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Full Paper

Elucidating the Genetic Architecture of Cooking and Eating Quality of Rice with Similar AAC by SNP Genotyping in ApoxIR64 Mapping Population

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Quality parameters are key indicators for selecting advanced breeding programs. As a core indicator of grain quality, amylose content (AC) does not always provide a comprehensive representation of the pasting profile or cooked rice quality. Apo and IR64 are rice varieties that belong to the intermediate AC class but exhibit distinct cooking and eating characteristics, whereas other grain components are known to be associated with these differences. This study investigated genetic variations in the pasting properties of rice associated with cooking and eating quality traits using genome-wide single-nucleotide polymorphisms (SNPs) in a mapping population derived from IR64 and Apo, which have similar apparent amylose content (AAC). Genome-wide SNPs identified 27 main-effect quantitative trait loci (QTLs) associated with cooking and eating quality parameters on seven chromosomes (1, 4, 7, 8, 9, 10, and 11). The results revealed novel QTLs for AC, pasting properties, and protein content in rice from a population with parents having similar AAC. Moreover, putative functional SNPs in the promoter and 5' untranslated region of the *Wx* gene were identified via in silico analysis. These findings will contribute to further understanding the genetics controlling the cooking and eating quality of rice, specifically the AC, pasting profile, and protein content.

Keywords: amylose content, eating and cooking quality, cooked rice texture, consumer preference

INTRODUCTION

Rice is consumed by more than half of the world's population and remains a primary source of carbohydrates. Rice quality dictates the price, consumer acceptance, and adoption by farmers for cultivation. Thus, cooked rice texture is one of the most important considerations in defining market segments for rice breeding (Paguirigan et al. 2018; Cuevas et al.2018). High-quality rice provides higher satiety, whereas poor-quality rice is often associated with impurities, firm and dry texture, poor cooking quality, and small broken grains. Rice is categorized into 18 types based on grain quality combinations, encompassing varieties from Asia, Australia, and major rice-producing nations in Europe, Africa, and the Americas (Calingacion et al. 2014). Amylose content (AC or AAC) defines major differences in cooking and eating quality; however, some rice varieties have similar AC but distinct qualities. In such cases, other quality indicators help classify rice into its respective types (Champagne et al. 2004).

The screening of advanced lines in a breeding program is primarily based on grain quality parameters, such as AC, pasting properties measured using a rapid visco analyzer (RVA), and protein content (Fitzgerald et al. 2009). While AC is often considered the primary factor determining the cooking and eating quality of rice, studies have revealed a more intricate genetic structure influencing this characteristic. This complex architecture encompasses variations in the proportions of macromolecules, degree of polymerization, protein content, and fiber content in rice. Protein content is an important factor associated with variability in the pasting profile of flour and the cooking properties of rice. Samples with the same AAC (Zhong 2005; Fitzgerald 2003; Martin and Fitzgerald 2002; Hamaker 1993; Cuevas et al. 2018) demonstrate that rice quality is significantly influenced by starch viscosity profiles (Bao et al. 2000). These profiles are typically generated with advanced generations of newly developed lines at later breeding stages (Panozzo and McCormick 1993). The cooking of rice grains begins with the addition of water and heat (Champagne, 2004), leading to chemical reactions that transform raw rice grains into a gel. The precise detection of pasting profiles has become more reliable and faster with the RVA developed by Newport Scientific Pty Ltd., Warriewood, Australia. Recent research has illuminated the structure of amylose and proteins, as well as their effects on cooking characteristics; however, rapid phenotyping methods have not thoroughly examined detailed phenotypic structures. Cuevas et al.. (2018) employed multivariate techniques, such as multinomial logistic regression and random forests, to categorize rice varieties into distinct cooking quality groups. They identified three clusters of rice samples based on 19 grain-quality traits. Clusters 1 and 2 included high-AC samples, and Cluster 3 comprised intermediate-AC samples. The study revealed that rheometry parameters, especially the storage modulus maximum (G'max) and slope 1 (S1), were crucial in distinguishing between clusters 1 and 2, a differentiation that traditional methods could not achieve. Their research offers valuable insights into the complexity of classifying rice cooking quality, showing that varieties within the same AC class can exhibit unique textural and sensory characteristics detectable through advanced analytical methods, such as rheometry. The availability of these detailed phenotypic data facilitates QTL mapping, allowing for more precise genetic selection to enhance the phenotypic selection of desirable traits. However, much remains unknown, and detailed gene annotation is needed to disclose the genetic architecture of various genes responsible for rice cooking and eating traits.

While previous studies have recognized the importance of the *Wx* gene in determining eating and cooking quality, most were based on comparisons between parents with significantly different amylose contents (AAC) and distinct *Wx* alleles. Consequently, our understanding of other genetic factors, such as minor genes or modifiers that influence quality parameters beyond *Wx* and/or *Alk* genes, remains limited. This knowledge gap necessitates further investigation into the genetic determinants of rice quality traits within varieties that share similar AAC profiles.

The simplest way to explore the genetics of pasting properties, aside from AC and the *Wx* gene, is to study the starch structure and grain quality of either (1) waxy rice varieties or (2) non-waxy rice varieties of the same AC class. Two such non-waxy varieties are Apo (IR55423-01 or NSIC Rc9) and IR64, both of which belong to the intermediate AC class but exhibit different cooking and eating qualities than each other. IR64 is a widely grown, high-yield cultivar with good cooking and eating quality traits. Apo is an upland cultivar with good agronomic traits and climate resilience owing to drought tolerance, but it exhibits poor cooking and eating quality.

Quantitative descriptive sensory profiling showed that despite being in the same AC class, trained panelists easily differentiated between the two varieties (Champagne et al. 1999). IR64 rice grains showed high sweetness, low astringency, and water-like metallic qualities, whereas Apo rice had strong sewer/animal, water-like metallic, astringent, and sour/silage attributes, with low sweetness (Champagne et al. 2010). Calingacion et al. (2014) confirmed these findings, associating IR64's pleasant profile with indole, octanal, and decanal (sweet, fruity aromas), and Apo's off-flavors with propene thiol, ocimene, heptanal, and hexanal (sewer/animal, sour/silage, and hay-like/musty aromas).

In this study, we used a mapping population between Apo and IR64, parents with similar AAC to identify genetic factors which influence the genetic basis of cooking and eating quality parameters, AC, and protein content of rice, along with the genetic mapping of the pasting profiles that used to measure cooking behaviours (e.g., cooking temperature is associated with pasting temperature) and predict texture (e.g., hardness) using a high throughput SNP-genotyping.

MATERIALS AND METHODS

Mapping population and field experiments

The mapping population consisted of 213 $F_{4:5}$ plants derived from a cross between IR64 (female parent) and Apo (male parent) developed by the Rainfed-Lowland Breeding Team of the Plant Breeding and Genetics Division at the International Rice Research Institute (IRRI) in the Philippines. Paddy grains were harvested, cleaned, and dried to a moisture content of 12% for various grain quality assays.

The research was conducted at the International Rice Research Institute (IRRI) in Los Baños, Laguna, Philippines, during the dry season of 2012 (DS2012) and the wet season of 2012 (WS2012). The soil of the experimental field was classified as Maahas clay loam soil. An Alpha lattice design was employed for the experiment, utilizing three replicates along with the drought-tolerant checks: Apo, Nagina 22, MTU1010, and the susceptible check IR64. This study implemented the cultural management practices outlined by Ghimire et al. (2011).

I. Phenotyping for Grain Quality

The paddy grains were then dehulled and polished. The milled samples were ground to a powder using a Udy Cyclone Mill (Udy Corporation, Fort Collins, CO, USA) for grain quality analysis.

a. Apparent Amylose Content (AAC) Analysis

The method outlined by Juliano (1971) was used to determine AAC. The experiment was conducted three times, and the average of the readings was obtained. The obtained curves were evaluated against standard curves generated from rice samples of various AAC classifications.

b. Viscosity or Pasting Viscosity Analysis

Rapid Viscosity Analysis (RVA) evaluates the viscosity changes in a water-flour mixture during heating over time. This technique can distinguish between varieties with identical AAC by identifying setback (SB), breakdown (BD), and consistency viscosity (CS) (Bao et al. 2007). A Rapid Visco Analyzer (RVA) equipped with Thermocline Windows control and analysis software, Version 1.2 (Newport Scientific, Australia) was employed to generate the viscosity profiles, following the standard protocol (AACC61-02) established by the American Association of Cereal Chemists (2000). The 12.5-minute thermal curve sequence consisted of: (1) a 1.0-minute incubation at 50°C; (2) an increase to 95°C with a 1.4-minute hold; and (3) a cool-down to 50°C with a 1.4-minute hold. The RVA viscosity profiles yielded several pasting parameters: pasting temperature (PsT), lift-off (LO) or retrogradation, peak viscosity (PV), trough viscosity (TV), breakdown (BD), setback (SB), final viscosity (FV), and peak time (PT). All measurements were expressed in rapid viscometer units (RVU), with 1RVU equivalent to

