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Comparative Analysis for Physicochemical and Pasting Characteristics of Advanced Non-Glutinous Rice Genotypes Under Irrigated Condition in Thailand

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Abstract

Improving grain quality alongside yield remains a primary objective in rice breeding, especially under irrigated systems in Thailand, where consumer demand for soft-textured, premium table rice continues to grow. This study evaluated physicochemical and pasting characteristics of ten advanced non-glutinous rice genotypes compared with high- and low-amylose checks across three irrigated environments during off-season 2024. Combined ANOVA revealed highly significant genotype, environment, and genotype × environment interaction effects, with genotypes contributing up to 94.30% of total variation for key quality traits. Grain breadth and elongation rate were predominantly influenced by environmental conditions. Principal component analysis showed that PC1 and PC2 explained 72.86% of total variance, separating genotypes based on amylose-driven starch properties and paste stability. High-amylose genotypes exhibited low peak viscosity and high setback, whereas low-amylose genotypes showed greater swelling, higher breakdown, and softer pasting behavior. Selected genotypes exhibited distinct quality profiles; specifically, DS24-Inter-8 and DS24-Inter-10 combined low-to-intermediate amylose (15.09–19.73%) with high gel consistency (84.78–96.22 mm). Interestingly, DS24-Inter-4 maintained high gel consistency (97.78 mm) despite a higher amylose content (26.39%), indicating a unique soft-cooking profile for high-amylose types. In contrast, DS24-Inter-7 and DS24-Inter-9 showed typical firmer, high-amylose characteristics. These contrasting quality profiles indicate that the genotypes were suitable for different utilization purposes depending on the desired physicochemical and textural attributes. Therefore, the advanced genotypes demonstrated stable and diverse quality profiles under irrigated conditions, warranting further multi-location and multi-season evaluation.

Keywords: crop improvement; genetic variability; apparent amylose content; peak viscosity; breakdown; setback; *Oryza sativa* L.

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1. Introduction

Rice (*Oryza sativa* L.) is a foundational global staple, supplying over 20% of caloric intake and sustaining rural livelihoods across Asia [1,2]. As production systems intensify and markets shift toward differentiated quality standards, grain quality has become a decisive factor shaping market value, particularly within premium non-glutinous or non-waxy rice markets of Southeast Asia [3]. Grain quality encompasses multiple dimensions, such as eating and cooking quality (ECQ), nutritional value, appearance traits, milling performance, and hygiene quality, with ECQ being the most influential attribute governing consumer acceptance [4–6].

The ECQ of rice is governed mainly by starch molecular composition and its functional behavior during gelatinization and cooling. Key physicochemical traits, including apparent amylose content (AAC), gel consistency (GC), gelatinization temperature (GT), and pasting properties, capture the structural organization of amylose and amylopectin and directly predict ECQ attributes such as cooked-rice hardness/softness, stickiness, cooking time, and retrogradation tendency [7,8]. At the molecular level, these quality-related traits are regulated by a coordinated network of *Starch Synthesis-Related Genes* (*SSRGs*), including *AGPase*, *SS*, *GBSSI*, *SBE*, and *DBE* [9]. The *Waxy* (*Wx*) locus is the principal determinant of AAC and GC [10,11], whereas GT is governed largely by *ALK/SSIIa*, which modulates amylopectin chain-length distribution and crystalline structure [12,13]. Additional *SSRGs*, such as *SBEI/BEIIb* and *ISA1*, contribute to amylopectin branching patterns, affecting swelling power, viscosity breakdown, and retrogradation behavior [14,15].

Instrumental profiling, particularly Rapid Visco Analyzer (RVA) analysis, provides standardized functional indicators of starch behavior during heating and cooling, including swelling capacity, paste stability under shear, and retrogradation tendency [16–19]. These RVA-derived parameters complement AAC, GC, and GT by translating starch structural differences into measurable rheological patterns that are directly relevant to ECQ. Across South and Southeast Asia, consistent associations among AAC, RVA parameters, and consumer sensory preference have been reported, supporting RVA as a practical tool for discriminating cooking-quality groups in rice germplasm [5,17,20,21]. Nonetheless, prior studies have focused primarily on landraces or traditional cultivars, resulting in limited understanding of quality expression in advanced non-glutinous breeding lines developed for irrigated production systems. Achieving stable ECQ across environments remains a major breeding challenge because the determinants of rice texture are largely established during grain filling, when endosperm starch is synthesized and deposited. Thermal and edaphic variation during this stage (e.g., temperature, radiation, irrigation regime, and nutrient availability) can modify amylose accumulation, amylopectin chain organization, and starch crystallinity, thereby shifting AAC, GT, and RVA pasting profiles and ultimately altering sensory texture attributes such as softness, stickiness, cooking time, and retrogradation tendency [22–24]. Consequently, multi-environment studies consistently report substantial genotype $\times$ environment (G $\times$ E) effects for these quality traits, leading to inconsistent ECQ performance across locations and seasons [4,25–27]. However, evaluations of advanced breeding lines under irrigated ecosystems remain limited.

Thailand's Central Plain, an internationally important irrigated rice production zone, is characterized by heterogeneous microclimates and spatially variable soil fertility, particularly during dry-season cultivation [28]. Although ECQ and starch properties have been documented for Thai rice cultivars, the stability and G $\times$ E interaction responses of advanced non-glutinous breeding lines, especially those targeting the growing demand for soft-cooking rice, remain insufficiently understood. This study aims to evaluate the physicochemical and pasting characteristics of advanced non-glutinous rice genotypes across multiple irrigated environments in Thailand to identify those combining soft-cooking ECQ attributes with stable performance for high-quality production systems.

2. Materials and Methods

2.1. Study Site Descriptions

The experiment was conducted at three representative irrigated rice-growing locations in the central plain of Thailand during the dry season: Pathum Thani

(14°03′54″ N, 100°38′46″ E), Suphan Buri (14°46′30″ N, 99°54′40″ E), and Chai Nat (15°15′19″ N, 100°02′17″ E). These sites were selected to represent major dry season irrigated production environments with contrasting soil and climatic conditions in Thailand's central plain. The study was carried out during the 2024 dry season (January–May 2024).

Monthly weather data, including maximum and minimum temperatures and cumulative rainfall, were obtained from nearby meteorological stations and are summarized in Figure S1. Across locations, daily temperatures during the grain-filling period ranged from 25.75 to 36.70 °C, with low rainfall typical of dry-season cultivation. All experimental fields were managed under controlled canal-based irrigation, and standing water depth was maintained at approximately 5–10 cm throughout the cropping period.

