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Genetic diversity and population structure of the mangrove lime (*Merope angulata*) in India revealed by AFLP and ISSR markers

Satya Narayan Jena[a], Sushma Verma[b], Kuttan Narayanan Nair[b,*], Awadhesh Kumar Srivastava[b], Sujata Misra[b], Tikam Singh Rana[b]

[a] Plant Molecular Biology & Genetic Engineering Division, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow-226001, India
[b] Plant Diversity, Systematics & Herbarium Division, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow-226001, India

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ABSTRACT

The mangrove lime, *Merope angulata* (Willd.) Swingle (Rutaceae) is a threatened mangrove associate species occurring sporadically in the tidal forests from Papua New Guinea to the east coast of India. It is an underutilized salt tolerant plant related to *Citrus*. We have applied AFLP and ISSR markers to estimate the extent of genetic diversity among 55 individuals of *M. angulata*, sampled from two natural populations (Bhitarkanika Wildlife Sanctuary) and one *ex situ* population (Jharkhali Mangrove Ecological Garden) in India. A combined analysis of both AFLP and ISSR markers revealed low polymorphism (42.93%) and a moderate heterozygosity and Shannon diversity index ($H = 0.393$, $I = 0.571$) in the species. The Jaccard genetic distance among 55 accessions ranged from 0.16 to 0.74 (average 0.43). Analysis of population genetic structure using Bayesian method in STRUCTURE software identified two genetic clusters. F_{ST} statistics and AMOVA results indicated low population differentiation with high gene flow. Haplotype indices and mismatch distributions indicated some evidence of expansion in the two natural populations. Outcrossing test revealed a predominantly out-breeding system with high outcrossing rate. The high gene flow detected among the populations indicates that *M. angulata* does not face any genetic drift, and hence no local genetic differentiation and population divergence. The highly fragmented state of its habitats warrants adequate measures for maintenance of genetic diversity in this species without further depletion. This is the first report on molecular analysis of genetic diversity and population structure of *M. angulata* from its native distributional range in India.

1. Introduction

The mangrove lime, *Merope angulata* (Willd.) Swingle is a rare and threatened mangrove associate species occurring in the tidal forests or brackish swamps from Papua New Guinea to Philippines, Java, Singapore, Malaysia, Myanmar, and to the east coast of India in West Bengal and Orissa (Swingle and Reece, 1967). *M. angulata* is the sole representative of the mono-specific genus *Merope* M. Roem., and also the only known mangrove member in the family Rutaceae. It is a small spiny tree or shrub, characterized by its simple, thick, leathery and aromatic leaves dotted with translucent glands; solitary (very rarely two) and bisexual flowers in the leaf axils; and the small lime like, three-loculed, triangular fruits containing large flattened seeds. It is a salt-tolerant plant with potential for use as a rootstock for grafting citrus fruit trees (Krueger and Navarro, 2007), and also has medicinal uses in Malaya and India (Jones, 1982).

In India, *M. angulata* has been recorded to grow in a very few isolated localities in the southern part of the Jharkhali islands of the Sunderbans (Chhota Herrobongha: Naskar and Mandal, 1999) and along the east coast of Orissa (Bhitarkanika and Mahandi delta: Thatoi and Biswal, 2008). The extant populations of this remarkable species are under severe threat due to conversion of its natural habitat for agriculture, aquaculture, tourism and other developmental works. Natural regeneration of this species is slow due to sparse seed production and poor seedling establishment (Thatoi et al., 2000). Consequently, *M. angulata* has been reported as critically endangered in Singapore (Davison et al., 2008), endangered in Malay peninsula (Jones, 1990), and rare and threatened in Indian Sunderbans (Naskar and Mandal, 1999) and in the east coast of Orissa (Thatoi and Biswal, 2008).

Despite its medicinal uses, genetic resource values and current threat status, no attempt has been made so far to assess the

* Corresponding author. Tel.: +91 0522 2297852/2205831–35x852; fax: +91 0522 2205836.
E-mail address: nairnbri@yahoo.co.in (K.N. Nair).

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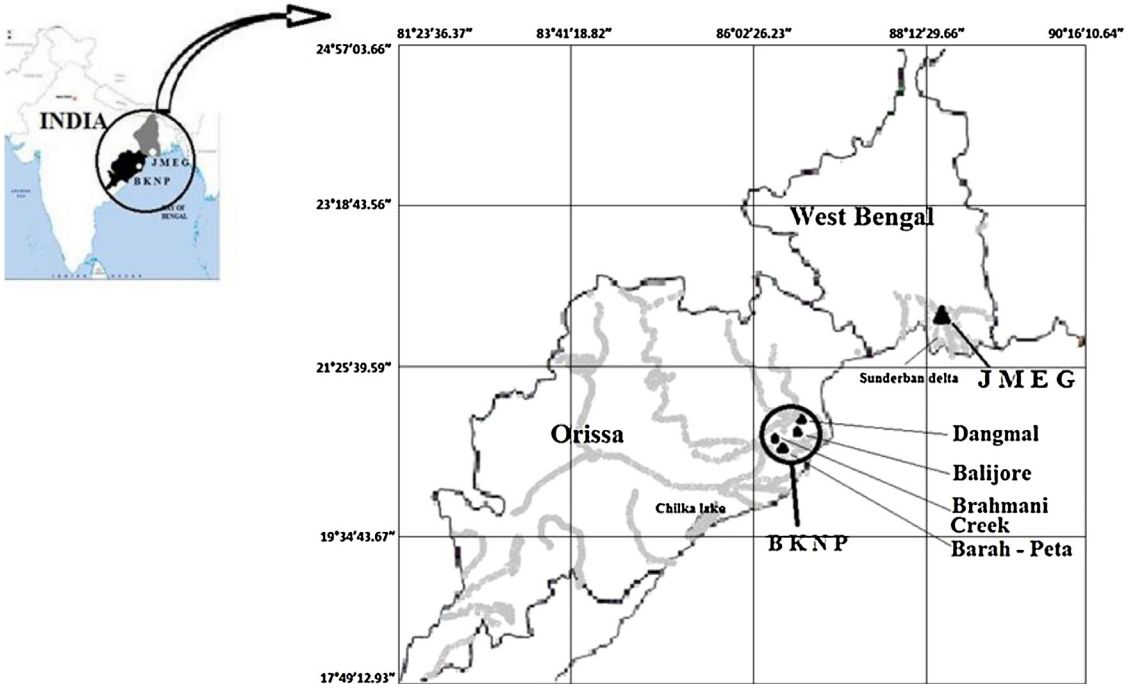

