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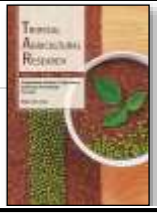

RESEARCH

Leaf Morphological and Molecular Characterization of Selected Accessions of Jackfruit (*Artocarpus heterophyllus* Lam) in the Field Gene Bank- Gannoruwa Sri Lanka

P.G.N.N. Dayarathna[1], V.N.S. Sirimalwatta[1,2]* and N.U. Jayawardana[1,2]

[1]*Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka*
[2]*Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Sri Lanka*

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Sirimalwatta, V.N.S.
https://orcid.org/0000-0001-8275-8269

ABSTRACT

Jackfruit *(Artocarpus heterophyllus* Lam) belongs to the family Moraceae. Selected jackfruit accessions in Gannoruwa Fruit Crop Research and Development Station were studied based on molecular markers and leaf morphology. The main objective of the study was to evaluate the morphological and molecular diversity of specific jackfruit accessions collected from an orchard in Udayalkattikulam Mullaitivu, Sri Lanka. Morphological studies were conducted on two recommended accessions (*Maharagama* and *Mandoor*) and 11 other accessions. Four accessions were subsequently selected for molecular analysis. Leaf morphology was studied using the descriptors provided by the International Plant Genetic Resources Institute (IPGRI). Molecular characterization focused on the Internal Transcribe Spacer (*ITS*) region in ribosomal RNA. Morphological data were analyzed using PAST 4.03. A scatter plot was obtained from the Principal Component Analysis (PCoA). *ITS* sequence data were analyzed by MEGA 11 and DnaSP 5.10. Based on the leaf morphology, accession ACC-17-267 was found to be morphologically distinct from other accessions, while Maharagama and ACC-15-27 showed close morphological similarity. The four accessions (ACC-1-275, ACC-3-265, ACC-7-249, ACC-8-271) selected for molecular analysis were closely related to *Mandoor* for their leaf morphology. Molecular analysis revealed no genetic distance between *Maharagama Mandoor* and ACC-7-249. According to the dendrogram resulting from MEGA 11, *Maharagama*, *Mandoor* and ACC-7-249 clustered into one group. Diversity analysis revealed that the selected Jackfruit accessions exhibited high haplotype diversity and moderate level of nucleotide diversity (Pi: 0.115). These findings suggest that the *ITS* locus is an effective marker for characterizing the genetic diversity of Jackfruit.

*Corresponding author- nipuni.siri@agri.pdn.ac.lk

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.), is a member of the family Moraceae which has 50 genera. of them, ~33 species and 8 genera are found in Sri Lanka (Peiris, 2003). Jackfruit is believed to have originated in the Western Ghats of India and thrives in tropical and subtropical areas with warm and humid climates (Clement and Weiblen, 2009).

In Sri Lanka, jackfruit is often consumed as a primary and secondary staple by the locals. Jackfruit is particularly important to Sri Lankans since it served as a much-needed alternative to Sri Lanka's primary staple- rice during times of food shortage and rationing, when starvation is looming threat (Medagoda, 2007). Several cultivars of jackfruit are cultivated in Sri Lanka including *Father long*, *Maharagama*, *Kothmale*, *Hirosh*, *Mandoor* and *Singapore* or Ceylon *Jackfruit* (Agalakumbura, 2021). Also, there are two fruit types have been identified in Jackfruit based on the firmness of the edible flesh (Pushpakumara and Harris, 2007). In Sri Lanka, the soft flesh (SF) type is known as *Wela* and the firm flesh (FF) type is referred to as *Waraka* (Medagoda, 2007). Consumers and growers generally prefer cultivating the firm flesh type over the soft flesh type due to qualities and higher export potential (Pushpakumara and Harris, 2007).

Understanding genetic diversity and conducting molecular characterization of crop plants are important for identifying superior genotypes for cultivation, developing effective sampling, management and conservation strategies, and promoting species as commercial crops. The diversity of jackfruit germplasm has primarily been assessed using morphological parameters which are highly influenced by environment factors (Zerega et al., 2010; Williams et al., 2017). However, morphological parameters often lack the necessary detail to differentiate organisms at higher taxonomic levels such as phyla, class, order, and family (Hall, 2004).