10cP (AACC 2000). For the mapping population, three grain quality parameters that indicate the cooking and eating quality of rice were used, including AAC, RVA, and protein content analysis using Kjeldahl N. Grain protein content was estimated using the Kjeldahl method (1983). A total of 2.5 g rice flour was placed in a tube and subjected to digestion using 25 ml of sulfuric acid with the addition of two Kjeldahl tablets. Subsequently, the grain samples were processed through steam distillation and titrated with sodium hydroxide to determine their protein content. The resulting nitrogen content of each sample was multiplied by a fixed factor (5.95) (Kjeldahl, 1983) to obtain the total protein content. The analysis was performed in triplicate for each sample, and the results were averaged across the replicates.

c. Gel consistency
Gel consistency measures the tendency of cooked rice to harden upon cooling. Rice classification using this parameter ranges from hard to soft, depending on the length of the gelatinized-starch paste. It is typically measured by the length of the gel formed from cooked rice flour, with longer gels indicating a softer texture (Chemutai et al. 2016; Sowbhagya et al1987). The gel consistency was measured using 0.1 g of flour, as described by Cagampang et al. (1973).

d. Texture Profile Analysis (TPA)
For each accession sample, 25 polished rice grains were cleaned thrice and immersed in 1 mL of Milli-Q water for 30 min in a test tube. The samples were then boiled for 20 min and maintained at 50°C to prevent retrogradation. The textural characteristics of the cooked rice (hardness, cohesiveness, springiness, and adhesiveness) were evaluated using a modified version of the method described by Lyon et al. (2000). The analysis was conducted using a Ta.XT-Plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK). The apparatus was fitted with an aluminum cylinder probe of 35 mm and a 5 kg load cell. For the measurements, the probe was positioned 15 mm above the base. Three whole cooked rice grains were placed in parallel on an aluminum plate base, centered beneath the probe, and compressed to 90% of their original height for each measurement. A TPA force-deformation curve was generated using a two-cycle compression test performed on the TPA. The instrument was set to operate at test and post-test velocities of 0.5 mm s^{-1}. Exponent Lite Software (version 3.0.5.0) was used to obtain and analyze the hardness (maximum force of the initial compression by the height of the first curve), adhesiveness (negative force area under the first bite), cohesiveness (A2/A1), and springiness (T2/T1) values. Adhesiveness is denoted by negative values, indicating the direction of movement of the probe. Consequently, the adhesiveness values were reported as absolute values. Texture experiments were conducted in triplicate with three biological replicates and three runs for each biological replicate.

II. Genotyping

a. SNP genotyping by using Illumina BeadXpress
Genome-wide SNPs genotyping was performed using Illumina BeadXpress with *an Indica-Indica* SNP chip containing 384-plex genome-wide SNPs (Thomson et al.2012). For each sample, 250 ng of DNA (50 ng/µL) and 160 pg of DNA were used per reaction. The signals were analyzed using the Alchemy software (Wright et al. 2010). All SNP data were scored using the Illumina Genome Studio software with a recommended no-call threshold (0.25) (Wright et al. 2010). SNPs with call rates lower than the recommended rate (90%) were removed, and 125 SNPs were retained for the QTL mapping.

b. In-silico Analysis of the genetic divergence of ApoxIR64
Genetic sequences of Nipponbare, a rice variety of low amylose class, IR64, Apo, and NSIC Rc 222, of intermediate and high amylose classes, were downloaded from the 3 K Rice Genome database. The Integrated Genome Viewer was used to investigate the details of LOC_OS06g4200, which encodes *Wx* genes.

III. QTL analysis
A linkage map was generated using 125 SNPs that were distributed over 12 chromosomes. Mapping analysis was performed using QTL-Cartographer (Basten, 2001) software. QTLs for different cooking and eating quality traits were detected following both regression and non-regression mapping approaches using composite interval mapping (CIM) and single marker analysis (SMA) (Kao et al. 1999). Interval mapping factors were determined using both forward and reverse methods. A 10 cM window was employed, with likelihood ratios calculated at 2 cM intervals. To establish a precise QTL detection threshold P-value ($P < 0.05$), a permutation rate of 1000 at 95% confidence interval or 1-LOD interval was defined by the left and right flanking markers where the LOD score dropped below 1. The likelihood ratio test was employed to ascertain phenotypic variance (R2) and LOD values, as well as to formulate a hypothesis incorporating dominance and additive effects (H3: H0). QTL significance was identified using a LOD score of 2.5 or higher (Kao et al. 1999).

RESULTS

Grain Quality and Genetic Divergence Analysis of Apo and IR64
The AAC of Apo and IR64, although significantly different, measuring 23.14 and 19.02, respectively, were still within the intermediate AC class. IR64 is at the higher end of the intermediate classification, and Apo is at the lower end, whereas the Gel Consistency for both is classified as soft (Figure 1, Table1); however, the cooking and eating qualities varied considerably.

Notably, the instrumental analysis of the cooked rice texture clearly distinguished between the two varieties. IR64 exhibited significantly higher instrumental hardness and cohesiveness, but lower adhesiveness than Apo (Figure 2). Although instrumental springiness did not differ significantly between the two, Apo showed a lower mean and greater variance than IR64 (Figure 2).

Table 1. One-way Analysis of Variance of selected garin quality parameters in Apo and IR64.

Grain Quality Attribute	df	Variety 1	Residuals 4
Amylose Content	Sum of Squares	25.5441	0.0705
	Mean Squares	25.5441	0.0176
	F-value	1448.6	
	Pf(>F)	0.000002846***	
Gel Consistency	Sum of Squares	73.5	70.833
	Mean Squares	73.5	17.708
	F-value	4.1506	
	Pf(>F)	0.1113	
Hardness	Sum of Squares	586368	20872
	Mean Squares	586368	5218
	F-value	112.37	
	Pf(>F)	0.0004482***	
Adhesiveness	Sum of Squares	2633.88	551.88
	Mean Squares	2633.88	137.97
	F-value	19.09	
	Pf(>F)	0.01198*	
Cohesiveness	Sum of Squares	0.0048735	0.0006813
	Mean Squares	0.0048735	0.0001703
	F-value	28.611	
	Pf(>F)	0.00589**	
Springiness	Sum of Squares	0.00003267	0.00101467
	Mean Squares	3.27E-05	2.54E-04
	F-value	0.1288	
	Pf(>F)	0.7378	
Pasting Temperature	Sum of Squares	4.9504	6.8667
	Mean Squares	4.9504	1.7167
	F-value	2.8837	
	Pf(>F)	0.1647	
Pasting Viscosity	Sum of Squares	60.167	25.333
	Mean Squares	60.167	6.333
	F-value	9.5	
	Pf(>F)	0.03685 *	
Peak Time	Sum of Squares	0.036296	0.047407
	Mean Squares	0.036296	0.011852
	F-value	3.0625	
	Pf(>F)	0.155	
Set Back	Sum of Squares	1584148	11369
	Mean Squares	1584148	2842
	F-value	557.37	
	Pf(>F)	1.908e-05 ***	
Final Viscosity	Sum of Squares	25350	3939.3
	Mean Squares	25350	984.8
	F-value	25.74	
	Pf(>F)	0.007113 **	
Trough Viscosity	Sum of Squares	121.5	4872
	Mean Squares	121.5	1218
	F-value	0.0998	
	Pf(>F)	0.7679	
Breakdown	Sum of Squares	874017	18171
	Mean Squares	874017	4543
	F-value	192.4	
	Pf(>F)	0.0001566 ***	
Peak Viscosity	Sum of Squares	1032520	13197
	Mean Squares	1032520	3299
	F-value	312.95	
	Pf(>F)	5.998e-05 ***	
Lift-Off	Sum of Squares	28981.5	729.3
	Mean Squares	28981.5	182.3
	F-value	158.95	
	Pf(>F)	0.0002278 ***	

*** Significant at 0.001 level, ns- not significant

The RVA profiles (Figure 3) showed that IR64 had a higher PV than Apo. Table 3 and Figure 4 present the differences in the RVA viscosity profiles of Apo and IR64, where lift-off (LO), peak viscosity, breakdown (BD), setback (SB), and final viscosity (FV) were significantly different, whereas the pasting temperature (PsT), trough viscosity (TV), and peak time were not.

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The consumer panelists determined that Apo possessed certain textural characteristics (Figure 5A). They were uncertain about the presence of an initial starchy coating, slickness, or stickiness on the surface of the cooked grains. However, a significant majority (at least 60%) concluded that Apo was rough and not sticky between the grains. Upon the first bite, Apo was perceived as not springy but rather cohesive and firm. While chewing, the panelists noted that the grains lacked mass cohesiveness and uniformity in bite, yet they were capable of absorbing moisture. After swallowing, the panelists observed loose particles left behind, but no tooth pack was present. Conversely, the panelists characterized the textural properties of IR64 as having a grain surface with an initial starchy coating and slickness, yet devoid of roughness (Figure 5B). The grains were sticky both to the lips and among themselves. Upon the first bite, the IR64 grains were described as springy, lacking cohesiveness and hardness. During mastication, the grains were noted for their cohesive mass and uniform bite, although they did not absorb moisture. Post-ingestion, IR64 did not leave residual loose particles or tooth pack.

