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Molecular characterization of *Syzygium cuminii* (wild *jamun*) from A&N Islands

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ABSTRACT

***Syzygium cuminii* (wild *jamun*) is one of the most important wild fruit tree having great medicinal value. The present investigation was undertaken for characterizing wild *jamun* genotypes using DNA marker technology. Twenty three genotypes of wild *Jamun* comprising of twenty one from Andaman and Nicobar Islands and two from mainland were selected for analysis. A set of 30 ISSR and 20 RAPD primers were taken for DNA fingerprinting, among them 17 ISSR and 19 RAPD primers produced 70 and 126 amplicons out of which 43 and 70 amplicons were polymorphic having 61.4 and 55.5% polymorphism, respectively. The maximum discriminating band was obtained from primer ISSR8 and OPA7. Cluster analysis divided the all genotypes into five clusters with both RAPD and ISSR markers. The present study has not only differentiated islands genotypes from mainland but even clustered genotypes separately. The genotype collected from Car Nicobar was distinct with both RAPD and ISSR markers.**

Key words: RAPD, ISSR, genetic diversity, *Syzygium*.

INTRODUCTION

Syzygium cuminii (wild *jamun*) has a very long history of use for various medicinal purposes and currently has a large market for the treatment of diabetes, chronic diarrhea and other enteric disorders, including its use as an antimicrobial (Migliato *et al*., 11). *Syzygium* is a genus of flowering plants that belongs to the myrtle family, Myrtaceae. The genus comprises about 1100 species and has a native range that extends from Africa and Madagascar through southern Asia east through the Pacific. Its highest levels of diversity occur from Malaysia to northeastern Australia, where many species are very poorly known and many more have not been described taxonomically. Some of the edible species of *Syzygium* are planted throughout the tropics worldwide. *Jamun* seeds and bark have been prescribed in Ayurvedic medicine for the treatment of diabetes and are also used as anti-inflammatory, antipyretic, astringent, and antidiarrheal agents (Ross, 14). The seed is also used in various alternative healing systems like Ayurveda, Unani and Chinese medicines for digestive ailments. The leaves and bark are used for controlling blood pressure and gingivitis, astringent, antiscorbutic, diuretic, antidiabetic, and in chronic diarrhea and enlargement of spleen (Achrekar *et al*., 1).

Assesment of genetic diversity of different populations is important to form a basis for conservation, genetic tree improvement and promotion or domestication of populations with desirable traits. Characterisation based on morphological traits is not very influensive in evaluating genetic distance, since the degree of divergence between genotypes at the phenotypic level is not necessarily correlated with a similar degree of genetic difference and morphological traits are influenced by environmental conditions or vary with development stage of plant. Similarly, isozymes have limitation of low levels of polymorphism (Asha *et al*., 2). DNA based markers, have overcome limitations of allozyme carry high numbers of alleles. This greatly contributes to the assessment of genetic relationships among and within populations (Esselman *et al*., 5; Vinod *et al*., 18). RAPD and Inter-Simple Sequence Repeats (ISSR) has been extensively used for the identification of either species or cultivars in a wide range of plants (Ahmad *et al*., 7; Mariniello *et al*., 10) as it does not require prior knowledge about the genome of plants and are simple to use, efficient and provide a quick method for identification of plants at any developmental stage (Conner *et al*., 3). ISSR amplifies inter-microsatellite sequences at multiple loci throughout the genome (Li *et al*., 9) and permits the detection of polymorphism in microsatellites and intermicrosatellite loci without previous knowledge of DNA sequences. The present study was undertaken to assess the genetic diversity present among the different genotypes of *Syzygium cuminii* reported in Andaman and Nicobar Islands using DNA (ISSR, RAPD) based markers. This is the first attempt to study the genetic diversity of *Syzygium cuminii* in island ecosystem of India using DNA markers.

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MATERIALS AND METHODS

Altogether 23 genotypes (Table 1) were collected from different parts of South Andaman Islands, Carnicobar, Havelock Islands and mainland India. The total genomic DNA was extracted from disease-free fresh young leafs young by CTAB method (Murray *et al*., 12) with slight modification. Purity of DNA was chequed by UV spectrophotometer and running in 1% agarose gel. The quantitation of DNA in RNA free sample was done using UV spectrophotometer.

