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BRIEF COMMUNICATION

# Assessment of genetic diversity in *Coscinium fenestratum*

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## Abstract

Random amplified polymorphic DNA (RAPD) markers were used to assess the genetic diversity of 14 individuals belonging to 7 populations of *Coscinium fenestratum* (Gaertn.) Colebr. (*Menispermaceae*). 18 decamer primers used for the analysis generated 99 scorable bands of which 79 were found to be polymorphic. Coefficient of similarity ranged from 0.6604 to 0.9809. Variation within population was slightly higher than between populations. Similarity between individuals within and between populations was found. Dendrogram was obtained by using unwieghed pair-group method analysis (UPGMA). Distinct accession also exhibited higher percentage of medicinally active compound.

*Additional key words*: berberine, critically endangered species, *ex situ* conservation, RAPD.

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*Coscinium fenestratum* (Gaertn.) Colebr. is a seed propagated dioecious woody liana belonging to the tribe *Coscinieae*, the smallest tribe of family *Menispermaceae*. This species is of particular interest due to its medicinal properties and presence of isoquinoline alkaloids particularly berberine which is known to have several biological activities (Warrier *et al.* 1994, Birdsall and Kelly 1997). 1997 IUCN Red list of threatened plants recorded the status of *C. fenestratum* as endangered in India, vulnerable in Vietnam, rare in Singapore and indeterminate in Sri Lanka (Walter and Gillet 1998). A number of reports have discussed the use of RAPD markers to study the polymorphism in populations (Jayanthi and Mandal 2001), cultivars (Das née Pal and Raychaudhuri 2003), clones (Singh *et al*. 2004) and even mapping the genome (Banerjee *et al.* 2001). RAPD is particularly suitable to the genetic analysis of rare and endangered organisms where often met with the availability of material and inability to detect polymorphic isoenzyme loci and has been employed by many scientists (Bartish and Nybom 1999, Fu *et al.* 2003). The present work is an exploratory analysis to measure the diversity existing within and between populations of *C. fenestratum* from seven different places of Western Ghats, India viz., Bonakkad, Braimore, Sinikkala, Kallar, Kottur, Mundakkayam and Kulathoopuzha. All these places are more than 20 km apart. In all the places except Bonakkad region, individuals of *C. fenestratum* found as scattered consisting of 3 - 5 individuals. In Bonakkad region 8 individuals were located within 1 $km^2$ area and were selected to analyze diversity within a population. No reproductively mature plant could be located in the nature and we do not know the sex of the species taken for analysis. There were no morphological variants observed in the samples taken for analysis. It takes 12 - 15 years for the plant to flower and fruit and most of the plants are destructively collected even before permitting them to reproduce. In India *C. fenestratum* is restricted to Western Ghats and is facing a population reduction above 80 % over a period of three generations and is on the verge of extinction (Ravikumar and Ved 2000).

Total genomic DNA was extracted from the mature leaves by following the modified Murray and Thompson (1980) method using cetyltrimethylammonium bromide (CTAB). Extraction buffer consisted of 2 % (m/v) CTAB, 100 mM Tris, pH 8.0, 20 mM $Na_2EDTA$, 1.4 M NaCl, 1.2 % PVP (m/v) and 1 % β-mercaptoethanol (v/v). Presence of PVP in the extraction buffer and subsequent double chloroform extraction helped to remove phenolics

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*Abbreviations*: RAPD - random amplified polymorphic DNA; UPGMA - unwieghed pair-group method analysis.
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and polysaccharides, respectively. Finally ethanol precipitated DNA was resuspend in TE buffer (10 mM Tris pH 8.0 and 1 mM $Na_2EDTA$). Amplification reactions were carried out in 0.025 $cm^3$ volumes. Each reaction mixture consisted of 0.0025 $cm^3$ 10× amplification buffer, 0.2 mM each of dNTPs , 0.5 U *Taq* DNA polymerase (*Promega*, Madison, WI, USA), 0.025 $cm^3$ $MgCl_2$, 15 pM of decamer primer (*Operon Technologies*, Alameda, CA, USA) and 50 ng of genomic DNA. Reaction was performed in DNA thermal cycler 480 (*Perkin Elmer*, Boston, MA, USA). A total of 18 primers were used for the present study (Table 1). The sequential steps followed were: First cycle of 2 min at 93 °C, 2 min at 35 °C and 2 min at 72 °C followed by 38 cycles of 1 min at 93 °C, 1 min at 36 °C and 2 min at 72 °C. The last cycle was for 10 min extension at 72 °C. The amplified products were resolved in 1.2 % agarose gel and stained with ethidium bromide. The bands were detected using *Alpha Chemimager* (*Alpha Innotech Corporation*, San Leandro, CA, USA). Amplified products, which were reproducible and consistent in performance, were scored for data analysis. Pairwise comparisons were calculated from the data matrix and analysis was carried out following UPGMA method using the *POPGENE* package version 1.32. Nei's original measures of genetic identity and genetic distance were calculated according to Nei (1972).