Genetic characterization of germplasm using molecular markers in addition to morphological characterization is useful because DNA molecules-based markers enable rapid detection of genetic variations and are less influenced by the environmental factors (Vicente and Fulton, 2003). Although molecular marker-based characterization is relatively expensive, it provides high speed and accuracy in the genetic characterization of germplasm (Mursyidin and Setiawan, 2023). Molecular markers that have been utilized most commonly in the assessment of the genetic diversity of jackfruit are RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), ISSR (Inter- Simple Sequence Repeat), and SSR (Simple Sequence Repeats). These markers target the variations or polymorphisms (Insertions, deletions, translocations, duplications, and point mutations) within a nucleic acid sequence between various individuals (Nakintu et al., 2019).

In Sri Lanka Jackfruit field gene banks are located at Gannoruwa (Central Provice) and Horana (Western Province). The Gannoruwa Plant Genetics Resources Center (PGRC) holds the national responsibility for conserving the genetic diversity of e food crop and their wild relatives in Sri Lanka. Its mandate includes planning and conducting plant exploration, collection, introduction, evaluation, documentation and conservation of the genetic diversity of food crops and their wild relatives for the benefit of present and future generations. The fruit crop research and development station at Gannoruwa maintains 11 jackfruit accessions originally collected from orchards at Udayalkattikulam, Mulllaitivu. However, the morphology and molecular diversity of these accessions are yet to be studied. These accessions were planted in 2018 at the Gannoruwa field gene bank. Currently, little is known about the genetic diversity of jackfruit in the country. This lack of knowledge poses risk to the crop's genetic resources, as farmers tend to selectively grow varieties based on market demand.

Nuclear markers, such as *ITS,* are widely used in molecular biology and genetic studies to analyse genetic diversity, phylogenetics, and evolutionary relationships among plant species. *ITS* regions are highly conserved across plant taxa and therefore, the amplified

ITS fragments can then be sequenced and analyzed to assess genetic variation and relationships among varieties or populations (Thinh et al., 2020). Due to the universality and simplicity of amplifying and showing a high mutation rate, the *ITS* provides better resolution in the estimation of the genetic diversity of most plants (Lee et al., 2017). Some plants that are successfully characterized using *ITS* marker are *Acanthopanacis*, *Anoectochilus*, *Dioscorea*, *Litsea*, *Uncaria*, and *Zanthoxylum* (Mursyidin and Setiawan, 2023).

Although a few studies have been conducted to identify the SF and FF types of jackfruit in Sri Lanka, to date, no one has attempted to estimate the genetic diversity of different jackfruit accessions in the country. Therefore, the current study was conducted to explore the morphological and genetic diversity of selected jackfruit accessions available at the field genebank-Gannoruwa Sri Lanka.

METHODOLOGY

Study was conducted at the Department of Agricultural Biology of the Faculty of Agriculture, University of Peradeniya from November 2023 to February 2024. There were 11 jackfruit accessions available at the Gannoruwa field gene bank collected from the orchard at Udayalkattikulam, Mullaitivu (ACC1-275, ACC3-265, ACC7-249, ACC8-271, ACC15-27, ACC16-276, ACC17-267, ACC24-225, ACC25-85, ACC26-250, ACC31-266). All were characterized morphologically and four accessions (ACC 1-275, ACC 3-265, ACC 7-249, ACC 8-271) were selected randomly for molecular characterization. Maharagama and Mandoor accessions available at the same orchard were selected as the recommended varieties.

Morphological analysis

Morphological analysis was performed for 11 accessions of jackfruit including two recommended cultivars using leaf morphological characters. The leaf morphological parameters including leaf shape, leaf apex shape, leaf base shape, leaf margin, leaf color petiole shape petiole grooves were measured from ten fully expanded representative leaves from one accession at same height and same maturity stage of each tree descriptively according to the descriptors developed for Jackfruit by the International Plant Genetic Resources Institute (IPGRI). Leaf length, leaf width and petiole length were measured using a ruler.