PCR reaction was performed in final volume of 20 µl containing 10x assay buffer 2.5 mM dNTPs, 0.5 unit of *Taq* DNA polymerase all from Bangalore Genei@, 10 pmols/reaction ISSR/RAPD primer and 100 ng of template DNA. ISSR was done with 30 primers of ISSR series (Clonitec) and RAPD was done with 20 primers of OPA, OPE, OPF and OPX series obtained from OPERON TECHNOLOGIES Inc. Alameda Calif. The PCR was performed by initial denaturation at 94°C for 5 min. followed by 45 cycle of denaturation at 94°C for one min., annealing at 37°C for one minutes, and extension at 72°C for two minutes and final elongation of at 72°C for 7 min. For ISSR the annealing temperature were taken as recommended by company. The PCR products were resolved on 1% Agarose gel (Bangalore Genei@) prepared in 1 × TAE buffer containing 0.5 µg/ml of the ethidium bromide at 100 V for 2.5 h.

All the genotypes were scored for presence and absence of the ISSR and RAPD bands and the data were entered into a binary matrix as discrete variables. 1 for presence and 0 for absence of character and this data matrix was subjected to further analysis. The 0/1 matrix was used to calculate similarity using Jaccard's coefficient. The resultant similarity matrix was employed to construct dendrogram using SAHN based UPGMA to infer genetic relationship (Rohlf, 13; Sneath *et al*., 16).

Table 1. Different genotypes of wild *Jamun* collected from different part of Andaman and Nicobar Islands.

Genotype code	Location
WJ 1	Gandhi park
WJ 2	Police line
WJ 3	VKV School
WJ 4	Dollygunj
WJ 5	Chauldari basti
WJ 6	Portmoot
WJ 7	CARI Mandir
WJ 8	Ferrargunj
WJ 9	CARI field
WJ 10	CARI Lab
WJ 11	Carbinscove
WJ 12	Tusnabad
WJ 13	Chauldari farm
WJ 14	Havelock
WJ 15	Car Nicobar
WJ 16	Bambooflat
WJ 17	Mount harriet
WJ 18	Chidiyatapu
WJ 19	Wandoor
WJ 20	Manpur
WJ 21	Maccapahad
WJ 22	Mainland 1 (GKVK, Bangalore)
WJ 23	Mainland 2 (National Park, Bangalore)

RESULTS AND DISCUSSION

A total of 30 ISSR and 20 RAPD primers were used to infer the genetic diversity among 23 different genotypes of *Syzygium cuminii*. Among 30 ISSR primers used, 17 produced amplification and produce a total of 70 amplicons across 23 genotypes of which 43 amplicons were found to be polymorphic with the level of polymorphism of 61.4% (Tables 1 & 2). The amplicons size with ISSR primers varies from 1.2 to 0.20 kb. The primers varied in the number of bands produced which ranged from 7 in ISSR 8 to 2 in ISSR 13 with an average of 4.1 bands per primer. Range of polymorphic bands per primer was 5 in ISSR 8 to 1 in ISSR 11. Dendrogram generated by UPGMA (Fig. 1) differenciated all the 23 genotypes at 44.8% similarity. Dendrogram could be divided into five clusters. Cluster one had genotypes of WJ 1, WJ 4, WJ 11 and WJ 14 at 81% similarity. Cluster two had genotypes WJ 2, WJ 3, WJ 5, WJ 6, WJ 7, WJ 8, WJ 9 and WJ 10 at 78% similarity. Cluster three had genotypes WJ 16, WJ 12 and WJ 13 at 76% similarity. Cluster four had genotypes of WJ 21, WJ 18, WJ 17 and WJ 19 at 70% similarity. Cluster had five genotypes WJ 15, WJ 20, WJ 22 and WJ 23 at 44.8% similarity.

Similar results were obtained from 19 RAPD primers which produced a total of 126 amplicons of which 70 amplicons were found to be polymorphic with 55.5% polymorphism. The amplicons size with RAPD primers varied from 0.35 to 2.5 kb. The primers varied in the number of bands produced which ranged from 11 in OPF2 to OPF5 with an average of 6.6 band per primer. Range of polymorphic band per primer was 7 in OPF2 to 2 in OPA11, OPQ1. Dendrogram generated by UPGMA (Fig. 2) differenciated all the 23 genotypes at 67% similarity. Dendrogram could be

Table 2. RAPD primers used and polymorphism generated.

