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RESEARCH ARTICLE

Haplo-pheno analysis of the *Rc* gene reveals association with yield parameters in pigmented rice

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Abstract

Traditional rice genotypes often have pigmented pericarps such as green, red, brown and black, while widely cultivated varieties typically exhibit white pericarps. Although white polished rice is generally preferred for consumption, pigmented rice offers notable health benefits due to its antioxidant properties. However, pigmented rice varieties typically have lower yield potential compared to white rice varieties. To address this limitation, we employed haplotype analysis to explore associations between pericarp pigmentation and yield traits. Since the *Rc* gene is a major regulator of pericarp colour in rice, the haplotypes of the *Rc* gene were characterized using 231 accessions from the 3K rice panel, categorizing them by three distinct pericarp colour scores (scores 0, 1 and 9). The score 0 consists of accessions with green, brown, red, light red, dark red and black pericarp colours. Score 1 includes non-pigmented rice accessions having white and off-white pericarp colours. Score 9 is a subset of score 0 and it includes accessions depicting various shades of red in the pericarp colour. The major haplotype H2, associated with score 0, was significantly enhanced (p value <0.05) in the number of panicles and tillers per plant. For pericarp colour score 1, the haplotype H2 was identified as superior, exhibiting significantly (p value <0.05) earlier flowering, reduced chaffy grains and increased tillers per plant. These findings suggest that haplotype-based breeding of pigmented rice varieties targeting superior haplotypes associated with increased yield traits offers a promising strategy to enhance the yield potential of coloured rice.

Keywords: diversity; haplotype analysis; pericarp colour; SNP variants

Introduction

White rice has long been the predominant type of rice grown and consumed globally. The majority of rice cultivars have white pericarps, while others have coloured pericarps that are red, brown, green, black or purple (1). Whole grain rice, mainly pigmented rice, is becoming more and more popular for its high nutritional value, unlike the milled or polished rice, which have significantly fewer health benefits (2, 3). Regular consumption of black rice offers several health benefits, including better heart health, improved longevity and reduced risks of atherosclerosis, hypertension, stroke and chronic diseases like diabetes, cancer and Alzheimer's. It also supports digestion, detoxification and has anti-inflammatory and anti-allergic properties (4, 5). The pigmentation in rice pericarps, such as black and red, is primarily attributed to anthocyanins and proanthocyanidins, respectively (6, 7). These compounds are known for their significant health benefits and are synthesized within the flavonoid biosynthesis pathway (8). Research into these biosynthetic pathways has identified two main categories of genes: structural and regulatory genes (9). Among these, the *Rc* gene plays a crucial role as a regulator of pericarp colour (10). The loss-of-function allele, *rc*, characterized by a 14-bp deletion in *Rc* resulting in a frameshift mutation and premature stop codon, causes pericarp pigmentation to change from red to white (11, 12). Conversely, restoration of the gene's reading frame by a G base deletion combined with the 14-bp deletion restores pericarp pigmentation to red (11, 12). Additionally, a one-base transversion also leads to premature termination of the *Rc* protein, resulting in white rice (13).

Given the health benefits associated with pigmented rice, there is a growing interest in developing functional foods with enhanced anthocyanin content (1). Even though the demand for black rice has increased, the yield potential of coloured rice is less than that of white or hybrid rice (1, 14). An ideal plant variety should fulfil the grain quantity and quality parameters, besides providing the necessary nutraceutical benefits for human health. Such dualpurpose rice varieties would not only meet the global demand for food security but also address rising health concerns through diet-based interventions. With the use of haplotype-based breeding, it is possible to combine superior alleles from multiple genes to develop high-yielding rice varieties that are better equipped to meet future food security and nutritional demands. A comprehensive study conducted on the 3K rice genome panel analysed 120 functionally characterized genes, including 87 genes related to grain yield and 33 genes associated with grain quality (15). The results uncovered significant genetic variation and identified superior haplotypes associated with key agronomic traits. These included improvements in early flowering, medium-duration flowering and bold grain size, highlighting the potential of haplotype-based strategies to accelerate genetic gain (15).

Although pigmented rice is rich in bioactive compounds such as anthocyanins, its lower yield compared to the widely cultivated white rice remains a significant limitation. This poses a challenge for breeders attempting to integrate desirable quality traits, like high anthocyanin content, with high yield potential in a single cultivar. Haplotype-based breeding is a promising strategy that allows for the creation of designer crop varieties by using and investigating haplotypes or superior alleles among target genes to produce novel traits in breeding programs. Therefore, it can be used as a tool to overcome the yield barriers in coloured rice. In this study, we conducted a haplotype analysis of the *Rc* gene, focusing on its association with agro-morphological and yield-related traits in rice. By identifying superior haplotypes, this research aims to support the development of parental lines for breeding high-yielding, pigmented rice varieties with improved nutritional profiles.

Materials and Methods

Plant materials

A total of 231 globally diverse rice (*Oryza sativa* L.) accessions were taken from the SNP-Seek Database (16). This panel comprised 12 groups of subpopulations, which include ind3 (63 accessions), indx (58 accessions), ind2 (36 accessions), ind1A (17 accessions), ind1B (15 accessions), aus (13 accessions), trop (11 accessions), admix (7 accessions), aro (6 accessions), japx (3 accessions), subtrop (1 accession) and temp (1 accession). Sowing of approximately 100 seeds of each accession was conducted in the nursery during the kharif season 2022, followed by transplanting in an augmented randomized complete block design and harvesting in December 2022 in the research field located at ICAR - Central Rice Research Institute, Cuttack.

