http://dx.doi.org/10.22161/ijeab/2.3.52 *ISSN: 2456-1878*

Deciphering Genetic Diversity in *Spathodea campanulata Beauv*. from South India Based On Randomly Amplified Polymorphic DNA Markers

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***Abstract*—** *The African tulip (Spathodea campanulata) is a monophyletic species native to tropical forests of sub-Saharan Africa belonging to family Bignoniaceae. Although its endemism in sub-Saharan Africa, it is listed as potential alien invasive species in Pacific, Indian and Caribbean, but also Singapore, Papua New Guinea and Australia. The expansion of its range from ornamental to invasive is being observed in South India. In this study, RAPD based DNA markers have been used to determine genetic diversity of the monophyletic species. About 14 populations were studied from 6 states of South India using 41 random decamer primers. The total number of 517 bands were scored which are generated by 41 primers producing 12.6 bands on an average per primer, of these 517 bands produced 379 were polymorphic showing 73% polymorphism revealing higher level of genetic polymorphism in the study population. The similarity coefficient ranged from 0.312 to 0.837 and the dendrogram constructed by the UPGMA based on Jaccard's similarity matrix farmed two clusters. In the present study, results showed the high degree of genetic diversity within the populations of S. campanulata supporting high degree of adaptation and its range expansion from ornamental to possible invasive species in South India.*

***Keywords*— Genetic diversity, Spathodea campanulata, invasion, South India, RAPD.**

I. INTRODUCTION

The African tulip tree (*Spathodea campanulata*) is a monophyletic species native to tropical forests in a broad area of sub-Saharan Africa belongs to family Bignoniaceae. It's also known as fountain tree, scarlet bell flower tree, squirt tree, syringe tree in English and neerukayi mara or ucche kayi mara in the local Kannada language of Karnataka, India. It is planted as an ornamental tree for its attractive glossy deep green pinnate leaves and glorious orange scarlet flowers. *Spathodea* is endemic in Angola, Ethiopia, Ghana, Kenya, Sudan, Tanzania, Uganda, Zambia and exotic to Colombia, Costa Rica, Cuba, India, Jamaica, Puerto Rico, Sri Lanka, Zanzibar [1].

S. campanulata has been introduced pan-tropically for its ornamental value, it was introduced to India in 1800's [2]. The extensive fruiting and the heavy masses of wind-dispersed seeds credited the capacity of being invasive to the species. It is invasive in many countries like Pacific, Indian and Caribbean but also Singapore, Papua New Guinea and Australia. Removal of trees is highly recommended, particularly when they are in close proximity to natural forests and aquatic ecosystems [3].

S. campanulata grows naturally in secondary forests and prefers wet areas especially the borders of dynamic water resources. It may also appear as a pioneer species in the native range, such as it being one of the species that naturally colonizes grasslands in Uganda. But, it invades both abandoned agricultural land and closed forest being dominant acquiring the weed status [4,5] and is a weed in coffee plantations in Cuba[6]. It is reported as highly invasive in Tahiti, French Polynesia in cloud forests up to 1300 m [7]. In southern part of India, we have observed the preliminary invasion of *S. campanulata* in barren lands and borders of the forest lines.

Introduced species are generally expected to have low genetic diversity due to population bottlenecking of introduction process and founder effect. Low genetic diversity is expected to limit adaptability to the new environment there by limiting the evolutionary potential of introduced population. This relationship between genetic diversity and population viability, in contrast to the success of invaders over native species, constitutes an apparent paradox in invasion biology [8,9].

http://dx.doi.org/10.22161/ijeab/2.3.52 *ISSN: 2456-1878*

Understanding Genetic diversity and population structure within introduced populations provide valuable information for assessing the adaptability and evolutionary potential of invasive species and explain successful invasions.

