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MOLECULAR EVALUATION OF DIVERSITY IN SOME INDIAN BIGNONIACEAE

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Genetic diversity in some Bignoniaceae growing in India was evaluated on molecular basis. RAPD-PCR amplification of genomic isolates purified from 12 selected genotypes (species) of the family was performed with the same protocol and conditions for all the 20 primers. Out of 354 bands produced by 20 primers used, there were 353 polymorphic bands and the percentage of polymorphism with these primers was 99.71. The dendrogram constructed on the basis of 354 amplified products generated by random amplification of polymorphic DNA showed the separation of 12 genotypes into 11 major groups at 0.52 Jaccard's coefficient. Only one species *Dolichandrone falcata* growing at two different places was found in same cluster at Jaccard's coefficient. However, at 0.3 scale of Jaccard's coefficient, these genera segregated into 10 groups and *Adenocalymma alliaceum* and *Tabebuia heterophylla* formed another group. These results showed high degree of variability among all the genera of family Bignoniaceae.

Keywords: RAPD-PCR amplification, genomic isolates, Jaccard's coefficient, polymorphism

Worldwide, the Bignoniaceae are mostly tropical trees or shrubs comprising of 120 genera and about 800 species (Gentry 1980, 1992, Lohmann 2002, 2004). In India the family is represented by 21 genera and 50 species chiefly found in western and southern parts and a few are found in Himalayan region also (Chauhan 2017). The members of the family are truly remarkable lineage of trees, shrubs, and wood lianas, rarely herbs or with suffruticose habit with large, brightly coloured flowers and unusual fruits. The evaluation of the members of the family Bignoniaceae has been done so far purely on the basis of their morphological and reproductive characters, the latter is influenced by environmental factors. Advances in molecular biology have introduced an alternative for genotype identification-DNA markers (Paterson *et al*. 1991). The development of PCR technique has greatly contributed to the spread of new genome analysis and it has allowed the introduction to the Random Amplification Polymorphic DNA (RAPD) approach for the molecular analysis of the genome.

Present investigation was undertaken to evaluate the genetic diversity in some Bignoniaceae growing in India on molecular basis. RAPD-PCR amplification of genomic isolates purified from 12 selected genotypes (species) of the family was performed.

MATERIALS AND METHODS

Molecular Analysis: Evaluation of 11 species growing at Agra (26° 44' N to 27° 25' N and 77° 26' E to 78° 32' E) and one at Gwalior (26.221521°N78.17802°E), India was carried out on molecular level using RAPD-PCR technique at the Phytotron Facility, Division of Genetics, Indian Agricultural Research Institute, New Delhi (Table 1).

RAPD-PCR-- Leaves (3/plant) from a bulk of 10 plants each from Agra and Gwalior were collected for DNA extraction. The collected leaves were kept in an icebox at –20°C freeze bricks and were immediately frozen with liquid nitrogen and stored at –80°C. Within next 24 hours the leaf samples were freeze dried in Holten freeze drier for 48 hours at –51°C to remove the moisture present in the tissues. The dried leaf tissue was ground to a fine powder in liquid nitrogen and transferred to 50 ml gamma radiation. Sterilized leaf tissue from each sample was used for DNA isolation.

a. *DNA extraction*-Total genomic DNA was isolated from samples using the protocol described by Prabhu *et al.* (1998). DNA was extracted from 50 mg of the ground tissue in a 1.5 ml ependrof tubes by adding 1 ml of (pre-heated at 95°C) extraction buffer and incubated

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Table 1: Species analyzed

Lane No.	Species	Location
1.	*Campsis grandiflora* (Thunb.) K.Schum.	Agra
2.	*Tecoma stans* (L.) Juss. ex Kunth	Agra
3.	*Millingtonia hortensis* L. f.	Agra
4.	*Kigelia africana* (Lam.) Benth	Agra
5.	*Pyrostegia venusta* Miers syn. *Bignonia venusta* Ker Gawl.	Agra
6.	*Dolichandrone falcata* Seem.	Gwalior
7.	*Dolichandrone falcata* Seem.	Agra
8.	*Adenocalymma alliaceum* (Lam.) Miers	Agra
9.	*Tabebuia heterophylla* (DC.) Britton	Agra
10.	*Jacaranda mimosifolia* D. Don	Agra
11.	*Fernandoa adenophylla* (Wall. Ex G. Don) Steenis syn. *Haplophragma adenophyllum* (Wall. ex G.Don)	Agra
12.	*Crescentia cujete* L.	Agra

at the same temperature for 10 minutes with agitation of the mixture by vortexing once in every two minutes and were then placed on ice for two minutes. The homogenate was transferred to remove cell debris at 12,000 rpm for 10 minutes. The supernatant was then transferred to fresh tubes containing 5l of RNAase (10 mg/ml) and incubated at 37°C for 20 minutes. To the content, 480 ml of cold isopropanol was added and mixed by rocking to precipitate the DNA. Centrifugation at 10,000 was done and supernatant was removed. The pellet formed was dissolved in 300 µl of H_2O by brief heating and flicking at 50°C. Preparation of DNA was obtained by addition of 30 µl of 3 M NaOAc and 600 ml of 95% ethyl alcohol. The tubes were rocked and centrifuged at 10,000 rpm for 5 minutes. The supernatant was decanted and pellet was washed with 400 µl of 70% ethanol and was centrifuged for 3 minutes at 10,000 rpm. Residue of ethanol was removed and the tubes were dried for 45 minutes by making the tubes stand up inverted. After drying, the pellet was suspended again in 100 µl of sterile water by flicking the tubes placed at45-50°C for 20-30 minutes and was stored at 4°C.