The skim sequence analysis of the *Wx* region (LOC_Os064200), otherwise known as granule-bound starch synthase(*GBSS1*), revealed an interesting divergence of Apo and IR64, which could also partly explain the reason behind the two being under the same intermediate AC class, with similar soft gel consistency but divergent cooking and eating profiles detected both through instrumental texture analysis (the present study) and consumer panel sensory analysis (*Fig 5)*. Figure 6 shows that IR64 and Apo share the known and published functional SNPs characteristic of *Wx (Int)*, but they differ in all the rest of the gene, particularly the promoter and 5' untranslated region-5' UTR (Table 2). The *IR64* allele is similar to *Wx(a)* overall, while the *Apo* allele is almost identical to the *Wx(b)* promoter and the other parts of the gene, except for the 5'UTR splice site variation, which seems to be different (Figure 6, Table 2). Interestingly, such variation in the 5'UTR splice site found in IR64 was not observed in NSIC RC222, a mega-variety known for its otherwise hard cooked rice texture upon retrogradation. Apart from these two SNPs, the Apo and IR64 alleles were completely distinct. The Apo allele is more similar to the *Wx(b)* group (for example, the Reference Nipponbare genome), whereas IR64 is more similar to the *Wx(a)* group. According to recent research by Hanashiro et al.(2008) and Crofts et al.(2019), a single nucleotide polymorphism (SNP) in intron 1 and exon 6 affects the production of extra-long amylopectin chains in rice. This genetic variation may partially account for the differences in cooking and eating qualities observed between the Apo and IR64

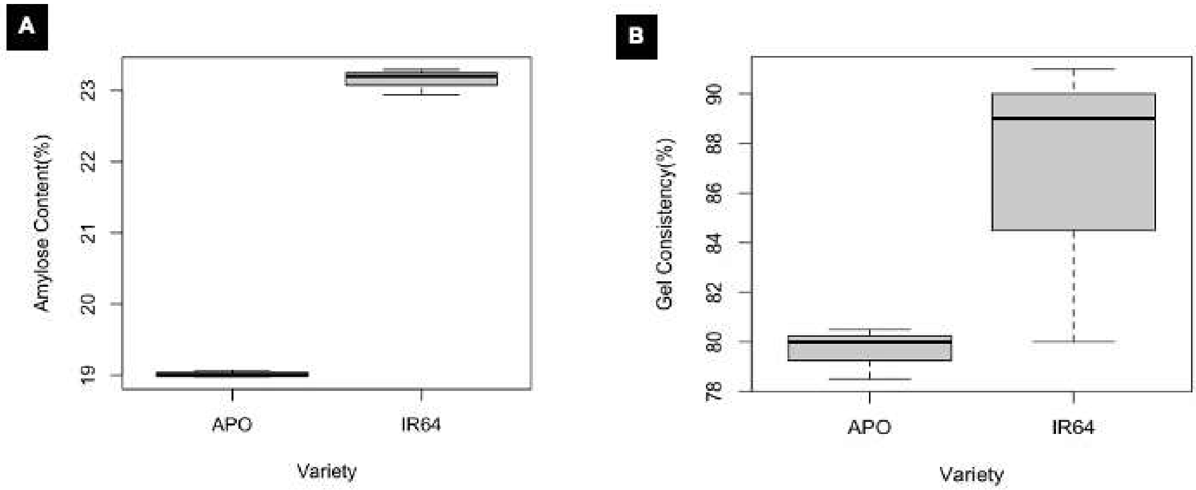

Figure 1. Amylose Content (A) and Gel Consistency (B) of Apo and IR64.

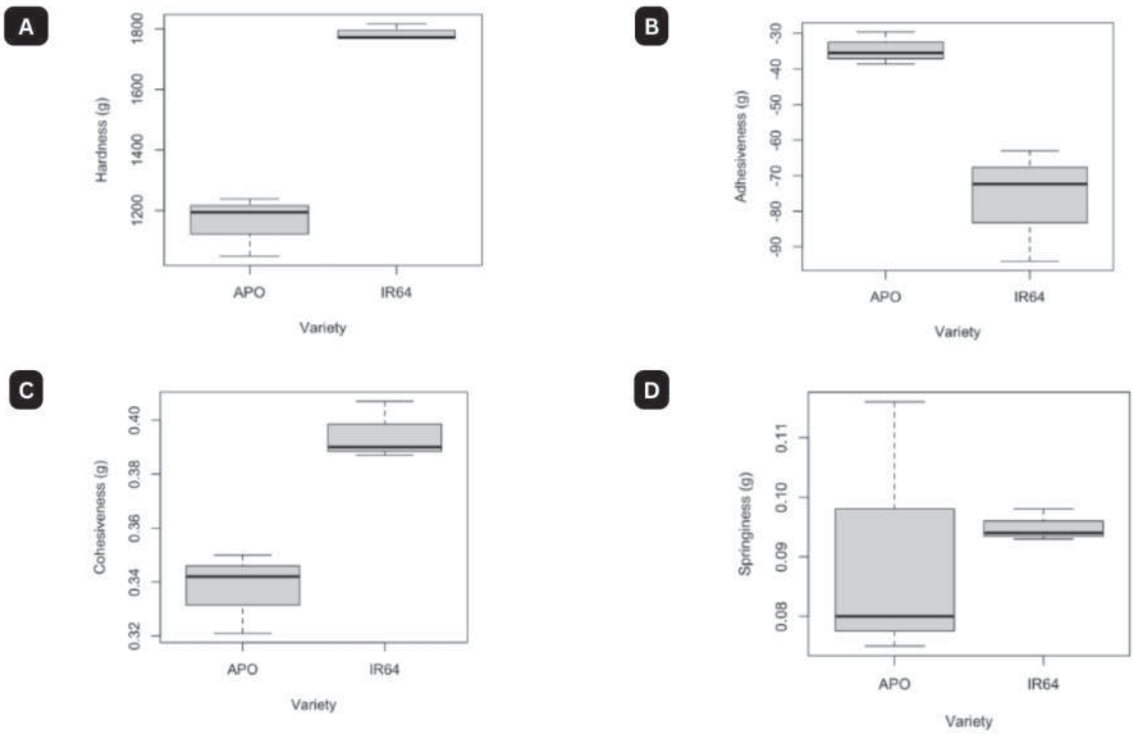

Figure 2. Differences in instrumental texture profiles of cooked apo and IR64 (A – hardness; B – adhesiveness; C – cohesiveness; D – springiness).

rice varieties, alongside other factors such as viscosity and instrumental texture profiles.

QTL mapping

Amylose content

This study identified three QTLs associated with AAC: two on chromosome 4 (qAM-4a and qAM-4b) and one on chromosome 11 (qAC-11). These QTLs accounted for 32% of the phenotypic variation (R2), with logarithm of odds (LOD) scores of 8.5 and 5.9, respectively (Table 3; Figure 6).

Protein content

This study identified four novel QTLs associated with protein content. Two of these QTLs were located on chromosome 1 and collectively accounted for 38% of the phenotypic variation (R2) (Table 3; Figure 6). The remaining two QTLs were found on chromosomes 10 and 11, explaining 10% and 39% of the phenotypic variation, respectively (Table 3). Notably, on chromosome 1, Apo alleles had a negative effect on protein content, with the total phenotypic variation attributed to these alleles being 38% (Table 2).

Pasting properties

This study identified several QTLs for various starch properties related to pasting properties that are associated with the cooking and eating properties of rice. For peak viscosity (PV), three QTLs, namely, qPV-7, qPV-9, and qPV-11, were found on chromosomes 7, 9, and 11. These QTLs accounted for 10%, 12%, and 6% of the phenotypic variance