Pericarp colour scoring

Ten seeds of each accession were randomly selected and manually dehusked. The colour of the pericarp of individual accession was visually ascertained and scored using three different scoring systems: 0, 1 and 9 as reported earlier (17). The accessions with pigmented pericarp like green, brown, red, light red, dark red and black were scored as 0 (68 accessions), provided in Supplementary Table 1. Accessions with white and off-white colours were scored as 1 (163 accessions). Considering the lesser number of accessions having green or black pericarp, a subset of the pericarp colour score 0, score 9 consisting of accessions with light red, red, dark red and brown pigmented pericarp (49 accessions), was considered after eliminating rice accessions with green or black pericarp.

Measurement of morphological parameters

The data were observed for three replications, which included plant height (cm), flag leaf length (cm) and flag leaf width (cm), total number of panicles per plant, panicle length (cm), number of spikelets, number of filled grains, number of chaffy grains, above ground biomass (g), harvest index (%) and single plant yield (g). The days to flowering and maturity were noted from the days after sowing (DAS). Parameters like plant height, flag leaf length, flag leaf width and panicle length were measured using a measuring scale. A tagging technique was used to identify the flowering dates. Parameters such as the number of spikelets, filled grains and chaffy grains were counted manually. Single plant yield and above-ground biomass were measured using a weighing balance. All the agro-morphological parameters were reported previously (18).

Genotypic data retrieval and phylogenetic tree analysis

Genotypic data for the *Rc* gene (Os07g0211500) and its 2kb promoter region were retrieved from the SNP-Seek Database. To enhance the statistical power, SNPs with > 20 % of missing alleles and heterozygous alleles were removed manually. After filtering, 55 SNPs were used for all the analyses. A phylogenetic tree was created using MEGA11 involving the neighbour-joining algorithm with 1000 bootstrap replicates (19). The principal component analysis for the genotypes was conducted using the GAPIT software (20). Tajima's D value was calculated using the TASSEL software (21).

Haplotype analysis

Further, the input file was manually prepared in table format according to the GeneHapR package manual (22). For annotation, the Nipponbare reference genome in gff3 file format was retrieved from the RAP database. The haplotype grouping was performed in the GeneHapR package. Minor haplotypes with less than five percent frequency were removed before further analysis. Major haplotype variants were mapped onto the *Rc* gene structure. The evolutionary relationships among the major haplotypes were studied and visualised in a haplotype network. Linkage disequilibrium (LD) between SNPs present in major haplotypes was calculated and visualised in LD blocks. Rare haplotypes and outliers were eliminated in order to identify superior haplotypes with highly significant phenotypic differences. For identifying significant *p*-values, t-tests between haplotypes were performed. The haplotype network, LD and t-tests were analyzed using the GeneHapR package in R software (22).

Results

Evolutionary relationship of the *Rc* gene

The evolutionary relationship of the *Rc* gene was analysed using phylogenetic tree analysis, revealing two main clusters (Cluster I and II) (Fig. 1). Cluster I is further subdivided into Cluster IA and Cluster IB. Notably, the majority of coloured (brown, black and red-shaded) rice accessions were grouped within Cluster IB, while Cluster II primarily consisted of white pericarp rice accessions. This clustering pattern suggests that the accessions have a common ancestral allele of the *Rc* gene associated with pericarp colour. The principal component analysis of the accessions revealed three major groups, PCI, PCII and PCIII, showing 60.18 %, 21.43 % and 8.45 % variation, respectively (Fig. S1). The Tajima's D value was found to be 1.815 (moderately high), indicating the presence of more intermediate-frequency alleles in the population.

