World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Morphological and molecular characterization of wax jambu (*Syzygium samarangense)*

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World Journal of Advanced Research and Reviews, 2025, 25(01), 725-729

Publication history: Received on 30 November 2024; revised on 08 January 2025; accepted on 10 January 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.25.1.0106

Abstract

An experiment was carried out at BAU-GPC and laboratory of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh to study morphological characteristics of Wax Jambu (*Syzygium samarangense*), genetic variability, characters association, correlation and path coefficient analysis of 5 accessions of Wax Jambu collected from BAU-GPC and from Indonesia during the period from February, 2012 to March, 2013. Random amplified polymorphic DNA (RAPD) was used to characterize these accessions at molecular level. In spite of having close resemblance, the morphotypes of 5 accessions of Wax Jambu were different from each other in respect of fruit color, shape and TSS percentage with different leaf characteristics. In respect of path analysis, fruit breadth, dry matter and percent moisture contributed towards maximum phenotypic and genotypic direct effect on fruit weight indicating its importance as a selection parameter. Molecular characterization was conducted to investigate the variability of 5 Wax Jambu accessions using random amplified polymorphic DNA (RAPD) markers. Out of 4 primers screened, 2 primers were selected which gave 23 clear and bright fragments. Between the two primers used, CCAACGTCGG showed the highest level of polymorphism (83.33%). Of the 23 bands, 19 (82.7%) were polymorphic. The estimation of Nei's (1972) genetic diversity for entire accessions of Wax Jambu was 0.3339 and Shannon's information index was 0.4952. The highest genetic distance (0.9861) was observed between the accessions BAU Jamrul 3 and Indonesian Jamrul. On the other hand, the lowest distance (0.1911) was found between the accessions BAU Jamrul 1 and BAU Jamrul 2 which revealed that there is a great genetic difference among the accessions. The present lend an adequate support to the potentiality of using molecular markers as supplementary to morphological descriptors in the identification process of different morphotypes and accessions of *Syzygium samarangense*

Keywords: *Syzygium samarangense*; Molecular parameters; Genetic conservation; Jamrul; RAPD markers

1. Introduction

The Wax Jambu *(Syzygium samarangense)* is a non-climacteric tropical fruit; other names are wax apple, rose apple, java apple and water apple. The color of the fruit is usually pink, light-red, red, green, sometimes greenish white or cream-colored. The species presumably originated in Malaysia and other south East Asian countries. The fruits of Wax Jambu are eaten raw with salt or cooked as a sauce. Ninety per cent or more of the fruit is edible. In Malaysia, there are about three species which bear edible fruits, namely the water apple *(Syzygium aquem),* Malay apple *(Syzygium malaccense)* and Wax Jambu *(Syzygium samaragense).* Wax Jambu fruit is rounder and oblong in shape. It has a drier flesh compare to the other spp of Syzygium. The compositions of Wax Jambu per 100 g edible portion are: water which is more than

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90%, protein 0.7 g, fat 0.2 g, carbohydrates 4.5 g, fiber 1.9 g, vitamin A 253 IU, vitamin B1 and B2 traces, vitamin C 8 mg, energy value 80 kJ/100 g [1]. Fruit growth and development are associated in the morphological, anatomical and physiological changes [2]. As the molecular process controls morphological characters, the differences in the morphological traits are the indication of differences in gene level. Thus, study of gene pattern for cultivar differentiation and progeny legitimacy will be more precious than the other methods. It is helpful for cultivar identification, gene mapping and so on. In Wax Jambu DNA-based molecular genetic methods serve as a potential tool in resolving classification problems [3],[4]. Different molecular markers have been used to study *Syzygium* species. Shakya R [5] made use of both RAPD and the SSR markers in the molecular characterization of *S. cumini* while AFLP was used in the study of *S. sayeri* [6]. In the phylogenetic analysis of another species from the Myrtaceae family.

An attempt was made therefore to evaluate using morphological and molecular parameters for genetic resource conservation.

2. Material and methods

Five accessions of *Syzygium samarangense* constituted the experimental materials. Morphological data was taken by both visual observation and measurement. The parameters were classified as leaf parameters (leaf length, leaf breadth, petiole length, color of leaf and leaf apex), flower characteristics (time of flowering, color of petal and anther, anthesis period, fruit setting time and days to maturity), fruit characteristics (fruit length and breadth, fruit color, fruit weight, percent moisture, dry matter content, total soluble solids, sweetness). Molecular characterization was done as follows:

2.1. DNA Extraction

DNA was extracted from fresh green tender leaves following the protocol as developed by Doyle and Doyle [7]. DNA samples were evaluated both quantitatively and qualitatively (whether it had higher molecular weight or there was substantial shearing or degradation) using 1% agarose gel stained with ethidium bromide. The samples were then further diluted to a uniform concentration of 25 ng/ µl. Then the samples were preserved in -20 0 C.