2.2. Soil Properties

Topsoil samples (0–15 cm) were collected from each site prior to land preparation using a composite sampling method adapted from standard soil analysis procedures. Five subsamples were collected in a zig-zag pattern across each field and combined to obtain one representative sample per location. Samples were air-dried, ground, and passed through a 2 mm sieve prior to analysis.

Soil pH, organic matter content, texture, and macronutrient levels (total N, available P, and exchangeable K, Ca, and Mg) were determined following established protocols, with analyses conducted at the Soil Science Laboratory, Department of Plant and Soil Sciences, Chiang Mai University. A summary of the physicochemical properties of soils at the three locations is presented in Table S1.

In brief, Pathum Thani soils were classified as clay, Suphan Buri soils as sandy loam, and Chai Nat soils as sandy clay loam, reflecting the inherent heterogeneity of irrigated lowland soils in Thailand's Central Plain.

2.3. Plant Materials and Experimental Design

Ten advanced non-glutinous rice breeding lines (designated DS24-Inter-1 to 10; where DS24 and Inter signify the 2024 dry season and advanced intermediate status, respectively) were evaluated. These lines were developed under the irrigated lowland breeding pipeline of the Ruamjai Pattana Kwamroo Research Station, Thailand. Two reference cultivars, RJP233088 (low-amylose) and RD85 (high-amylose), served as contrasting ECQ checks (Table 1).

Table 1. Non-glutinous rice genotypes were used in this study.

No.	Genotypes	Generation	Parental Lines	
			Female	Male
1	DS24-Inter-1	F_{11}	improved from A-line	IRRI
2	DS24-Inter-2	F_{11}	selected from modified mass selection population	
3	DS24-Inter-3	F_{11}	selected from modified mass selection population	
4	DS24-Inter-4	F_{11}	selected from modified mass selection population	
5	DS24-Inter-5	F_{11}	selected from modified mass selection population	
6	DS24-Inter-6	F_{11}	selected from modified mass selection population	
7	DS24-Inter-7	F_{12}	improved from A-line	advanced line (F_8)
8	DS24-Inter-8	F_{12}	advanced line (F_8)	advanced line (F_8)
9	DS24-Inter-9	F_{11}	advanced line (F_8)	advanced line (F_8)
10	DS24-Inter-10	F_{11}	improved from A-line	advanced line (F_8)
Check varieties				
11	RD85		Rice Department, Thailand	
12	RJP233088		Ruamjai Pattana Kwamroo Research Station	

The experiment was arranged in a randomized complete block design (RCBD) with three replications per site. Twenty-five-day-old seedlings were transplanted at 25 × 25 cm spacing into four-row plots (5 × 1 m). Crop management followed Thai Rice Good Agricultural Practices (GAP) [29] to ensure consistency across environments.

At maturity, ten representative panicles per genotype per replication were harvested. Grain processing and milling followed standard procedures, and milled rice flour was prepared and stored at −20 °C prior to analysis of GC, ASV, AAC, and RVA pasting properties.

2.4. Measurement of Grain Dimensions (Length, Breadth, and Shape)

For each genotype and replication, thirty fully developed head rice kernels were randomly selected for grain-dimension measurements. Grain length and breadth (mm) were measured using a digital Vernier caliper with 0.01 mm precision, following standard IRRI grain-quality evaluation procedures [30]. Measurements were conducted at approximately 14% grain moisture content.

Based on kernel length, genotypes were classified as extra-long (>7.50 mm), long (6.61–7.50 mm), medium (5.51–6.60 mm), or short (<5.50 mm), according to IRRI classification criteria [30].

Grain shape was expressed as the length-to-breadth (L/B) ratio, calculated as:

$$L/B = \frac{L\ (\mathrm{mm})}{W\ (\mathrm{mm})} \quad (1)$$

where L is the average grain length (mm) and W is the average grain width (mm). Based on the L/B ratio, grains were categorized as slender (>3.0), medium (2.1–3.0), bold (1.1–2.0), or round (<1.1) following IRRI standards [20].

2.5. Determination of 1000 Grain Weight

The 1000-grain weight was determined by randomly counting and weighing 1000 head rice kernels per genotype at approximately 14% moisture content, following standard grain-quality evaluation procedures [20].

2.6. Evaluation of Grain Chalkiness

Grain chalkiness was evaluated using 100 head rice kernels per genotype and replication. Kernels were visually scored under uniform lighting on a six-point scale (0–5) based on the proportion of chalky area on the endosperm surface, adapted from the IRRI Standard Evaluation System [30]. A chalkiness value was calculated as:

$$\text{Chalkiness value} = \sum(Ci \times Xi)/100, \quad (2)$$

where C_i is the chalkiness score and X_i is the number of kernels at each score. Chalkiness was classified as low (0–1.0), medium (1.10–1.50), medium-to-high (1.60–1.90), or high (2.0–5.0).

2.7. Determination of Gel Consistency

GC was determined following the method described by Cagampang et al. [31], as detailed in the IRRI Standard Evaluation System [30]. Briefly, rice flour samples were gelatinized, allowed to cool horizontally, and the gel length (mm) was measured after a fixed setting period. Based on gel length, samples were classified into hard (26–40 mm), medium (41–60 mm), and soft (>60 mm) gel consistency classes.

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2.8. Determination of Gelatinization Temperature by Alkali Spreading Value

GT was determined to use the ASV test following the IRRI Standard Evaluation System [30]. Six intact milled rice kernels per genotype were incubated in 1.7% (*w*/*v*) KOH at 30 °C for 23 h. Kernel spreading was visually scored on a 1–7 scale, with higher ASV indicating lower gelatinization temperature.

2.9. Determination of Elongation Ratio

Elongation ratio (ER) was determined as the ratio of the mean length of cooked rice kernels to that of uncooked kernels, following standard procedures [32]. Measurements were performed on ten randomly selected kernels per genotype.

2.10. Determination of Apparent Amylose Content

AAC was determined using the iodine-binding colorimetric method described by Juliano [33], as detailed in the IRRI Standard Evaluation System [30]. Rice samples were classified as low (10–19%), intermediate (20–24%), or high (>25%) amylose types according to standard criteria.

