Structural and Functional Insights into *Rc* Allelic Variation Governing Pericarp Pigmentation in Sri Lankan Traditional Rice Germplasm

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The *Rc* gene encodes a basic helix-loop-helix (bHLH) transcription factor that regulates proanthocyanidin biosynthesis and pericarp pigmentation in rice. Allelic variation at this locus determines red, brown, and white pericarp colours. Pericarp colour was visually evaluated using the Rice Germplasm Characterization Catalogue (PGRC) in previous studies (33 pigmented and 14 non-pigmented). This study evaluated *Rc* allelic diversity and its structural-functional implications in 47 Sri Lankan traditional rice varieties using *Nipponbare* as a reference. The *Rc* gene sequences of 47 Sri Lankan rice varieties present in the International Rice Research Institute's 3000 Rice Genome Project (IRRI 3K RGP) database were retrieved from Rice SNP-Seek database. Cluster analysis of the amino acid sequences (AA) identified nine distinct clusters, from which 24 representative varieties were selected based on amino acid length and colour (19-pigmented, 5-non-pigmented). Protein lengths ranged from 351 to 668 amino acids and correlated with pericarp colour. Full-length alleles (664–668 AA) were associated with 11 pigment varieties, while truncated alleles (351–473 AA) were linked to white and seven pigment pericarp varieties. ProtParam, SWISS-MODEL homology modelling, and PyMOL analyses revealed significant differences in physicochemical properties, molecular weight, aliphatic index, and secondary structures according to length. Domain (NCBI CDD) and motif (Motif Scan) analyses showed that all white pericarp varieties possessed only one domain and two motifs. Four white varieties carried the known 14-bp deletion at position 1408 in exon 6. Notably, *Kuruluwee* variety lacked this deletion but contained a C1353A substitution that introduced a premature stop codon. Sixteen pigmented varieties had two domains and four motifs, including the critical bHLH transcriptional activation-like domain, while light brown/striped varieties (6) typically showed three motifs. Two pigmented exceptions (*Galawaka handeran*, and *Kula karuppan*) with only one domain and two motifs were further analysed, revealing unique SNP(A897G) in *Galawaka handeran* that likely compensate for the missing domains to restore function. Statistical analyses confirmed a significant association between motif count and pericarp colour (Kruskal-Wallis test, $p = 0.007$).

Keywords: allelic variation, bHLH transcription factor, pericarp pigmentation, proanthocyanidin, *Rc* gene