b. Quantification of DNA-The concentration of the DNA in the extract was measured with Unico™ UV- 2100 spectrophotometer. The quantity of DNA was calculated as follows:

$$DNA\ Quantity = \frac{50 \times A_{260} \times \text{dilution factor}}{(ng/\mu l)\ 1000}$$

Where

A_{260} = *O.D. value at 260 nm wavelength*

1 O.D. = 50 µg / ml

The DNA was diluted to a final concentration of 5 ng/µl for RAPD.

c. RAPD analysis-The random sequence twenty two decamer oligonucleotides from Operon Kits (Operon Technologies, Inc., Alameda, CA, USA) were used for amplification of extracted DNA (Table 2). PCR reactions were performed in a volume of 20 □l containing 1x PCR buffer, 2mM magnesium

chloride, 200 M each of the four dNTPs, 0.75 units/reaction of Taq polymerase, 0.2 µM of primers and 15 ng of genomic DNA (Williams *et al.* 1990).

Amplification reactions were performed in Thermal cycler. Thermal cycler was programmed for 1 cycle of 1 ½ minute at 95°C followed by 44 cycles each of 30 seconds at 95°C, 1 minute at 35°C and 1 minute at 72°C for denaturation, annealing and primer extension phase, respectively. The last cycle was followed by 7 minutes at 72°C for final extension.

d. Agarose gel electrophoresis: 4 µl of 2x tracking dye bromophenol blue was added to 20 µl volume of PCR product, mixed well and were loaded in the wells. A 100 bp ladder (MBI Fermentus) was used as a molecular weight standard. The gels were stained in ethedium bromide solution (1 µg/ml). These gels were visualized and photographed under UV Tran illuminator using gel documentation system. RAPD bands were then scored as present or absent.

e. Cluster analysis of banding pattern: The scoring of bands was done by measuring the distance of the bands from the well and (1) sign was given for the presence and (0) for the absence of the band.

f. Calculation of Similarity/Jaccard's coefficient: Jaccard's coefficient is quantitative estimates describing the association or resemblance between the associations or resemblance between banding patterns. The similarity coefficient of each variety/genotype was calculated using the formula:

$$\text{Similarity Index} = \frac{\Sigma \text{ Similar band (No. of Similar bands)}}{\Sigma \text{ Similar bands} + \Sigma \text{ dissimilar bands (No. of dissimilar bands)}} \times 100$$

RAPD Amplification: PCR amplification of genomic isolates and purified from 12 selected genotypes (genera) of the family Bignoniaceae (Table 1) was performed with the same protocol and conditions for all the 20 primers (Table 2). The PCR products were checked with (2% w/v) agrose gel electrophoresis and the bands were scored manually. The bands were recorded as presence (1) or absence (0) for each primer and genotype. The amplification profiles generated by each primer were compared and the relative molecular size of each band was examined by comparing with DNA size.

RESULTS AND DISCUSSION

1. Primer OPB-01: A total of 16 bands were produced by this primer in all the species (Fig.1A). The product size ranged between 600-1500 bp. All the bands were polymorphic. Therefore, this primer showed 100% polymorphism. No band was produced in *Crescentia cujete* with primer OPB-01.

2. Primer OPB-02: This primer produced 14

Table 2: Primers used in the RAPD analysis with their sequence.

S.N.	Primer name	Primer Sequence
1.	OPB-01	GTTTCGCTCC
2.	OPB-02	TGATCCCTGG
3.	OPC-04	CCGCATCTAC
4.	OPC-05	GATGACCGCC
5.	OPC-12	TGTCATCCCC
6.	OPG-01	CTACGGAGGA
7.	OPG-02	GGCACTGAGG
8.	OPL-01	GGCATGACCT
9.	OPL-02	TGGGCGTCAA
10.	OPL-03	CCAGCAGCTT
11.	OPP-02	TCGGCACGCA
12.	OPV-01	TGACGCATGG
13.	OPAB-15	CCTCCTTCTC
14.	OPAB-16	CCCGGATGGT
15.	OPAC-01	TCCCAGCAGA
16.	OPAC-02	GTCGTCGTCT
17.	OPAE-01	TGAGGGCCGT
18.	OPAE-02	TCGTTCACCC
19.	OPAF-01	CCTACACGGT
20.	OPAF-02	CAGCCGAGAA

bands of 500-1440 bp size (Fig.1B). All the bands were polymorphic in nature. Therefore, this primer showed 100% polymorphism. *Pyrostegia venusta* produced maximum number of bands (7). However, *Crescentia cujete* failed to produce any band with this primer.

3. Primer OPC-04: A large number of bands (25) were produced by primer OPC-04 (Fig.1C). The amplicon size ranged between 400-1500 bp. Maximum number of bands (10) were produced in *Millingtonia hortensis* and *Fernandoa adenophylla.* On the other hand, a unique band of 450 bp was produced in *Tabebuia heterophylla.* However, no bands were produced by *Crescentia cujete*. All the bands produced were polymorphic in nature. Primer OPC-04 showed complete similarity in both the plants of *Dolichandrone falcata* growing at Agra and Gwalior.