2.11. Measurement of Pasting Properties

Pasting properties were measured using an RVA (model 3-D; Newport Scientific, Warriewood, Australia) following Li et al. [34]. Rice flour (3.0 g, adjusted to 14% moisture basis) was mixed with 25 mL distilled water in an RVA canister. The suspension was stirred at 960 rpm for 10 s, then maintained at 160 rpm throughout the test.

The standard heating-cooling cycle consisted of equilibration at 50 °C for 60 s, heating to 95 °C over 4 min, holding at 95 °C for 2 min, cooling to 50 °C over 4 min, and holding at 50 °C for 2 min. Peak viscosity (PV), trough viscosity (TV), final viscosity (FV), breakdown viscosity (BD), setback viscosity, peak time (PeT), and pasting temperature (PaT) were recorded automatically in Rapid Visco Units (RVU). To facilitate comparison with SI units, a conversion factor of 1 RVU = 12 centipoise (cP) may be applied, according to the manufacturer's specifications. Each sample was analyzed in triplicate.

2.12. Statistical Analysis

Prior to combined analysis, data from each environment were tested for homogeneity of error variances using Levene's test following Gomez and Gomez [35]. As error variances were homogeneous (α = 0.05), a combined analysis of variance (ANOVA) across the three environments was performed using the following linear model:

$$\boldsymbol{Y_{ijk}} = \boldsymbol{\mu} + \boldsymbol{E_k} + \boldsymbol{R_{j(k)}} + \boldsymbol{G_i} + (\boldsymbol{GE})_{\boldsymbol{ik}} + \boldsymbol{e_{ijk}} \quad (3)$$

where $\boldsymbol{Y_{ijk}}$ is the observed value of genotype $\boldsymbol{i}$ in replication $\boldsymbol{j}$ of environment $\boldsymbol{k}$;

$\boldsymbol{\mu}$ is the overall mean;
$\boldsymbol{E_k}$ is the fixed effect of environment $\boldsymbol{k}$ (k = 1–3);
$\boldsymbol{R_{j(k)}}$ is the effect of replication $\boldsymbol{j}$ nested within environment $\boldsymbol{k}$;
$\boldsymbol{G_i}$ is the effect of genotype $\boldsymbol{i}$ (i = 1, . . ., 12);
$(\boldsymbol{GE})_{\boldsymbol{ik}}$ is the G × E interaction; and
$\boldsymbol{e_{ijk}}$ is the residual error of mean.

For inference, genotype, environment, and genotype × environment interaction were treated as fixed effects, while replication nested within environment was treated as a random blocking factor to account for field variability. Mean comparisons among genotypes were conducted using Fisher's least significant difference (LSD) test at α = 0.05. All univariate statistical analyses were performed using STATISTIX software (version 10.0; Analytical Software, Tallahassee, FL, USA).

Multivariate relationships among physicochemical and RVA traits were explored using principal component analysis (PCA) implemented in GraphPad Prism version 10.2.3 (GraphPad Software, San Diego, CA, USA). Variables were autoscaled (z-score standardized), and PCA was conducted based on the correlation matrix. The major contributing variables were identified by calculating Pearson's correlation coefficients between individual traits and the first two principal components, with effect sizes for PC1 and PC2 estimated according to Ongrak et al. [36]. To assess uncertainty in variance explained by the principal components, bootstrap resampling (10,000 iterations) was performed using MATLAB R2025b (MathWorks, Natick, MA, USA) to generate 95% confidence intervals.

3. Results

3.1. Variance Components for Physicochemical and Pasting Characteristics

A combined analysis of variance revealed significant ($p < 0.05$) effects of genotype, environment, and G × E interaction for all physicochemical and pasting traits, except for the L/B ratio, for which G × E interaction was non-significant (Table 2). Genotypic effects were the predominant source of variation, explaining 41.13–94.30% of the total sum of squares for most traits, including ACC, GC, ASV, and pasting viscosity parameters, indicating strong genetic control over these quality attributes. In contrast, GR showed a greater influence on environmental variation, accounting for 42.87% and 46.59%, respectively. For PaT, genotype (41.13%) and G × E (30.75%) contributed nearly equally to the total variation, indicating differential thermal behavior across environments. These variance patterns were supported by location-specific means, which confirm the relative stability of key starch-quality traits across irrigated environments while revealing environmental modulation of selected parameters (Tables S2–S4). Thus, the high proportion of genetic variance for most traits indicates that the tested genotypes expressed consistent quality-related characteristics across locations, whereas traits associated with grain dimensions were more sensitive to environmental differences.

Table 2. Combined analysis of variance for grain physical and chemical characteristics of non-glutinous rice genotypes evaluated across three irrigated locations in Thailand, off-season 2024.

df [1]	2	11	22	66	
Characters	Mean Squares				C.V. (%)
	Environment (E)	Genotype	Genotype × E	Pooled Error	
Grain length	0.86 ** (19.92) [2]	0.45 ** (57.38)	0.04 ** (10.91)	0.01 (10.58)	1.7
Grain breadth	0.29 ** (42.87)	0.03 ** (21.55)	0.01 ** (14.84)	0.00 (19.32)	3.3
Grain length-breadth ratio	0.64 ** (25.33)	0.19 ** (41.72)	0.02 (10.79)	0.02 (21.55)	3.56
1000-grain weight	15.61 ** (11.24)	13.62 ** (53.93)	2.93 ** (23.22)	0.48 (11.32)	2.93
Chalky grain	9.31 ** (23.72)	3.88 ** (54.34)	0.68 ** (19.00)	0.03 (2.30)	10.27
Gel consistency	1812 ** (5.06)	4373 ** (67.08)	390.98 ** (12.00)	161 (14.82)	15.97
Alkali spreading value	2.134 ** (2.11)	17.34 ** (94.30)	0.31 ** (3.32)	0.01 (0.25)	1.74
Elongation ratio	0.21 ** (46.59)	0.01 ** (12.60)	0.01 ** (26.71)	2×10^{-3} (13.74)	2.93

Table 2. *Cont.*

df [1]	2	11	22	66	
Characters	Mean Squares				C.V. (%)
	Environment (E)	Genotype	Genotype × E	Pooled Error	
Apparent amylose content	23.92 ** (2.23)	173.41 ** (88.97)	3.17 ** (3.25)	1.66 (5.10)	6.08
Peak viscosity	3,196,783 ** (12.02)	3,804,682 ** (78.67)	218,830 ** (9.05)	1893 (0.23)	1.44
Trough viscosity	580,545 ** (3.71)	2,527,300 ** (88.73)	102,380 ** (7.19)	1609 (0.34)	4.77
Breakdown	194,880 ** (2.12)	1,444,345 ** (86.50)	88,893 ** (10.65)	1537 (0.55)	1.06
Final viscosity	215,561 ** (3.08)	1,150,478 ** (90.62)	37,268 ** (5.87)	389 (0.18)	1.39
Peak time	0.44 ** (5.43)	1.08 ** (72.94)	0.13 ** (17.55)	7×10^{-3} (2.74)	1.37
Pasting temperature	66.29 ** (22.21)	22.32 ** (41.13)	8.34 ** (30.75)	0.48 (5.31)	0.79

** significant at the 0.01 probability level. [1] df; degree of freedom. [2] Number within the parentheses is percentage of sum of squares to total sum of squares.

