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Bouea oppositifolia – A fast disappearing native mango genetic resource from Andamans: Morphological and molecular evidences

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ABSTRACT

Andaman is a genetically diverse hot spot of native wild mangoes. The taxon *Bouea oppositifolia* is an extremely rare, shy reproducing wild mango. Until recently only three reproducing individuals have been located in the South Andaman. In terms of morphological features it closely resembles to *Mangifera andamanica*. In this study we highlight some morphological features and molecular evidences that substantiates the uniqueness of this taxon. We deployed SSR markers to understand its genetic relatedness with *M. andamanica*. Sixty SSR primers were used of which about 15 were polymorphic. The study resulted in 61 SSR products of which 29 were polymorphic (47.5%). Analysis indicates that *B. oppositifolia* is 43% genetically similar to *M. andamanica*. Possibilities of conservation and domestication in *B. oppositifolia* are discussed.

Key words: *Bouea oppositifolia*, characterization, *Mangifera andamanica.*

INTRODUCTION

Studies in Asia indicate that forest genetic resources are on a rapid decline trend and in some regions their future looks jeopardized (Anon, 3&4). According to the Convention of Biological Diversity (CBD), individual countries are responsible for conservation and sustainable use of their biological diversity (Anon, 2, 5). In India, programmes on cataloguing forest genetic resources and bioprospecting has gained considerable momemtum. These are being prioritised on the basis of economical demand, taxonomical rarity and threat factors. Furthermore, the Millenium Ecosystem Assesment (MA) considers that forest genetic resources involved in provisioning are of primary importance to the well being of humankind (Anon, 6).

The genus *Mangifera* commonly referred to as mango belongs to the family Anacardiaceae. South East Asia is the centre of diversity of mangoes with over 30 taxa (Mukherjee, 12, 13). In India, the Andaman and Nicobar group of islands is a Genetically Diverse Hot Spot (GDHS) for wild mangoes. The islands are rich in biodiversity with extremely fragile habitats harbouring over 25,00 angiosperm taxa of which about 245 are endemic (Ahlawat, 1). The native wild mangoes in the region are *Mangifera andamanica, M. camptosperma*, *M. griffithi, M. nicobarica* and *M. indica* (Mukherjee, 13). In this report we highlight an extremely rare mango taxon namely *Bouea oppositifolia* (Roxb.) from the South Andaman. It is a rare genetic resource that has got eroded over the time. Currently, it has been surveyed and spotted only in three places within South Andamans. In this study we have tried to understand the genetic relatedness between *Mangifera* and *Bouea* using morphological and molecular markers.

MATERIALS AND METHODS

Both *B. oppositifolia* and *M. andamanica* were surveyed and sampled in South Andamans. Their taxa were identified in the field with the assistance of Botanical Survey of India, Port Blair, Andamans and IPGRI descriptors for mangoes (Seeds from the said resources were collected, progeny raised and assembled at the Horticulture and Forestry Division in the Central Agricultural Research Institute (CARI) Campus, Port Blair (11°41'13.04" N; 92°43'30.16" E). Samples of *M. andamanica* were collected from Chouldhari (MA1) 11°37'55" N; 92°41'29"E, Chidiyatapu (MA2) 11°29'33" N; 92°42'27" E and Shoal Bay (MA3) 11°51'10" N; 92°44'24" E, while *B. oppositifolia* was sampled from Naya Shar (BO1) 11°34'57" N; 92°41' 29" E and Chidiyatapu (BO2 and BO3) 11°29'33" N; 92°42'27" E.

Genomic DNA sample from young leaves was extracted using modified Cetyl Trimethyl Ammonium Bromide (CTAB) method and quantified using spectrophotometer (Brown *et al*., 7). A set of 100 microsatellite primers (UBC Primer set # 9) were procured from Clonitec Inc., USA and seven (Table 1) primers were obtained from Sigma-Aldrich Inc., USA

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Table 1. List of SSR primers used for characterization of six mango species.