S. No.	Primer	Sequence 5'-3'	% of GC content	Polymorphism (%)
1.	OPF 1	ACGGATCCTG	60	62.5
2.	OPF 2	GAGGATCCCT	70	63.6
3.	OPF 3	CCTGATCACC	60	57.1
4.	OPF 4	GGTGATCAGG	60	50.0
5.	OPF 5	CCGAATTCCC	60	50.0
6.	OPF 6	GGGAATTCGG	60	57.1
7.	OPF 7	CCGATATCCC	60	42.8
8.	OPF 8	GGGATATCGG	60	50.0
9.	OPF 9	CCAAGCTTCC	60	57.1
10.	OPA 6	GGTCCCTGAC	70	75.0
11.	OPA 7	GAAACGGGTG	60	60.0
12.	OPA 8	GTGACGTAGG	60	50.0
13.	OPA 9	GGGTAACGCC	70	62.5
14.	OPA 10	GTGATCGCAG	60	57.1
15.	OPA 11	CAATCGCCGT	60	33.3
16.	OPQ 1	GGGACGATGG	70	40.0
17.	OPQ 2	TCTGTCGGTC	60	50.0
18.	OPQ 3	GGTCACCTCA	60	50.0
19.	OPQ 4	AGTGCGCTGA	60	60.0

divided into five clusters. Cluster one groupped WJ 1, WJ 2, WJ 3, WJ 4, WJ 5, WJ 8, WJ 9, WJ 10 and WJ 11 at 84% similarity. Cluster two had genotypes WJ 7, WJ 12, WJ 13 and WJ 14 at 83% similarity. Cluster three had genotypes WJ 16, WJ 17, WJ 18, WJ 19, WJ 20 and WJ 21 at 82% similarity. Cluster four had genotypes WJ 22 and WJ 23 at 73% similarity. Cluster five was with genotypes WJ 6 and WJ 15 at 67% similarity.

Assessment of genetic diversity of cultivated crop plants is very important to select proper genotypes for any hybridization programme. It is an important tool of crop improvement programme and can also be helpful in protecting the biodiversity of various agro- economically important varieties of crops. RAPD and ISSR marker systems are routinely being used in ecological, evolutionary, taxonomical, phylogenic and genetic studies of plant sciences (Escribano *et al*., 4; Iqbal *et al*., 6). Overall comparison of ISSR and RAPD was indicative of greater efficiency of ISSR and RAPD markers for diversity assessment.

In the present study 23 genotypes collected from the same geographical regions normally grouped together and depict very high similarity (44.8% with ISSR and 67% with RAPD). This high genetic similarity suggested that there is more gene flow with in the agro ecological zone. High gene flow may be due to random mating with very little selection. These findings are in accordance with Shakya *et al.* (15) in *Syzygium cuminii*, and Kingdom *et al.* (8) in *Annona* spp. Selection of germplasm on the basis of the *Syzygium cuminii* dendrogram can be used for collection of appropriate parental material to improve horticultural traits. In our study genotypes WJ 15 collected from Car Nicobar Island showed maximum diversity with other Island genotypes and grouped together with mainland genotypes. Hence, this genotype can be utilized to generate sufficient genetic variability by crossing with other Island genotypes. Similarly, both the genotypes of the mainland showed significantly more diversity then among local genotypes.

In conclusion this is the first report of *Syzygium cuminii* using molecular markers in Bay Islands. Two markers (namely RAPD and ISSR) were valuable for the determination of genetic diversity and relationships amongst 23 genotypes of *Syzygium cuminii* collected from A&N Islands and mainland India. Characterization based on the ISSR molecular markers was found to be more efficient

