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RESEARCH ARTICLE

GENETIC DIVERSITY OF *FICUS KRISHNAE* C.DC. AND *FICUS BENGHALENSIS* L.

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ABSTRACT

The status of *Ficus krishnae* remains ambiguous since more than 90% of seedlings become *F.benghalensis* on germination. This prompted the present genetic study to provide a better insight into the species status. The genetic similarity matrix generated by ISSR data indicates that *F. benghalensis* is genetically more diverse than *F. krishnae*. Genetic data analysis suggests separate species status to *F. krishnae* which is in the process of evolution.

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INTRODUCTION

Ficus krishnae, fondly known as "Krishna bor" is a medium sized sacred tree with a legendary theme of being served as Krishna's buttercup with several medicinal properties (Anonymous, 1956). Prain (1906) pointed out the similarities of *F. krishnae* with *F. benghalensis* while Corner (1965) considered it as a variety of *F. benghalensis*. Tiwari *et al.* (2015) after studying its morphology, anatomy and cytology, reinstated the status of *F. krishnae* as a species and further confirmed by Mahima *et al*. (2021) by DNA barcoding. The natural occurrence of this species is meager although *Ficus krishnae* profusely set seeds. According to Biswas (1935) *F. krishnae* seeds on germination produced only 10% of true breed while the rest 90% were of the horticultural variety of *F. benghalensis.* Considering the growing demand for this horticultural species, seed studies were carried out for conservation and species status ascertainment (Anilkumar *et al*, 2008). With this back ground, a molecular marker analysis was carried out to determine the extent of genetic diversity in the natural population of these species.

MATERIALS AND METHODS

Sampling of materials: Total of 12 accessions of *Ficus* comprising four accessions of *F. benghalensis*, six of *F. krishnae* and two of morphologically intermediate types were collected from different parts of Kerala in Southern India. Samples were collected in a manner that the accessions are more than 5 km distance from each other.

Genomic DNA isolation and ISSR: Total genomic DNA from the young leaves was isolated following the modified Murray and Thompson (1980) method using CTAB. After ethanol precipitation DNA was resuspended in 100µl of 1xTE buffer (pH 8.0). The DNA was quantified spectrophotometrically by taking the absorbance at 260 nm. ISSR assay was carried out in 25µl volume containing 50ng template DNA, 0.2mM dNTP's, 10mM Tris-Hcl, 1.5mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 1.0 U Taq DNA polymerase (Finnzymes, Finland), 10 µM primers (The University of British Columbia, Vancouver, Canada). PCR reactions were performed using an MJ Research thermal cycler PTC-100 with following amplification conditions: initial denaturation of 2 min at 94 C, 2 min at 36 °C and 2 min at 72 °C, followed by 38 cycles of 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min and a final extension at 72oC for 7 min. The amplified products were fractionated in 1.4% agarose gel (1x TBE) followed by EtBr staining. The gel image was documented using a gel documentation system (Alpha Imager, USA).

Genetic data analysis: Amplification with each random primer was repeated two times and those primers that produced reproducible and consistent bands were selected for data generation. Reproducible ISSR products were scored against the presence or absence of a fragment and denoted as' +' or' -' respectively. Dice coefficient of similarity defined as 2a/2a+u, where 'a' is the number of positive matches and 'u' is the number of non matches was computed using the WINDIST software (Yap et al. 1996). The scored binary matrix was analyzed for the construction of phenogram and determination of confidence limits by bootstrap analysis using the WINBOOT software (Yap et al. 1996).

The populations from which the samples taken for the present analysis were assumed to be in Hardy-Weinberg equilibrium implying that the populations are at random mating.

RESULTS

ISSR polymorphism and genetic variation: A total of 17 random primers were used for the estimation of genetic variation in *F. benghalensis* and *F. Krishnae* and the putative intermediates (Table 1). Out of 119 products generated, 77 were found to be polymorphic (64.71 % polymorphism).

The similarity matrix developed using the WINDIST software showed that similarity index ranges from 0.73 to 0.96 with mean value of 0.82 thereby suggesting low levels of genetic variability in the species (Table 2). At the interspecific level, extent of variability was found to be relatively more in *F. benghalensis* as similarity index is from 0.73 to 0.97 (mean value 0.88) whereas in *F. krishnae* the similarity index ranged from 0.82 to 0.96 with mean value of 0.91.

Species specific bands: Out of the 15 ISSR primers used, species specific band was generated only by primer 849. An amplicon of 1.7 kb size generated by this primer was found to be unique to *F. krishnae* while no such product was found in *F. benghalensis* (Fig. 2).