S. No.	Primer	Primer sequence (5´-3´)	GC content (%)	Scorable bands	Polymorphic band (s)	Size range (bp)
1.	UBC-07	AGAGAGAGAGAGAGAGT	47.05	6	3	553-1358
2.	UBC-10	GAGAGAGAGAGAGAGAT	47.05	4	2	597-968
3.	UBC-12	GAGAGAGAGAGAGAGAA	47.05	4	1	612-1357
4.	UBC-14	CTCTCTCTCTCTCTCTA	47.05	4	3	600-1238
5.	UBC-16	CACACACACACACACAT	47.05	4	1	627-1118
6.	UBC-36	AGAGAGAGAGAGAGAGYA	44.44	4	2	722-1188
7.	UBC-40	GAGAGAGAGAGAGAGAYT	44.44	5	3	600-1238
8.	UBC-55	ACACACACACACACACYT	44.44	4	2	550-850
9.	UBC-56	ACACACACACACACACYA	44.44	3	2	612-1300
10.	UBC-80	GGAGAGGAGAGGAGA	60.00	5	2	500-1138
11.	UBC-835	AGAGAGAGAGAGAGAG	50.00	3	1	400-685
12.	UBC-841	GAGAGAGAGAGAGAGAGA	50.00	4	2	450-750
13.	UBC-844	CTCTCTCTCTCTCTCTCT	50.00	3	1	400-700
14.	UBC-868	GAAGAAGAAGAAGAAGAA	33.33	4	2	455-925
15.	UBC-881	GGGTGGGGTGGGGTG	78.57	4	3	486-1356

used. Amplification was performed in 25 µl reaction mixture which consisted 25 ng of genomic DNA, 10X reaction buffer with 15 mM $MgCl_2$, 2.5 mM each of dNTPs, 0.2 mM UBC-microsatellite primer (University of British Columbia, Canada) and 1 unit of *Taq* DNA polymerase (Bangalore Genei Pvt. Ltd, Bengaluru). The reaction was carried out in a thermal cycler (PTC-200, MJ Research Inc. USA). The DNA amplification programme was set to a 4 min. initial denaturation at 94°C followed by 45 cycles of 1 min. at 94°C for denaturation, 1 min. at 42°C for annealing, 2 min. at 72°C for extension and ended with a final 10 min. extension at 72°C (Charters *et al.*, 8). Amplified products were resolved on metaphor agarose (2%) (Cambrex Bioscience Rockland, USA) with 100 bp DNA ladder using 1X TBE buffer for 3 h (5 V/cm). The gel was visualized using Gelstar nucleic acid gel stain (Lonza Rockland, USA) and documented (Vilber Lourmat, France).

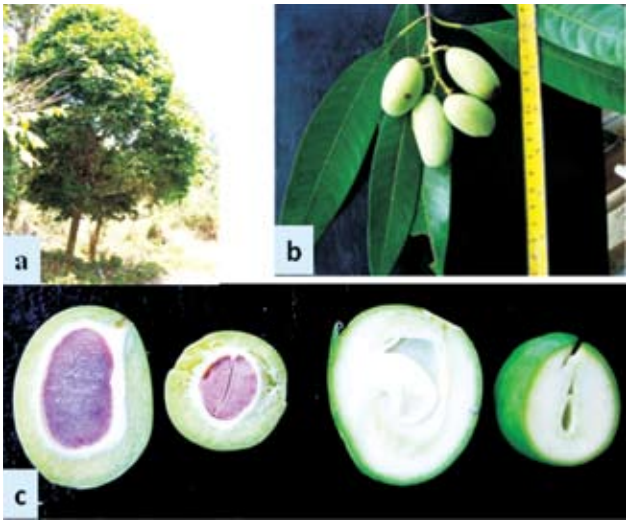

Fig. 1. a. Habit of *B. oppositifolia*, b. Unripe matured fruits of *B. oppositifolia*, c. T.S and C.S of the unripen fruits in *B. oppositifolia* and *M. andamanica.*

RESULTS AND DISCUSSION

Bouea oppositifolia is a tree that grows up to 27 m in height, with light brown, fissured bark (Fig. 1). Branchlets are often smooth, hanging and angular or flattened. It is often mistaken as a minor variant of *M. andamanica* by field foresters because of its striking similarity in terms of appearance of habit. However, *B. oppositifolia* has some unique morphological characteristics. A major taxonomical characteristic is that phyllotaxy is opposite in *B. oppositifolia*, while in *Mangifera* it is alternate. It flowers during October to November and fruits are dispersed between February and March. Flowering is profuse and the taxon is adapted to entomophily. Fruit set is also limited and the bearing is very shy. The immature fruits (drupe) in both species look alike and closely resemble each other. However, the cotyledons are brilliant violet

colour in *B. oppositifolia,* while cotyledons are white in *M. andamanica*. On ripening the fruits become golden yellow in colour in both the species. Currently in the South Andaman region, *B. oppositifolia* has become extremely rare. The rarity could be due to anthropogenic or forestry operations in the region. At present, only three reproductively matured plants have been documented in the region. Its close relative *B. macrophylla*, which is a very popular fruit tree that occurs in Thailand and Sumatra (Ediathong *et al.*, 9).