4. Primer OPC-05: Electrophoretic pattern of different species of the family Bignoniaceae showed presence of 21 bands with the primer OPC-05 and the band size ranged between 360-1490 bp (Fig.1D). There was no monomorphic band present and primer showed 100% polymorphic band number present and primer showed 100% polymorphism. *Campsis grandiflora* and *Adenocalymma alliaceum* produced maximum (9) bands each. Maximum (three) unique bands of 600, 1040 and 1310 bp were produced by *Fernandoa adenophylla*. While, *Crescentia cujete* failed to produce any band with this primer also. The plants of *Dolichandrone falcata* growing at Agra and Gwalior showed complete similarity with this primer also.

5. Primer OPC-12: This primer produced 27 bands and the amplification size ranged between 400-1430 bp (Fig.1E). All the bands were polymorphic in nature. There were 7 unique bands. The genotypes of *Dolichandrone falcata* growing at Gwalior and *Pyrostegia venusta* at Agra produced 2 unique bands each of 460 & 620 bp and 560 & 1430 bp respectively. Single unique band of 500 bp was produced by *Campsis grandiflora* of 1020 bp by *Fernandoa adenophylla* and of 1390 bp by *Millingtonia hortensis* On the other hand, *Kigelia africana* and *Crescentia cujete* failed to produce any bands and *Dolichandrone falcata* (Gwalior) and *Fernandoa adenophylla* produced highest number of 10 bands.

6. Primer OPG-01: Primer OPG-01 produced only 8 bands of 600-1380 bp size (Fig.1F). There was 100% polymorphism and there were no monomorphic bands. *Pyrostegia venusta* at 800 bp and *Campsis grandiflora* at 1380 bp produced unique bands. Maximum number of bands (7) was produced in *Pyrostegia venusta.* However, *Tecoma stans, Kigelia africana, Dolichandrone falcata* (at Agra and Gwalior both), *Adenocalymma alliaceum*, *Tabebuia heterophylla* and *Crescentia cujete* failed to produce any bands.

7. Primer OPG-02: The amplification size ranged between 320-1410 bp. Primer OPG-02 produced 24 bands in which all the bands were polymorphic (Fig.1G). However, 2 unique bands of 320 and 1390 bp were produced by *Millingtonia hortensis* and *Campsis grandiflora* respectively. Highest numbers of bands (10) were produced by *Pyrostegia venusta* while *Adenocalymma alliaceum* produced only 2 bands. *Dolichandrone falcata* plants growing both at Gwalior and Agra produced 8 similar bands. However, the plant of this species growing at Agra produced one additional band of 980 bp.

8. Primer OPL-01: A total number of 16 bands of size ranging between 400-1500 bp were produced by this primer (Fig.1H). All the bands were polymorphic in nature and only one unique band of 1500 bp was produced by *Millingtonia hortensis*. *Pyrostegia venusta* produced maximum number of bands (8), *Dolichandrone falcata* (Agra) produced 5 bands and showed similarity with the species growing at Gwalior. However, this genotype produced 2 extra bands of 700-790 bp in addition to those produced by the plants growing at Gwalior.

