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SHORT COMMUNICATION

Molecular Phylogeny of Critically Endangered *Crinum woodrowii* Baker (Amaryllidaceae) from Bhimashankar, District Pune, Maharashtra, India

Sanjay Jagtap
Dept. of Botany, Elphinstone College, Mumbai (MS), India
sanjayjagtap64@gmail.com; +91 9969421282

Abstract

Crinum woodrowii Baker is a critically endangered bulbous plant belonging to family Amaryllidaceae found only at Kates point, Mahabaleshwar. It is endemic to Kates Points, Mahabaleshwar in Satara district of Maharashtra. It was recollected in 2012 from Vahigaon, Bhimashankar and in 2014 in Katraj. *Crinum woodrowii* is used as ornamental as well as medicinal herb. The scented flowers may be used in perfume and pharmaceutical industry. In present findings, the molecular phylogenetic study i.e. DNA finger printing of the *C. woodrowii* was determined. A consensus phylogenetic tree that compared known sequences of *C. woodrowii* from the current collection suggested that the specimens in our collection were closely related to genus *Crinum*.

Keywords: *Crinum woodrowii*, endangered plant, Mahabaleshwar, phylogenetic study, DNA finger printing.

Introduction

Crinum woodrowii Baker is a critically endangered bulbous plant belonging to family Amaryllidaceae found only at Kates point, Mahabaleshwar. It is endemic to Kates Points, Mahabaleshwar in Satara district of Maharashtra, India, North Western Ghats of Maharashtra State. Northern Western Ghats are home to three endemic species and 1 from viz. *Crinum brachynema* herb, *Crinum eleonorae* f. eleonorae Blattt. & McCann., *Crinum eleonorae* f. purpurea Blattt. & McCann., *Crinum woodrowii* Baker. According to Punekar *et al.* (2004) all these four taxa are confirmed to Mahabaleshwar and surrounding areas in Maharashtra. As per Lekhak and Yadav (2011) *Crinum woodrowii* has an extended distribution from Mahabaleshwar to Radhanagari (Kolhapur district) in the main ranges of Northern Western Ghats. It was recollected by Pethe and Tillu in (2012) at river banks of village Vahigaon in Thane district of Maharashtra. I have also surveyed the distribution of *C. woodrowii* Baker from Bhimashankar Wild Life Sanctury, Bhimasankar Hills, District Pune from Maharashtra in 2012. Similarly Sing and Garg (2014) collected it from Katraj Ghats in Pune district. The International Union for Conservation of Nature and Natural Resources (IUCN) status of *Crinum woodrowii* is critically endangered. *Crinum woodrowii* is used as ornamental as well as medicinal herb. The scented flowers may be used in perfume and pharmaceutical industry. Considering the above facts, this spiece of investigation focuses on the molecular phylogenetic study i.e. DNA finger printing of the *C. woodrowii*

Materials and methods

Sampling: Fresh samples of bulbs of *Crinum woodrowii* Baker were collected during monsoon (August, 2012) from hill slopes of Bhimashankar Wildlife Sanctuary in Pune district regions of Western Ghats of Maharashtra (Fig. 1 and 2). The plants were identified and authenticated using herbarium collection at Botany research laboratory, DST-FIST School of Life Science, SRTM University, Nanded (MS) and Dept. of Botany Walchand College, Solapur. Fresh bulbs were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The dried material was ground into coarse powder using mechanical grinder. This coarse powder was sieved by 1 mm pore size sieve. The powder was stored in airtight containers at room temperature till further study of DNA profiling.

Fig. 1. Plant of *Crinum woodrowii* Baker.

Fig. 2. Bulbs of *C. woodrowii* Baker.

DNA extraction and quantification: DNA extraction was carried out using HiPurA Plant Genomic DNA Miniprep Purification Spin kit (Himedia, MB507). Concentration of DNA was determined using UV-1800 spectrophotometer (Schimadzu Corporation A11454806498). The DNA was stored at -200°C till further use.

PCR amplification: The DNA isolated from plant was subjected to polymerase chain reaction (PCR) amplification using Biometra thermal cycler. 2f-5'GGCAAAGAGGGAAGATTTCG3' and 4r-3'CCATAAGCATATCTTGAGTTGG5' sequencing primer were used for amplification. The PCR reaction mix contained 2.5 µL of 10X buffer, 1 µL of each primer, 2.5 µL of 2.5 mM of each dNTP, 2.5 Units of Taq DNA polymerase and 1 µL Template DNA and 8.5 µL nuclease free water. The PCR amplification cycle consisted of, a cycle of 5 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 50°C, 2 min at 72°C and additionally 1 cycle of 7 min at 72°C.

Gel electrophoresis: Gel electrophoresis of the amplified product was performed using 1.0% agarose (Seakem, 50004L) to analyze the size of amplified PCR product. The size obtained was approx. 500 bp for *rpoC1* region.