Molecular analysis

Tender emerging leaves of selected jackfruit accessions (Maharagama, Mandoor, ACC-1-275, ACC-3-265, ACC-7-249, ACC-8-271) were collected from Gannoruwa field gene bank in the morning and after covering with aluminum foil, leaves were brought to the laboratory. Leaf surfaces were washed with sterile water and then wiped with 70% ethanol and stored at 4°C zip-lock bags for further processing.

Genomic DNA of the selected *Artocarpus* were extracted using CTAB (Cetyl Trimethyl Ammonium Bromide) method developed by Doyle and Doyle (1987) with some modifications. Concentration and purity of extracted DNA were determined by using Nanodrop Spectrophotometer. Purified DNAs were then amplified by ITS primers (forward 5'-AGA GCC GTG ATC TTC GTT GC- 3', reverse 5'-CCG ATT GAA TGG TCC GGT GA-3') in PCR. The cycle parameters were initial denaturation (94°C for 5 m); denaturation (94°C for 30 s); annealing (58.5°C for 30 s); extension (72°C for 45 s); and final extension (72°C for 7 m). A 20 µl PCR solution mixture was prepared with 10 µl of 2x Go Taq® Green Master Mix, 1 µl of forward and reverse primers and 1-3 ng of template DNA (4 µl). The PCR products were visualized in agarose gel (1%) electrophoresis and purified PCR products were sequenced using the Sanger method by Sanger Genelabs of Genelabs Medical (Pvt) Ltd in Nawala, Sri Lanka.

