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Molecular Characterization and Genetic Diversity of *Blumea* Species Using RAPD Marker

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ABSTRACT
The objective of the present study was to find out the genetic relationship within the species of *Blumea* of family Asteraceae through random amplified polymorphic DNA (RAPD) marker. Genetic analysis was made by using 10 arbitrary primers which revealed a total 292 polymorphic fragments. The genetic similarity was evaluated on the basis of presence or absence of bands. High degree of polymorphism was observed among the samples, suggesting the degree of genetic variability. Collectively all primers data was used for construction of dendrogram. All primers gave amplification products and had from (OPX-07) - 23 bands to 42 bands (OPB-12) with an average 29.9 bands per primers. Thus, these RAPD markers have the potential for assessment of genetic variation and phylogenetic analysis within the species of *Blumea*. The results of the current study have been found to be useful for assessing genetic diversity, genetic relationship and phylogenetic analysis.

KEY WORDS: *BLUMEA*, RAPD MARKER PCR, GENETIC DIVERSITY, POLYMORPHISM, DENDROGRAM.

INTRODUCTION

Genus *Blumea* belonging to the family Asteraceae is one of the important medicinal plant found in the tropical and sub-tropical zones of Asia, especially the Indian Subcontinent and Southeast Asia. A few species are found in Australia and still fewer in Africa. The plants of this genus are mostly relatively small weeds. In India, the species are distributed throughout the country from South to North up to the Himalayas at about 2,000 m elevations. The maximum diversity of the species lies in the North-East region followed by the Peninsular region and Andaman and Nicobar Islands. Many species of genus *Blumea* are used in traditional medicine with higher therapeutic values (Tamilarasi and Thirugnanasampandan, 2014).

Use of DNA markers for the identification of genetic diversity, can be useful in identifying genetic structure as well as diversity among species of genus. Due to their simplicity, reliability and cost effectiveness PCR based markers are in demand (Parita et al., 2018). Different PCR based techniques have been developed during the last two decades. The random amplified polymorphic DNA (RAPD) is simple, cost-effective and a powerful tool in the analysis of plant genome characterization (Bardakci, 2001). RAPD technique has been widely used in many plant species for varieties analysis, population studies and genetic linkage mapping (Prasad, 2014, Mishra et al., 2018, Mostofa et al., 2020. Dobhal1 and Kumar, 2021).

The present work was aimed to assess the genetic diversity of five species of *Blumea* (coded as in Table-I) which are *Blumea axillaris* (Lam.) DC, *Blumea eriantha* DC, *Blumea fistulosa* (Roxb.) Kurz, *Blumea lacera* (Burm. f.) DC and *Blumea oxyodonta* DC. coded as (Table-I) using Random Amplified Polymorphic DNA (RAPD) markers.

MATERIAL AND METHODS

Plant material collection and total DNA extraction: Leaf samples of five selected species of *Blumea* were collected from Amravati, Maharashtra. Fresh and disease-free young leaf samples were taken in Ziplock plastic bag with silica gel and transported to laboratory. The plant materials were stored at -70° C (Remi Queek freezer). DNA was isolated by CTAB extraction method (Murray and Thompson, 1980). 100 mg of leaf tissue was used to extract genomic DNA. The quality of a genomic DNA was checked by agarose gel electrophoresis for this 5 uL of the sample was loaded in each well and quantity with Qubit fluorometer (Invitrogen USA) PCR amplification (Fig.-1). Total 10 primers were used

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for each sample. A 25.0µl reaction mixture for PCR was prepared containing PCR buffer DNTPs, Tag polymerase, primers and sample of DNA.

RAPD analysis :RAPD PCR amplification: The prepared reaction mix was subjected to PCR amplification in which different thermal profile was set for 10 primer types, where total 35 cycle programmed for amplification was run using PCR machine. Thermal profile used for PCR amplification involved an initial denaturing step (95°C) followed by 35 cycles. (Denaturation on at 95°C, primer annealing at respective temperature, and primer execution at 72°C). Final step at 72°C was carried out for polishing the end of PCR products. Finally at the end these PCR amplified products were resolved electrophoretically where 2.0% (w/v) agarose gel was used for RAPD.

Analysis of RAPD data: Analysis of number of base substitutions per site from between sequences were conducted using the Maximum Composite Likelihood model (Tamura et al., 2004). This analysis involved 5 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 402 positions in the final dataset. Evolutionary analysis was conducted in MEGA X (Kumar et al., 2018).

Table 1. Job code for each Blumea species and number of band generated

Sr. No.	Species Name	Job Code	Number of Bands Generated
1	*Blumea fistulosa* (Roxb.) Kurz	11041	88
2	*Blumea laciniata* (Roxb.) DC	11042	38
3	*Blumea eriantha* DC	11043	54
4	*Blumea axillaris* (Lam.) DC	11044	80
5	*Blumea oxyodonta* DC	11045	39