Medicinally active compound of *C. fenestratum* is berberine (Siwon *et al.* 1980), which is present in all parts of the plant (Narasimhan and Nair 2004). Quantitative analysis of berberine was done in the samples from stem pieces (0.5 cm diameter) by HPLC as described elsewhere (Narasimhan and Nair 2004).

A total of 99 bands were generated from 18 decamer primers of which 79 was found to be polymorphic (88.76 % polymorphism) (Table 1). The primer OPX 5 generated maximum number of bands. None of the primers were monomorphic. The similarity values ranged from 0.6604 to 0.9809 and the average of coefficient of similarities was 0.8851 and shows a moderate degree of variation in this critically endangered species (Table 2). A low coefficient of similarity is attributed to broad genetic base in the origin of the species and was demonstrated by Padmesh *et al.* (1999) in *Andrographis paniculata* accessions where the mean coefficient of similarity was 0.60. Mean coefficient of similarity among the 8 individuals in the Bonakkad population was 0.8402 showing relatively more genetic variation within population. A low genetic variation occurs due to fragmented population and reproductive failure resulting in limited gene flow, population differentiation and genetic erosion.

UPGMA dendrogram of Nei's genetic distances revealed four distinct clumps A, B, C and D and revealed a distinct occupation of KUBOT 5491 (clump A) and KUBOT 5494 (Clump D) (Fig. 1). Such distinct accessions are inevitable for maintaining the conservation of total genetic variation in *ex situ* conservation of

Table 1. Polymorphisms obtained with different primers in the RAPD analysis of *C. fenestratum.*

| Primer | Total number of bands | Number of polymorphic bands |
|---|---|---|
| OPP-03 | 2 | 1 |
| OPP-04 | 9 | 8 |
| OPP-01 | 7 | 5 |
| OPP-20 | 7 | 5 |
| OPP-18 | 7 | 4 |
| OPP-17 | 8 | 6 |
| OPP-02 | 2 | 2 |
| OPW-02 | 5 | 5 |
| OPX-19 | 4 | 1 |
| OPX-17 | 6 | 5 |
| OPX-10 | 2 | 1 |
| OPX-13 | 5 | 5 |
| OPX-16 | 3 | 2 |
| OPX-06 | 5 | 4 |
| OPX-05 | 9 | 8 |
| OPX-20 | 6 | 6 |
| OPX-04 | 5 | 5 |
| OPX-12 | 7 | 6 |

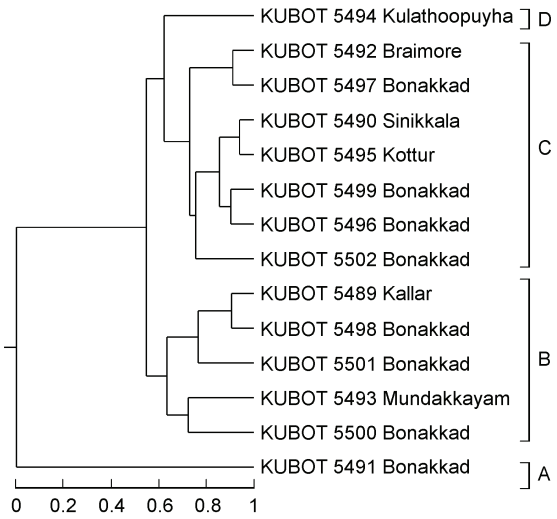


Fig. 1. UPGMA dendrogram showing the relationship among 14 plants of *Coscinium fenestratum* sampled from different places of Western Ghats, India.

*C. fenestratum*. Bonakkad plants were found in 4 clumps.