3.2. Physical Characteristics

3.2.1. Grain Length and Breadth

Highly significant differences were observed among genotypes for both grain length and breadth (Table 3). DS24-Inter-1 (7.23 ± 0.24 mm) exhibited the greatest grain length, comparable to the long-grain check RD85 (7.28 ± 0.11 mm). Several advanced lines, including DS24-Inter-4, DS24-Inter-10, DS24-Inter-6, and DS24-Inter-9, also displayed long kernels, whereas DS24-Inter-2 had significantly shorter grains than both checks. Based on IRRI classification, all genotypes were categorized as long-grain types, showing suitability for consumer preferences in Thailand and export markets. Grain breadth differed among genotypes, with DS24-Inter-6 showing the lowest value, which is consistent with a narrower grain shape.

Table 3. Grain physical characteristics of non-glutinous rice genotypes evaluated across three irrigated locations in Thailand, off-season 2024.

Genotypes	Grain Length (mm)	Grain Breadth (mm)	Length-Breadth Ratio	1000-Grain Weight (g)	Grain Chalkiness
DS24-Inter-1	7.23 ± 0.24 [a 1]	1.98 ± 0.11 [a]	3.66 ± 0.21 [bc]	13.03 ± 1.09 [bc]	1.98 ± 0.75 [bc]
DS24-Inter-2	6.65 ± 0.27 [d]	1.92 ± 0.15 [a–d]	3.48 ± 0.18 [de]	11.97 ± 0.92 [de]	1.59 ± 0.86 [cde]
DS24-Inter-3	6.74 ± 0.20 [cd]	1.97 ± 0.08 [ab]	3.43 ± 0.14 [e]	11.93 ± 1.58 [de]	1.48 ± 0.74 [de]
DS24-Inter-4	7.06 ± 0.22 [b]	1.92 ± 0.10 [a–d]	3.68 ± 0.17 [bc]	12.75 ± 1.28 [bcd]	1.22 ± 0.63 [ef]
DS24-Inter-5	6.83 ± 0.15 [c]	1.91 ± 0.10 [bcd]	3.59 ± 0.16 [cd]	12.24 ± 1.41 [cd]	1.07 ± 0.46 [f]
DS24-Inter-6	7.02 ± 0.19 [b]	1.78 ± 0.11 [e]	3.94 ± 0.17 [a]	10.61 ± 0.46 [f]	1.91 ± 0.57 [bc]
DS24-Inter-7	6.75 ± 0.25 [cd]	1.86 ± 0.06 [d]	3.63 ± 0.13 [c]	11.18 ± 0.86 [ef]	2.31 ± 0.45 [b]
DS24-Inter-8	6.70 ± 0.18 [cd]	1.89 ± 0.12 [cd]	3.56 ± 0.20 [cd]	11.24 ± 0.55 [ef]	1.64 ± 0.22 [cd]
DS24-Inter-9	7.00 ± 0.13 [b]	1.95 ± 0.09 [abc]	3.59 ± 0.11 [cd]	10.42 ± 1.26 [f]	2.11 ± 1.00 [b]
DS24-Inter-10	7.05 ± 0.21 [b]	1.96 ± 0.13 [ab]	3.61 ± 0.20 [c]	13.44 ± 1.13 [b]	0.41 ± 0.32 [g]
RD85	7.28 ± 0.11 [a]	1.94 ± 0.11 [abc]	3.76 ± 0.24 [b]	14.45 ± 1.51 [a]	0.85 ± 0.30 [f]
RJP233088	6.63 ± 0.12 [d]	1.95 ± 0.06 [abc]	3.41 ± 0.15 [e]	13.31 ± 1.20 [b]	2.75 ± 0.51 [a]
Mean	6.91	1.92	3.61	12.21	1.61
F-test	**	**	**	**	**
C.V. (%)	2.08	3.76	3.69	8.31	26.58

** significantly difference at $p \leq 0.01$. [1] Means followed by the same letters in the same column are not significantly different at $p \leq 0.05$ as determined by LSD.

3.2.2. Grain Length-Breadth Ratio and Grain Shape

Significant differences in L/B ratio were detected (Table 3). DS24-Inter-6 (3.94 ± 0.17) showed the highest ratio, indicating a distinctly slender grain morphology, exceeding that of RD85. Conversely, DS24-Inter-2 and DS24-Inter-3 exhibited the lowest ratios, similar to the soft-cooking check RJP233088. All genotypes were classified as long grain; nevertheless, their degree of slenderness differed markedly, which may affect milling quality and market acceptance.

3.2.3. 1000-Grain Weight

A highly significant variation in 1000-grain weight was observed across genotypes (Table 3). All advanced lines recorded lower grain weight than RD85 (14.45 ± 1.51 g), reflecting differences in grain density and endosperm development. However, DS24-Inter-10, DS24-Inter-1, and DS24-Inter-4 were statistically comparable to RJP233088, indicating moderate grain filling. The lowest values were observed in DS24-Inter-8, DS24-Inter-7, and DS24-Inter-9, suggesting lighter kernels typical of soft-textured rice.

3.2.4. Grain Chalkiness

Grain chalkiness differed significantly among genotypes (Table 3). All advanced lines exhibited lower chalkiness than RJP233088, confirming improved grain transparency. The highest chalkiness levels appeared in DS24-Inter-7, DS24-Inter-9, DS24-Inter-1, and DS24-Inter-6, whereas DS24-Inter-10 (0.41 ± 0.32) recorded exceptionally low chalkiness, comparable to the high-quality check RD85. Genotypes were classified into four chalkiness groups: low (2 genotypes), medium (3 genotypes), medium to high (4 genotypes), and high (3 genotypes). This variation highlights opportunities for selecting lines with improved appearance quality suitable for premium markets.