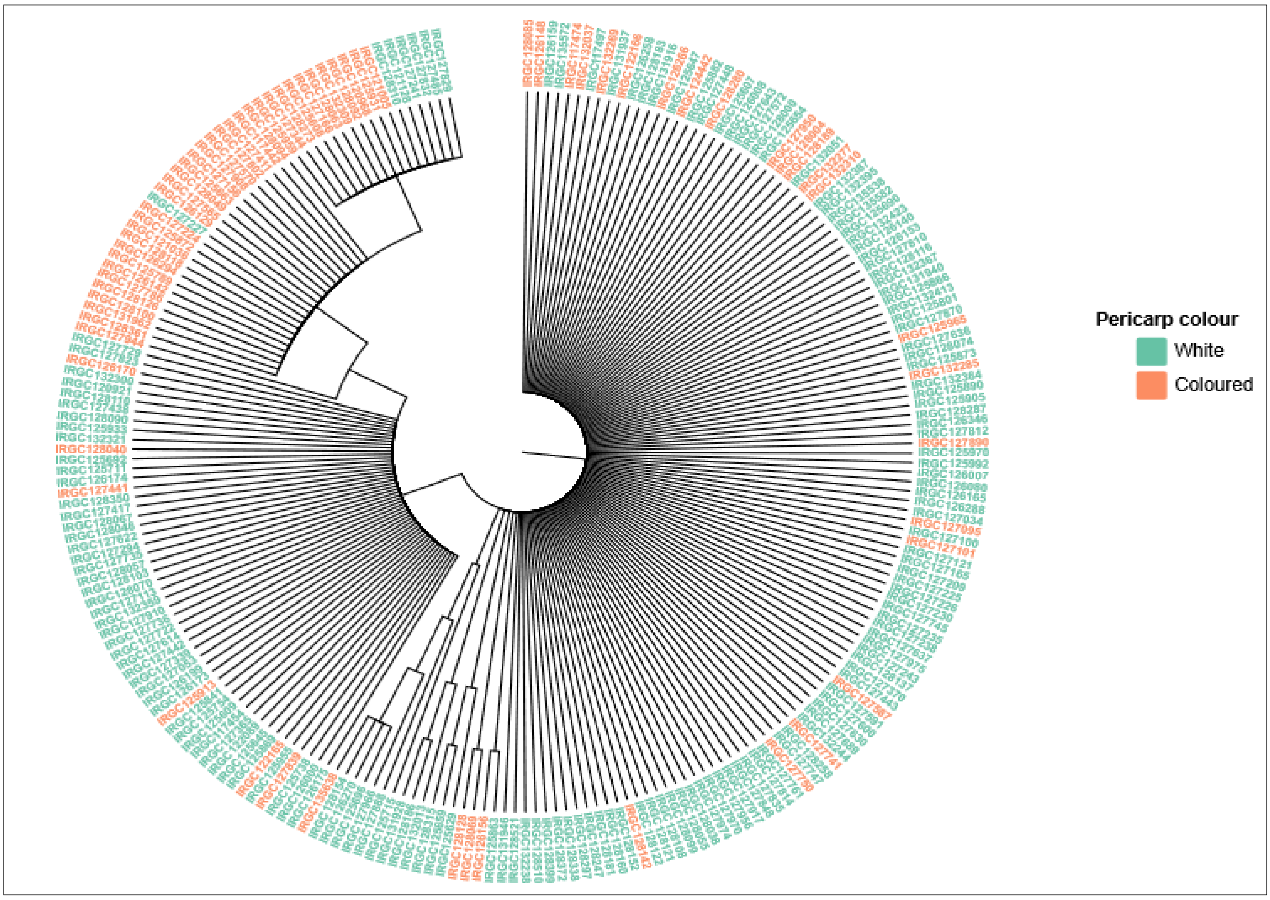


Fig. 1. Phylogenetic tree of the *Rc* gene. Different colours represent different pericarp colour scores (light orange colour for 0 coloured accessions and light green for 1 white accessions).

Haplotype-phenotype associations in pigmented pericarp (Score 0)

A total of 55 genetic variants were identified within the *Rc* gene with the colour score 0 (pigmented pericarps such as green, brown, red, light red, dark red and black). The haplo-pheno analysis showed 35 haplotypes (H1 to H35), among which three haplotypes (H1 to H3) were major (more than 5 % frequency). The haplotype H1 consists of 9 accessions, followed by H2 with 4 accessions and H3 with 3 accessions. The Nipponbare reference allele and alternate allele of each SNP were mentioned in the first column of the matrix (Fig. 2A). Each SNP position and variation was mapped onto the *Rc* gene structure (Fig. 2B). A haplotype network was constructed on major haplotypes to visualise the relationships among them (Fig. 2C). Only one SNP variation was found between H2 and H3. The LD heatmap represents the strong LD along the *Rc* gene, suggesting that the SNPs are inherited together (Fig. 2D). Among the 16 traits, seven traits were significantly different between major haplotypes and are visualized in combined violin and box plots (Fig. 3). Significant differences ($p \leq 0.05$) between haplotypes H1 and H3 were observed for seven traits (panicles per plant, tillers per plant, chaffy grains, flag leaf width, leaf area, above ground biomass and harvest index). Similarly, a significant difference ($p \leq 0.05$) between haplotypes H2 and H3 were observed for the flag leaf area. The mean value of H2 was found to be higher than H1 and H3 for seven traits including plant height, panicle length, panicles per plant, flag leaf length, leaf area, biomass and single plant yield (Table 1). These identified genomic variants and their linked regions can be used to select the aforementioned phenotypic traits.

Distribution of functional haplotypes for non-pigmented pericarp (Score 1)

The haplo-pheno analysis showed 59 haplotypes with the colour score 1 (consisting of accessions with white and off-white pericarp), among which three are major haplotypes (H1 to H3) with more than 5 % frequency. The haplotype H1 consists of 52 accessions, H2 consists of 12 accessions and H3 consists of 8 accessions (Fig. 4A). Each SNP position and variation was mapped onto the *Rc* gene model (Fig. 4B). A haplotype network was constructed on major haplotypes to visualise the relationships among them (Fig. 4C). Only one SNP variation was found between H2 and H3. The strong LD along the *Rc* gene indicates the combined inheritance of the SNPs (Fig. 4D). The combined violin and box plots illustrate the phenotypic distributions of significant traits among the major haplotypes (H1 to H3) (Fig. 4E-G). Significant differences ($p \leq 0.05$) between haplotypes H1 and H2 were observed for days after flowering, tillers per plant and chaffy grains. Additionally, a significant difference was also observed between haplotype H1 and H3 for the tillers per plant. The mean value of H2 was observed to be higher compared to H1 and H3 for eight agronomic traits, including days for flowering, plant height, panicles per plant, tillers per plant, chaffy grains, spikelet fertility, single plant yield and harvest index (Table 1).