Fig. 1. Distribution map of *Merope angulata* showing locations of sample collections made in Bhitarkanika National Park (BNP) in Orissa and Jharkhali Mangrove Ecological garden (JMEG) in West Bengal of India.

genetic diversity and population genetic structure in *M. angulata*. Estimation of the extent and pattern of genetic diversity in a threatened species like *M. angulata* is critically important for developing appropriate methods for its *ex situ* and *in situ* conservation. Genetic diversity assessment of a species at intra and inter-populations level will also help infer the population genetic structure, breeding systems, gene flow and the genetic bottlenecks, if any, due to habitat fragmentation or other spatial or temporal barriers. PCR-based DNA marker techniques have been successfully used in genetic diversity assessment in several plant species of biological, economic and conservation interests, including mangroves. Among the multi-allelic markers, AFLP (Vos et al., 1995) and ISSR (Zietkiewicz et al., 1994) are highly polymorphic markers having high resolving power and reproducibility. AFLP and ISSR, either singly or in combination, have been applied in many studies to evaluate genetic diversity and population genetic structure of several endangered mangrove and their associates (Triest, 2008; Deng et al., 2009; Jian et al., 2010).

In the present study, we applied AFLP and ISSR markers to estimate the extent of genetic diversity and the local population genetic structure in two natural populations (50 accessions) and an *ex situ* collection (five accessions) of *M. angulata*, sampled respectively from Bhitarkanika National Park (Orissa) and Jharkhali Mangrove Ecological Garden (West Bengal) in India. The study was also undertaken to evaluate the relative efficiency of the two markers in detecting polymorphisms and other genetic parameters in the sampled populations of *M. angulata* and to discuss the implications of present findings for conservation and management of this species in India.

2. Materials and methods

2.1. Plant sampling and DNA extraction

The plant samples of *M. angulata* were collected from different localities from the creeks/channels of tidal forests and brackish swamps of Baitarani-Brahmani delta (core zones of Bhitarkanika National Park [BNP], Orissa) and also from an *ex situ* collection maintained at the Jharkhali Mangrove Ecological Garden (JMEG) along the Sunderban delta at Jharkhali (West Bengal) in the east coast of India (Fig. 1). A random sampling strategy was followed, and the collected samples included plants in reproductive as well as vegetative phases. The collection sites in BNP included four scattered mangrove patches at Brahmani creek, Balijore, Bankual and Barah–peta. The average distance between the first three locales was less than 5 km and all the 33 plant samples collected from these three locales in and around Dangmal were considered as a single population (DANG). The fourth BNP locale (Barah-peta) was located at a distance of more than 10 km farther from DANG and all the 17 samples from this locale were designated as a separate population (BARA). JMEG is an *ex situ* mangrove conservation centre maintained by the Calcutta Wildlife Society in a 10 ha area in the inter-tidal zones on the northern bank of the river Chhota Herobhanga in the central portion of the Indian Sunderban at Jharkhali in Basanti Island (about 260 km away from BNP, Orissa). JMEG holds a germplasm collection of about 53 species of mangroves or mangrove associates of the Sunderbans, including *M. angulata*. Five accessions sampled from among the 15 adult plants of *M. angulata* maintained at JMEG were included in the present analysis as representatives of the species from the Sunderbans (JMEG). The JMEG accessions of *M. angulata* were 10 year old and were originally collected from the wilderness of mangrove forests from the eastern bank of the Bidya River. Thus, a total of 55 accessions representing two natural populations and one *ex situ* collection of *M. angulata* were considered in the present investigation (Table 2). Samples for DNA extraction and other analyses were collected randomly from plants located at a distance of about 10 m in each locality. Leaf tissues were stored dry at room temperature over silica gel at the time of collection. Voucher specimens have also been prepared for all the collected materials and are housed in the herbarium of CSIR-National Botanical Research Institute (LWG), Lucknow, India.

Total genomic DNA was extracted from silica-gel dried leaves of individual accession of *M. angulata* following the CTAB method (Doyle and Doyle, 1990). Qualitative and quantitative assessment