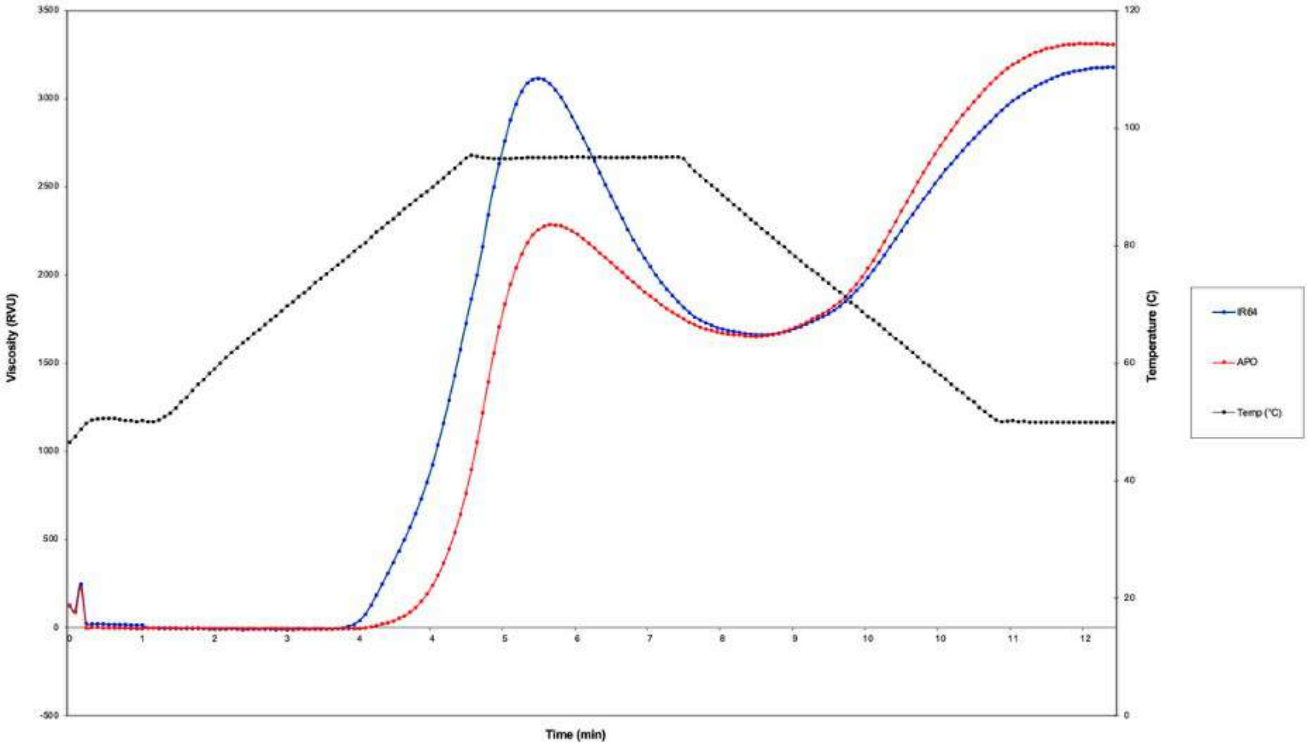

Figure 3. RVA graph of Apo and IR64.

*PV-Peak Viscosity TV-Trough Viscosity FV- Final Viscosity. Other RVA parameters (BD, LO and SB) can be derived from these three.

Table 2. Single nucleotide polymorphisms between Apo and IR64 from 5' UTR splice to Transcription Start Site.

Position	Ref (Nipponbare)	IR64	Apo	Comments
1765761	T	G	G	5'UTR splice site variant
1765668	0	-2	2	5'UTR SSR (non-functional)
1765622				Transcription start
1765448	C	T	C	
1765438	G	A	G	
1765415	A	G	A	
1764700	G	A	G	
1764641	C	T	C	
1764553	T	C	T	
1764516	T	G	T	
1764460	A	G	A	Possible CCAAT box

(R2), with corresponding LOD scores of 6.5, 5.5, and 8.5, respectively (Table 3; Figure 1). Breakdown was associated with two QTLs: qBD-1 on chromosome 1 and qBD-4 on chromosome 4, explaining 8% and 5% of the phenotypic variance, respectively, with LOD scores of 6.2 and 5.5, respectively (Table 3; Figure 6). Final viscosity was linked to two QTLs (qFV-1 and qFV-10) on chromosomes 1 and 10, accounting for 14% and 12% of the phenotypic variance, respectively, with LOD scores of 5.5 and 7.5, respectively (Table 3; Figure 6). Setback was associated with two QTLs, qSB-10 and qSB-11, on chromosomes 10 and 11, explaining 8% and 29% of the phenotypic variance, respectively, with LOD scores of 8.0 and 8.5, respectively (Table 3; Figure 6). Analysis identified six QTLs associated with peak time, which were distributed across three chromosomes. Chromosome 1 harbored three QTLs, chromosome 8 contained two QTLs, and chromosome 9 contained one QTL. These QTLs explained varying proportions of the phenotypic variance (R2): 18%, 22%, 36%, 12%, 13%, and 14%, respectively. The corresponding LOD scores for each QTL were 5.5, 6.5, 7.2, 6.0, 5.0, and 6.9, respectively (Table 3; Figure 6). Two QTLs on chromosome 11 were linked to pasting temperature, accounting for 5% and 8% of the phenotypic variance (R2) with LOD scores of 7.0 and 5.5, respectively (Table 3; Figure 6), respectively. Chromosomes 1 and 11 each harbored a QTL (qLO-1 and qLO-11) responsible for 12% and 14% of the phenotypic variance, respectively, with corresponding LOD scores of 5.0 and 6.0. The IR64 alleles located on chromosomes 1 and 11 enhanced starch retrogradation by 58% (Table 3; Figure 6).

QTL mapping

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This study identified three QTLs associated with AAC: two on chromosome 4 (qAM-4a and qAM-4b) and one on chromosome 11 (qAC-11). These QTLs accounted for 32% of the phenotypic variation (R2), with logarithm of odds (LOD) scores of 8.5 and 5.9, respectively (Table 3; Figure 6).

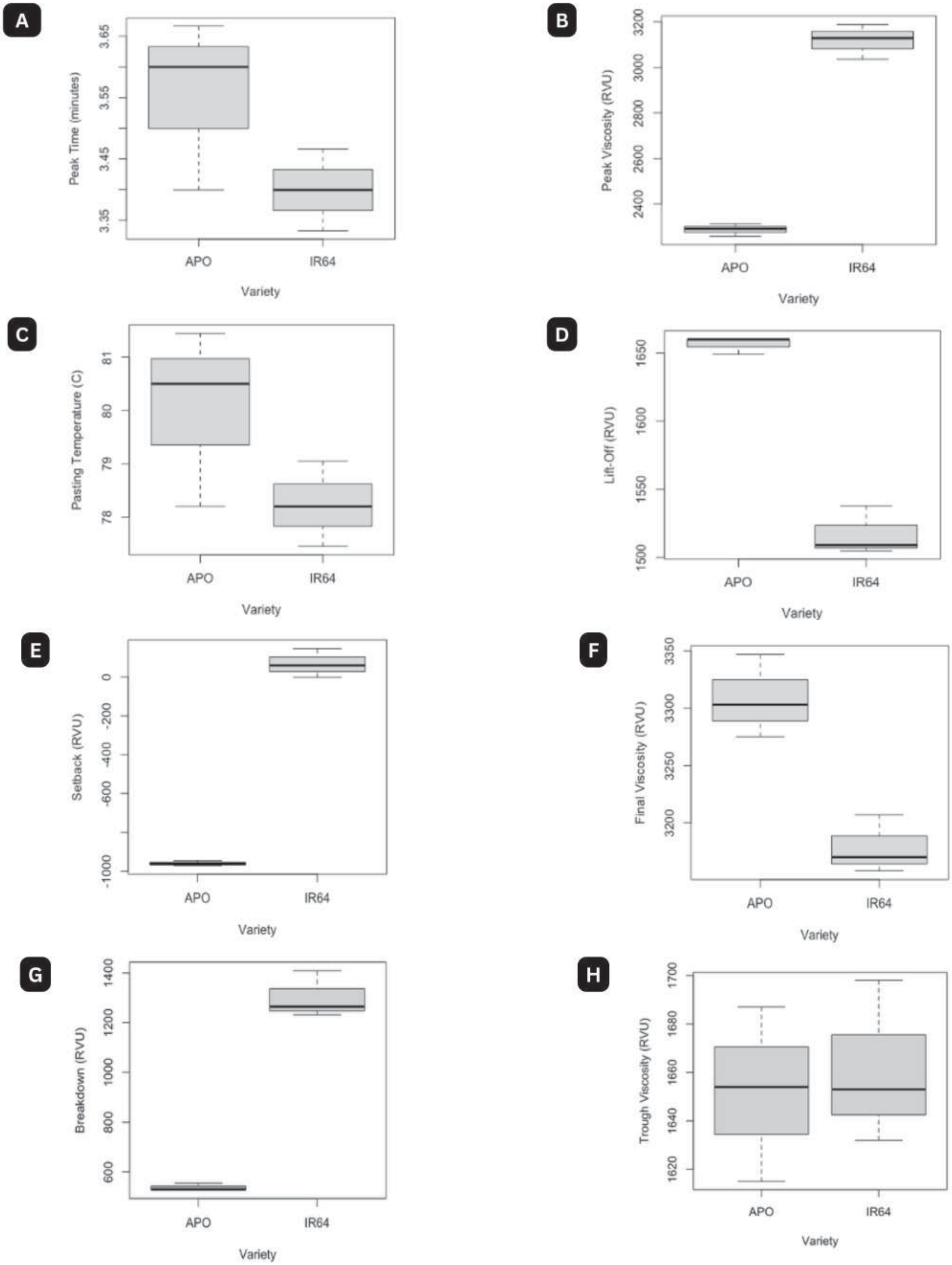

Figure 4. Box plots of RVA parameters of Apo and IR64 (A- Pasting Temperature, B- Lift-Off, C- Peak Viscosity, D - Peak Time, E - Setback, F- Final Viscosity, G- Breakdown, H- Trough Viscosity).