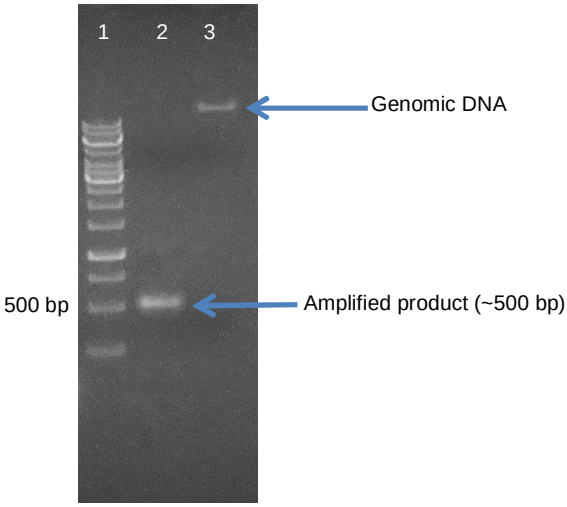
DNA sequencing: The PCR product was purified using AxyPrep PCR Clean up kit (Axygen, APPCR-50). It was further sequenced using Applied Biosystems 3730xl DNA Analyzer USA and chromatogram was obtained. For sequencing of PCR product 2f-5'GGCAAAGAGGGAAGATTTCG3' sequencing primer was used.

Bioinformatic analysis: DNA sequence was submitted to NCBI Gene Bank (http://www.ncbi.nlm.nih.gov/) and NCBI's web-based BLAST algorithm was constructed using the default settings (Felsenstein, 1985; Saitou and Nei, 1987; Tamura *et al.,* 2011).

Results and discussion

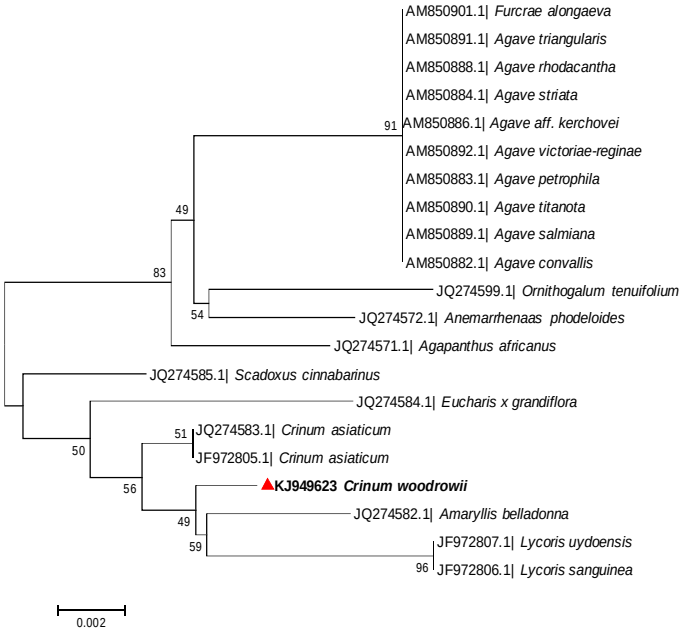
PCR amplification of *rpoC1* gene in *C. woodrowii* Baker is shown in Fig. 3. We confirmed the identity of the current species using molecular phylogenetic methods. Model Test in MEGA 6 suggested that models JC (BIC=1389.073, AIC= 1136.538, in L-531.063 explained the nucleotide patterns in the *ropC1* gene sequences. A consensus phylogenetic tree (Fig. 4) that compared known sequences of *Crinum wooodrowii* from the current collection suggested that the specimens in our collection were closely related to genus *Crinum* as per UNESCO World heritage list of Western Ghats.

Fig. 3. Amplification of *rpoC1* gene in *C. woodrowii* Baker.



Lane 1: 1 Kb DNA marker of Fermentas (#SM0311)
Lane 2: Amplified PCR product of *C. woodrowii*
Lane 3: Genomic DNA of *C. woodrowii*
1 Kb DNA marker (Top to bottom): 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 750, 500 and 250 bp.

Fig. 4. Phylogenetic tree for *Crinum woodrowii* Baker using sequence of *rpoC1* region.



Conclusion

The molecular phylogeny of *Crinum woodrowii* was determined by analyzing plastid *rpoC1* region sequences. On the basis of position of sequence of *Crinum woodrowii* in the phylogenetic tree, the plant showed closest similarity with *Amaryllis belladonna.* On the basis of morphology and phylogeny the given plant is *C. woodrowii* belonging to family Amaryllidaceae. Molecular phylogeny is an authentic tool in taxonomic identification and authentication. A new location leads to save these critically endangered plants by propagating in nursery conditions. The conservation and reintroduction of this species to suitable habitats is an urgent need. It will also help in ethano-pharmacologicial studies pertaining to properly identified plant species.

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References

1. Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolut*. 39: 783-791.
2. Lekhak, M.M. and Yadav, S.R. 2011. Karyotype studies in two critically endangered and endemic Crinum species (Amaryllidaceae) from Northern Western Ghats of India. *Nucleus*. 54(1): 25-30.
3. Pethe, J. and Tillu. 2013. A range extension for critically endangered plant *Crinum woodrowii* Baker. *J. Bombay. Nat. Hist. Soc.* 110(2): 169-170.
4. Punekar, S.A., Kavade, S.P., Datar, M.N., Lakshminarasimhan, P. and Rao, P.S.N. 2004. *Crinum woodrowii* Baker (Amaryllidaceae), hitherto assumed to be extinct, rediscovered after a century from Mahabaleshwar, India. Scientific Correspondence 1050. *Curr. Sci*. 87: 8.
5. Saitou, N. and Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evolut.* 4: 406-425.
6. Singh, R. and Garg, A. 2014. Discovery of the critically endangered bulbous herb *Crinum woodrowii* (Amaryllidaceae) and ITS neotypification. *Taprobanica.* 7(2): 68-71.
7. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. *Mol. Biol. Evolut.* 28(10): 2731-2739.