Out of the 60 primers tested in initial screening only 15 were found to amplify scorable and reproducible banding patterns (Table 1, Fig. 2). Amplification using 15 SSR primers yielded 61 products of which 29 were polymorphic (47.5%). The GC content of the primers also varied from 33.33 to 78.57. In order to be included as scorable markers, bands had to be sufficiently intense and different in size from neighbouring bands to preclude any ambiguity in scoring (Sharon *et al.,* 14). Interestingly, the bands (alleles) 1317, 1050 and 834 bp was observed uniformly in all the three *Bouea oppositifolia* accessions only with the primer $(GGGTG)_3$ of UBC-881. The most polymorphic primers were UBC-7, UBC-40 and UBC-881 with products ranging from sizes 400 to 1358 bp. The primer UBC-881 resulted in monomorphic products in sizes of 834, 1050 and 1317 bp in all the three *B. oppositifolia* accessions that can be used as species-specific molecular marker in future. A higher level of polymorphism was earlier explained using the SSR primers $(GAA)_6$ of UBC-868 (Jingjie *et al*., 10). The PCR products generated were converted into binary data and analyzed using NTSYS-PC. 2.0 (Numerical Taxonomy System, Applied Biostatistics, Inc, USA) software package and a dendrogram was generated using UPGMA cluster analysis (Fig. 3).

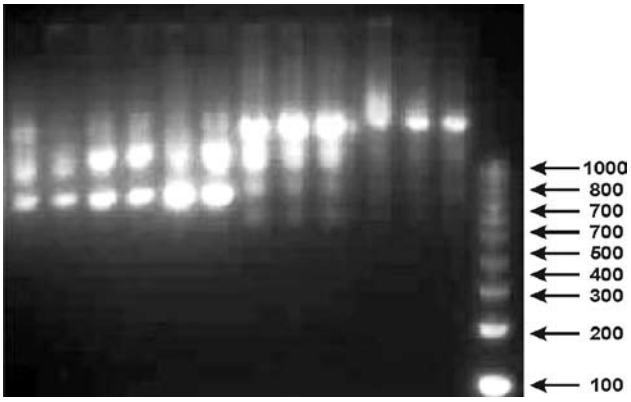

Fig. 2. PCR amplified products of SSR primers UBC-835 lanes 1-3 *M. andamanica* lanes 4-6 *B. oppositifolia* and UBC-840 lanes 7-9 *B. oppositifolia*, lanes 10-12 *M. andamanica*, lane 13-100 bp DNA ladder.

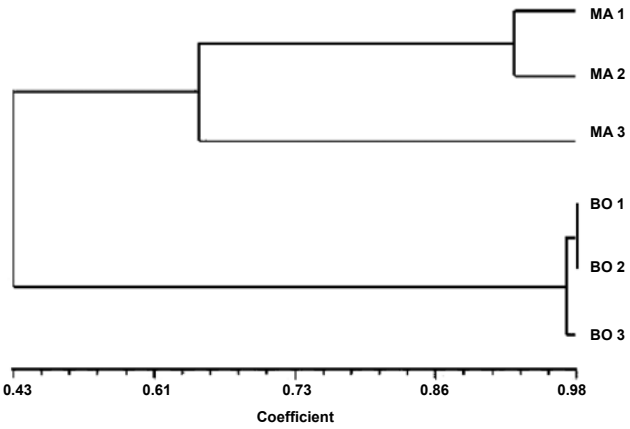

Fig. 3. UPGMA dendrogram showing genetic relationships with in the accessions of *M. andamanica* L. and *B. oppositifolia* Roxb.

The result of this study suggests that *B. oppositifolia* and *M. andamanica* are two unique genetic resources with substantial genetic variation and polymorphism across the taxa. *B. oppositifolia* is 43% genetically similar to *M. andamanica* (Fig. 3). The results obtained from SSR analysis have been widely used to assess genetic diversity and identification of specific markers in several economically important plants and are reliable than the other markers (Manimekalai *et al*., 11). In terms of intra-population variation it appears that *B. oppositifolia* is genetically highly related when compared to *M. andamanica*. The three different *B. oppositifolia* accessions divided into two clusters but exhibited a very high similarity co-efficient value of 0.97 between them. The three *M. andamanica* accessions used in the study also grouped into two clusters; however their coeffiecient of similarity was lower (0.64). The sub-cluster consisting accessions MA1 and MA2 were genetically highly related with a co-efficient value of 0.92. It is interesting to note that in case of *B. oppositifolia* one of the Chidiya Tapu accessions BO2 was found to be genetically close to BO1 (Naya Shar), which is spatially 10 Km away than its co-occurring relative BO3. Though mango is a basically an entomophilous cross-pollinated crop, earlier workers have reported the possibility of self-pollination in some cultivars. In the current study we find that three *B. oppositifolia* accessions observed are highly isolated in their respective sites and the chance for cross-pollination among these individuals is extremely remote. Thus, it is understandable that the shy reproductive output is a resultant of self-pollination. The low genomic variation noticed among the *B. oppositifolia* accessions showed that the levels of diversity loss very low and limited in their founder

populations. Though mango is a cross-pollinated crop, earlier workers have reported the possibility of self-pollination in some cultivars (Wagle, 15).