9. Primer OPL-02: Maximum number of

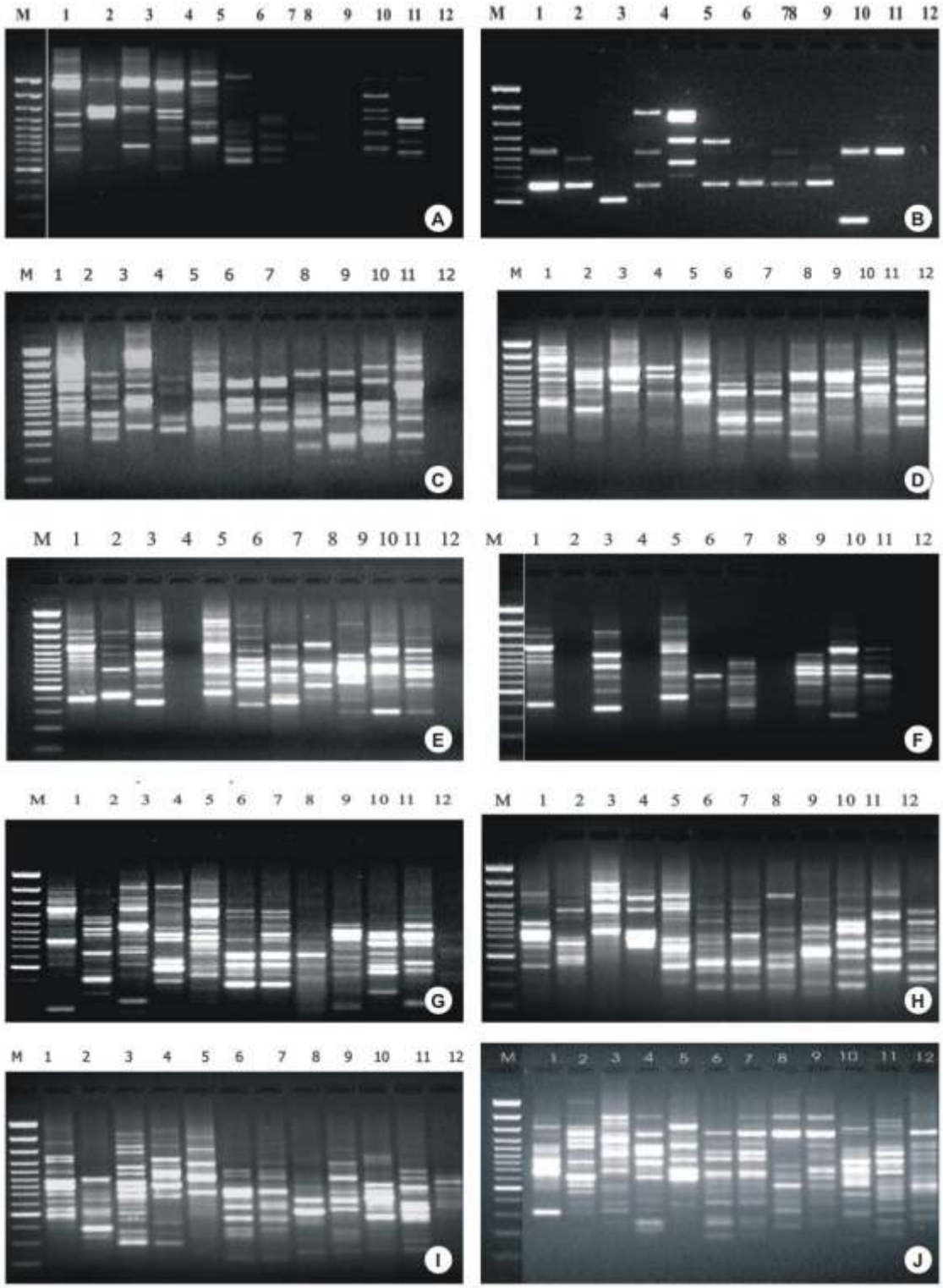

Figure 1. Representative RAPD profile of 12 members of the family Bignoniaceae generated by 10 primers. **A**. OPB-01, **B.**OPB-02, **C.** OPC-04, **D**.OPC-05, **E.**OPC-12, **F.**OPG-01, **G**. OPG-02, **H.**.OPL-01, **I**.OPL-02, **J.** OPL-03.

bands (39) was produced by this primer (Fig. 1I). Their amplican size ranged between 320-1480 bp. All the bands were polymorphic in nature. Five unique bands were produced by *Fernandoa adenophylla* at 410 bp, *Dolichandrone falcata* (Gwalior) at 450 bp, *Kigetia africana* at 530 bp, *Jacaranda mimosifolia* at 1210 and *Pyrostegia venusta* at 1400 bp. Maximum number of bands (17) were produced by the *Millingtonia hortensis.* On the other hand, *Adenocalymma alliaceum* produced only 3 bands. The plants of *Dolichandrone falcata* growing both at Agra and Gwalior exhibited a slight difference with this primer.

10. Primer OPL-03: A total number of 20 bands of 290-1500 bp size were produced (Fig.1J). There was no monomorphic band and *Tecoma stans* produced a unique band of 1500 bp size. On the other hand, in *Campsis grandiflora* and *Crescentia cujete* one band of 490 bp was lacking which was present in all the other species. *Fernandoa adenophylla* produced highest (13) bands. *Dolichandrone falcata* plants growing at Gwalior produced 9 bands. The plant of this species growing at Agra also showed similar 9 bands but in addition to these there were 2 more bands of 1160 and 1400 bp.

11. Primer OPP-02: This primer produced only 16 bands and their size ranged between 500-1500 bp and there was 100% polymorphism (Fig.2A). *Millingtonia hortensis* produced maximum (8) bands along with a unique band of 1500 bp. *Crescentia cujete* failed to produce any band. *Dolichandrone falcata* plants growing at both Gwalior and Agra showed similarity in banding pattern but there was one additional band of 1240 bp was present in the genotype of Agra.

12. Primer OPV-01: The amplicon size of 17 bands produced with this primer ranged between 380-1460 bp (Fig.2B). There was a single monomorphic band at 650 bp. In *Jacaranda* a band of 1060 bp was absent while it was present in other 11 species. However, only in *Kigelia africana* and *Fernandoa adenophylla* 570 bp band was absent. *Campsis grandiflora* produced maximum number of bands (10). The plants of *Dolichandrone falcata* growing at Agra and Gwalior exhibited more or less similar banding pattern except only 2 bands of 700 and 1150 bp were additionally present in the plant of Agra.

13. Primer OPAB-15: Thirteen bands of 400-1420 bp size were produced by this primer (Fig. 2C). Out of these only one unique band of 1400 bp was produced by *Millingtonia hortensis*. There was 100% polymorphism. *Tecoma stans*, *Jacaranda mimosifolia* and *Crescentia cujete* produced only 2 bands each. On the other hand, plants of *Dolichandrone falcata* at Agra and *Adenocalymma alliaceum* produced 8 bands. On the other hand, *Dolichandrone falcata* plant at Gwalior produced only 4 bands and these were similar to those of Agra plant.