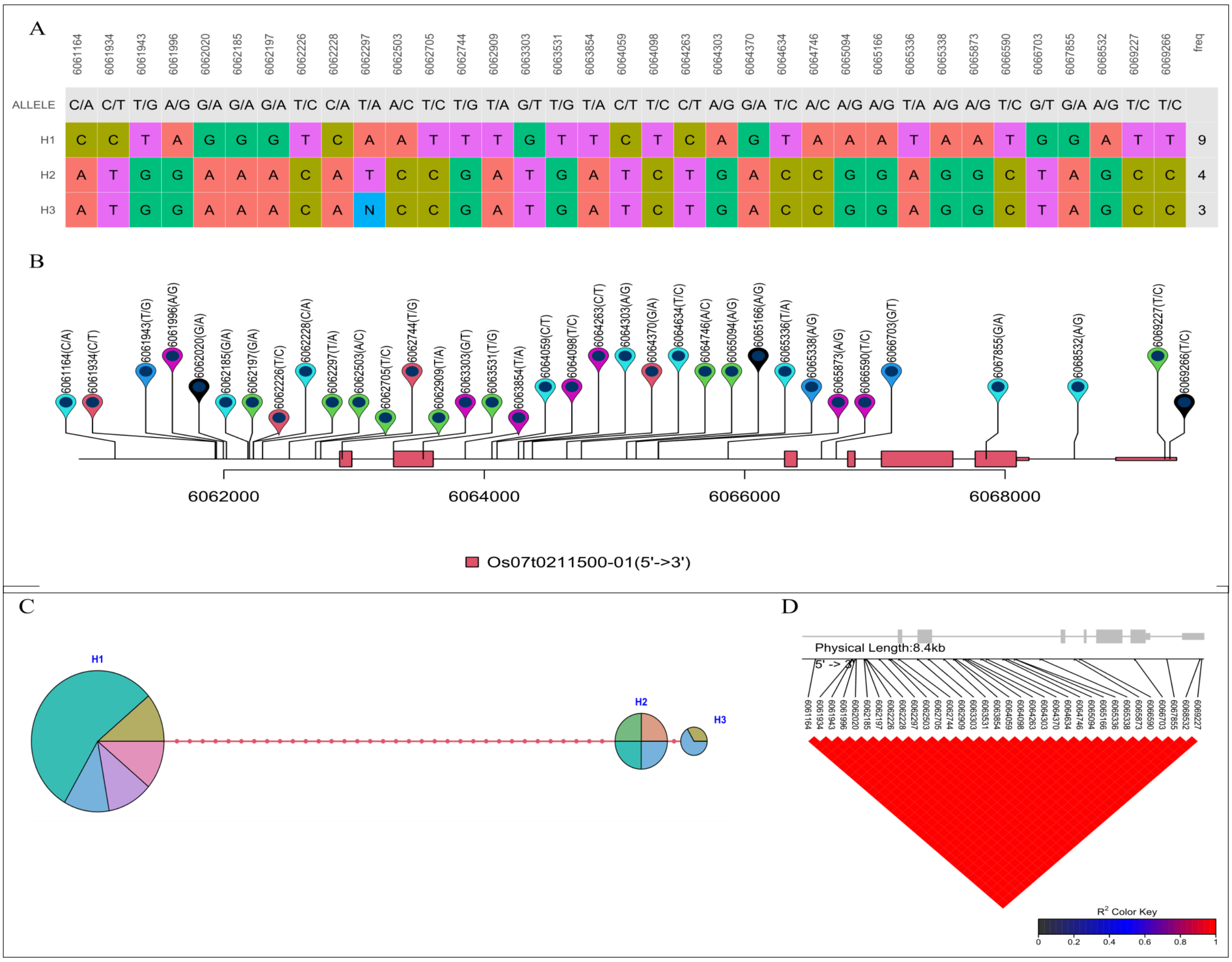
Haplotype effects of pigmented pericarp (Score 9)

The haplo-pheno analysis showed 29 haplotypes with the colour score 9 (consisting of rice accessions with red shaded pericarp), of which three are major haplotypes–H1, H2 and H3, consisting of 4, 3 and 3 accessions respectively (Fig. 5A). The SNP positions and variations were mapped onto the *Rc* gene model (Fig. 5B). A major haplotype network represented the haplotype H1 with a larger circle showing the four constituent sub-populations (Fig. 5C).

Table 1. Trait-wise mean differences of superior haplotypes across three different colour scores

Traits	Colour score 0			Colour score 1			Colour score 9		
	H1	H2	H3	H1	H2	H3	H1	H2	H3
Days for flowering (DFF)	105.67 ± 6.37	102.50 ± 6.24	74.67 ± 10.10	101.29 ± 2.10	90.75 ± 2.55	99.00 ± 4.74	102.50 ± 6.24	74.67 ± 10.11	95.33 ± 14.77
Plant height (PH)	123.43 ± 6.09	114.49 ± 8.59	116.97 ± 8.32	117.29 ± 2.37	110.94 ± 6.07	121.48 ± 7.75	114.49 ± 8.59	116.97 ± 8.32	122.48 ± 12.62
Panicle length (PL)	22.18 ± 1.07	23.23 ± 2.21	20.99 ± 1.50	22.65 ± 0.39	22.91 ± 0.64	23.68 ± 1.03	23.23 ± 2.21	20.99 ± 1.50	22.26 ± 2.52
Panicles per plant (PP)	8.11 ± 0.66	10.92 ± 2.15	10.33 ± 0.33	8.15 ± 0.32	9.58 ± 0.70	9.42 ± 0.68	10.92 ± 2.15	10.33 ± 0.33	7.56 ± 1.75
Flag leaf length (FLL)	31.26 ± 1.40	35.85 ± 3.15	24.97 ± 3.68	28.55 ± 0.69	28.44 ± 1.45	31.38 ± 1.23	35.85 ± 3.15	24.97 ± 3.68	30.06 ± 2.80
Flag leaf width (FLW)	1.44 ± 0.07	1.34 ± 0.11	1.02 ± 0.11	1.38 ± 0.03	1.28 ± 0.06	1.32 ± 0.05	1.34 ± 0.11	1.02 ± 0.11	1.38 ± 0.16
Leaf area (LA)	31.99 ± 2.10	33.98 ± 3.81	17.54 ± 0.47	28.12 ± 1.05	26.02 ± 1.98	29.42 ± 1.93	33.98 ± 3.81	17.54 ± 0.47	29.98 ± 5.69
Tillers per plant (TP)	9.15 ± 0.75	11.50 ± 1.58	11.78 ± 0.44	9.33 ± 0.37	11.31 ± 0.76	11.25 ± 0.69	11.50 ± 1.58	11.78 ± 0.44	8.44 ± 2.00
Filled grains (FG)	84.56 ± 7.49	65.42 ± 8.53	75.89 ± 4.55	83.83 ± 3.92	78.64 ± 7.02	70.29 ± 8.13	65.42 ± 8.53	75.89 ± 4.55	84.67 ± 15.18
Chaffy grains (CG)	17.04 ± 2.66	12.17 ± 2.25	10.33 ± 1.15	21.19 ± 1.42	15.17 ± 2.50	16.29 ± 3.45	12.17 ± 2.25	10.33 ± 1.15	11.00 ± 3.27
Total number of grains (TG)	101.59 ± 8.02	77.58 ± 7.77	86.22 ± 4.45	105.02 ± 4.44	93.81 ± 7.87	86.58 ± 7.78	77.58 ± 7.77	86.22 ± 4.45	95.67 ± 16.00
Spikelet fertility (SF)	83.05 ± 2.48	83.52 ± 3.96	87.95 ± 1.41	79.53 ± 1.24	83.99 ± 2.34	80.61 ± 3.96	83.52 ± 3.96	87.95 ± 1.41	88.43 ± 3.46
Biomass (BM)	79.37 ± 6.87	80.27 ± 20.01	48.06 ± 9.21	72.25 ± 4.03	65.30 ± 8.07	82.56 ± 11.44	80.27 ± 20.01	48.06 ± 9.21	80.98 ± 17.20
Single plant yield (SPY)	16.90 ± 1.22	18.17 ± 3.95	17.25 ± 2.83	15.97 ± 1.03	17.69 ± 1.75	16.57 ± 2.29	18.17 ± 3.95	17.25 ± 2.83	19.71 ± 1.72
Harvest index (HI)	22.84 ± 2.93	24.66 ± 4.14	36.53 ± 2.14	23.61 ± 1.35	28.79 ± 2.37	21.45 ± 2.67	24.66 ± 4.14	36.53 ± 2.14	26.17 ± 4.68
Hundred seed weight (HSW)	2.20 ± 0.11	2.17 ± 0.46	2.16 ± 0.29	2.29 ± 0.06	2.26 ± 0.16	2.14 ± 0.19	2.17 ± 0.46	2.16 ± 0.29	2.22 ± 0.11