2.2. Amplification of RAPD markers by PCR

The RAPD technique is based on the polymerase chain reaction (PCR). A target DNA sequence is exponentially amplified with the help of arbitrary primers, a thermostable DNA polymerase, deoxy nucleotide tri-phosphates, magnesium chloride and reaction buffer. The reaction involves repeated cycles, each consisting of a denaturation, a primer annealing and an elongation step. In the first step the DNA is made single stranded by raising the temperature to 94 0C (denaturation). In the second step, lowering of the temperature at 36 0C annealing temperature, the primer binds to their target sequences on the template DNA (annealing step). In the third cycle, temperature is chosen as where the activity of the thermostable Taq DNA polymerase is optimal, i.e., usually 72 0C. The polymerase then extends the 3' ends of the DNA-primer hybrids towards the other primer-binding site. Since this happens at both primer-annealing sites on both the DNA strands, the target fragment is completely replicated. Repeating these three step cycles 40 to 50 times results in the exponential amplification of the target between the 5' ends of the two primer binding sites. Amplification products are separated by agarose gel electrophoresis and visualized by ethidium bromide staining.

2.3. Primer selection

Primers were evaluated on the basis of intensity or resolution of bands, repeatability of markers, consistency within individuals and potentiality for discriminating different accessions. To confirm the reproducibility of RAPD markers, the selected two primers were screened two times on the same samples necessary, especially for Taq polymerase.

2.4. DNA amplification and electrophoresis for final PCR

DNA amplification reactions were carried out in l0 µl each sample containing 4 µl of genomic DNA working sample, l µl Taq buffer, 1 µl dNTPs (250 µM), 0.25 µl selected primers, 0.2 µl Taq DNA polymerase and 3.55 µl de-ionized water. A pre-mixture was prepared in the order to reaction buffer, dNTPs, selected primers, and sterile de-ionized water. Then Taq DNA polymerase was added and mixed properly, and centrifuged for 5 -10 sec at 4 0C temperature and at 6000 rpm. After that 4 µl of genomic DNA working sample and 6 µl pre-prepared mixture was taken in the PCR tubes and centrifuged for 5 -10 sec at 4 0C temperature and 6000 rpm. PCR was performed at initial denaturation at 93 0C for 4 min followed by 40 cycles of 1min denaturation at 94 0C, l min annealing at 36 0C and 2 min extension at 72 0C with a final extension of 72 0C for l0 min using a Thermal Cycler (Eppendorf Thermal Cycler). After completion of cycling program, reactions were held at 4 0C. The PCR products were visualized under UV light and photographed using Gel Gam Polaroid camera. The size of amplification products was estimated from hyper ladder II.

2.5. RAPD data analysis and dendrogram construction

Following electrophoresis, the sizes of amplification products were estimated by comparing the migration of each amplified fragment with that of a known size fragments of molecular weight markers: DNA hyper ladder II (Bioline) as base pair ladder. All distinct bands or fragments (RAPD markers) were thereby given identification numbers according to their position on the gel and scored visually on the basis of their presence (l) or absence (0), separately for each individual and each primer. The scores obtained using all primers in the RAPD analysis were then combined to create a single data matrix. This was used for estimating polymorphic loci, Nei's [8] gene diversity, genetic distance (D) and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among the populations using computer program POPGENE of Version 1 .31 [9].

3. Results and discussion

The materials of 5 accessions of *Syzygium samarangense* were screened with four RAPD primers. Among those two primers had shown polymorphism in their amplification profile in 1.5% agarose gel. Table 1 containing the sequences and information of the primers is provided. Between the two primers 5' CCAACGTCGG 3' showed highest polymorphism (83.33%) among the 5 accessions. The frequencies of polymorphic band obtained varied in the two primers. The values of gene diversity and Shannon's information index for different accessions of Wax Jambu across all loci are shown in Table 2. The estimate of Nei's [8] genetic diversity for entire accessions of Wax Jambu was 0.33 and Shannon's information index was 0.49.

