Junior Science Communications
Faculty of Applied Sciences, UiTM Shah Alam
https://journal.uitm.edu.my/ojs/index.php/JSC

E-ISSN 2637-0689
Vol 69 2026

Colloquium on Applied Sciences 1 2026
19-22 January 2026, Faculty of Applied Sciences, UiTM Shah Alam, Malaysia

Morphological and Genetic Identification of Rambutan Cultivars (*Nephelium Lappecium*)

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Structured Abstract

Background: Rambutan (*Nephelium lappaceum*) is one of the exotic fruit native to southeast Asia countries with 52 varieties currently registered in Malaysia. High quality varieties became increasingly importantto strengthenand revitalise the Malaysian rambutan industry. This study aimed to comprehensively characterise the phenotypic traits and identify the genetic species of rambutan accessions collected from Kampung Selamat, Tasek Gelugor, Pulau Pinang and Kampung Padang Balang, Selangor Malaysia.

Methods: Genomic DNA was extracted using a modified (CTAB) protocol with adjustable reagent composition. The use of a consistent and reliable molecular biomarker is essential for distinguishing cultivars and elucidating genetic relationships. In this study, polymerase chain reaction (PCR) were performed using a chloroplast DNA biomarker, the ribulose-bisphosphate carboxylase (rbcL) gene. Bioinformatic analyses included multiple sequence alignment, pairwise distance estimation and phylogenetic tree construction.

Results: The primary morphological characteristic distinguishing the rambutan cultivars was the colour of the outer peel. The unknown sample exhibited a bright red peel with yellow and orange shades on the spines, whereas the Deli cultivar showed a deep red colour and the Gading cultivar ranged from yellow to golden yellow. Two sequence datasets were subjected to BLAST analysis against the NCBI GenBank database, and both were identified as highly similar to the same eight genetic variants. Multiple sequence alignment of a total of ten sequences revealed that the rbcl gene region was highly conserved, with bootstrap support values ranging from 8% to 71%. Phylogenetic analysis using the neighbour-joining method demonstrated that all sequences clustered within the same species. The formation of several groups or subclades reflects their similar genetic patterns, further supporting this classification.

Conclusion: Analysis using the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene revealed near-identical sequences between the Deli cultivar and the unknown samples. However, the findings indicate that the rbcL gene lacks sufficient resolution for discriminating specific cultivars within the *Nephelium* species. Therefore, further studies employing additional high-resolution molecular markers or the development of cultivar-specific primers are recommended to improve the accuracy of rambutan cultivar identification.

Keywords: Rambutan, CTAB protocol, rbcL, phylogenetic analysis

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