Values represent Mean ± Standard error.



	6061164	6061934	6061943	6061996	6062020	6062185	6062197	6062226	6062228	6062297	6062503	6062705	6062744	6062909	6063303	6063531	6063854	6064059	6064098	6064263	6064303	6064370	6064634	6064746	6065094	6065166	6065336	6065338	6065873	6066590	6066703	6067855	6068532	6069227	6069266	freq
ALLELE	C/A	C/T	T/G	A/G	G/A	G/A	G/A	T/C	C/A	T/A	A/C	T/C	T/G	T/A	G/T	T/G	T/A	C/T	T/C	C/T	A/G	G/A	T/C	A/C	A/G	A/G	T/A	A/G	A/G	T/C	G/T	G/A	A/G	T/C	T/C	
H1	C	C	T	A	G	G	G	T	C	A	A	T	T	T	G	T	T	C	T	C	A	G	T	A	A	A	T	A	A	T	G	G	A	T	T	9
H2	A	T	G	G	A	A	A	C	A	T	C	C	G	A	T	G	A	T	C	T	G	A	C	C	G	G	A	G	G	C	T	A	G	C	C	4
H3	A	T	G	G	A	A	A	C	A	N	C	C	G	A	T	G	A	T	C	T	G	A	C	C	G	G	A	G	G	C	T	A	G	C	C	3

Fig. 2. Haplotype analysis of the *Rc* gene based on pericarp colour score 0. **A.** Major haplotype chart, **B.** SNP variations located in the *Rc* gene, **C.** haplotype network and **D.** Linkage disequilibrium blocks.