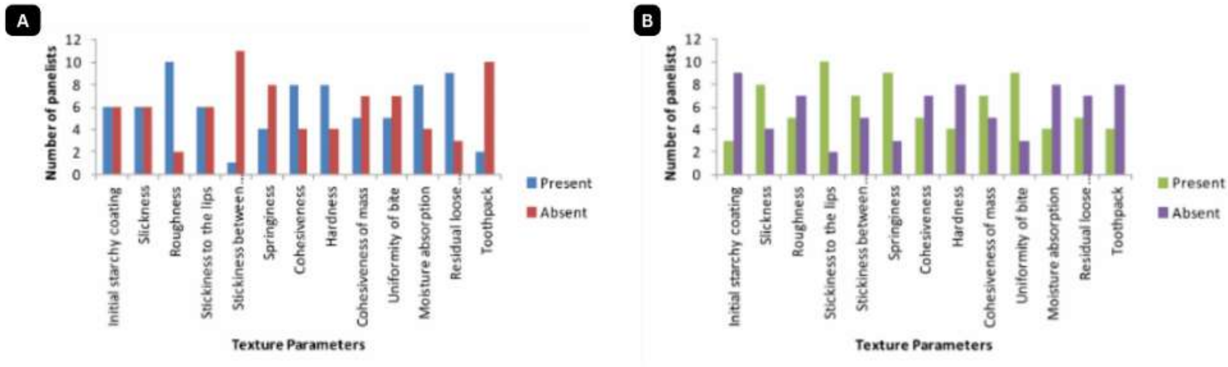

Figure 5. Number of panelists who voted for the presence or absence of textural attributes in A. Apo and B. IR64.

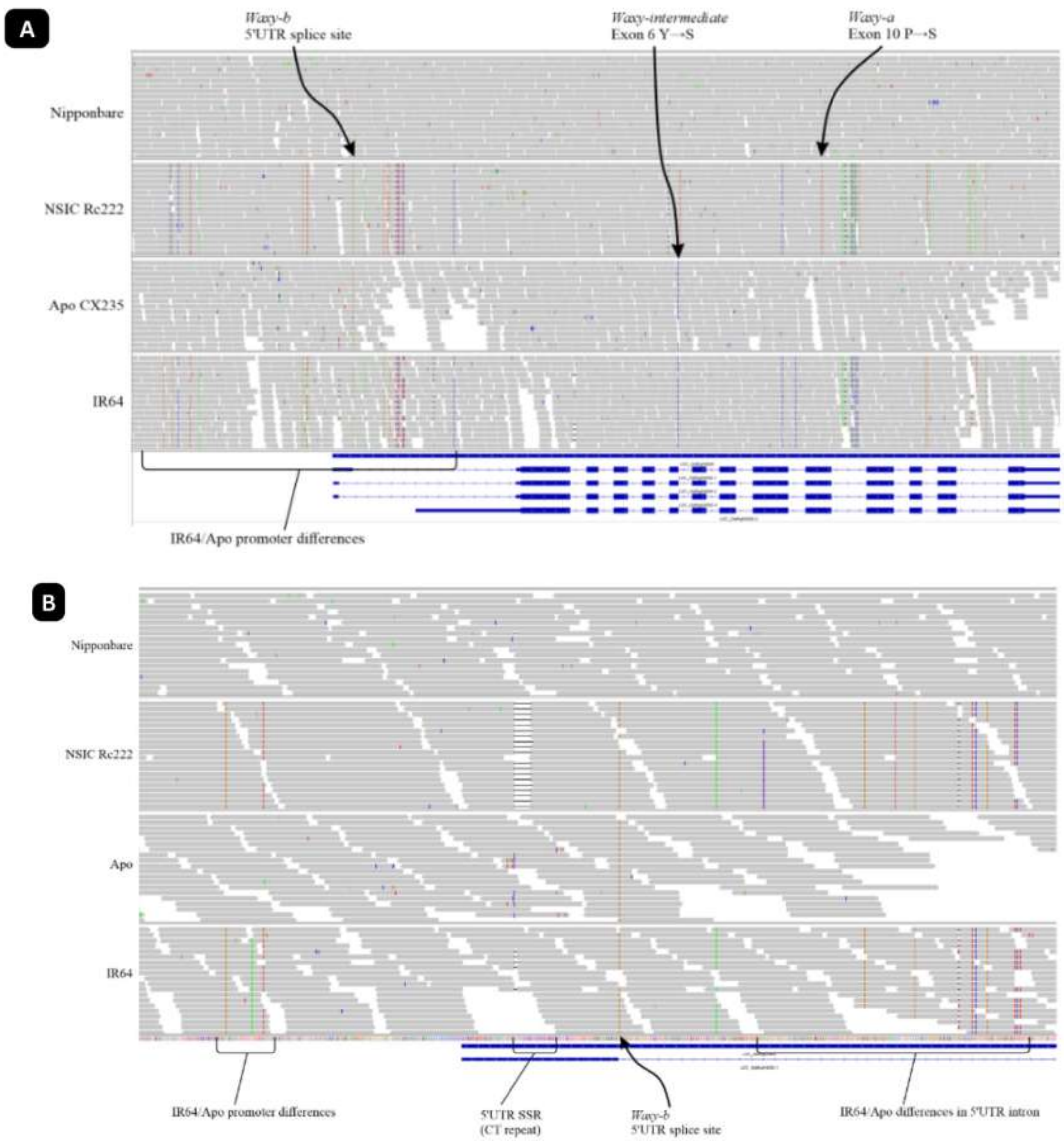

Figure 6. A. Skim sequence view of the LOC_Os06g04200 (Wx) region coding for the Granule-bound starch synthase (GBSS) gene. Genotypes used were Nipponbare (Low AC), NSIC Rc222, APO, and IR64 (Intermediate AC). B. Zoomed in view of the promoter and 5' UTR of the LOC_Os06g04200 (Wx) region

Protein content

This study identified four novel QTLs associated with protein content. Two of these QTLs were located on chromosome 1 and collectively accounted for 38% of the phenotypic variation (R2) (Table 3; Figure 6). The remaining two QTLs were found on chromosomes 10

Table 3. Distribution of QTLs for different cooking and eating quality traits detected by QTL-Cartographer. "+" and "–" values indicate the directions of additive effects of alleles from IR64 and APO, respectively.

Traits	QTL	Linkage Group	Distance(cM)	Marker Interval	LOD	Additive	R2
Amylose content (AC)	*qAC4*	4	14	id4007105-id4002562	8.5	10.2	18
	q4C4	4	48	id4011774-id4006135	5.9	14.6	14
	qAC11	11	9	id11004240-wd11001469	6.5	-6.82	9
Protein content (PC)	*qPC1a*	1	14	id1004348-id1008684	5.5	-7.75	24
	qPC1b	1	35	id1022408-id1023892	6.8	-10.5	14
	qPC8	8	35	id8003309-id8001854	5.8	-12.1	27
	qPC10	10	18	id10002602-id10005538	6.5	17.5	10
	qPC11	11	10	id1109687-id1101505	12	11.5	39
Peak viscosity (PV)	*qPV7*	7	8	id7000519-id7002859	6.5	14.4	10
	qPV9	9	47	id9002721-id9003720	5.5	50.5	12
	qPV11	11	20	id1104398-id1104812	8.5	5.5	6
Breakdown (BD)	*qBD1*	1	16	id1023892-id1028304	6.2	4.55	8
	qBD4	4	8	id4002562-id4006135	5.5	-8.35	5
Final viscounty (FV)	*qFV1*	1	12	id1014853-id1020828	5.5	22.2	14
	qFV10	10	1	id10001624-id10001251	7.5	32.5	12
Set back (58)	*qSB10*	10	48	id1000524-id1006963	8	-19.3	8
	qSB11	13	10	id1009687-id1031505	8.5	-22.4	29
Peak time (PT)	*qPT1a*	1	17	id1000711-id11000727	5.5	24.2	18
	qPTI b	1	20	id1004348 id1008686	6.5	22.3	22
	qPTI c	1	38	id3010526-id1030609	7.2	31.1	36
	qPT8a	8	15	id8001477-id8002194	6	20.1	12
	qPT8b	8	10	id8001223-id8001477	5	15.2	13
	qPT9	9	25	id9003720-id9004168	6.9	23.7	14
Pasting temp (PST)	qPs721a	11	5	id11000133-id110515	7	10.1	5
	qPs721b	11	45	id3007488-011001784	5.5	15.2	8
Lift-off (LO)	*qPLO11*	11	30	id1004240-id1001464	5	27.1	12
	qpLO1	1	11	id1014853-id1020828	6	21.4	14