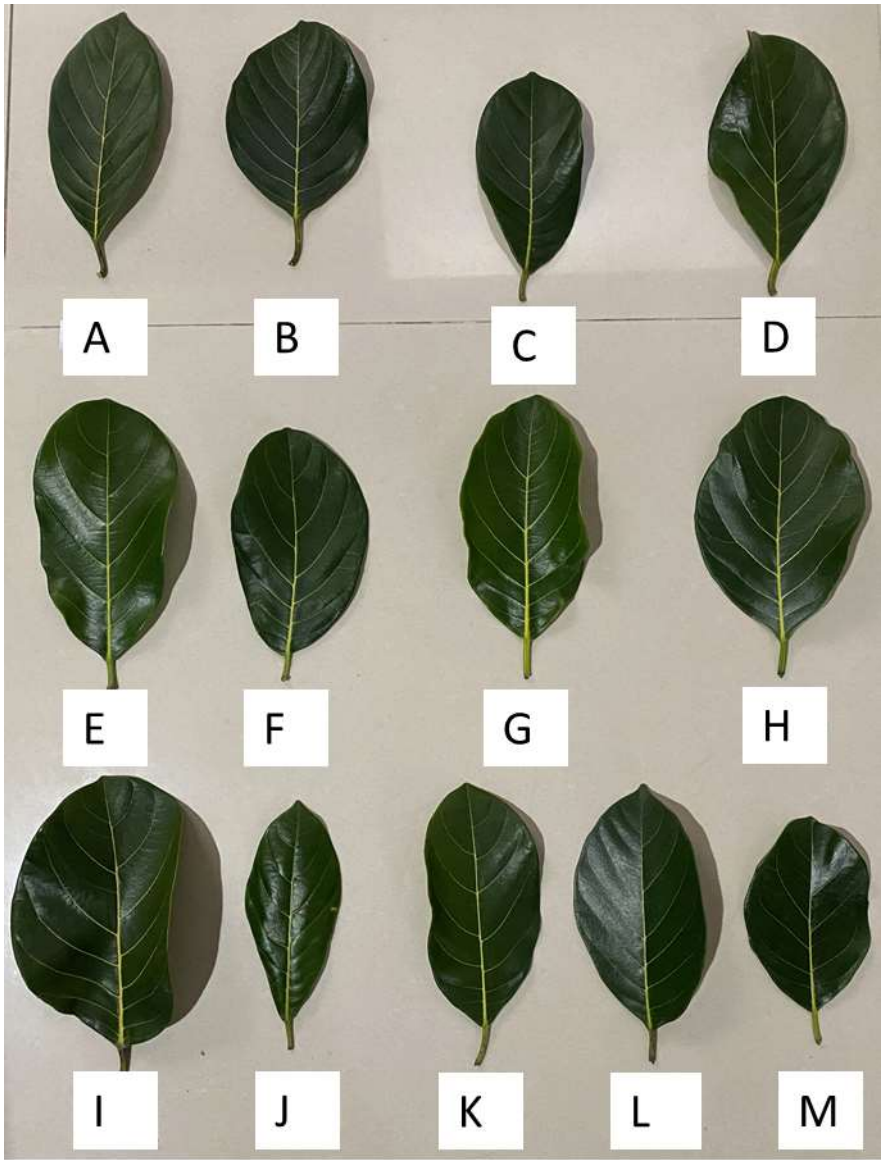

Figure: 01- Leaf Morphology of selected Jack fruit accessions (A- Maharagama, B- Mandoor, C-ACC-1-275, D- ACC-3-265, E-ACC-7-249, F- ACC-8-271, G- ACC-15-27, H- ACC-16-276, I-ACC-17-267, J-ACC-24-225, K-ACC-25-85, L-ACC-26-250, M-ACC-31-266)

Data analysis

Quantitative parameters of leaves were subjected to ANOVA procedure and the qualitative morphological parameters of leaves were descriptively assessed. Leaf morphological data were analyzed using clustering and ordination methods. Hierarchical Cluster Analysis (CA) and Principal Component Analysis (PCA) were performed using the statistical software PAST (Version 4.03). The characters were defined as either ordinal, nominal, binary, continuous or as unspecified prior to the analyses. Cluster Analysis (CA) was applied to the matrix of all variables to obtain preliminary information about the grouping of specimens based on overall morphological similarity. The cluster solution was selected from the best suitable algorithm where Euclidean distance was used to calculate the similarity measures with the 'paired group' (UPGMA) option and the Single Linkage algorithm with the highest Cophenetic correlation value.

ITS sequences of *Artocarpus* were reconstructed and analyzed manually by the MEGA 11 software. All were then aligned using

Multiple Sequence Alignment (MSA) and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram was generated with the Jukes-canter model as the best fitting model. The phylogenetic tree was evaluated by bootstrap statistics (1000 replicates) and pairwise distance analysis was done by the same software. Haplotype and haplotype diversity were analyzed by DnaSp Ver. 5.10.

RESULTS AND DISCUSSION

Selected jackfruit accessions were cultivated at *Gannoruwa* Fruit Crop station in 2018 and the plants were obtained from grafting from the mother plant. All the accessions were about 2m-3m in height and all were at the vegetative stage and no reproductive structures (flowers/ fruits) were observed at the time of data collection. Therefore, only leaf morphology characters were evaluated.

According to the observed results, leaf length, width and petiole length are not significantly different in each accession. Cluster Analysis (CA) was applied to the matrix of all variables associated with leaf morphology. The cluster solution was selected from the best suitable algorithm where Euclidean distance was used to calculate the similarity measures with the 'paired group' (UPGMA) option. The dendrogram resulted from the CA (cophenetic correlation coefficient = 0.94) recognized two distinct diversity clusters.

According to the dendrogram (Figure 02), ACC-15-27 and *Maharagama* cluster together. With respect to the leaf morphology parameters (Table 01), both accessions have narrowly elliptic leaf blade with acute apex, cuneate leaf base with flattened petiole with grooves. Whereas ACC-8-271, ACC-3-265, ACC-26-250, Mandoor, ACC-25-85, ACC-1-275, ACC-16-276 and ACC-7-249 form another clade. Most of the accessions are related to *Mandoor* accession with respect to the leaf morphology.

As a similar study, leaf morphology of selected SF and FF jackfruit accessions in Sri Lanka were studied by Karunarathna *et al*, (2018). According to the recorded data, the SF leaves have significantly longer tips compared to the FF type and the leaf shape was found to be elliptical in SF type and obovate in FF type indicating its potential applicability in differentiating trees of two different flesh types (Karunarathna et al., 2018).