Clumping of accessions from distinct places like Kallar, Mundakkayam and Bonakkad region (Clump B) and Sinikkala, Braimore, Kottur and Bonakkad region (Clump C) were observed. More than 95 % of similarity was observed between KUBOT 5493 and KUBOT 5489; KUBOT 5490 and KUBOT 5495; KUBOT 5497 and KUBOT 5492; KUBOT 5502 and KUBOT 5490; KUBOT 5499 and KUBOT 5490; KUBOT 5496 and KUBOT 5495; KUBOT 5496 and KUBOT 5499; and KUBOT 5498 and KUBOT 5489. Jayanthi and Mandal (2001) concluded that such a high similarity between

Table 2. Similarity matrix of 14 individual plants of *Coscinium fenestratum*.

| KUBOT | 5494 | 5489 | 5493 | 5491 | 5492 | 5490 | 5495 | 5497 | 5502 | 5499 | 5501 | 5496 | 5500 | 5498 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5494 | 1.0000 | 0.8641 | 0.8945 | 0.7765 | 0.8652 | 0.9087 | 0.8760 | 0.8426 | 0.8966 | 0.9222 | 0.7443 | 0.8895 | 0.7932 | 0.8161 |
| 5489 | | 1.0000 | 0.9512 | 0.7491 | 0.9139 | 0.8913 | 0.8946 | 0.9083 | 0.8412 | 0.9056 | 0.9198 | 0.9377 | 0.9101 | 0.9703 |
| 5493 | | | 1.0000 | 0.7401 | 0.9121 | 0.8898 | 0.8603 | 0.9068 | 0.8965 | 0.9037 | 0.8391 | 0.8756 | 0.9151 | 0.8784 |
| 5491 | | | | 1.000 | 0.6904 | 0.7351 | 0.7290 | 0.6915 | 0.7708 | 0.7332 | 0.6604 | 0.7573 | 0.6679 | 0.7326 |
| 5492 | | | | | 1.0000 | 0.9262 | 0.9056 | 0.9718 | 0.9001 | 0.9262 | 0.8718 | 0.8980 | 0.8560 | 0.9048 |
| 5490 | | | | | | 1.0000 | 0.9809 | 0.9420 | 0.9616 | 0.9750 | 0.8464 | 0.9496 | 0.8116 | 0.8702 |
| 5495 | | | | | | | 1.0000 | 0.9306 | 0.9265 | 0.9441 | 0.8568 | 0.9508 | 0.7763 | 0.8734 |
| 5497 | | | | | | | | 1.0000 | 0.9249 | 0.9214 | 0.8891 | 0.8949 | 0.8284 | 0.8961 |
| 5502 | | | | | | | | | 1.0000 | 0.9306 | 0.7779 | 0.8801 | 0.8094 | 0.7940 |
| 5499 | | | | | | | | | | 1.0000 | 0.8580 | 0.9695 | 0.8512 | 0.8997 |
| 5501 | | | | | | | | | | | 1.0000 | 0.9020 | 0.8794 | 0.9348 |
| 5496 | | | | | | | | | | | | 1.0000 | 0.8451 | 0.9462 |
| 5500 | | | | | | | | | | | | | 1.0000 | 0.8798 |
| 5498 | | | | | | | | | | | | | | 1.0000 |

distantly located plants probably arises due to the fact what we see now is only a few plants of once existed large population. The present study is also in agreement with this observation.

It is of interest to note that genetically distinct accession KUBOT 5491 was also stands distinct in terms highest berberine content (0.79 % of dry mass) followed by KUBOT 5494, which is having 0.57 % of berberine. Amount of berberine present in other samples were 0.40 % (KUBOT 5492), 0.39 % (KUBOT 5497), 0.42 % (KUBOT 5490), 0.34 % (KUBOT 5495), 0.37 % (KUBOT 5499), 0.30 % (KUBOT 5496), 0.42 % (KUBOT 5502), 0.28 % (KUBOT 5489), 0.37 % (KUBOT 5498) 0.27 % (KUBOT 5501), 0.39 % (KUBOT 5493) and 0.45 % (KUBOT 5500).

As the rate of natural propagation is too low in this endangered species compared to its indiscriminate exploitation, immediate conservation management programmes need to be implemented to conserve the existing genetic diversity. It is also strongly felt that efforts should be made to conserve this species through an international collaborative effort in the Indo-Malayan region to preserve the plant's diversity for the posterity.

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