brahmi. In comparison to traditional morphological markers, the molecular approach to plant genotype identification appears to be more effective because it directly accesses hereditary information and enables the relationship between the individuals (Paterson *et al*., 1991). There is no evidence available for the use of ISSR markers with this genotypes. Polymerase chain reaction (PCR)-based DNA markers such as ISSR markers were used to assess the genetic diversity among the accessions Tripathi *et al*. (2012); Bansal *et al.* (2014); Ravi (2016). The ISSR analysis is a highly helpful molecular method for investigating population genetics on a variety of plant species as well as for distinguishing species, cultivars, or populations of the same species.

MATERIAL AND METHODS

Plant materials: A total of 22 accessions of *B. monnieri* were collected from various locations of South India and maintained at Horticultural Research Station, Venkataramannagudem.

DNA extraction: Total genomic DNA was extracted using a modified CTAB method based on the protocol of Doyle and Doyle (1990). Young leaves were used for DNA extraction from each plant of every accession. Quality of DNA was tested by submerged horizontal agarose gel (0.8 %) electrophoresis and visualized with UV light.

PCR analysis: PCR amplifications were performed in a programmable thermocycler. Each sample was amplified in a reaction mixture containing 50 ng genomic DNA, Taq polymerase, 1 × PCR buffer with 2.5 mM $MgCl_2$ and 0.2 Mm of each dNTP mixture, 15 pmol of ISSR primers. Cycling parameters for ISSR were adjusted to 4 min at 94°C for pre-denaturation,41 cycles each of 1 min at 94°C, for denaturation, 45 sec for annealing at 50/ 55°C, 90 sec at 72°C for extension and a final extension at 72°C for 5 min. After cooling to 4°C, amplified products were stored at -20°C until electrophoresis. Amplified products were separated on 1.5 % agarose gel in 1 × TE buffer with 100 bp ladder to determine the size of amplified DNA fragments. Gels were run for 90 min at 70 V, stained with ethidium bromide and documented with gel documentation system.

Data analysis: Evaluation of fragment patterns was carried out by similarity index. Reproducible bands were scored manually as '1' or '0' for presence or absence of the bands. Polymorphic information content (PIC) values were calculated for ISSR primer according to the formula: $PICi = 2f(1 - fi)$

Where, PICi is the polymorphic information content of the locus I; fi is the frequency of the amplified fragments;1-fi is the frequency of non-amplified fragments. The ISSR data generated were used to calculate pairwise similarity co-efficients (Jaccard, 1908) using the similarity for qualitative data of NTSYS-pc version 2.11a (numerical taxonomy and multivariate analysis system) software package (Rohlf, 2002). The similarity matrix values obtained were subjected to the cluster analysis using Jaccard's similarity approach with the help of DARwin version 6.

RESULTS AND DISCUSSION

The ISSR analysis was carried out using 20 primers for DNA amplifications through PCR, out of which only 19 primers responded to all the accessions (Table 1). The number of bands produced per primer ranged from 5 (ISSR 810, 811, 823) to 9 (ISSR 881). ISSR amplification pattern amplified by primer ISSR 807 illustrated in Fig. 1. Among 20 primers used 19 were found to be polymorphic and the lowest polymorphism 33.3% was shown by ISSR 836.

Table 1: The list of ISSRs used for analysis of brahmi accessions.

Sr. No.	Primer	Sequence (5'-3')	Annealing temperature
1.	807	AGAGAGAGAGAGAGAGT	52
2.	808	AGAGAGAGAGAGAGAGC	52.5
3.	810	GAGAGAGAGAGAGAGAT	53
4.	811	GAGAGAGAGAGAGAGAC	52
5.	812	GAGAGAGAGAGAGAGAA	52
6.	814	CTCTCTCTCTCTCTCTA	51.5
7.	815	CTCTCTCTCTCTCTCTG	51
8.	822	TCTCTCTCTCTCTCTCA	52.5
9.	823	TCTCTCTCTCTCTCTCC	55.5
10.	824	TCTCTCTCTCTCTCTCG	53
11.	825	ACACACACACACACACT	52
12.	827	ACACACACACACACACG	53.3
13.	834	AGAGAGAGAGAGAGAGYT	53
14.	836	AGAGAGAGAGAGAGAGYA	51.5
15.	840	GAGAGAGAGAGAGAGAYT	52
16.	842	GAGAGAGAGAGAGAGAYG	53
17.	844	CTCTCTCTCTCTCTCTRC	52.5
18.	859	TGTGTGTGTGTGTGTGRC	53.5
19.	876	GATAGATAGACAGACA	55.3
20.	881	GGGTGGGGTGGGGTG	55.0

It was observed from the Table 2 that maximum PIC value (0.992) was observed for ISSR 814, where ISSR 808 showed the minimum PIC value (0.089). Similar results reported by Tripati *et al.* (2012) on PIC content in brahmi which ranged from 0.419 to 0.836. Higher PIC value more will be the usefulness of primer hence, primer ISSR 814 was found to be highly informational as more number of accessions can be differentiated by using this primer. Among the 22 accessions studied APBm-3 was found to have the least genetic distance of 0.111 with APBm-22 and showed maximum genetic divergence. The APBm-9 and APBm-12 accessions had a high genetic similarity of 1.000 and showed minimum genetic divergence. Similar results of similarity index were reported by Kumar *et al.* (2013) ranged from 0.70 to 0.95, Tripati *et al*. (2012) ranged from 0.179 to 0.945.

Similarity matrix values obtained for all the 22 brahmi accessions were subjected to the cluster analysis using Jaccard's similarity approach with the help of DARwin version 6 and a dendrogram was generated based on genotypic differences and the results are presented.