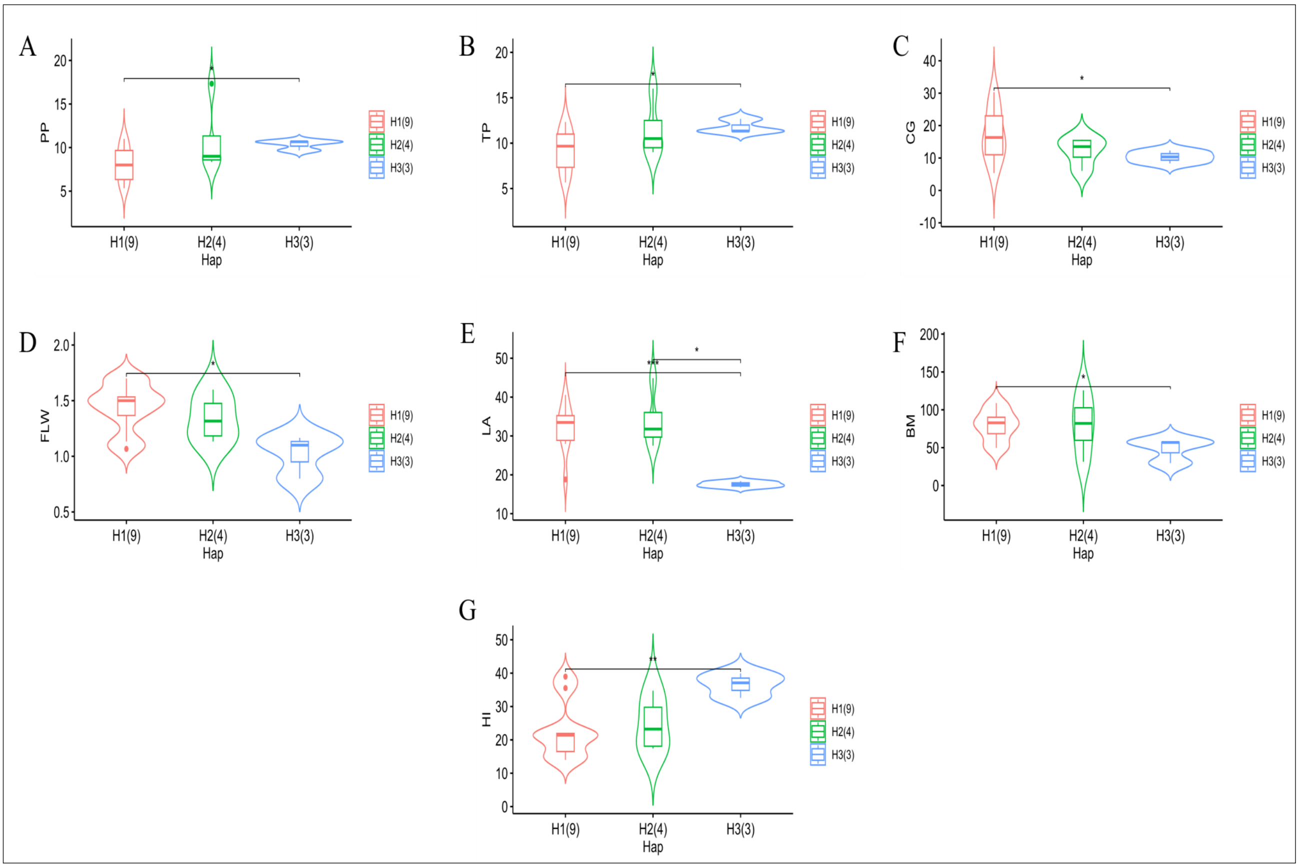
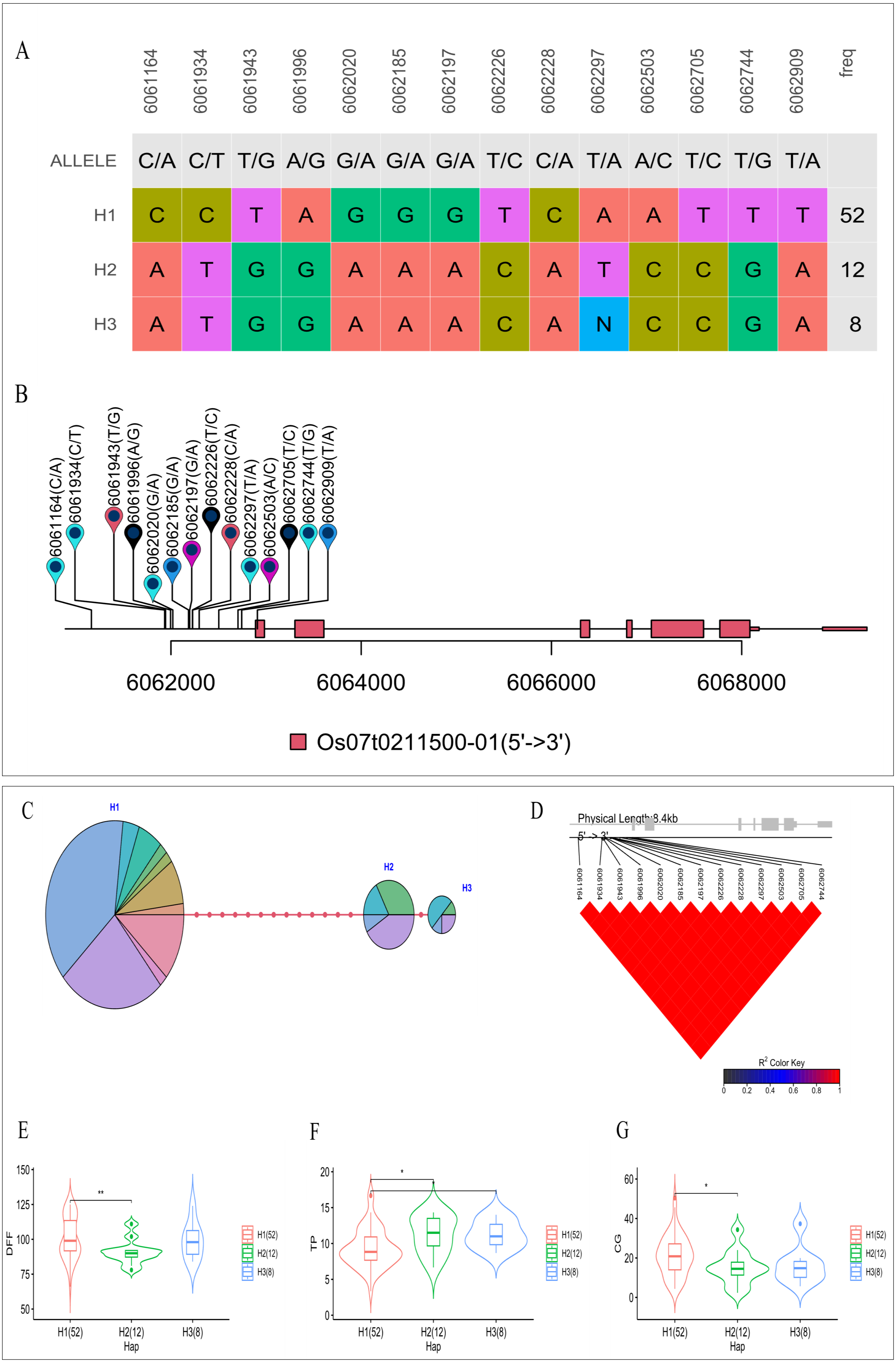
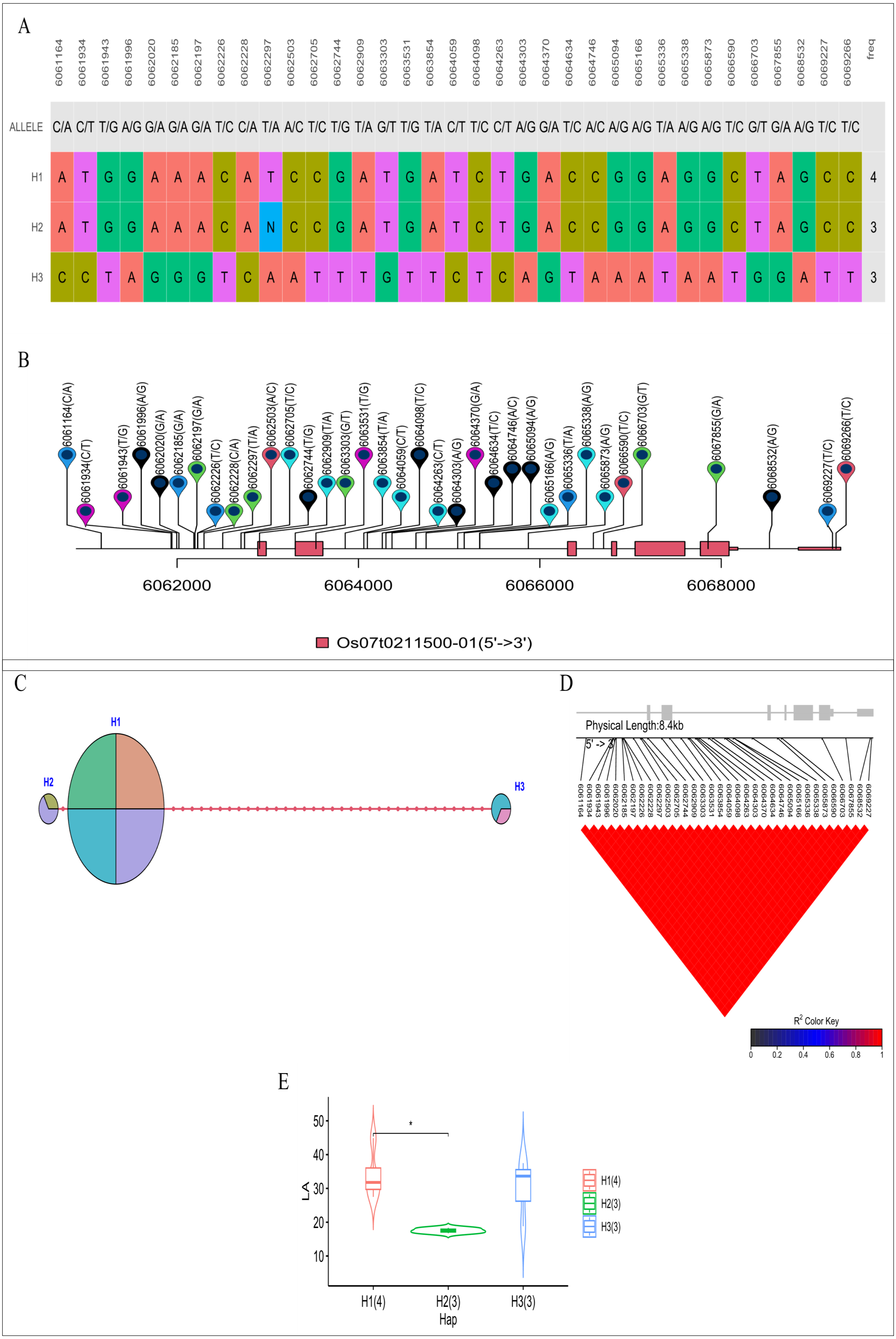