3.1. Phylogenetic dendrogram

UPGMA dendrogram based on Nei's (1972) [8] genetic distance indicated the phylogenetic relationship and segregation patterns among 4 Wax Jambu germplasm of Bangladesh and one germplasm of Indonesia. The 5-germplasm used in the present investigation grouped into two major clusters, 'a' and 'b' (Fig. 1). Cluster 'a' forming with only one Wax Jambu accession, Indonesian Jamrul collected from Indonesia. Other 4 Wax Jambu accessions were formed into cluster 'b' which subsequently separated into two sub-clusters, 'c' and 'd'. Three Wax Jambu accessions were formed in sub-cluster 'c' which might be due to morphological and molecular distinctness from other germplasm resources and to that of some extent of similarity within these three Wax Jambu accessions. Sub-cluster 'd' formed with only one Wax Jambu accession and this was BAU Jamrul 3 which indicated distinctness of the germplasm from those in sub-cluster 'c'. The germplasm resources of Wax Jambu sourced from BAU, Mymensingh were found to be present in cluster 'b' specifically sub-cluster 'c' supporting geographical similarity, although all the clustering patterns in the present study did not accord with geographical sources due to having genetically diversed germplasm resources.

Table 1 Characteristics of RAPD primers

Primer code	Sequences (5′-3′)	Total number of bands scored	Polymorphic band	Proportion of polymorphic loci (%)
OPA17	GAAACGGGTG	11	9	81.81
OPA19	CCAACGTCGG	12	10	83.33

Table 2 Summary of genetic diversity (h) statistics and Shanon Information Index (I) for all loci.

Locus	(h)	(I)	Locus	(h)	(I)
OPA17-1	0.3200	0.5004	OPA19-2	0.4800	0.6730
OPA17-2	0.0000	0.0000	OPA19-3	0.3200	0.5004
OPA17-3	0.4800	0.6730	OPA19-4	0.4800	0.6730
OPA17-4	0.4800	0.6730	OPA19-5	0.3200	0.5004
OPA17-5	0.4800	0.6730	OPA19-6	0.0000	0.0000
OPA17-6	0.3200	0.5004	OPA19-7	0.4800	0.6730
OPA17-7	0.3200	0.5004	OPA19-8	0.3200	0.5004

OPA17-8	0.4800	0.6730	OPA19-9	0.3200	0.5004
OPA17-9	0.0000	0.0000	OPA19-10	0.3200	0.5004
OPA17-10	0.3200	0.5004	OPA19-11	0.4800	0.6730
OPA17-11	0.3200	0.5004	OPA19-12	0.3200	0.5004
OPA19-1	0.3200	0.5004			

Table 3 Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values

	G1	G2	G3	G4	G5
G1	****	0.8261	0.5652	0.6522	0.6522
G2	0.1911	****	0.7391	0.6522	0.4783
G3	0.5705	0.3023	****	0.4783	0.2174
G4	0.4274	0.4274	0.7376	****	0.5652
G5	0.4274	0.7376	0.9861	0.5705	****

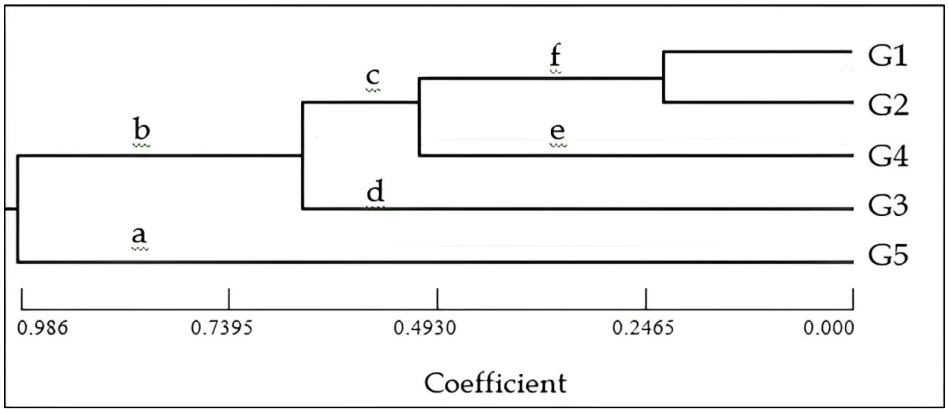

Figure 1 Dendrogram of clustering of *Syzygium samarangense* by UPGMA using molecular data

4. Conclusion

From the result of the experiment the following conclusion can be drawn:

- A wide variability is present among the studied accessions of Wax Jambu.
- Selection of superior genotypes from the collected accessions will be helpful to increase production through breeding programme which in turn could improve varietal characteristics.

In this study, the RAPD profiles of studied Wax Jambu accessions would be useful in monitoring genetic stability. However, further studies on molecular characterization using other molecular techniques such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) should be tried in order to generate markers for future breeding programme. Also, further pharmacological and Phytochemical studies are required for systemic investigation of this plant.

Compliance with ethical standards

Disclosure of Conflict of interest

No conflict of interest is disclosed.

Statement of ethical approval

'The present research work does not contain any studies performed on animals/humans' subjects by any of the authors.

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