3.2.5. Gel Consistency

Significant differences in GC were detected (Table 4). The softest (longest gel length) values were observed in DS24-Inter-9, DS24-Inter-4, DS24-Inter-7, and DS24-Inter-10, all of which did not differ significantly from either check variety. In contrast, DS24-Inter-1, DS24-Inter-3, and DS24-Inter-2 exhibited markedly shorter gel lengths, indicative of firmer cooked texture. Based on GC classification, rice lines were grouped into medium (3 genotypes) and soft (9 genotypes) categories, aligning with Thai consumer preference for soft, cohesive rice.

Table 4. Gel consistency, alkali spreading value, and elongation ratio of non-glutinous rice genotypes evaluated across three irrigated locations in Thailand, off-season 2024.

Genotypes	Gel Consistency (mm)	Alkali Spreading Value	Elongation Ratio
DS24-Inter-1	44.78 ± 17.85 [d] [1]	5.06 ± 0.09 [e]	1.46 ± 0.05
DS24-Inter-2	52.67 ± 19.43 [d]	5.71 ± 0.32 [d]	1.49 ± 0.08
DS24-Inter-3	47.89 ± 17.22 [d]	6.33 ± 0.81 [c]	1.46 ± 0.05
DS24-Inter-4	97.78 ± 4.66 [ab]	7.00 ± 0 [a]	1.50 ± 0.09
DS24-Inter-5	67.22 ± 27.82 [c]	5.13 ± 0.16 [e]	1.46 ± 0.08
DS24-Inter-6	67.56 ± 18.47 [c]	5.66 ± 0.52 [d]	1.44 ± 0.16
DS24-Inter-7	97.00 ± 9.00 [ab]	3.00 ± 0 [g]	1.43 ± 0.03
DS24-Inter-8	84.78 ± 25.33 [b]	6.67 ± 0.50 [b]	1.44 ± 0.05
DS24-Inter-9	100.00 ± 0 [a]	3.99 ± 0.03 [f]	1.46 ± 0.03
DS24-Inter-10	96.22 ± 7.64 [ab]	5.08 ± 0.13 [e]	1.50 ± 0.09
RD85	100.00 ± 0 [a]	4.00 ± 0 [f]	1.45 ± 0.20
RJP233088	97.89 ± 6.33 [ab]	2.62 ± 0.33 [h]	1.45 ± 0.05

Table 4. *Cont.*

Genotypes	Gel Consistency (mm)	Alkali Spreading Value	Elongation Ratio
Mean	79.48	5.02	1.46
F-test	**	**	ns
C.V. (%)	18.34	5.54	4.58

ns: not significant; ** significantly difference at $p \leq 0.01$. [1] Means followed by the same letters in the same column are not significantly different at $p \leq 0.05$ as determined by LSD.

3.2.6. Alkali Spreading Value

Substantial variation in ASV was observed (Table 4). DS24-Inter-4 (7.00) showed the highest ASV, indicating low GT. DS24-Inter-8 and DS24-Inter-3 also displayed high ASV values in contrast, DS24-Inter-7 (3.00) recorded the lowest ASV, corresponding to a high GT, and was lower than both check varieties. Based on ASV, genotypes were classified into low (3 genotypes), medium (7 genotypes), and high (2 genotypes) GT groups, demonstrating meaningful variation in starch thermal properties.

3.2.7. Elongation Ratio

No significant differences were detected among genotypes for ER (Table 4), suggesting that kernel expansion upon cooking remained largely stable across genetic backgrounds and environments.

3.3. Apparent Amylose Content

Genotypes differed significantly in their AAC (Table 5). The highest AAC values were observed in DS24-Inter-4 and DS24-Inter-9 (26.39 ± 1.96% and 25.59 ± 1.69%), though both remained slightly lower than the high-amylose check RD85 (28.15 ± 1.40%). At the lower end, DS24-Inter-10 and DS24-Inter-5 exhibited AAC values around 15%, characteristic of soft-textured rice. Genotypes were classified into low (5 genotypes), medium (3 genotypes), and high (4 genotypes) AAC groups, offering a useful range of starch profiles for developing both soft-cooking table rice and high-amylose types suited to processed-food applications.

Table 5. Apparent amylose content and pasting viscosity properties of non-glutinous rice genotypes evaluated across three irrigated locations in Thailand, off-season 2024.