Table 1. List of primers and its sequences used for ISSR analysis and the number of bands produced by them

Primers	Sequence 5' 3'	No. of bands produced	No. of polymorphic bands
811	GA GAGA GA GAGAGA GA C	8	2
818	CA CA CA CA CA CA CA A	4	3
827	AC AC AC AC AC AC AC AC G	5	1
860	TG TG TG TG TG TG TG TG RA*	7	7
880	GG AG AG GAGA GG AG A	14	13
834	AG AG AG AG AG AG AG AG YT**	7	2
843	CT CT CT CT CT CT CT CT RA*	4	4
853	TC TC TC TC TC TC TC TC RT*	6	5
840	GA GA GA GA GA GA GA GA YT**	12	9
844	CT CT CT CT CT CT CT CT RC**	11	9
830	TG TG TG TG TG TG TG TG G	2	0
852	TC TC TC TC TC TC TC TC RA*	4	1
855	AC AC AC AC AC AC AC AC YT**	6	2
856	AC AC AC AC AC AC AC AC YA**	5	2
847	CA CA CA CA CA CA CA CA RC	5	3
848	CA C A CA CA CA CA CA CA RG*	11	8
849	GT GT GT GT GT GT GT GT YA**	8	6
Total No. of bands		**119**	**77**

* R= (A,G) **Y= (C,T)

Table 2. Similarity matrix of *Ficus* varieties analyzed based on Dice Coefficient

1.00											
1.00	1.00										
0.80	0.80	1.00									
0.92	0.92	0.80	1.00								
0.84	0.84	0.91	0.84	1.00							
0.95	0.95	0.83	0.95	0.90	1.00						
0.82	0.82	0.73	0.82	0.81	0.85	1.00					
0.74	0.74	0.68	0.74	0.73	0.78	0.89	1.00				
0.82	0.82	0.73	0.78	0.81	0.85	0.96	0.84	1.00			
0.85	0.85	0.85	0.89	0.91	0.91	0.87	0.79	0.83	1.00		
0.64	0.64	0.81	0.68	0.77	0.68	0.65	0.63	0.60	0.74	1.00	
0.57	0.57	0.75	0.62	0.71	0.62	0.59	0.54	0.54	0.68	0.94	1.00
A-01	**A-02**	**A-03**	**A-04**	**A-05**	**A-06**	**A-07**	**A-08**	**A-09**	**A-10**	**A-11**	**A12**

A-01 to A-06 – *F. krishnae* accessions, A-07 – A-10 *F. benghalenss* accessions and A11-A12 – *F.* intermediate type accessions.

On an average, the primers generated 7 products and 4.53 polymorphism per primer. The number of products generated by these arbitrary decamer primers was found to range from 2 to 14 with primer 880 giving the maximum (14) and primer 830 giving the minimum (2) number of amplicons. While primer 860 produced 100% polymorphism complete monomorphism was observed with the primer 830 which incidentally produced only two amplicons. A representative ISSR profile developed by the primer 834 is shown as Figure 1.

Cluster analysis: All the twelve accessions of *Ficus* broadly clustered into two separate groups where the clad is supported by high bootstrap values. In cluster I, all accessions of *F. Krishnae* formed a separate subcluster Ia while accessions of *F. benghalensis* except one formed subcluster Ib (Fig 2). The subclusters where supported by 46 and 64.8 % confidence limits. The two morpho intermediates formed cluster II at a confidence interval limit of 96.3%.

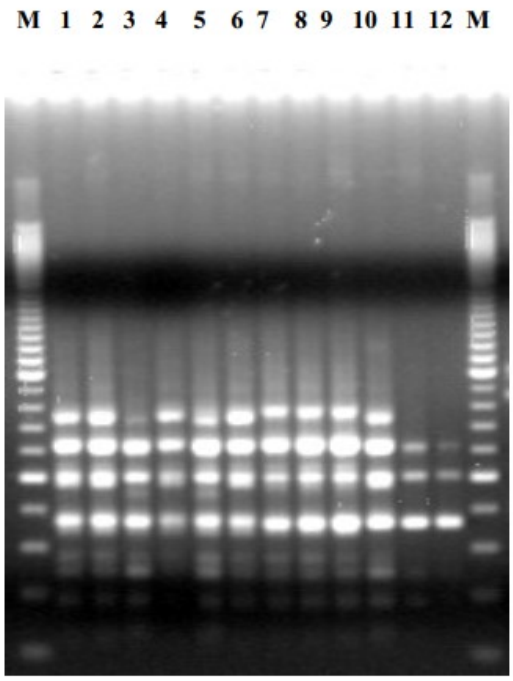

Fig. 1. Gel electrophoresis of amplification products obtained with the ISSR primer 834 1-6 *F. krishnae*; 7-10 *F. benghalensis* 11-12 intermediate types and M –marker