The analysis of the population genetic structure through Randomly Amplified polymorphic DNA (RAPD) based markers proved potential in various species. The RAPD technique is quick, require no prior genetic information and relatively inexpensive method which is extensively used to analyze the genetic variability in plants. Despite of lower reproducibility, the RAPD method has become popular and highly practical due to its cost-effectiveness with studies involving a smaller number of populations [10,11]. The present effort is a first attempt to decipher the genetic diversity of *S. campanulata* populations from different states of south India.

II. MATERIALS AND METHOD

2.1. Sample collection:

Young leaves from the study populations were collected in zip lock bags and brought the laboratory in ice box. On arrival to the laboratory leaf samples were washed in 70% ethyl alcohol and blotted between sterile blotting papers. Leaf samples were wrapped in aluminium foil, labeled and stored at -80ºC until DNA isolation.

2.2. Isolation of DNA from leaf samples

The CTAB lysis buffer [12] with certain modification was used for the total DNA isolation from leaf samples. Briefly, the CTAB buffer contained 2% w/v CTAB (HiMedia), 100 mM Tris–HCl (pH = 8.0; HiMedia), 20 mM EDTA (pH = 8.0; HiMedia, 1.4 M NaCl (HiMedia) and PVP 2%. The pH of the lysis buffer was adjusted to 5.0 prior to sterilization by autoclaving. The β-Mercaptoethanol (2%) was added freshly and lysis buffer was prewarmed at 65ºC in a water bath (Grant, India) prior to use. The leaf samples were ground to a fine powder with the help of liquid nitrogen using mortar and pestle. About 2g of powder was ground into a paste in pre warmed CTAB lysis buffer and incubated at 65ºC for 60 minutes. The lysate was cooled to room temperature and extracted with phenol:chloroform:isoamylalcohol (25:24:1) and DNA was precipitated with isopropanol [13]. The DNA pellet obtained was washed in 70% alcohol, air dried and dissolved in 200µl of $T_{10}E_1$ buffer. The genomic DNA samples isolated were separated on 0.8% agarose (SeaKem) gel prepared in 1X TAE in a horizontal electrophoresis Unit. Quality and quantity of DNA were assessed by Biophotometer (Eppendorf) measuring the absorbance at 260nm/280nm.

2.3. PCR Amplification

A total number of 60 decamer primers (Eurofin genomics) belonging to OPA, OPB and OPC series were used for the amplification. The PCR reaction was performed in a thermal cycler (Eppendorf, Germany) with the reation volume of 20µl containing 100ng of genomic DNA, 5pm primer, 100µm of dNTP mix (Fermentas), 1.5mMMgCl_2, 1X Taq Buffer, 1U Taq DNA polymerase (New England Biolabs) and the total volume was adjusted to 20µl using molecular biology grade water. The cycling conditions were set as follows: initial denaturation temperature set at 95°C for 45 sec followed by 40 cycles of denaturation temperature set at 95°C for 20 sec. The annealing temperature was set depending on the standardized annealing temperature 0f 37°C for 15 sec for all primers. The extension temperature was set at 68°C for 1 min and the final extension for 10 min temperature was set at 68°C.

2.4. Agarose gel electrophoresis

The bands amplified using random decamer primers were separated on agorae gel. The gels were prepared using 1.5% agarose dissolved in 1X TAE buffer. 0.5µl of ethidium bromide (10mg/ml) was added to stain bands for better visualization. The PCR products were loaded along with the gel loading dye (Bangalore Genei). The PCR amplicons (bands) were visualized using G;Box Chemi gel documentation unit (Syngene).

2.5. Scoring and Statistical analysis

Only brisk and sharp bands were considered as scorable bands and used for scoring. Each amplified product was considered as a unit character and the populations were scored for the presence (1) or absence (0) of a band on the gel. The cluster analysis was performed and dendrogram was drawn using NTSys PC (version 2.0) by Unweighted Pair Group Method with Arithmetic mean (UPGMA) and similarity matrix was deduced using Jacard coefficient [14].