Dendrogram based on ISSR similarity matrices separated the 22 brahmi accessions which were grouped into five main clusters (Fig. 2). Cluster I contains APBm-1, APBm-3, APBm-11, APBm-16. Cluster II comprises of APBm-2. Cluster III includes APBm-4, APBm-5, APBm-6, APBm-21, APBm-22. Cluster IV includes APBm-7, APBm-8, APBm-9, APBm-10, APBm-12, APBm-13, APBm-14, APBm-15, APBm-17, APBm-18, APBm-20. Cluster V contains APBm-19.

Table 2: The list of ISSR's and the degree description of the polymorphism obtained in brahmi accessions.

Sr. No.	Primer	Total number of amplicons	No. of Polymorphic amplicons	Polymorphism (%)	PIC
1.	807	7	5	71.4	0.750
2.	808	7	4	57.1	0.089
3.	810	5	3	60.0	0.948
4.	811	5	4	80.0	0.535
5.	812	7	6	85.7	0.926
6.	814	7	5	71.4	0.992
7.	815	8	6	75.0	0.868
8.	822	8	5	62.5	0.926
9.	823	5	4	80.0	0.471
10.	824	6	6	100.0	0.967
11.	825	8	5	62.5	0.254
12.	827	6	5	83.3	0.651
13.	834	8	4	50.0	0.254
14.	836	6	2	33.3	0.899
15.	840	7	4	57.1	0.868
16.	842	8	6	75.0	0.981
17.	859	8	6	75.0	0.793
18.	876	8	6	75.0	0.174
19.	881	9	7	77.7	0.535

Where, PIC- Polymorphic information content.

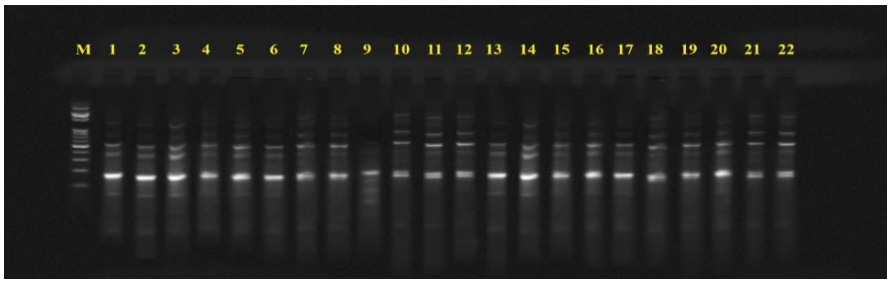

Fig. 1. ISSR profile generated for brahmi accessions by primer 807, where = 100 bp DNA ladder.

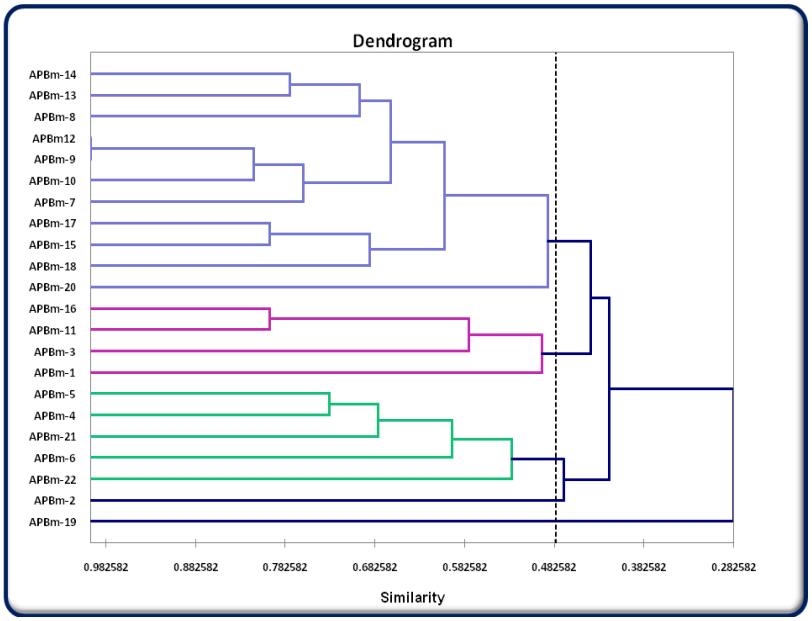

Fig. 2. Jaccard's dendrogram of brahmi accessions using ISSR markers.

Inter cluster distances. Inter cluster distance values ranged from 1.767 to 2.960. Maximum (2.960) distance was observed between cluster V and III, which shows that there is more genetic diversity between the two clusters when compared to other clusters. Minimum (1.767) distance was observed between cluster IV and I, which shows less genetic diversity between these clusters (Table 3).

Table 3: Inter cluster distances of brahmi accessions.

Cluster	I	II	III	IV	V
I	0	2.278	2.224	1.767	1.920
II		0	2.315	2.735	2.236
III			0	1.982	2.960
IV				0	2.398
V					0

CONCLUSIONS

It can be concluded that ISSR markers may be an useful tool for the identification of Brahmi. The present findings can help the genetic variation analysis among different accessions of Brahmi. The accessions showed a considerable level of genetic diversity, indicating a high genetic variability in the population. The genetic variability in a gene pool is normally considered as being the major resource available for breeding programs.

FUTURE SCOPE

Through molecular characterization, the level of genetic variability among brahmi accessions was assessed. This information could serve as the foundation for future breeding programmes and a finer molecular analysis with more ISSRs and SSRs. It is necessary to conduct additional research on QTL mapping to improve brahmi accessions by marker-assisted breeding for the selection of elite genotypes and to accelerate the breeding plan.

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