14. Primer OPAB-16: 16 bands were produced by this primer and two of these bands produced by *Campsis grandiflora* (770 bp) and *Tecoma stans* (1500 bp) were unique and all the bands were polymorphic in nature (Fig.2D). *Campsis grandiflora* produced maximum 8 bands. On the other hand, *Crescentia cujete* failed to produce any band. *Dolichandrone falcata* (Gwalior) showed similarity in 2 bands with that of Agra plant, but the plant growing at Gwalior showed the presence of an extra band of 510 bp which was absent in the plant of Agra.

15. Primer OPAC-01: A total number of 23 bands of 310-1460 bp were produced by this primer in different genera of the family (Fig. 2E). There was complete polymorphism. Four unique bands were produced one each by *Jacaranda mimosifolia* (310 bp), *Pyrostegia venusta* (400 bp), *Millingtonia hortensis* (1420 bp) and *Kigelia africana* (1460 bp). Maximum number of bands (11) was produced by the genus *Kigelia africana*. However, *Crescentia cujete* failed to produce any bands. The plants of *Dolichandrone falcata* growing both at Agra and Gwalior exhibited dissimilarity with this primer.

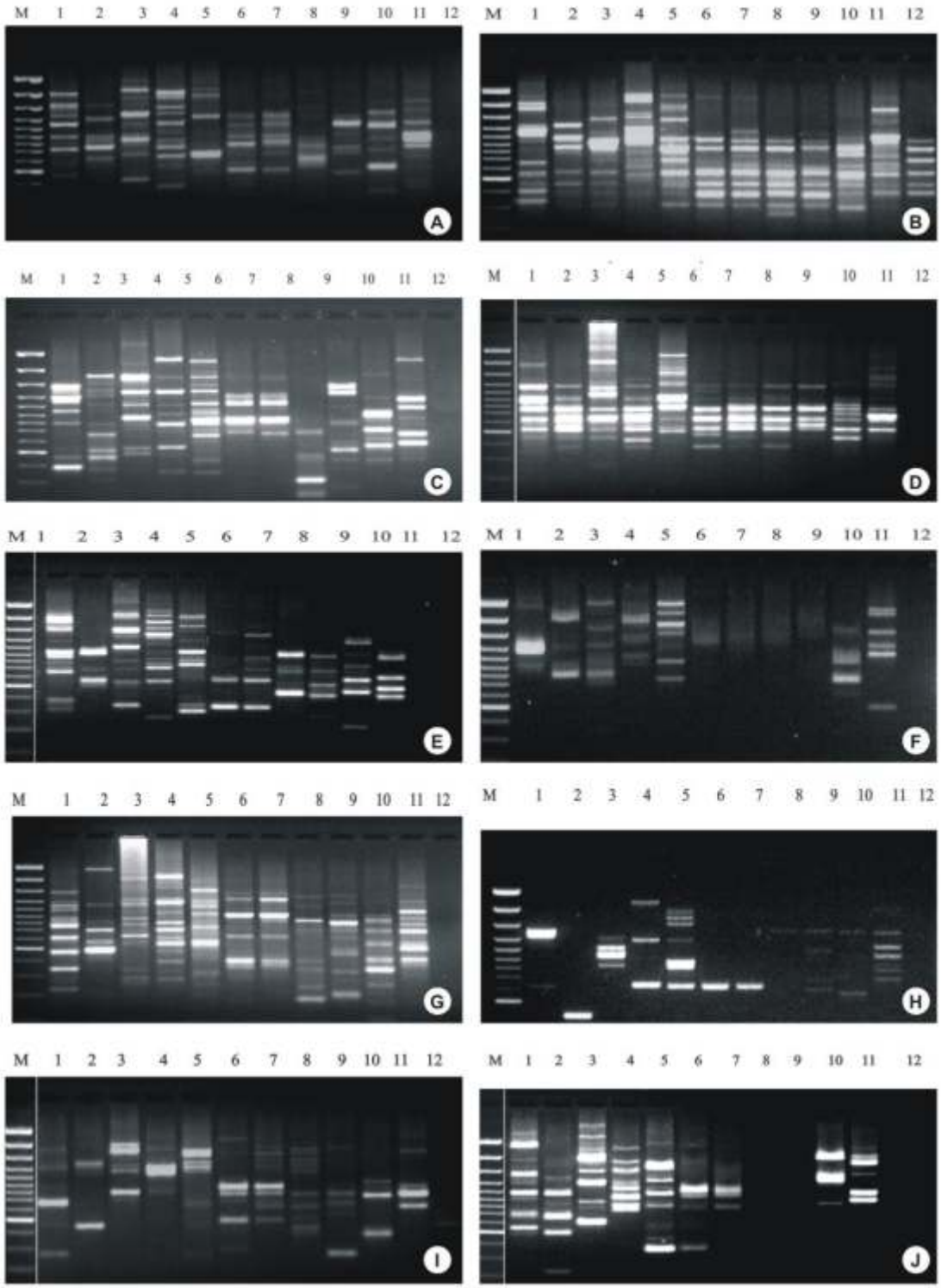

Figure 2. Representative RAPD profile of 12 members of the family Bignoniaceae generated by 10 primers. **A.** OPP-02, **B.**OPV-01, **C.** OPAB-15, **D.**OPAB-16, **E.**OPAC-01, **F.**OPAC-02, **G.** OPAE-01, **H.**OPAE-02, **I.** OPAF-01, **J.** OPAE-02.