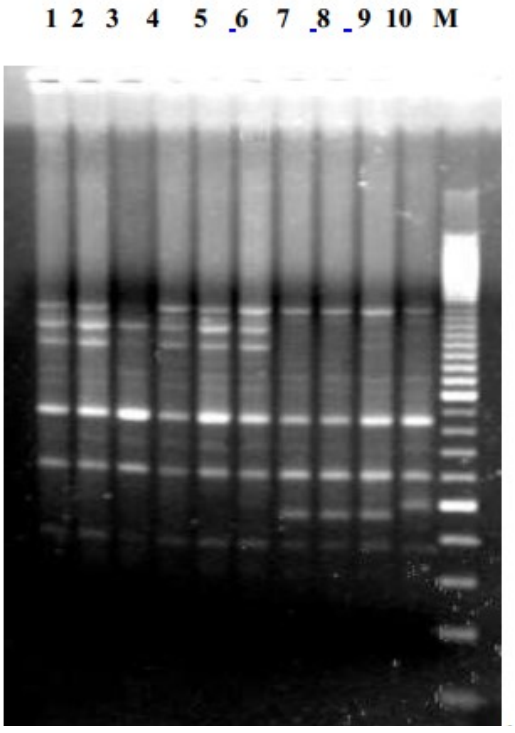

Figure 2. Gel electrophoresis of amplification unique products obtained with the ISSR primer 849 1-6 *F. krishnae* ; 7-10 *F. benghalensis* and M -marker

DISCUSSION

The ISSR data generated out of the limited number of accessions provide an insight into the extent of diversity in between *F. benghalensis* and *Ficus krishnae* for a cue on their taxonomic status. The phenogram developed from the ISSR data showed alignment of species under two different groups which strongly suggest the existence of variability at the genetic level. Although the level of intravarietal variability is low in the varieties *F.benghalensis* (mean GS= 0.88) and *F.krishnae* (mean GS= 0.91) intervarietal variability is

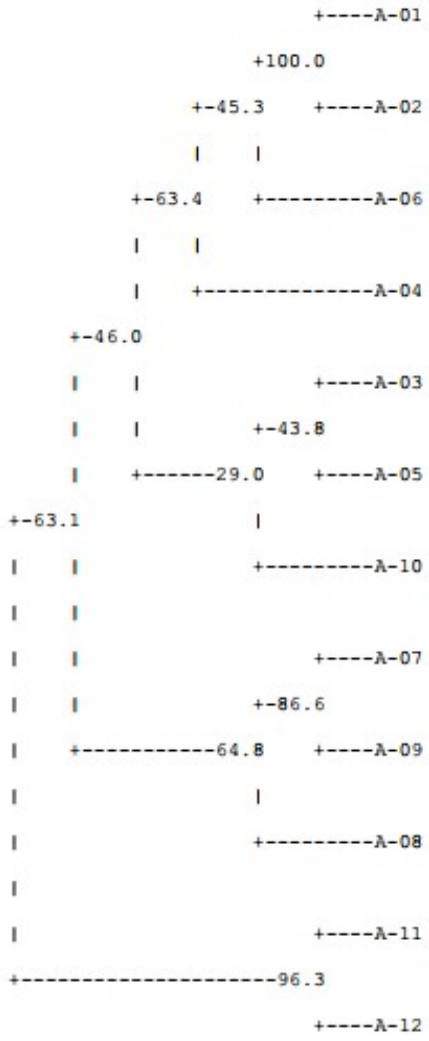

Fig 3. Phenogram based on UPGMA analysis *Ficus* varieties. Numbers at the clad indicates bootstrap values

substantially high (mean GS= 0.82) as an indicator of genetic diversity. Rout and Aparajita (2009) studied the genetic relationships between 21 species/varieties of *Ficus* in India and found that *F. krishnae* and *F. benghalensis* are grouped together but in separate subcluster with morphological variance. The unique species specific band generated by ISSR primer may be used to develop species specific marker in future. The observation that seeds of *F. krishnae* on germination results more of *F. benghalensis* rather than true breed may be attributed to the presence of the accessory chromosome in the somatic cells. In *F. krishnae*, besides the normal complement of 2n= 26, presence of 1-2 small eukaryotic chromosomes is also reported by Sheila Joshi and Raghuvanshi S.S. (1970). While the frequency of the accessory chromosome is 30% in somatic cells as high as 54% was noted in meiotic cells. The presence of intermediate types with gradation in the size and pattern of the leaf lamina the result of the number and frequency of occurrence of the accessory chromosomes. The estimated genetic diversity due to the occurrence of accessory chromosomes, which is reflected by natural intermediate types, specific anemophily and seed characters, *F. krishnae* is in the process of evolution.

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Conflict of Interest

The authors have no conflict in publishing the paper in the Journal – International Journal of Current Research.

Bullet points

- 90% of the seeds of *Ficus Krishnae* on germination become seedlings of *Ficus benghalensis.*
- *Ficus benghalensis* is more genetically diverse that *Ficus krishnae*.

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