Even though *B. oppositifolia* and *M. andamanica* apparently resemble each other, this study has provided substantial morphological and genomic evidences in support of their current taxonomical status. It has also overruled the suspicion of suggesting *B. oppositifolia* as a minor variant of *M. andamanica*. The study also resulted in identification of SSR markers that can further be sequenced and utilized for identification of the genus. The results also shows that the use of SSR markers is more reliable and suitable for molecular profiling of different *Mangifera* and *Bouea* accessions from different geographical locations. It also makes us to understand spatial distance versus genetic distance could be independent. There exists tremendous scope for mango domestication using both the species. The immatured fruits of *B. oppositifolia* are very crispy and hence could be a potential source to pickle industry. With adequate care and choice of the right rootstock breeding *B. oppositifolia* can be domesticated in to a very productive horticultural resource. In posterity the high genetic relatedness among individuals could be a major bottleneck in *B. oppositifolia*. It is important to carry out intensive reconnaissance survey to locate more individuals. It would be highly desirable to conduct control pollination programs among unrelated individuals in order to improve heterozygosity.

REFERENCES

1. Ahlawat, S.P.S. 2001. Biodiversity of Andaman and Nicobar Islands. *J. Andaman Sci.* **17**: 1-2.
2. Anonymous. 1992. *UNEP, United Nations Environmental Program. Convention on Biological Diversity*, UNEP/BIO/Conf/L.
3. Anonymous. 1999. *State of the World's Forests. Food and Agricultural Organization of the United Nations*, Rome, Italy.
4. Anonymous. 2000. ITTO/RCFM, Technical guidelines for the establishment and management of *ex situ* conservation stand of tropical timber species. International Timber Trade Organization, Yokahama, Japan and Regional Centre for Forest Management, Kaula Lumpur, Malaysia, 2000.
5. Anonymous. 2005. Millennium Ecosystem Assessment 2005. **In**: *Ecosystems and Human Well Being*; *Biodiversity Synthesis*, World Resources Institute, Washington, 85 p.
6. Anonymous. 2006. IPGRI, *Descriptors for Mango* (*Mangifera indica* L.). International Plant Genetic Resources Institute, Rome, Italy.
7. Brown, S.M., Hopkins, M.S., Mitchell. S.E., Senior, M.L., Wang, T.Y., Dunean, R.R., Gonzalez-Candelas, F. and Kresovich, S. 1996. Multiple method for the identification of polymorphic simple sequence repeats (SSRs) in sorghum (*Sorghum bicolor* (L.) Moench). *Theor. Appl. Genet*. **93**: 190-98.
8. Charters, Y.M., Robertson, A., Wilkinson, M.J. and Ramsay, G. 1996. PCR analysis of oilseed rape cultivars (*Brassica napus* L. ssp. *oleifera*) using 5´- anchored simple sequence repeat (SSR) primers. *Theor. Appl. Genet*. **92**: 442-47.
9. Eiadthong, W., Yonemori, K., Sugiura, A., Utsunomiya, N. and Subhadrabandhu, S. 1999. Identification of mango cultivars of Thailand and evaluation of their genetic variation using amplified fragments b simple sequence repeat (SSR) anchored primers. *Scientia Hort.* **82**: 57-66.
10. Jingjie, H., Makota, N., Andonio, G.L., Toshikazy, K. and Tatsuhito, F. 2003. Genetic analysis of sweet potato and wild relatives using Inter-Simple Sequence Repeats (ISSRs). *Breed. Sci.* **53**: 297-304.
11. Manimekalai, R. and Nagarajan, P. 2006. Assessing genetic relationship among coconut (*Coconus nucifera* L.) accessions using inter simple sequence repeat markers. *Scientia Hort.* **108**: 49-54.
12. Mukherjee, S.K. 1948. The variation of mango and its classification. *Bull. Soc. Bengal*, **2**: 101-33.
13. Mukherjee, S.K. 1949. A monograph on the genus *Mangifera* Linn. *Lloydia*, **12**: 73-136.
14. Sharon, D., Adato, A., Mhameed, S., Lavi, U., Hillel, J., Gomalka, M., Epplen, C. and Epplen, J.T. 1995. DNA fingerprints in plants using simple-sequence repeat and minisatellite probes. *Hort. Sci.* **30**: 109-12.
15. Wagle, P.V. 1929. A preliminary study of the pollination of the Alphonso mango. *Agri. J. India*, **24**: 259-26.

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