16. Primer OPAV-02: A total number of 13 bands of 600-1480 bp were produced by this primer (Fig. 2F). *Fernandoa adenophylla* and *Jacaranda* produced unique bands of 600 bp and 700 bp respectively. No monomorphic bands were produced in any of the species investigated. Highest number of bands (6) was produced by *Fernandoa adenophylla*. On the other hand, the genotypes of *Dolichandrone falcata* (Agra and Gwalior), *Adenocalymma alliaceum*, *Tabebuia heterophylla* and *Crescentia cujete* failed to produce any bands**.**

17. Primer OPAE-01: Only 8 bands of 650-1400 bp were produced by the primer OPAE-01 (Fig. 2G). *Millingtonia hortensis* produced three unique bands of 780, 1100 and 1400 bp and only one band of 650 bp was produced by *Tecoma stans*. All these bands were polymorphic in nature. Both the genotypes of *Dolichandrone falcata* growing at Agra and Gwalior, *Adenocalymma alliaceum*, *Tabebuia heterophylla*, *Jacaranda mimosifolia* and *Crescentia cujete* failed to produce any bands.

18. Primer OPAE-02: Only 10 bands of size ranging between 500-1380 bp were produced by this primer (Fig. 2H). *Pyrostegia venusta* produced 3 unique bands of 800, 980 and 1310 bp, *Jacaranda mimosifolia*, *Millingtonia hortensis* and *Tecoma stans* produced single unique band each of 500, 600 and 1000 bp respectively.

19. Primer OPAF-01: Primer OPAF-01 produced only 8 bands of 450-1400 bp size (Fig. 2I). Seven unique bands were produced, of which 3 of 450, 550 and 1300 bp were produced by *P venusta*, 2 bands of 730 and 1400 bp by *Campsis grandiflora* and 2 of 1100 and 1250 bp by *Fernandoa adenophylla, Campsis grandiflora* and *Pyrostegia venusta* produced only 3 bands each, while, the other remaining species failed to produce any bands.

20. Primer OPAF -02: This primer reproduced 20 bands of 300-1500 bp amplicon size (Fig. 2J). All these bands were polymorphic in nature. However, there were a total number of 6 unique bands. Two of these unique bands of 620 and 1500 bp were produced by *Campsis grandiflora*. On the other hand, *Tecoma stans*, *Pyrostegia venusta* and *Millingtonia hortensis* each produced one unique band each of 300, 530 and 700 bp. Maximum number of bands (8) were produced by *Millingtonia hortensis*. On the other hand, *Adenocalymma alliaceum*, *Tabebuia heterophylla* and *Crescentia cujete* failed to produce any bands. The plant of *Dolichandrone falcata* growing at Gwalior produced four bands as compared to only three bands produced by the plant of this species growing at Agra.marker (MBI, Fermentus).

Polymorphism: The polymorphism observed among different species of the family with the help of twenty random primers is shown in the Table 3

Table 3 shows that a total of 354 bands were produced by 20 primers used, while there were 353 polymorphic bands polymorphic bands among these 354 bands and the percentage of polymorphism with these primers was 99.71. Primer OPL-02 produced maximum number of bands (39) and showed 100% polymorphism. This was followed by the primers OPC-12 and OPC-04, producing 27 and 25 bands respectively exhibiting 100% polymorphism. On the other hand, minimum polymorphism (94.11%) was recorded with the primer OPV-01 which generated a total of 17 bands with 16 showing polymorphism. On the other hand, the primers OPG-01, OPAE-01 and OPAF-01 produced minimum number of bands (8/primer). However, all of these bands were of polymorphic nature.

A dendrogram was constructed on the basis of 354 amplified products generated by random amplification of polymorphic DNA (Fig. 3). This showed the separation of 12 cultivars into 11 major groups at 0.52 Jaccard's coefficient. Only one genus i.e. *Dolichandrone falcata* (Tecomeae) growing at two different places i.e. at Gwalior and Agra was found in the same cluster at 0.52 Jaccard's coefficient. However, at 0.3 scale of Jaccard's coefficient, these genera were segregated into 10 groups. *Millingtonia hortensis* (Bignonineae) *and*

Table 3: Analysis of the polymorphism obtained with random primers among various species of Bignoniaceae.

S. N.	Primers	Total Number of Bands (a)	Number of Polymorphic Bands (b)	Polymorphism = b/a x 100 (%)
1.	OPB-01	16	16	100
2.	OPB-02	14	14	100
3.	OPC-04	25	25	100
4.	OPC-05	21	21	100
5.	OPC-12	27	27	100
6.	OPG-01	8	8	100
7.	OPG-02	24	24	100
8.	OPL-01	16	16	100
9.	OPL-02	39	39	100
10.	OPL-03	20	20	100
11.	OPP-02	16	16	100
12.	OPV-01	17	16	94.11
13.	OPAB-15	13	13	100
14.	OPAB-16	16	16	100
15.	OPAC-01	23	23	100
16.	OPAC-02	13	13	100
17.	OPAE-01	8	8	100
18.	OPAE-02	10	10	100
19.	OPAF-01	8	8	100
20.	OPAF-02	20	20	100
	TOTAL	354	353	99.71