III. RESULTS AND DISCUSSION

The present study is a first attempt to decipher the genetic diversity of *S. campanulata* from Karnataka, Maharashtra, Telangana, Tamilnadu, Goa and Kerala states belonging to South India. The DNA samples with A260/A280 ratio ranging between 1.6-1.8 were used for the PCR amplification. A total number of 60 decamer primers were tested for 14 plants populations (Table 1), out of which 41primers gave good amplification and the same were used for the analysis of genetic diversity (Table 2). The OPB-14 primer amplified highest number of bands (18) with 55% polymorphism and OPC-15 showed the least number of bands (7) with 100% polymorphism. The total number of 517 bands was scored which were generated by 41 primers producing 12.6 bands on an average per primer. Out of these 517 bands produced 379 were

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polymorphic showing 73% polymorphism revealing higher level of genetic diversity in the study population. The primers used in the present study proved very informative suggesting the future possibility of using same for the study of genetic diversity of *S. campanulata* populations.

Table.1: Locations of Spathodea campanulata sample collection

Sl. No.	**Name of the Place**	**State**	**Latitude**	**Longitude**
1	Hyderabad	Telangana	17.4930° N	78.3906° E
2	Gulberga	Karnataka	17.3297° N	76.8343° E
3	Belgaum	Karnataka	15.8839° N	74.5175° E
4	Dharwad	Karnataka	15.4404° N	74.9852° E
5	Gadag	Karnataka	15.4337° N	75.6477° E
6	Kolhapur	Maharashtra	16.6780° N	74.2555° E
7	Sirsi	Karnataka	14.6061° N	74.8484° E
8	Karwar	Karnataka	14.8185° N	74.1416° E
9	Panjim	Goa	15.4909° N	73.8278° E
10	Mangalore	Karnataka	12.8170° N	74.9231° E
11	Cochin	Kerala	09.9312° N	76.2673° E
12	Bangalore	Karnataka	13.0777° N	77.5805° E
13	Mysore	Karnataka	12.3052° N	76.6376° E
14	Chennai	Tamilnadu	13.0660° N	80.2832° E

Table.2: Primers used, amplified products and percent polymorphism noted in the study

Sl. No.	Primers	Primer Sequence (5’ à 3’)	Number of amplified products (a)	Number of polymorphic products (b)	Percent polymorphism (b/a X 100)
1	OPA-01	CAGGCCCTTC	15	14	93%
2	OPA-02	TGCCGAGCTG	16	12	75%
3	OPA-03	AGTCAGCCAC	14	13	93%
4	OPA-04	AATCGGGCTG	12	12	100%
5	OPA-06	GGTCCCTGAC	9	9	100%
6	OPA-07	GAAACGGGTG	17	16	94%
7	OPA-08	GTGACGTAGG	13	13	100%
8	OPA-09	GGGTAACGCC	12	8	67%
9	OPA-10	GTGATCGCAG	15	10	75%
10	OPA-11	CAATCGCCGT	16	16	100%
11	OPA-13	CAGCACCCAC	13	13	100%
12	OPA-14	TCTGTGCTGG	14	14	100%
13	OPA-17	GACCGCTTGT	14	9	64%
14	OPA-18	AGGTGACCGT	15	5	33%
15	OPA-19	CAAACGTCGG	16	16	100%
16	OPB-01	GTTTCGCTCC	14	12	88%
17	OPB-02	TGATCCCTGG	13	10	77%
18	OPB-03	CATCCCCCTG	12	11	92%
19	OPB-04	GGACTGGAGT	14	13	93%
20	OPB-06	TGCTCTGCCC	15	12	80%
21	OPB-07	GGTGACGCAG	16	12	75%
22	OPB-09	TGGGGGACTC	13	11	85%
23	OPB-11	GTAGACCCGT	12	9	75%
24	OPB-13	TTCCCCCGCT	17	15	88%