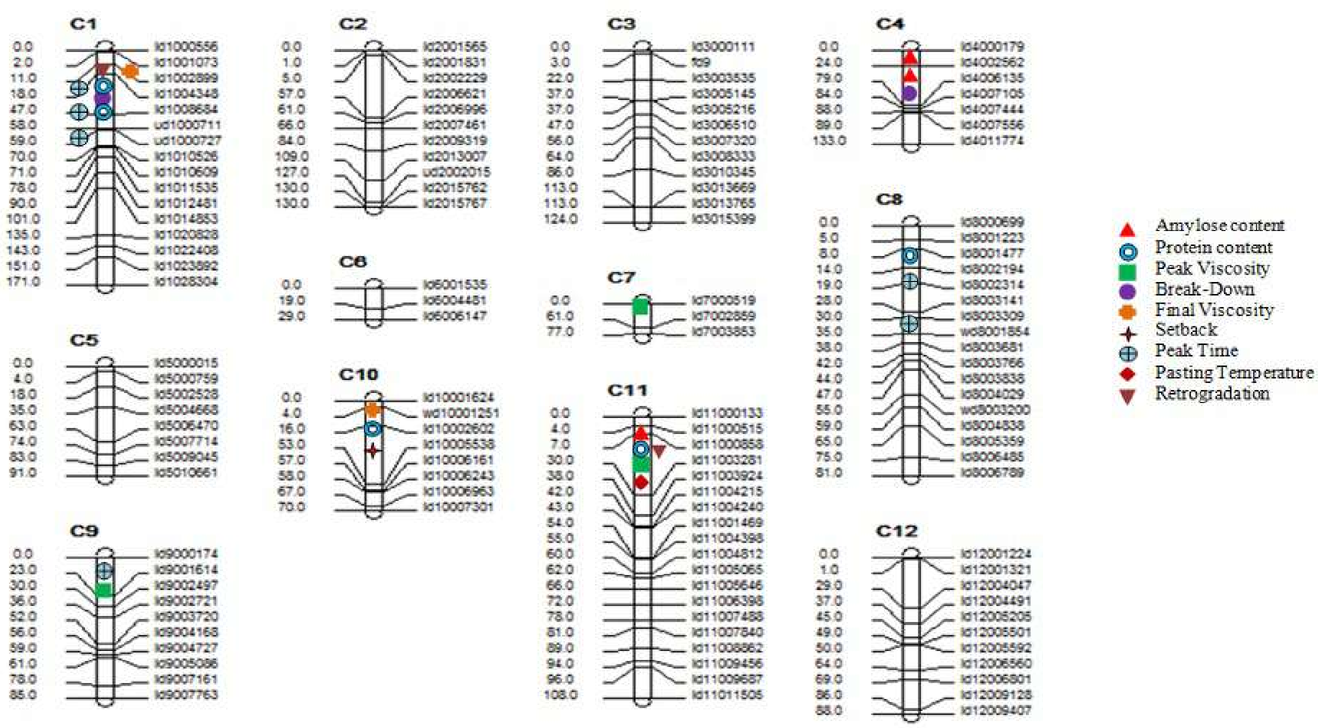

Figure 7. Rice linkage map with QTLs detected for different traits linked to different genome-wide SNPs.

and 11, explaining 10% and 39% of the phenotypic variation, respectively (Table 3). Notably, on chromosome 1, Apo alleles had a negative effect on protein content, with the total phenotypic variation attributed to these alleles being 38% (Table 2).

Pasting properties

This study identified several QTLs for various starch properties related to pasting properties that are associated with the cooking and eating properties of rice. For peak viscosity (PV), three QTLs, namely, qPV-7, qPV-9, and qPV-11, were found on chromosomes 7, 9, and 11. These QTLs accounted for 10%, 12%, and 6% of the phenotypic variance (R2), with corresponding LOD scores of 6.5, 5.5, and 8.5, respectively (Table 3; Figure 1). Breakdown was associated with two QTLs: qBD-1 on chromosome 1 and qBD-4 on chromosome 4, explaining 8% and 5% of the phenotypic variance, respectively, with LOD scores of 6.2 and 5.5, respectively (Table 3; Figure 6). Final viscosity was linked to two QTLs (qFV-1 and qFV-10) on chromosomes 1 and 10, accounting for 14% and 12% of the phenotypic variance, respectively, with LOD scores of 5.5 and 7.5, respectively (Table 3; Figure 6). Setback was associated with two QTLs, qSB-10 and qSB-11, on chromosomes 10 and 11, explaining 8% and 29% of the phenotypic variance, respectively, with LOD scores of 8.0 and 8.5, respectively (Table 3; Figure 6). Analysis identified six QTLs associated with peak time, which were distributed across three chromosomes. Chromosome 1 harbored three QTLs, chromosome 8 contained two QTLs, and chromosome 9 contained one QTL. These QTLs explained varying proportions of the phenotypic variance (R2): 18%, 22%, 36%, 12%, 13%, and 14%, respectively. The corresponding LOD scores for each QTL were 5.5, 6.5, 7.2, 6.0, 5.0, and 6.9, respectively (Table 3; Figure 6). Two QTLs on chromosome 11 were linked to pasting temperature, accounting for 5% and 8% of the phenotypic variance (R2) with LOD scores of 7.0 and 5.5, respectively (Table 3; Figure 6), respectively. Chromosomes 1 and 11 each harbored a QTL (qLO-1 and qLO-11) responsible for 12% and 14% of the phenotypic variance, respectively, with corresponding LOD scores of 5.0 and 6.0. The IR64 alleles located on chromosomes 1 and 11 enhanced starch retrogradation by 58% (Table 3; Figure 6).

DISCUSSION

Starch, which comprises 90% of the dry weight of rice grains, primarily influences their cooking and eating characteristics. The distinct cooking and eating qualities of Apo and IR64 were apparent from the detailed grain quality measurements. Earlier studies have shown that IR64 rice grains have high sweetness, low astringency, and water-like metallic qualities, whereas Apo rice was characterized by strong sewer/animal, water-like metallic, astringent, and sour/silage attributes, with low sweetness (Champagne et al. 2010). Additionally, Calingacion et al. (2014) confirmed these findings, associating IR64's pleasant profile with indole, octanal, and decanal, which give sweet, fruity aromas, while off-flavor compounds, including propene thiol, ocimene, heptanal, and hexanal, giving sewer/animal, sour/silage, and hay-like/musty aromas, were associated with Apo. Consumer sensory analysis found that most consumer panelists perceived Apo as rough and not sticky between grains. On the first bite, the Apo was judged to be non-springy, cohesive, and hard. While chewing, the panelists described the grains as lacking cohesiveness of mass and uniformity of bite but able to absorb moisture. After swallowing, residual loose particles were observed, but no toothpack was observed. Most panelists perceived that IR64's grain surface had an initial starchy coating and slickness without roughness. The grains were sticky to the lips and to each other. At first bite, the IR64 grains were springy but not cohesive or hard. While chewing, IR64 had cohesiveness of mass and uniformity of bite but did not absorb moisture. After swallowing, IR64 had no residual loose particles or toothpack. This study highlights the intricate genetic factors that govern cooking and eating quality. The apparent amylose content is not the sole determinant of the culinary attributes of rice. Amylose content ranges from 1-35% in rice and is a key determinant of cooking and eating quality, with varieties discriminated by at least ten functional SNPs in the Wx locus. Rice varieties are classified based on their AAC (%) content: (1) waxy = 1-2% characterized by a 23-bp deletion in the Wx locus (Wx allele) (Wanchana et al.2003); (2) very low amylose 2-9% attributed to Wx1-1, Wxmp, Wxmq, and Wxop/hp (Ando et al.2010; Zhou et al.2021a; Zhang et al.2021b); (3) low amylose = 10-19% while Wxb and Wxmw/la Ando et al. 2010; Zhou et al.2021a; Zhang et al.2021b; (4) intermediate = 20-25% Wx(int)/Wx(a) (intermediate/high amylose) (Chen et al.2012);(5) high amylose = 25-33%controlled by *Wxlv* and *Wxa* alleles, which result in different starch viscosities (Zhang et al.2019a). The G-T SNP at the 5′-leader intron splice site distinguishes between waxy and low amylose varieties, and those with intermediate and high amylose content (Chen et al.2012). Varieties with intermediate and high AC have the sequence AGGTATA at this site, whereas those with low AC have AGTTATA. However, no functional SNPs are known to distinguish the different cooking and eating qualities of rice varieties within the same intermediate ACC class. The varying cooked and eating rice texture of IR64 and Apo may be explained by the putative functional SNPs in the promoter and 5'UTR that were shown in this study and the differing ratios of short and long chains, which vary based on GBSS alleles and/or starch synthesizing genes (Crofts et al. 2019). During cooking, AC negatively affects adhesiveness Misra et al.(2018), which explains the significantly lower adhesiveness observed in IR64 resulting in softer, cooked rice texture of the variety. IR64 had a higher PV than Apo, where PV represents the equilibrium between the shear and swelling of starch granules (Santamaria et al. 2021). The higher peak viscosity (PV) of IR64 contributes to its softer, stickier cooked texture and superior eating quality compared to Apo, consistent with findings from a previous study by Peng et al.(2021). This is because of the higher proportion of amylopectin short chains and relatively lower proportion of amylopectin long chains in the starch structure, leading to increased swelling power and water absorption during cooking, resulting in a softer and more cohesive texture (Zhu et al. 2023).

Rice genotype classification and cooking quality assessment often rely on viscosity curves as a valuable tool (Fitzgerald et al. 2003). The ability of starch granules to absorb water and remain intact after gelatinization is indicated by the peak viscosity (Singh et al.2009). The difference between the final (FV) and peak viscosities (PV), known as setback (SB), is linked to grain amylose content and is an indicator of the firmness of cooked rice, with higher values indicating a firmer texture (Cuevas et al. 2012). FV reflects the influence of starch gelatinization and the cooked rice viscosity properties. A higher amylose content and firmer cooked rice texture correspond to a more positive setback. Retrogradation is associated with a higher final viscosity, whereas a lower final viscosity is related to a softer gel. Liftoff (LO), another term for retrogradation, is the difference between the FV and the trough viscosity (TV), indicating gel consistency upon cooling.