Kigelia africana (Crescentieae) formed one group; *Adenocalymma alliaceum* (Lam.) Miers (Tribe-Bignonieae) and *Tabebuia heterophylla* (Tecomeae) formed another group with *Tecoma stans* (Tecomeae) near to it and *Campsis grandiflora* (Bignineae) and *Fernandoa adenophylla* (Tecomeae) made another group. *J. mimosifolia* (Oroxyleae) was near to this group. *Crescentia cujete was* at the top of the dendrogram near to group 8 and 9. The formation of groups in the dendrogram clearly showed a high degree of variability among different genera of family Bignoniaceae. However, there is no variation among the same species (*Dolichanrone falacta*) growing at different places. Jain *et al.* (1999) have evaluated the genetic diversity and genome fingerprinting of *Pandorea* (Bignoniaceae) by RAPD and inter-SSR PCR. They have taken five *Pandorea jasminoides* (Lindl.) K. Schum.and eight *Pandorea pandorana* (Andrews) Steenis cultivars. Twenty-five random primers generated 375 polymorphic products with a mean number of 15 polymorphic bands per primer. The mean number of polymorphic products obtained by inter-SSR amplification of genomic DNA revealed higher genetic variability among the cultivars of *Pandorea pandorana* than among those of *Pandorea jasminoides.* These studies showed that it would be possible to take advantage of existing genetic variability of *P. pandorana* to enhance the narrow genetic base of *P. jasminoides* by interspecific crosses.

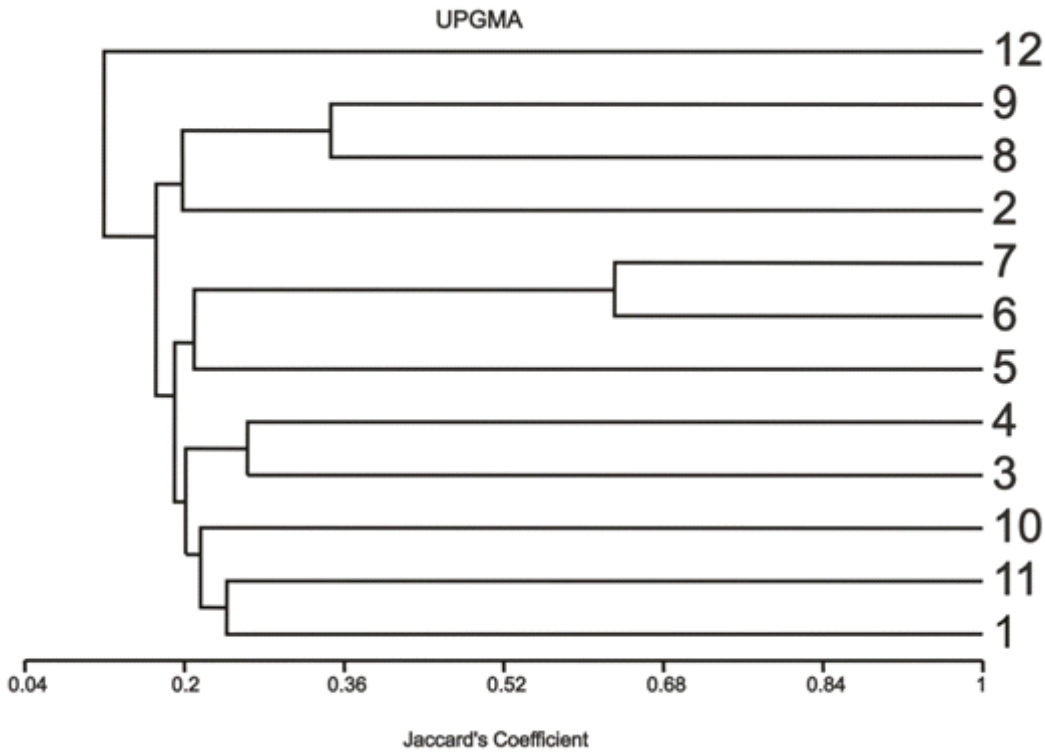

Figure 3. Dendrogram constructed on the basis of 354 amplified products generated by random amplification of polymorphic DNA of 12 members of the family Bignoniaceae by 20 primers showing the separation of 12 cultivars into 11 major groups at 0.52 Jaccard's coefficient.

The molecular studies made by Rana (2004) on *Kigelia pinnata* (Bignoniaceae) growing at Agra and Dehra Dun exhibiting variation in fruit formation exhibited slight genetic diversity and polymorphism. This may be due to differences in the climatic conditions of two places.

Lohmann (2006) used chloroplast (*ndhF*) and nuclear (*PepC*) DNA sequences to construct the phylogeny of tribe Bignonieae (Bignoniaceae). This study was under taken by Lohmann (2006) due to the presence of a large and morphologically diverse clade of neotropical lianas in the tribe Bignonieae of the family Bignoniaceae. According to her despite being a conspicuous component of the neotropical flora, the systematics of the tribe has remained uncertain due to confusing patterns of morphological variation within the group. Individual analysis of *ndhF* and *PepC* were highly similar to one another, yet localized differences in the placement of six species suggest some conflict between data sets. Combined analyses result in the trees that are consistent with those from the individual analysis and provide greater support for the suggested relationships. Lohmann (2006) is of the opinion that the phylogeny provides important new insight into the systematic of the tribe. It identifies 21 strongly supported species groups, eight of which broadly correspond to currently recognized genera. In addition, each of these 21 species group is supported by logical synapomorphies. The consistency between morphological and molecular data suggests that the current phylogeny provides a solid framework for a formal revision of the generic-level classification and for addressing other aspects of the biology of the tribe Bignonieae.