http://dx.doi.org/10.22161/ijeab/2.3.52 *ISSN: 2456-1878*

25	OPB-14	TCCGCTCTGG	18	10	55%
26	OPB-17	AGGGAACGAG	13	7	54%
27	OPB-18	CCACAGCAGT	11	10	91%
28	OPB-19	ACCCCCGAAG	15	11	73%
29	OPC-01	TTCGAGCCAG	10	6	60%
30	OPC-02	GTGAGGCGTC	9	9	100%
31	OPC-07	GTCCCGACGA	7	7	100%
32	OPC-08	TGGACCGGTG	11	9	82%
33	OPC-09	CTCACCGTCC	10	2	20%
34	OPC-10	TGTCTGGGTG	12	1	8%
35	OPC-12	TGTCATCCCC	9	5	55%
36	OPC-14	TGCGTGCTTG	8	2	25%
37	OPC-15	GACGGATCAG	7	2	28%
38	OPC-16	CACACTCCAG	9	1	11%
39	OPC-18	TGAGTGGGTG	8	3	37%
40	OPC-19	GTTGCCAGCC	9	4	44%
41	OPC-20	ACTTCGCCAC	14	5	36%

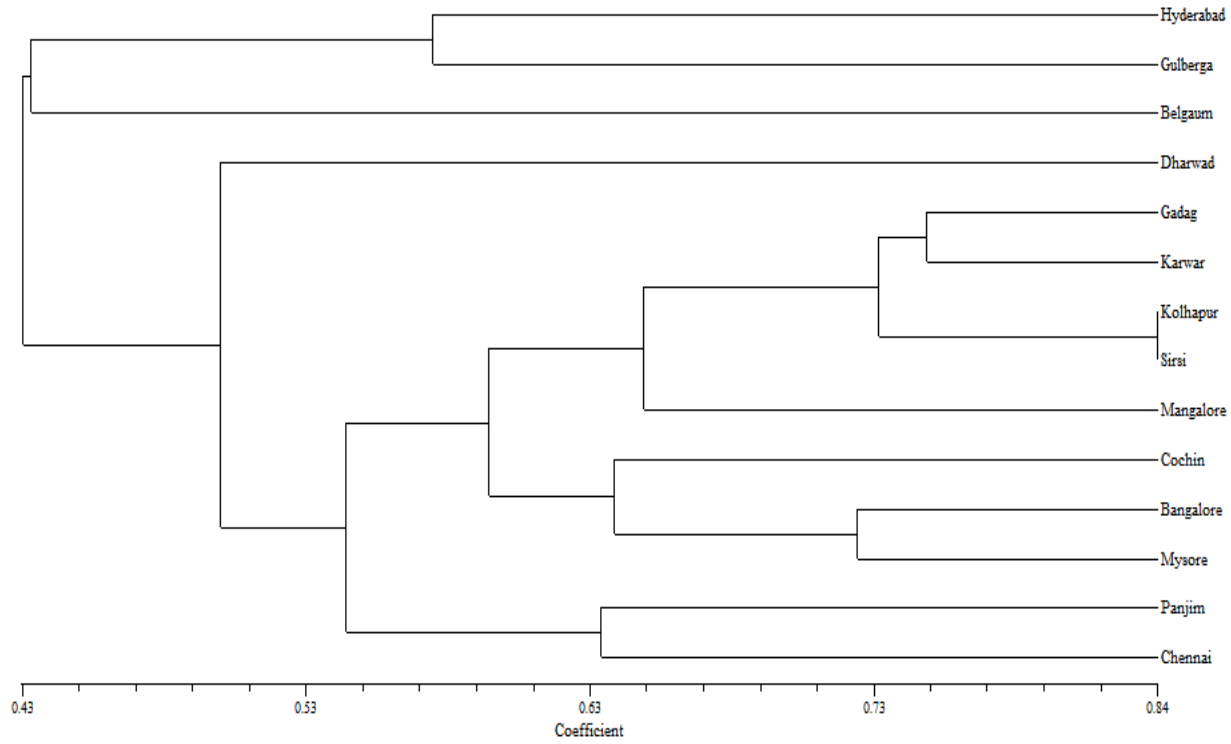

Fig.1: UPGMA Dendrogram showing genetic diversity in 14 population of S. Campanulata from South India

Based on the sample matching co-efficient (Jaccard index), a genetic similarity matrix was constructed using the binary data resulted from scoring presence or absence of bands to assess the genetic similarity among 14 populations of *S. campanulata*. The similarity coefficient ranged from 0.312 to 0.837 (Table 3). Interestingly lowest genetic similarity (0.312) was found in population belonging to Dharwad and Belagavi which are comparatively near in its geographical locations with respect to Sirsi and Kolhapur populations (0.837) which are geographically distinct.