Similar Results were observed in the scatter diagram obtained from the Principal Components Analysis (PCA) (Figure 3). The first four (principal) eigenvalues recovered from the PCA (5.90, 1.27, 0.41 and 0.20) accounted for 98% of the total variance (74.07%, 15.97%, 5.15% and 2.65% respectively). The leaf length and the leaf width were the most contributing characters for component 1 (PC 1) and component 2 (PC 2) respectively.

According to the UPGMA obtained from MEGA 11 for ITS sequenced region (Figure:04), *Mandoor*, *Maharagama* and ACC-7-249 form a one clade proving they are more closely related to each other with compared to ITS region. Also, ACC-8-271, ACC-3-265 and ACC-1-275 form separate clades.

Pairwise genetic distances for ITS sequences between selected Jackfruit accessions (Table: 02) shows that the distances between *Maharagama- Mandoor*, Maharagama-ACC-7-249 and Mandoor-ACC-7-249 are 0.000, indicating they are very closely related. According to the morphology data, *Maharagam* accession separate from *Mandoor* and other selected four accessions and form two separate clades, in molecular phylogenetic tree *Maharagama*, *Mandoor* and ACC-7-249 form a one clade and separate from other three accessions.

Table 01: Variation among the morphological parameters of leaves of the selected jackfruit accessions

Accession	Leaf blade shape	Leaf apex shape	Leaf base shape	Leaf margin	Leaf colour	Petiole shape	Petiole grooves(present/absent)	Leaf length	Leaf width	Petiole length
Maharagama	Narrowly elliptic	Acute	Cuneate	Entire	Light green	Flattened	Present	18.366[a]	8.23[a]	2[a]
Mandoor	Elliptic	Acuminate	Rounded	Entire	Light green	Flattened	Present	17.63[a]	8.93[a]	2.73[a]
AAC-1-275	Elliptic	Acuminate	Rounded	Entire	Light green	Flattened	Present	18.1[a]	8.06[a]	2.03[a]
ACC-3-265	Elliptic	Acuminate	Rounded	Entire	Light green	Flattened	Present	18.66[a]	9.6[a]	2.1[a]
ACC-7-249	Elliptic	Acuminate	Rounded	Entire	Light green	Flattened	Present	19.66[a]	9.5[a]	1.8[a]
ACC-8-271	Elliptic	Acute	Rounded	Entire	Light green	Flattened	Present	17.46[a]	9.26[a]	1.7[a]
ACC-15-27	Narrowly elliptic	Acute	Cuneate	Undulate	Light green	Flattened	Present	19.6[a]	9.46[a]	2.36[a]
ACC-16-276	Elliptic	Acute	Rounded	Undulate	Light green	Flattened	Present	18.5[a]	9.93[a]	2.26[a]
ACC-17-267	Broadly elliptic	Acuminate	Rounded	Entire	Light green	Flattened	Present	22.53[a]	14.4[a]	2.96[a]
ACC-24-225	Narrowly elliptic	Acute	Cuneate	Entire	Light green	Flattened	Present	15.73[a]	6.53[a]	1.76[a]
ACC-25-85	Elliptic	Acute	Rounded	Entire	Light green	Flattened	Present	18.43[a]	8.73[a]	2.36[a]
ACC-26-250	Elliptic	Acuminate	Rounded	Entire	Light green	Flattened	Present	18.33[a]	9.16[a]	2.16[a]
ACC-31-266	Elliptic	Acuminate	Oblique	Entire	Light green	Flattened	Present	15.33[a]	8.66[a]	1.66[a]