Li (2008) elucidated the phylogeny of *Chilopsis* D. Don. and *Catalpa* Scopoli (Bignoniaceae) on the basis of sequences of chloroplast *ndhF* and the nrDNA ITS region. In

Bignoniaceae, *Chilopsis* and *Catalpa* are most closely related as sister genera. The results of this investigation supported section *Macrocatalpa* (Griseb.) of the West Indies and section *Catalpa* of eastern Asian and North American continents. Within section *Catalpa, Catalpa ovata* of eastern Asia for a clad with North American species, *C. speciosa* and *C. bignonioides,* while the other eastern Asian species comprise a clade where *C. duclosuxii* is sister to the clad of *C. bungei* and *C. fargesii*. The Caribbean species of *Catalpa* diverged early from continental species. Still, Li (2008) feels that more studies are needed to test whether the phylogenetic pattern is common in eastern Asian-North American disjunct genera with species in the West Indies.

Olmstead *et al.* (2009) made phylogenetic analyses of chloroplast sequences (*rbcL*, *ndhF*, *trnL-F*) in order to infer evolutionary relationships in Bignoniaceae and to revise its classification. They recognized eight clades as tribes (Bignonieae, Catalpeae, Coleeae, Crescentieae, Jacarandeae, Oroxyleae, Tecomeae, Tourrettieae); additional inclusive clades are named informally. Jacarandeae and Catalpeae are resurrected; the former is sister to the rest of the family, and the latter occupies an unresolved position within the "core" Bignoniaceae. They included the tribe Eccremocarpeae in Tourrettieae. Past classifications recognized a large Tecomeae, but according to Olmstead *et al.* (2009), this tribe is paraphyletic with respect to all other tribes. They reduced Tecomeae to a clade of approximately 12 genera with a worldwide distribution in both temperate and tropical ecosystems. Two large clades, Bignonieae and Crescentiina, account for over 80% of the species in the family. Coleeae and Crescentieae are each included in larger clades, the Paleotropical alliance and Tabebuia alliance, respectively; each alliance includes a grade of taxa assigned to the traditional Tecomeae. Their parsimony inference suggested that the family originated in the neotropics, with at least five dispersal events leading to the Old World representatives.

The results of the present investigation and those of others presented in support clearly indicated a high degree of variability among all the genera of family Bignoniaceae. However, there was no variation among same species (*Dolichandrone falcata*) growing at places with geographical distinction.

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REFERENCES

Chauhan S V S 2017 *Bignoniaceae in India.* Presidential address. 27th Annual Conference of Indian Association of Angiosperm Taxonomy held at University of Delhi, Delhi, 10-12 November, 2017.

Gentry A H 1980 Bignoniaceae- Part I (*Crescentiae & Tourettieae*). *Flora Neotropica* Monograph, **25** 1-130.

Gentry A H 1992 Bignoniaceae. Part II (Tecomeae) *Flora Neotropica* Monograph. **56** 1-370.

Jain A, Apparand C and Bhalla P L 1999. Evaluation of genetic diversity and genome fingerprinting of *Pandorea* (Bignoniaceae) by RAPD and inter-SSR PCR. *Genome* **42**(4) 714-719.

Li J 2008. Phylogeny of *Catalpa* (Bignoniaceae) inferred from sequences of chloroplast *ndhF* and nuclear ribosomal DNA. *J Syst. Evol.* **46**(3) 341-348.

Lohmann LG 2002. Bignoniaceae. In: Henderson A and Mori S (eds.). *Flowering*

plant families of tropical America. New York: New York Botanical Garden Press.

Lohmann L G 2004. Bignoniaceae (Trumpet-Creeper Family). In: Smith N,Mori SA,Henderson A, Stevenson DW and Heald SV (eds.). *Flowering Plants of the Neotropics*. Princeton University Press, Princeton, New Jersey. Pp. 51-53.

Lohmann L G 2006. Untangling the phenology of neotropical lianas (Bignonieae, Bignoniaceae). *Am. J. Bot.* **93**(2) 304-318.

Olmstead R G, Zjhra M L, Lohamann L G, Susan O, Grose S O, Andrew J and Eckert A J 2009. A molecular phylogeny and classification of Bignoniaceae. *Am. J. Bot.* **96(9)** 1731-1743.

Paterson AH, Tanksley SD and Sorrels ME 1991. DNA markers in plant improvement. *Adv Argon* **46** 39-99.

Prabhu K V, Somers D J, Rakow G and Gugel RK 1998. Molecular markers linked to white rust resistance in mustard (*B. juncea). Ther. Appl. Genet.* **97** 865–870.

Rana Anita 2004. *Ultrastructural and biochemical studies in Kigelia pinnata DC. in relation to plant reproductive success.* D.Sc. Thesis, Dr. B. R. Ambedkar University, Agra.

Williams J G, Kubelik A R, Livak K J, Rafalski J A and Tingey S V 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* **18** 6531-6535.