Fig. 3. Combined violin and box plots representing significant differences between major haplotypes for the pericarp colour score 0. **A.** panicles per plant, **B.** tillers per plant, **C.** chaffy grains, **D.** flag leaf width, **E.** leaf area, **F.** biomass and **G.** harvest index.



	6061164	6061934	6061943	6061996	6062020	6062185	6062197	6062226	6062228	6062297	6062503	6062705	6062744	6062909	freq
ALLELE	C/A	C/T	T/G	A/G	G/A	G/A	G/A	T/C	C/A	T/A	A/C	T/C	T/G	T/A	
H1	C	C	T	A	G	G	G	T	C	A	A	T	T	T	52
H2	A	T	G	G	A	A	A	C	A	T	C	C	G	A	12
H3	A	T	G	G	A	A	A	C	A	N	C	C	G	A	8

Fig. 4. Haplotype analysis of the *Rc* gene based on pericarp colour score 1. **A.** Major haplotype chart, **B.** SNP variations located in the *Rc* gene, **C.** haplotype network, **D.** Linkage disequilibrium blocks, **E.** Functional haplotypes of days for flowering, **F.** tillers per plant and **G.** chaffy grains.



A

	6061164	6061934	6061943	6061996	6062020	6062185	6062197	6062226	6062228	6062297	6062503	6062705	6062744	6062909	6063303	6063531	6063854	6064059	6064098	6064263	6064303	6064370	6064634	6064746	6065094	6065166	6065336	6065338	6065873	6066590	6066703	6067855	6068532	6069227	6069266	freq
ALLELE	C/A	C/T	T/G	A/G	G/A	G/A	G/A	T/C	C/A	T/A	A/C	T/C	T/G	T/A	G/T	T/G	T/A	C/T	T/C	C/T	A/G	G/A	T/C	A/C	A/G	A/G	T/A	A/G	A/G	T/C	G/T	G/A	A/G	T/C	T/C	
H1	A	T	G	G	A	A	A	C	A	T	C	C	G	A	T	G	A	T	C	T	G	A	C	C	G	G	A	G	G	C	T	A	G	C	C	4
H2	A	T	G	G	A	A	A	C	A	N	C	C	G	A	T	G	A	T	C	T	G	A	C	C	G	G	A	G	G	C	T	A	G	C	C	3
H3	C	C	T	A	G	G	G	T	C	A	A	T	T	T	G	T	T	C	T	C	A	G	T	A	A	A	T	A	A	T	G	G	A	T	T	3

Fig. 5. Haplotype analysis of the *Rc* gene based on pericarp colour score 9. **A.** Major haplotype chart, **B.** SNP variations located in the *Rc* gene, **C.** haplotype network, **D.** Linkage disequilibrium blocks and **E.** Superior haplotypes for leaf area.