This study identified major and minor effect QTLs for all the cooking and eating quality parameters, including AC, pasting properties, and total protein content, located on different rice chromosomes, including 1, 4, 7, 8, 9, 10, and 11. Because IR64 and Apo belong to the intermediate AAC class, they share the same *Wx*$^{(int)}$/*Wx*$^{(a)}$ (intermediate/high amylose) (Chen et al. 2012). However, to date, the functional G-T SNP found in the 5' UTR of the *Wx* locus can only discriminate low from intermediate/high AC classes, but it cannot discriminate varieties within the same intermediate amylose class, with different cooking and eating properties. Therefore, variations in cooking and eating quality may be attributed to differences in starch structure and other alleles that affect the pasting profile and protein content. Rapid viscosity analysis showed that Apo had a lower peak viscosity compared to IR64, based on the pasting profile or cooked-rice texture and its characteristics after retrogradation.

Three QTLs related to AAC were identified in this study: two located on chromosome 4 (*qAC-4a* and *qAC-4b*) and one on chromosome 11 (*qAC-11*). The QTL for AC identified in this research is also situated on chromosome 4, as previously noted by Li et al. (2004) concerning amylose content. Various positions on chromosome 4 have been associated with QTLs for AC (Chattopadhyay et al. 2023; Nie et al. 2024). Nie et al. (2024) pinpointed two QTLs on chromosome 4, specifically qAC4-1 at 5,779,943 bp and *qAC4-2* between 14,068,488 bp and 14,178,161 bp. The stability of *qAC4-2* across two environments suggests minimal environmental impact. Chattopadhyay et al. (2023) identified *qAC4.1* and *qAC4.2* between 33,596,398 bp and 35,110,870 bp, which explained approximately 25% of the phenotypic variance for amylose content and overlapped with QTLs for grain protein content. This study uncovered QTLs for amylose content on chromosome 4 between 4,002,562 and 4,011,774 bp, indicating a potential new QTL for AC. Additionally, Jiang et al. (2024) found *qAC11*, a significant QTL for amylose content on chromosome 11, within the range of 5,456,564–5,535,796 bp, accounting for 19.40% of phenotypic variance. Three genes closely linked to amylose content were identified: LOC_Os11g10170-flavin monooxygenase; LOC_Os11g10180-OsFBX412 - F-box domain containing protein; and LOC_Os11g10200-OsFBX413 - F-box domain containing protein.

A major quantitative trait locus (QTL) for protein was discovered on chromosome 11, explaining 39% of the phenotypic variance (LOD=12), located between 1,009,687 bp and 1,101,150, suggesting the presence of key genes influencing protein content. Yun et al. (2016) identified a QTL for protein content between markers RM202 and RM206 on chromosome 11. Qin et al. (2009) located two QTLs on chromosome 11, *qPC11.1* and *qPC11.2*, with phenotypic variances of 22.10% and 6.92% and LOD scores of 4.90 and 2.75, respectively. Zhang et al.(2008) reported QTLs linked to glutelin content on chromosomes 2, 10, 11, and 12. This study detected two QTLs, *qPC1a* and *qPC1b*, on the same chromosome where QTLs associated with prolamin were previously reported by Zhang et al.2008 and Park et al.2019. Peng et al. (2014) identified qPC1 on chromosome 1's long arm within a 6.7-kb region aligned with marker PB13. This QTL is linked to the OsAAP6 gene, encoding an amino acid transporter that boosts grain protein content by regulating nutrient assimilation. Several studies have shown a QTL for protein content on chromosome 8 (Weng et al.2006; Zheng et al.2011; Yun et al.2014; Pradhan et al.2019; Nayak et al.2022). Pradhan et al. (2019) found qPC8 within the marker interval C483-C259G, detected at 21 days DAF with 12.94% and 28 DAF with 8.53% phenotypic variance. Kinoshita et al. (2017) reported qPC8 positioned between 3.0–8.6 Mb explaining 8.5–13.3% phenotypic variance, aligning with the current study's detected qPC8 between 8,003,309 and 8,001,854 bp, but with a higher phenotypic variance of 27%, indicating grain protein content is influenced by environmental factors. Wang et al. (2017) identified qPC10.1 and qPC10.2 between 7,659,738 and 17,723,490 bp, accounting for 8.1% and 6.7% of phenotypic variance, respectively. Yang et al. (2019) cloned *qGPC-10* between markers RM5758 and RM467, identifying OsGluA2 as a candidate gene linked to *qGPC-10*, crucial for protein storage. In contrast, this study located a QTL for grain protein content on chromosome 10 between 1,000,553 and 1,000,260 bp, potentially representing a novel locus.
A QTL for PV was identified at this locus, confirming the allelic interaction between protein content and pasting properties (Tan et al.2001). Research identified a quantitative trait locus (QTL) for peak viscosity on chromosome 7, consistently detected over multiple years between markers RM445 and RM418. This QTL accounts for 9.2% phenotypic variance with a LOD score of 3.2 (Wang et al.2007). Zhang et al. (2013) located a peak viscosity QTL, *qPKV7-1*, between 25,294,951 and 30,357,780 bp on chromosome 7, demonstrating an average additive effect of -14.15% across environments. Hsu et al. (2014) mapped QTL *qPKV7* near RM6420, explaining 6.7% phenotypic variance with an LOD score of 3.51

and a negative additive effect of -132.70 cP influenced by the TNG 78 allele. The current study identified *qPV7-2* between 7,002,859 and 7,000,519 bp, accounting for 10% phenotypic variance with a LOD Score of 6.5 and a positive additive effect of 14.4%. These varying additive effects may result from allelic interactions within the locus.

On chromosome 9, Shar et al. (2020) detected *qPKV9*, a peak viscosity QTL positioned between 7,888,509 and 14,373,074 bp. This QTL explained 9.42% phenotypic variance with a LOD score of 3.5 and a negative additive effect of -230.50 cP, attributed to the female parent, YK17. The present study identified a peak viscosity QTL on chromosome 9 between 9,002,721 and 9,003,720 bp, aligning with Shar et al. (2020) findings. However, this study observed a positive additive effect, potentially due to both parents belonging to the same apparent amylose content (AAC) class. Xu et al. (2015) reported *qPV9* between markers RM409 and RM257, explaining 7.04% phenotypic variance with a LOD score of 3.42 and an additive effect of 9.3. An isoamylase 3 (*ISA3*) gene, coding for an enzyme that hydrolyzes starch branch linkages, was found near this locus. Cho et al. (2013) identified QTL qPV9 between markers RM5688 and RM444, accounting for 9% phenotypic variation with a LOD Score of 2.67 and an additive effect of 25.6. QTLs for breakdown (*qBD9*) and setback (*qSB9*) were also detected in the same chromosomal position. Shar et al. (2020) identified two QTLs for peak viscosity on chromosome 11 with overlapping marker intervals between RM206 and RM5926 in two environments. The physical location of *qPKV11* in the first environment is 22,480,812 to 28,957,587 bp, LOD= 5.54, physical variance of 14.55, and additive effect of -253.35. Meanwhile, in the second environment, the location is 384,707 to 2,256,681 bp, LOD=4.50, phenotypic variance of 12.17%, and additive effect of -186.66. The QTL for peak viscosity in this study is located between 1,100,481 and 1,004,398 bp, which is situated within the chromosomal position of Shar et al. (2020), but has a higher LOD score of 12, a positive additive effect of 11.5, and phenotypic variance of 39%, confirming the interallelic interaction within the locus. This locus may also contain a major gene that significantly influences the trait. On chromosome 1, the QTLs for FV and LO shared the allele for protein content (Table 3), validating the allelic interaction between starch properties and protein content, which influences rice gel consistency after cooking (Tian et al. 2009). For BD on chromosome 1, IR64 alleles increased the trait by 4.5%, whereas Apo alleles on chromosome 4 decreased it by (-8.3) (Table 3). The BD locus on chromosome 4 is linked to AAC, supporting its role in regulating starch pasting properties (Li et al.2004). Shar et al. (2020) detected a QTL for breakdown viscosity, *qBDV1* on chromosome 1 between 3,947,852 to 5,287,494 bp with LOD=3.23, 10.77% phenotypic variance, and –94.60 cP negative additive effect. Bao et al. (2008) detected *qBDV-1* between markers RM1349 and RM246 with LOD= 3.04 and 6.68% phenotypic variance, and -66.71 cp negative additive effect. In contrast, *qBDV-1* showed a positive additive effect of 20.7 (Bao et al. 2000). The *qBD1* in this study is found between 1,023,892 to 1,028,304 with LOD=6.2, 8% phenotypic variance, and 4.55 positive additive effect, implying a novel minor QTL for breakdown viscosity (Table 3). Earlier studies reported the presence of QTL for breakdown viscosity on chromosome 1 only in one environment (Bao et al.2000; Zhang et al.2008; Shar et al.2020). This indicates that allele interaction for breakdown viscosity on chromosome 1 is highly sensitive to the environment, evident by varying additive effects.