The dendrogram constructed by the UPGMA based on Jaccard Similarity Matrix farmed two clusters A & B (Fig. 1). Major Cluster A comprised the populations of Dharwad, Gadag, Karwar, Kolhapur, Sirsi, Mangalore, Cochin, Bangalore, Mysore, Panjim and Chennai. Minor cluster B comprised the populations of Hyderabad, Gulberga and Belgaum. Cluster A showed the close relatedness between Kolhapur and Sirsi populations with

highest genetic similarity index of 0.837, while Panjim and Chennai populations fell into the outer group with a similarity index of 0.635. Cluster B depicted the close relatedness of Hyderabad and Gulberga populations with a similarity index of 0.573 and Belgaum fell in the outer group. The late acting self incompatibility nature of the *S. campanulata* [15,16] and cross pollination by birds and bats [1] may be attributed to the higher genetic diversity in the monophyletic species.

Table.3: Similarity index among 14 populations of S. Campanulata based on Jaccard coefficient

	Hyderabad	Gulberga	Belgaum	Dharwad	Gadag	Kolhapur	Sirsi	Karwar	Panjim	Mangalore	Cochin	Bangalore	Mysore	Chennai
Hyderabad	1													
Gulberga	0.573	1												
Belgaum	0.404	0.452	1											
Dharwad	0.396	0.491	**0.312**	1										
Gadag	0.433	0.514	0.387	0.559	1									
Kolhapur	0.376	0.451	0.465	0.477	0.745	1								
Sirsi	0.389	0.441	0.436	0.533	0.753	0.**837**	1							
Karwar	0.416	0.413	0.400	0.433	0.753	0.727	0.717	1						
Panjim	0.426	0.506	0.421	0.560	0.582	0.624	0.616	0.596	1					
Mangalore	0.397	0.375	0.485	0.431	0.628	0.646	0.665	0.663	0.522	1				
Cochin	0.400	0.480	0.466	0.584	0.604	0.591	0.629	0.602	0.613	0.590	1			
Bangalore	0.416	0.421	0.427	0.442	0.614	0.613	0.614	0.657	0.532	0.614	0.648	1		
Mysore	0.401	0.390	0.414	0.427	0.563	0.548	0.516	0.673	0.529	0.487	0.632	0.728	1	
Chennai	0.334	0.483	0.464	0.525	0.502	0.491	0.480	0.505	0.635	0.492	0.552	0.523	0.519	1

IV. CONCLUSION

The global challenge of invasive plant species has been increasingly recognized in the past two decades, while climate change and increased global trade have served to accelerate plant invasion [17]. The total genetic diversity of a species has key implications for its long-term survival and continued evolution revealing the insight of the levels and distribution of genetic diversity which is important for designing management strategies for the species [16]. In the present study, results showed the high degree of genetic diversity within the populations of *S. campanulata* supporting its range expansion from ornamental to possible invasive species in South India.

A species being a single taxon by definition, populations of a particular species may exhibit both phenotypic and genotypic diversity. Therefore the successful management of this diversified taxon may be greatly improved by understanding specific genetic variations of the species resulting in phenotypic variation attributable to genetic variation and/or plasticity [18]. Despite the use of morphological markers, there are no reports on molecular phylogeny and genetic diversity of monophyletics species *S. campanulata*. Hence, RAPD based molecular markers can be efficiently used to decipher the genetic polymorphism and to establish the management strategy for invasive species like *S. campanulata.*

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