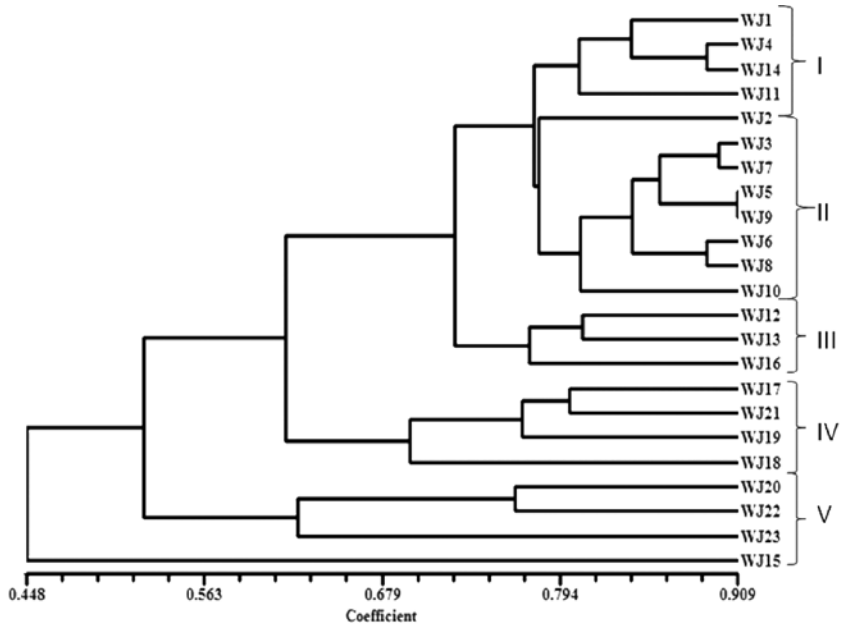

Fig. 1. Dendrogram showing genetic diversity amongst 23 genotypes of wild *jamun* by ISSR primers.

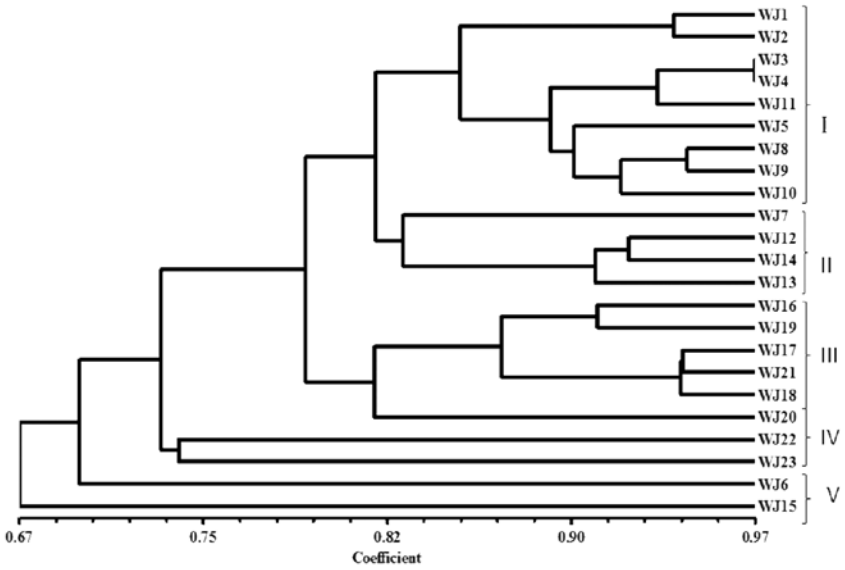

Fig. 2. Dendrogram showing genetic diversity amongst 23 wild *jamun* genotypes by RAPD primers.

than RAPD with common geographical backgrounds. Furthermore, ISSR marker was superior to RAPD marker with respect to the percentage-detection of polymorphism and discrimination between the more related genotypes of Islands. The data obtained in this study provided valuable genetic information, especially in the absence of comprehensive studies on the *Syzygium cuminii*.

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Table 3. ISSR primers used and polymorphism generated.

ISSR Primer No.	Sequence (5'-3')	Polymorphism (%)
7	AGAGAGAGAGAGAGAGT	75
8	AGAGAGAGAGAGAGAGC	71.4
9	AGAGAGAGAGAGAGAGG	60
10	GAGAGAGAGAGAGAGAT	66.6
11	GAGAGAGAGAGAGAGAC	33.3
12	GAGAGAGAGAGAGAGAA	75
13	CTCTCTCTCTCTCTCTT	00
18	CACACACACACACACAG	66.6
20	GTGTGTGTGTGTGTGTC	60
22	TCTCTCTCTCTCTCTCA	50
23	TCTCTCTCTCTCTCTCC	75
24	TCTCTCTCTCTCTCTCG	60
25	ACACACACACACACACT	50
28	TGTGTGTGTGTGTGTGA	80
32	CACACACACACACACACG	66.6
37	ACACACACACACACACAT	00
39	TGTGTGTGTGTGTGTGAA	75

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