A QTL named *qBD4* was identified by Zhang et al. (2017) on chromosome 4 in multiple environments, located between markers G264 and G177, with an average LOD score of 2.99, explaining 11% of phenotypic variance and showing a positive additive effect of 12.99. Yao et al. (2017) detected *qBD4* in a single environment between markers RM1359 and RM1155, with a LOD score of 3.56, accounting for 8.54% of phenotypic variance and demonstrating a positive additive effect of 80.21 cP. The current research uncovered a new minor QTL for BD, *qBD4.2*, on chromosome 4, located between positions 4,002,562 and 4,006,135, with a LOD score of 5.5, explaining 5% of phenotypic variance, and a negative additive effect of 8.35.Two QTLs for pasting temperature were identified on chromosome 11, with IR64 alleles increasing the trait by 10% and 15% at both the loci (Table 3). These results showed the quantitative nature and the interaction effects of some alleles regulating the pasting properties of rice. Previous studies have reported the involvement of minor genes in the control of various viscosity parameters (Bao et al.2000). This suggests that starches with high peak viscosity (PV) are prone to having elevated breakdown values, resulting in fragile gels. These weak gels are susceptible to disintegration when exposed to heat and shear forces (Singh et al.2006; Tsakama et al.2010). Zhao et al. (2011) identified four QTLs for final viscosity in separate locations on chromosome 1 linked with markers RM580, RM1, RM1387, and RM1067 with distances of 68.2 cM, 79.4 cM, 126.1, and 181.8, respectively, and one QTL for final viscosity was detected on chromosome 10 linked with marker RM171 with 58.1 cM. The RVA traits that overlap on markers from chromosome 1 are peak time viscosity (PK), trough viscosity (TV), and setback viscosity (SB), while on chromosome 10 are PV, PK, and SB. This suggests pleiotropic effects among RVA traits. In this study, qFV1 is located between 1,014,853 and 1,020,828 bp along with LOD=5.5, 14% phenotypic variance, and 22.2 positive additive effects. Meanwhile, qFV10 is located between 1,000,125 and 1,000,162 bp with LOD=7.5, 12% phenotypic variance, and 32.5 positive additive effects. High additive effects for FV might be due to interallelic interaction and pleiotropic effects.

Shar et al. (2021) found QTL qSBV10 for setback viscosity (SB) on chromosome 10 located between 21,181,946 and 22,314,708 with LOD=5.4, 14.80% phenotypic variance, and 147.72 cP additive effects. In a different chromosomal position on chromosome 10, a QTL influencing setback viscosity was detected between 10. 0.8–2.12 mB. These QTLs for SB on chromosome 10 were consistently observed across

multiple environments. The QTL qSB10 in this study is located between 1,000,524 and 1,006,963 with LOD=8, 8% phenotypic variance, and -19.3 additive effect, indicating that this is a novel QTL for SB.
Yao et al. (2017) detected QTL *qSBV11* which was observed across multiple environments and showed LOD scores of 7.69, 5.75, and 13.11, corresponding to phenotypic variances of 6.16%, 7.53%, and 33.92%, respectively. The additive effects were measured as -137.77, -63.83, and -135.31 cP. Only one study has reported a QTL for setback viscosity (*qSBV11*) on chromosome 11. Although Yao et al. (2020) did not specify the chromosomal position of *qSBV11*, this study validates the presence of QTL for SB on chromosome 11. In this study, the *qSB11* is located between 1,009,687 and 1,011,505 with LOD=8.5, 29% phenotypic effect, and -22.4 additive effect. This almost coincides with the QTL for protein also found in this study. Earlier studies showed that protein content is negatively correlated to peak viscosity and hot paste viscosity (Tan and Corke, 2002).

This study identified two QTLs for pasting temperature on chromosome 11. *qPsT11a* is located between 1,100,133 and 1,105,150 bp, with LOD=7, explaining 5% of phenotypic variance and 10.1 additive effect. *qPsT11b* is located between 1,007,488 and 1,100,784 bp, with LOD=5.5, accounting for 8% of phenotypic variance and 15.2 additive effect. This QTL codes for a putative expressed ATCHX gene. While early studies detected *qPaT11*, a QTL for pasting temperature on chromosome 11 located within 384,707 to 2,844,332 bp with LOD=5.92, 16.34% phenotypic variance, and 1.56 additive effect (Shar et al. 2020). Shar et al. (2020) also detected a QTL for peak time on chromosome 1 between 21,903,124 and 23,930,646 bp with LOD=5.43, 11.86% phenotypic variance, and 0.12 additive effects. This study detected three QTLs for peak time on chromosome 1. *qPT1a* is located between 1,000,711 and 1,000,727 bp with LOD=5.5, 18% phenotypic variance, and 24.2 positive additive effect. *qPT1b* is located between 1,004,348 and 1,008,684 bp with LOD=6.5, 22% phenotypic variance, and 22.3 positive additive effect. *qPT1c* is located between 1,010,546 and 1,010,609 bp with LOD=7.2, 36% phenotypic variances, and 31.1 positive additive effects. Interallelic interaction results in high additive effects.

Research by Zhang et al. (2017) revealed a QTL for peak time viscosity on chromosome 8, with a LOD score of 3.49, explaining 20.47% of phenotypic variance, and an additive effect of 0.13. Yan et al. (2014) identified *qBTime8* between markers RM310 and RM547, showing a LOD score of 5.94, accounting for 13.8% of phenotypic variance, and a -0.03 additive effect, potentially harboring alleles that reduce peak time. The *qBTime8* locus overlaps with several other QTLs, including *qAC-8* (amylose content), *qGC-8* (gel consistency), *qBTemp-8* (peak temperature), and *qBAtime-8* (viscosity peak time), all displaying negative additive effects. These traits relate to eating and cooking quality, and their consistent negative additive effects might be attributed to linkage. These QTLs are only observed in one environment, suggesting that peak time viscosity QTLs are highly influenced by environmental factors (Yan et al. 2014; Zhang et al, 2017). The current investigation uncovered two QTLs for peak time viscosity at distinct chromosomal locations. The first, *qPT8a*, is situated between 8,001,477 and 8,002,194 bp, with a LOD score of 6, explaining 14% of phenotypic variance, and showing +20.1 additive effects. The second, *qPT8b*, is located between 8,001,223 and 8,001,477 bp, with a LOD score of 5, accounting for 13% of phenotypic variance, and exhibiting +15.2 additive effects. These QTLs are in proximity, with lead SNPs at 8,001,836 bp and 8,001,350 bp, respectively, separated by only 486 bp. The proximity of these major QTLs suggests a strong genetic linkage influencing the peak time viscosity.
Multiple studies have established QTLs for key RVA parameters including peak viscosity (PV), trough viscosity (TV), breakdown (BD), setback (SB), pasting temperature (PT), and peak time (PsT). However, no QTL had previously been reported for lift-off viscosity. This study identified two novel QTLs associated with lift-off viscosity on chromosomes 11 and 1. The chromosome 11 QTL, *qLO11*, is positioned between 1,004,240 and 1,001,464 bp, while the chromosome 1 QTL, *qLO1*, is located between 1,014,853 and 1,020,828 bp. These QTLs demonstrate LOD scores of 5 and 6, respectively, and explain 12% and 14% of phenotypic variance. Both QTLs exhibited positive additive effects, contributing 27.1 and 21.6 to lift-off viscosity, suggesting that they may be major QTLs influencing this trait.

The genetic influence of the waxy locus and its modifier genes on the biochemical variation in starch-related properties remains ambiguous, as more than 18 genes are involved in various stages of starch biosynthesis (Tian et al. 2009, Sreenivasulu et al.2022).

QTLs located on chromosomes 1, 4, and 11 encompass genes related to starch synthesis (SSRGs) that play a role in starch production. Further investigation is needed to elucidate how these genes influence starch, and consequently, the quality of rice in terms of eating and cooking quality of rice varieties within intermediate AC classes. Additional QTLs with significant effects, such as those found on chromosomes 1, 8, and 9, may encode other enzymes involved in starch biosynthesis that require identification. Furthermore, given that some viscosity parameters are interconnected and share common alleles, a thorough examination of the molecular and biochemical mechanisms underlying the correlations between these traits is necessary.

CONCLUSION

We used the mapping population derived from ApoxIR64 to identify QTLs that determine the cooking quality. A total of 27 QTLs were detected in eight linkage groups across all environments in two consecutive seasons. In this study, QTLs were identified to be associated with amylose content, protein content, and viscosity profile properties including peak viscosity, breakdown, pasting time, and lift-off. Some novel QTLs were reported here for protein content (*qPC4.1, qPC4.2* and *qPC10.1*), viscosity profiles including breakdown (*qBD1, qBD4.2*), setback (*qSB10*) and lift-off *qLO1 and qLO11*). Most of the QTLs detected had low additive effects, showing the complex nature of the genetic architecture controlling these cooking property-related traits. Interestingly, the SNPs in the promoter region before the 5' UTR of *the Wx* gene, where the CT repeats occur, warrant further investigation that could lead to a more thorough understanding of the *role of Wx* in the cooking and eating profile of rice, especially those within the same amylose class. These studies provide good suggestions for MAS to improve the properties of cooked rice. Fine mapping and gene validation studies are recommended for subsequent research to provide a theoretical basis for the discovery of functional markers for cooking and eating quality traits that could be used to replace the alleles of otherwise good varieties in terms of yield and climate resilience but have undesirable cooking and eating qualities using modern breeding approaches.

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