Genotypes	AAC [1] (%)	PV (RVU) [2]	TV (RVU)	BD (RVU)	FV (RVU)	Setback (RVU)	PeT (min)	PaT (°C)
DS24-Inter-1	20.35 ± 1.93 [e] [3]	284.94 ± 16.68 [bc]	194.92 ± 7.94 [c]	90.02 ± 10.54 [b]	322.19 ± 10.82 [c]	117.01 ± 3.81 [c]	5.94 ± 0.08 [d]	88.06 ± 1.06 [cde]
DS24-Inter-2	20.97 ± 1.27 [e]	270.59 ± 41.97 [cd]	193.82 ± 17.22 [c]	81.90 ± 23.56 [b]	316.70 ± 12.25 [cd]	119.65 ± 8.43 [c]	6.07 ± 0.13 [bcd]	87.38 ± 2.31 [d–g]
DS24-Inter-3	22.40 ± 1.73 [d]	276.59 ± 30.81 [cd]	193.31 ± 13.48 [c]	83.65 ± 17.94 [b]	320.63 ± 3.99 [c]	119.28 ± 9.55 [c]	6.04 ± 0.09 [cd]	86.67 ± 1.98 [e–h]
DS24-Inter-4	26.39 ± 1.96 [b]	166.43 ± 24.92 [f]	157.62 ± 19.52 [f]	8.82 ± 5.78 [e]	292.74 ± 18.12 [e]	130.09 ± 3.03 [b]	6.23 ± 0.15 [b]	87.98 ± 1.86 [c–f]
DS24-Inter-5	15.52 ± 1.91 [g]	315.75 ± 45.87 [a]	187.76 ± 22.95 [cd]	125.70 ± 25.19 [a]	289.47 ± 11.44 [e]	93.44 ± 12.34 [d]	5.57 ± 0.07 [e]	87.18 ± 2.02 [d–h]
DS24-Inter-6	16.88 ± 1.35 [f]	316.85 ± 19.41 [a]	187.33 ± 11.08 [cd]	129.52 ± 17.72 [a]	263.12 ± 3.17 [g]	70.86 ± 10.40 [f]	5.61 ± 0.04 [e]	85.88 ± 0.91 [h]
DS24-Inter-7	24.96 ± 1.26 [c]	227.27 ± 28.87 [e]	206.13 ± 22.50 [b]	20.34 ± 8.45 [e]	342.98 ± 15.05 [b]	136.75 ± 2.23 [b]	6.57 ± 0.39 [a]	89.34 ± 2.02 [bc]
DS24-Inter-8	19.73 ± 1.87 [e]	217.30 ± 21.87 [e]	171.65 ± 7.91 [e]	47.30 ± 13.16 [d]	307.30 ± 5.83 [d]	133.52 ± 2.09 [b]	6.14 ± 0.13 [bc]	91.11 ± 1.10 [a]
DS24-Inter-9	25.59 ± 1.69 [bc]	262.62 ± 25.65 [d]	217.47 ± 6.20 [a]	43.97 ± 21.35 [d]	381.37 ± 18.09 [a]	166.02 ± 14.26 [a]	5.99 ± 0.15 [cd]	86.26 ± 1.98 [gh]
DS24-Inter-10	15.09 ± 0.60 [g]	302.32 ± 23.36 [ab]	179.66 ± 17.57 [de]	131.18 ± 15.45 [a]	269.18 ± 7.01 [fg]	81.76 ± 9.36 [e]	5.51 ± 0.03 [e]	86.56 ± 1.21 [fgh]
RD85	28.15 ± 1.40 [a]	153.14 ± 31.51 [f]	140.74 ± 18.06 [g]	16.72 ± 11.20 [e]	311.61 ± 26.96 [cd]	161.43 ± 15.43 [a]	6.48 ± 0.54 [a]	88.44 ± 3.70 [bcd]
RJP233088	17.92 ± 1.32 [f]	231.89 ± 15.74 [e]	170.72 ± 11.25 [e]	61.29 ± 5.47 [c]	274.90 ± 6.32 [f]	91.78 ± 12.37 [d]	5.67 ± 0.04 [e]	89.83 ± 0.91 [ab]
Mean	21.16	252.14	183.43	70.03	307.68	118.47	5.99	87.89
F-test	**	**	**	**	**	**	**	**
C.V. (%)	6.7	7.59	5.35	18.89	4.04	6.81	3.23	1.74

** significantly difference at $p \leq 0.01$. [1] AAC: Apparent amylose content; PV: Peak viscosity; TV: Trough viscosity; BD: Breakdown; FV: Final viscosity; PeT: Peak time; PaT: Peak temperature. [2] RVU = Rapid Visco Units. For conversion to SI units (centipoise, cP), 1 RVU is equivalent to 12 cP. [3] Means followed by the same letters in the same column are not significantly different at $p \leq 0.05$ as determined by LSD.

3.4. Pasting Viscosity Properties

All RVA-based pasting characteristics differed significantly among genotypes (Table 5). PV ranged from 153.14 ± 31.51 RVU (RD85) to 16.88 ± 1.35 RVU (DS24-Inter-6). High-AAC genotypes such as RD85 and DS24-Inter-9 displayed low PV and BD but high FV and setback, indicating restricted starch swelling and stronger retrogradation tendencies. In contrast, low-AAC genotypes, including DS24-Inter-5, DS24-Inter-6, and DS24-Inter-10, showed high PV and BD but lower setback values, reflecting greater granule swelling and reduced retrogradation. PeT averaged 5.99 min, while PaT varied across genotypes, with DS24-Inter-8 exhibiting the highest thermal requirement (91.11 °C). These contrasting profiles clearly differentiate the genotypes into functional quality groups aligned with consumer-preferred cooking textures.

3.5. Principal Component Analysis and Correlation

PCA was performed using ten key quality attributes to assess multivariate relationships among physicochemical and pasting properties. The first two principal components (PC1 and PC2) explained 72.86% of the total variance (Figure 1). PC1 accounted for 51.73% (95% CI: 40.35–75.79), driven mainly by variables strongly correlated with this axis, including AAC ($r = 0.91$), PeT ($r = 0.90$), and setback viscosity ($r = 0.89$) (Table 6). PC1 therefore predominantly captured differences in starch composition and retrogradation tendency. PC2 explained 21.13% (95% CI: 12.84–36.20), with TV ($r = -0.87$) and FV ($r = -0.79$) showing strong negative associations, reflecting variation related to paste stability and viscosity recovery.