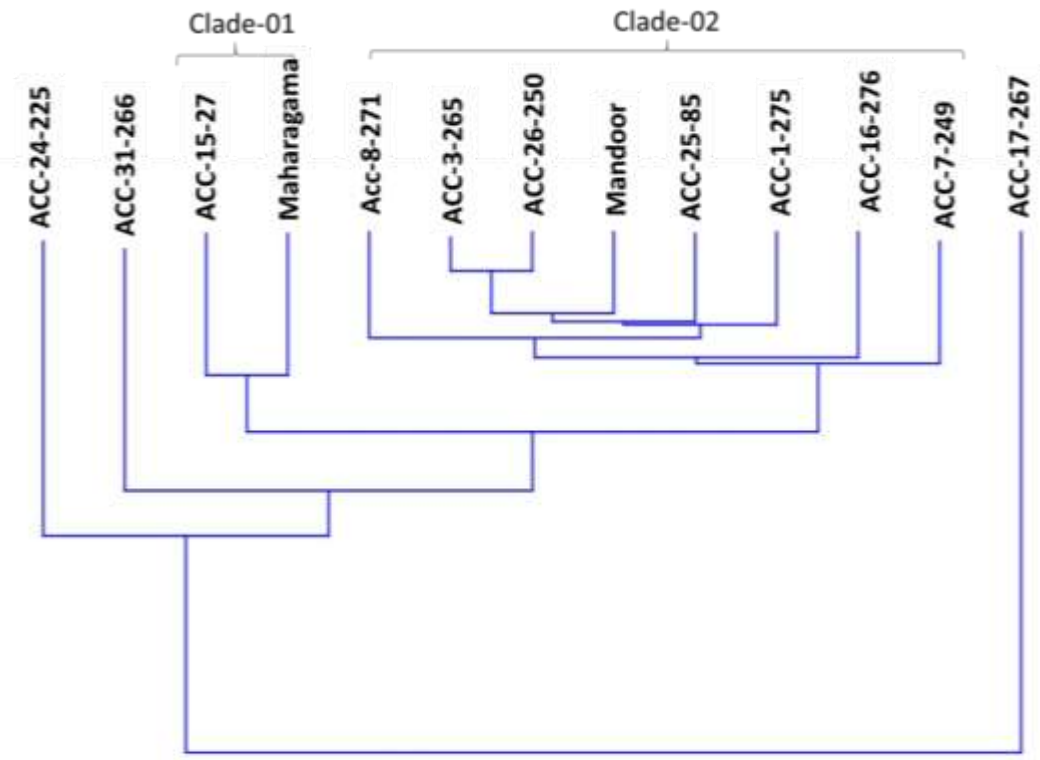

Figure: 02 Dendrogram resulting from cluster analysis forms two main clades

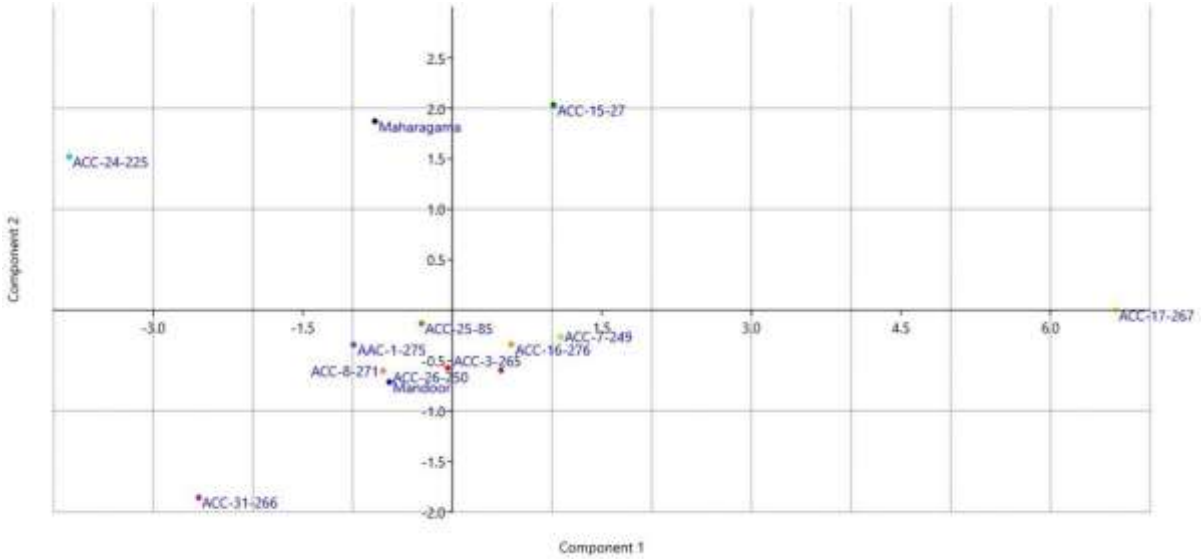

Figure: 03-Grouping of selected jackfruit accessions based on Principal Component Analysis (PCA).