Only one SNP variation was found between H1 and H2. The strong LD along the *Rc* gene indicates that the SNPs are inherited together (Fig. 5D). The combined violin and box plots illustrate the phenotypic distributions of leaf area across the three haplotypes, with a significant difference ($p \leq 0.05$) observed between haplotypes H1 and H2 (Fig. 5E). The mean value of H3 was found to be higher than H1 and H2 for seven key yield related traits, including flag leaf width, filled grains, total number of grains, spikelet fertility, biomass, single plant yield and hundred seed weight (Table 1).

Discussion

Traditional rice accessions exhibit pigmented pericarps, including green, yellow, red, purple, brown and black (10). Pigmented pericarps have antioxidant properties that provide various health benefits by preventing cellular oxidation (10). Haplotype analysis of the *Rc* gene has revealed a characteristic 14-bp deletion present in 97.9 % of cultivated rice accessions with white pericarps (13). In contrast, this deletion is absent in wild or cultivated rice accessions with red pericarps (6, 23). Previously, haplotype network analysis of the *Rc* gene conducted on 179 accessions showed four haplotypes (Rc-H1, Rc-H2, Rc-H3 and Rc-H4) with a haplotype diversity of 0.7076 (24). The Rc-H3 comprised wild rice accessions with red pericarps and was proposed as the ancestral haplotype, while the other three haplotypes likely evolved from it. Notably, Rc-H2 included genotypes with green, red and white pericarps. The presence of a common *Rc* haplotype in both Japonica and Indica groups supports the hypothesis of a monophyletic origin of cultivated rice (13). Furthermore, a second mutation in the *Rc* gene, known as the Rc-S allele, has been identified as responsible for white pericarp in the Aus subpopulation. Despite the widespread cultivation of white pericarp varieties, functional *Rc* haplotypes associated with red pericarps are still found in wild rice populations cultivated in parts of India (24). In another study, 32 rice accessions were evaluated for pericarp colour and antioxidant properties (10). Haplotype analysis was performed based on three critical sites regulating the functionality of the *bHLH* protein, which is involved in pericarp pigmentation. This analysis identified three haplotypes: H1, H2 and H3. Among them, H1, comprising red pericarp accessions, showed significantly higher levels of total flavonoids, total phenolic content, radical scavenging activity and proanthocyanidins compared to H2 and H3, which were primarily associated with white pericarps.

Purple pericarp rice is generally reported to have lower yield compared to white pericarp rice, primarily due to its reduced sink size (25). Historically, pigmented rice varieties have not been widely cultivated, resulting in limited breeding efforts directed toward their improvement. To overcome the yield barriers associated with pigmented rice, haplotype-based breeding offers a promising strategy. The application of haplotype-based approaches in rice breeding holds significant potential for improving crop productivity, enabling plants to withstand stresses while maintaining stable and high yields across diverse environments (26). By identifying and utilizing superior haplotypes associated with key yield-related traits, it is possible to develop pigmented rice varieties that combine high nutritional value with improved agronomic performance. In haplotype-based selection, the superior haplotype is determined by trait-specific criteria (27, 28). For traits such as panicles per plant, tillers per plant, flag leaf length, flag leaf width, leaf area, biomass and harvest index, haplotypes with higher mean values are considered superior. Conversely, for traits like chaffy grains and days to flowering, haplotypes with lower mean values are considered superior.

In the present study, haplotype analysis of the *Rc* gene was performed based on pericarp colour scores. For accessions with a pericarp colour score of 0, haplotype H3 was identified as the superior haplotype for traits such as panicles per plant, tillers per plant and harvest index. For pericarp colour score 1 (indicative of non-pigmented pericarp), haplotype H2 emerged as the superior haplotype for days to flowering, tillers per plant and number of chaffy grains. In accessions with a pericarp colour score of 9 (typically indicating intense pigmentation), haplotype H3 was found to be superior for the leaf area trait. Crossing accessions carrying these superior haplotypes across different pericarp colour scores may facilitate the development of high-yielding pigmented rice varieties. This approach has the potential to simultaneously enhance pericarp pigmentation and yield, contributing to both nutritional improvement and agricultural productivity.

Conclusion

Our findings reveal that specific *Rc* gene haplotypes are associated with favourable agronomic traits across different pericarp colour categories. Notably, for accessions with pericarp colour score 0 (pigmented accessions), haplotype H3 emerged as superior with a significantly increased number of tillers, panicles per plant and harvest index that are critical for improving yield potential. Similarly, pericarp colour score 1 (white/off-white) haplotype H2 was identified as superior, exhibited significantly earlier flowering, reduced chaffy grains and improved tillering capacity. These superior haplotypes could serve as valuable genetic resources for breeding high-yielding rice varieties with desirable pericarp pigmentation and agronomic traits.

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Authors' contributions

KK wrote the original draft. SS[1] did the formal analysis. GN did the phenotyping. PC prepared the methodology. AM reviewed and edited the manuscript. SS[2] provided the resources. SM supervised the work. DL conceptualized the manuscript. All authors read and approved the manuscript [SS[1] - Sabarinathan Selvaraj and SS[2] - Sanghamitra Samantaray].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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