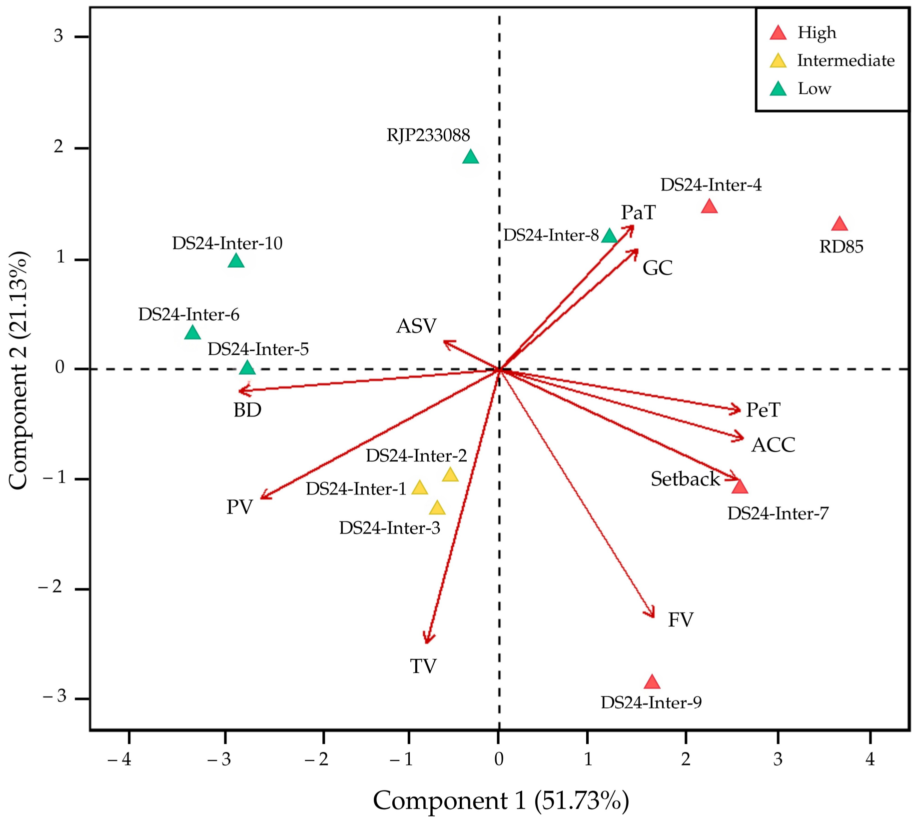

Figure 1. Score plot and loading plot of the first two principal components obtained from principal component analysis using grain physical and chemical characteristics of non-glutinous rice genotypes evaluated across three irrigated locations in Thailand, off-season 2024. ACC: Apparent amylose content; GC: Gel consistency; ASV: Alkali spreading value; PV: Peak viscosity; TV: Trough viscosity; BD: Breakdown; FV: Final viscosity; PeT: Peak time; PaT: Peak temperature. (green, yellow, and red triangle: low, medium, and high AAC, respectively).

Table 6. Pearson correlation coefficients between original variables and principal components, based on the combined data of non-glutinous rice genotypes evaluated across three irrigated locations in Thailand, off-season 2024.

Variables	PC1 (51.73%)	PC2 (21.13%)
Gel Consistency	0.52 [1]	0.38
Alkali spreading value	−0.21	0.09
Apparent amylose content	0.91	−0.22
Peak viscosity	−0.90	−0.41
Trough viscosity	−0.28	−0.87
Breakdown	−0.98	−0.07
Final viscosity	0.58	−0.79
Setback	0.89	−0.35
Peak time	0.90	−0.13
Pasting temperature	0.50	0.46

[1] Correlation strength was classified as strong (>0.70), moderate (0.40–0.69), and weak (<0.40).

In the score plot, RD85, DS24-Inter-4, DS24-Inter-7, and DS24-Inter-9 were located on the positive side of PC1, corresponding to their association with higher AAC and setback viscosity (Figure 1). DS24-Inter-6, DS24-Inter-5, and DS24-Inter-10 clustered on the negative side of PC1, aligned with higher PV and BD, indicating stronger swelling capacity and paste disintegration behavior. Genotypes DS24-Inter-1, DS24-Inter-2, and DS24-Inter-3 were positioned near the center-left region of the plot, indicating intermediate and relatively balanced physicochemical and pasting characteristics. Along with PC2, DS24-Inter-4 and RD85 plotted positively and were associated with higher GC and PaT, whereas DS24-Inter-9 plotted strongly negatively, reflecting its association with higher FV and distinct paste stability behavior. Thus, the PCA separated the genotypes into three functional groups: (i) high-AAC/high-retrogradation types: RD85, DS24-Inter-4, DS24-Inter-7, and DS24-Inter-9; (ii) intermediate, balanced types: DS24-Inter-1, DS24-Inter-2, and DS24-Inter-3; and (iii) low-AAC, high-swelling viscous types: RJP233088, DS24-Inter-5, DS24-Inter-6, DS24-Inter-8, and DS24-Inter-10. These groupings provide a multivariate basis for selecting genotypes with contrasting starch-functionality profiles under irrigated lowland conditions.

4. Discussion

The multi-environment evaluation revealed clear and systematic patterns in the genetic, physicochemical, and rheological characteristics of advanced non-glutinous rice genotypes, highlighting the relative contributions of genotype, environment, and their interactions under irrigated lowland ecosystems in Thailand. Although trait means were not presented by individual location, the substantial environmental variance observed for grain breadth and elongation rate (Table 2) is consistent with site-to-site contrasts in soil properties (Table S1) and seasonal microclimatic conditions (Figure S1), which are known to influence grain expansion and endosperm packing during grain filling. The variance component analysis (Table 2) demonstrated that most physicochemical traits, particularly AAC, GC, GT (assessed by ASV), and RVA-derived pasting parameters, were predominantly governed by genotypic effects, accounting for 41.13–94.30% of the total variation. Whereas some traits showed moderate environmental responsiveness due to substantial G × E contributions (Table 2). The strong genetic contribution aligns with previous reports that *Wx*- and *SSIIa*-driven starch traits exhibit high heritability across indica and japonica backgrounds [37–39]. This interpretation should be regarded as a bounded inference, as the present study did not explicitly model location-specific means for each trait. Future analyses incorporating per-location estimates will be required to quantify the direct contribution of soil and climate variables to grain-quality expression.

Environmental variation further contributed to the strong plasticity observed in grain breadth and elongation rate (Table 2). The three irrigated environments differed markedly in thermal regime and rainfall distribution (Figure S1), with all sites reaching high daytime temperatures of 36–40 °C during grain filling, conditions known to accelerate grain-filling rate and disrupt amyloplast organization [40]. Suphan Buri, which exhibited the highest cumulative rainfall (274.5 mm) and sandy-loam texture (Table S1), likely experienced substantial fluctuations in root-zone moisture, promoting inconsistent cell expansion and kernel thickening. In contrast, the clayey, high-EC soil of Pathum Thani may have imposed osmotic constraints on endosperm expansion. These thermal and edaphic contrasts are consistent with previous reports showing that temperature, soil water availability, and nutrient status interactively regulate grain filling, starch deposition, and kernel morphology in rice [41]. The significant G $\times$ E interaction to PaT further reflects the sensitivity of *SSIIa*-mediated gelatinization behavior to environmental conditions such as heat and nitrogen supply during grain development [42,43]. Overall, while starch composition traits remained genetically stable, grain-physical and thermal-pasting traits clearly responded to the heterogeneous environmental conditions across irrigated ecosystems.

Grain weight varied significantly (Table 3), suggesting differences in endosperm filling and assimilating partitioning among genotypes. Variation in 1000-grain weight among the evaluated genotypes likely reflects differences in grain size, endosperm development, and assimilate partitioning, which are collectively regulated by multiple grain size-related loci such as *GS3*, *GW2*, and *qSW5* [44–46]. Grain chalkiness varied markedly among the evaluated genotypes (Table 3). Low-chalkiness lines such as DS24-Inter-10 and DS24-Inter-4 exhibited improved grain translucency, which has been associated with more uniform endosperm development and tighter starch organization, contributing to superior milling quality [47,48]. In contrast, higher chalkiness observed in DS24-Inter-7 and DS24-Inter-9 may be attributed to disrupted starch accumulation during late grain filling, as chalk formation is highly sensitive to environmental stresses, particularly elevated temperature and fluctuating water availability, that interfere with orderly endosperm development and starch granule packing [49].