Table 2. Details of RAPD Primers with banding profile used in the present study

S. No.	Primer Code	Nucleotide sequence (5'-3')	TNB	PB	MB	PP
1.	OPA11	CAATCGCCGT	32	31	1	96.875
2.	OPA07	GAAACGGGTG	28	26	2	92.8571
3.	OPB01	GTTTCGCTCC	24	24	0	100
4.	OPB12	CCTTGACGCA	24	23	1	95.8333
5.	OPA04	AATCGGGCTG	34	33	1	97.0588
6	OPM05	GGGAACGTGT	42	41	1	97.619
7	OPM06	CTGGGCAACT	33	33	0	100
8	OPX01	CTGGGCACGA	27	27	0	100
9	OPX07	GAGCGAGGCT	32	32	0	100
10	OPB10	CTGCTGGGAC	23	22	1	95.6522
Total			299	292	7	--

TNB-Total numbers of bands, PB-Polymorphic band, MB-Monomorphic bands, PP-Percentage polymorphism

Dendrogram was drawn using MEGA X software. Briefly all the RAPD bands were scored in binary format. Collectively all primers data was used for construction of UPGMA tree (Iruela et al., 2002). The evolutionary history was inferred using the UPGMA method (Sneath and Sokal, 1973).

RESULT AND DISCUSSION

Total 10 RAPD primers were used for screening of five species of *Blumea*. All primers gave amplification products and had from (OPX-07) - 23 bands to 42 bands (OPB-12) with an average 29.9 bands per primers (Table -2). Among 10 RAPD primers tested all produced bands that were polymorphic across all the samples, 6 RAPD markers OPA07, OPA04, OPB01, OPB10, OPB12 and OPX07 produced monomorphic bands in the sample (Fig.- 2-11). Distance matrix values using Jaccard's coefficient based on RAPD markers ranged from 0.116 between 11045 to

0.689 between 11043 (Table-3). Similarity matrix values ranged from 0.311 between 11043 to 0.884 between 11045 (Table-4).

Table 3. Jaccard's Distance Matrix

11042				
11045	0.116			
11041	0.299	0.337		
11044	0.337	0.361	0.510	
11043	0.200	0.225	0.689	0.349

Table 4. Jaccard's Similarity Matrix

11042				
11045	0.884			
11041	0.701	0.663		
11044	0.663	0.639	0.490	
11043	0.800	0.775	0.311	0.651

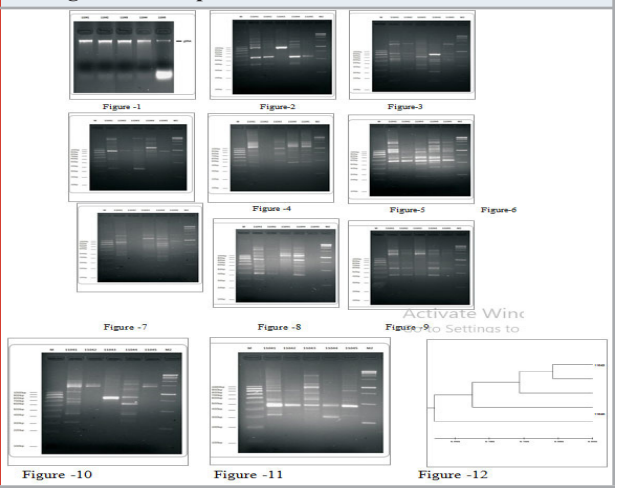
The dendrogram obtained clearly indicated two clusters (Fig. 12). Smaller one having one species-11041 and the larger cluster that could be further divided into four different sub clusters. The optimal tree with the sum of branch length = 0.79751903 was shown. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and were in the units of the number of base substitutions per site. This analysis involved 5 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 402 positions in the final dataset.

RAPD markers have been used in several studies for DNA fingerprinting and phylogenetic analysis (Ramakrishnan et al., 2016; Kumar et al., 2018; Sawsan et al., 2020; Omri et al., 2021). They have been used as effective tools to analyze genetic diversity in many species of family Asteraceae, (Elizabeth et al., 2000 and Geleta et al., 2007). In genus *Blumea* some researchers have made an attempt to evaluate genetic diversity using RAPD markers system. Pornpongrungrueng et al., (2007) studied evolutionary relationship in *Blumea* with sequences of cpDNA and nrDNA. Pang et al., (2014) used AFLP markers to determine genetic diversity of *Blumea balsamifera*.

The genetic distance estimated by authors with Jaccard similarity coefficient index showed low variability among genotypes. RAPD analysis revealed a little genetic variation in micropropagated plants of *Blumea mollis* (Tamilarasi and Thirugnasampandan, 2014). YingBo et al., (2016) did comparative analysis of SRAP and AFLP markers for genetic diversity of *Blumea balsamifera* In their opinion AFLP molecular markers is more suitable to estimate genetic diversity of *Blumea balsamifera* because it has more polymorphic sites and higher markers characteristic index.

Genomic DNA was amplified using ten RAPD primers and all were reproducible however, five primers were used for RAPD analysis out of which primers 2 and 5 showed more bands reported by Tamilarasi and Thirugnasampandan, (2014) in *Blumea mollis*. The findings of the current investigation revealed the successful utilization of RAPD markers for assessment of genetic diversity of *Blumea* species.

Figure 1 to 12: 1% (w/v) Agarose Gel electrophoresis of genomic DNA isolated 11041-11045 samples. 5 uL of the sample was loaded in each well.
RAPD profile for all samples with a single primer in a gel (For ten Primers).
RAPD based dendrogram representing genetic relationship among 5 *Blumea* species



CONCLUSION

The result of the current study found to be useful for assessing genetic diversity, genetic relationship and phylogenetic analysis.

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