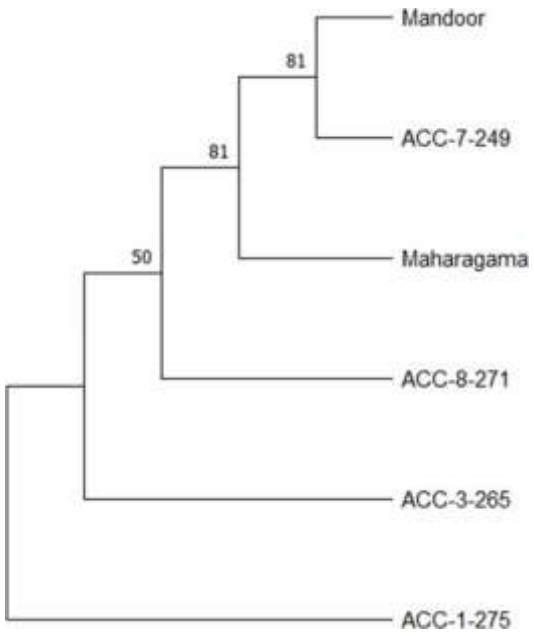

Figure 04: UPGMA dendrogram based on Jaccard similarity coefficient generated for ITS sequenced data showing the relationship of selected Jackfruit accessions to recommended accessions. Values on nodes indicate the bootstrap analysis 1000 times.

Table 02: Pairwise genetic distances for ITS sequences between selected Jackfruit accessions

	Maharagama	Mandoor	ACC-1-275	ACC-3-265	ACC-8-271	ACC-7-249
Maharagama						
Mandoor	0.000					
ACC-1-275	0.239	0.236				
ACC-3-265	0.015	0.015	0.134			
ACC-8-271	0.014	0.011	0.182	0.023		
ACC-7-249	0.000	0.000	0.161	0.008	0.012	

A total of six haplotypes were observed for ITS sequence of Jackfruit and overall haplotype diversity was Hd: 1.0. Haplotype Diversity measure the genetic variation within a population based on the distribution and frequency of haplotypes. Haplotype is a specific combination of alleles at multiple loci along a chromosome or DNA sequence that are inherited together from a single parent (Daley et al., 2024). High haplotype diversity suggests a complex evolutionary history with diverse ancestral contributions, including mutations, genetic drift, migration, and selection events low haplotype diversity suggests isolation, genetic bottlenecks, or founder effects, where a small number of individuals with limited genetic variation establish a new population. Haplotype diversity 0 indicates low diversity, meaning all individuals in the population have the same haplotype and 1 indicates high diversity, meaning each individual in the population has a unique haplotype (Buntjer et al., 2015).

Nucleotide diversity for Jackfruit accessions was Pi: 0.115. Nucleotide diversity is the genetic variation within a population at the nucleotide level. Nucleotide diversity measures the average nucleotide divergence between sequences in the population. Higher

nucleotide diversity values indicate greater genetic variation within the population which can enhance the population's ability to adapt to environmental changes and reduce the risk of extinction. Lower values suggest lower genetic diversity (Islam et al., 2022).

CONCLUSION

Morphological results showed that ACC-17-267 morphologically different from other accessions. *Maharagama* and ACC-15-27 closely related to each other. Also, selected four accessions for molecular analysis were morphologically related with *Mandoor*. According to the molecular data observed, no genetic distance between *Maharagama, Mandoor* and ACC-7-249 accessions were observed. Also, they form a separate taxon. Diversity analysis results revealed that the selected Jackfruit accessions has a higher haplotype diversity and low nucleotide diversity. Therefore, the ITS locus can be effectively employed to characterize the genetic diversity of Jackfruit. As future directions, it is recommended to extend the study with other possible molecular markers to study further on genetic diversity and incorporation of other morphological characters (inflorescence characters, fruit characters, seed characters etc.) to obtain best morphological characterization results.

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