Variation in GC, ASV, and ER among genotypes (Table 4) reflects differences in starch molecular architecture, particularly amylopectin chain-length distribution. Genotypes exhibiting soft GC and high ASV, such as DS24-Inter-4 and DS24-Inter-10, are indicative of lower GT and loosely packed crystalline structures, traits strongly associated with functional variation at the *ALK/SSIIa* locus [12,39,50]. Natural allelic differentiation of *SSIIa* (*ALKa* and *ALKb*) has been shown to alter amylopectin fine structure, enzyme activity, and gelatinization behavior, thereby modulating cooking quality independent of AAC [39,50]. These findings support the observed differentiation in thermal and textural properties among the advanced non-glutinous rice genotypes evaluated in this study.

Pasting properties further highlighted starch structural diversity among the tested genotypes and their implications for ECQ. High-amylose genotypes such as RD85 and DS24-Inter-9 exhibited low PV and BD but high FV and setback (Table 5), reflecting restricted starch granule swelling and strong chain reassociation during cooling. These characteristics were typically associated with firmer cooked texture and faster retrogradation. In contrast, low-amylose genotypes, including DS24-Inter-5, DS24-Inter-6, and DS24-Inter-10, showed higher PV and BD but lower setback, indicating greater starch swelling and reduced retrogradation, which are hallmarks of soft-cooking rice increasingly favored by consumers. These contrasting RVA profiles were consistent with established relationships among AAC, gelatinization behavior, and sensory texture reported in Thai rice cultivars and other indica germplasm [21,51,52].

Multivariate analysis using PCA (Figure 1; Table 6) provided an integrated view of starch-related quality variation among the tested genotypes. PC1, accounting for 51.73% of the total variance, was strongly associated with apparent amylose content, setback viscosity, and peak time (Table 6), reflecting differences in starch composition and retrogradation behavior. Genotypes with high AAC, such as RD85, DS24-Inter-7, and DS24-Inter-9, clustered positively along PC1, consistent with firmer texture, delayed gelatinization, and higher retrogradation potential after cooling, attributes commonly linked to reduced eating softness [19,53]. PC2 explained an additional 21.13% of the variance and was primarily related to paste stability, as indicated by strong negative correlations with TV and FV (Table 5). Genotypes such as DS24-Inter-9, characterized by high TV and FV, plotted negatively along PC2, indicating greater paste stability but reduced smoothness. In contrast, DS24-Inter-8 and DS24-Inter-10 plotted positively on PC2, which was consistently associated with higher GC and lower FV across the evaluated locations (Tables S2–S4). These physicochemical properties are key indicators of starch swelling and softer cooking quality favored in soft-cook rice [21,47,48].

Overall, PCA effectively discriminated genotypes into distinct quality-oriented groups based on starch compositional and rheological attributes, reinforcing its value for identifying lines with contrasting ECQ profiles. The high-AAC/high-retrogradation cluster (RD85, DS24-Inter-4, DS24-Inter-7, and DS24-Inter-9) is expected to maintain firmer texture after cooling, supporting utilization where structural integrity is required (e.g., rice noodles, extruded snacks, and starch-based gels). Whereas the low-AAC/high-swelling cluster (RJP233088, DS24-Inter-5, DS24-Inter-6, DS24-Inter-8, and DS24-Inter-10) corresponds to soft-cooking texture traits increasingly preferred in premium table rice markets. Intermediate genotypes (DS24-Inter-1, DS24-Inter-2, and DS24-Inter-3) showed comparatively stable, moderate physicochemical profiles, which may be advantageous for breeding programs targeting balanced quality across irrigated environments. The results further indicate that most physicochemical and cooking-quality traits in these advanced genotypes were predominantly under genetic control, supporting their suitability for irrigated lowland ecosystems in Thailand. Nevertheless, traits exhibiting pronounced environmental sensitivity should be validated through expanded multi-environment trials to ensure quality stability under irrigated production systems.

5. Conclusions

This study demonstrates that grain-quality variation among advanced non-glutinous rice genotypes is largely governed by genetic factors, whereas environmental effects primarily influence grain dimensions. Multivariate analysis distinguished genotypes according to starch composition and paste stability. Genotypes with contrasting amylose levels exhibited distinct pasting behaviors, reflecting differences in swelling and retrogradation. Remarkably, several advanced genotypes expressed soft-cooking profiles characterized by favorable gelatinization and GC, aligning with the increasing demand for soft-textured rice in both domestic and international markets. These differentiated quality attributes support targeted utilization and reinforce the importance of selecting stable, high-quality genotypes for irrigated rice production systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/crops6020031/s1, Figure S1: Total rainfall and minimum and maximum temperature during crop growth in the off- season 2024. (**a**) Pathum Thani: (**b**) Chai Nat: (**c**) Suphan Buri; Table S1: Soil properties across tested locations; Table S2: Mean performance of 12 rice genotypes for gel consistency and alkali spreading value evaluated across individual locations during off-season 2024; Table S3: Mean performance of 12 rice genotypes for apparent amylose content evaluated across individual locations during off-season 2024; Table S4: Mean performance of 12 rice genotypes for pasting viscosity properties evaluated across individual locations during off-season 2024.

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Abbreviations

The following abbreviations are used in this manuscript:

AAC	Apparent amylose content
ALK	*Amylose-Low Kernel* gene
ANOVA	Analysis of Variance
ASV	Alkali spreading value
BD	Breakdown
Cis	Confidence intervals
C.V.	Coefficient of Variation
EC	Electrical Conductivity
ECQ	Eating and cooking quality
ER	Elongation ratio
FV	Final viscosity
GAP	Good Agricultural Practices
GC	Gel consistency
G × E interaction	Genotype × Environment interaction
GT	Gelatinization temperature
IRRI	International Rice Research Institute
LSD	Least Significant Difference
PaT	Peak temperature
PCA	Principal component analysis
PeT	Peak time
PV	Peak viscosity
RCBD	Randomized complete block design
RVA	Rapid Visco Analyzer

RVU	Rapid Visco Units
SSIIa	*Soluble Starch Synthase Iia* gene
SSRG	*Starch Synthesis-Related* Gene
TCW	Thermocline for Windows
TV	Trough viscosity

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