Indian J. Genet. Plant Breed., (2025); 85(2): 188-197

https://doi.org/10.31742/ISGPB.85.2.2

ISSN: 0975-6906

www.isgpb.org

RESEARCH ARTICLE

Novel marker haplotypes and starch quality parameters in relation to glycaemic response of temperate rice (*Oryza sativa* L.)landraces and germplasm

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Abstract

The study investigates the quality of various rice genotypes, focusing on the identification of rice (*Oryza sativa* L.) genotypes with low GI to promote healthy dietary options. Given the link between amylose content and GI, evaluation was carried out for grain and kernel characteristics, amylose content, pasting and thermal properties. Significant variability in amylose content, with NR-AB 1 showing the lowest at 12.42% and Pusa Basmati 1509, the highest at 25.01%, was recorded. Pasting properties evaluated through RVA showed a wide range of viscosities, indicating differences in the thickening and water-binding capacities of the rice flour from various genotypes. Molecular marker analysis and allelic profiling at loci controlling starch properties further provided a genetic perspective on rice quality. The research offered valuable insights for rice breeders, food scientists, and the culinary industry, where the low GI genotypes were identified, which can be further promoted for product development.

Keywords: Rice, quality, gene, markers, glycaemic index, amylose

Introduction

Rice quality has broadly been explained as being contextually intrinsic or extrinsic (Demont and Ndour 2015). The intrinsic properties refer to grain quality traits such as color, grain shape and size, head rice (HR), softness, and aroma, while extrinsic attributes cover packaging, labeling, and branding. Rice quality remains guided by the consumer preference and socio-cultural stratification of rice-eating populations. These traits are mostly related to the starch composition of the rice endosperm and its packaging. Besides the taste, texture, and other organoleptic properties, starch digestibility and glycaemic response have become an important criterion of both intrinsic and extrinsic rice quality.

The glycaemic index (GI) was conceived by David Jenkins in the year 1981. It was proposed to classify the carbohydrate-based foods according to their glucose response after consumption for two hours. It is presented as a value relative to that of reference food such as glucose or white bread. Rice depicts a wide range of variation in GI from as low as 48 up to 92 with an average of 64. This indicates that rice can be a part of a healthy diet for the average consumer if genotypes with low GI are identified and promoted. The amylose content of the rice greatly influences the GI (Milleret al.1992), so its estimation provides a quick and inexpensive procedure for the prediction of glycaemic response. Amylose content in rice is conditioned by the Waxy (*Wx*) locus that codes for granule-bound starch Synthase (GBSS) protein (Chen et al. 2008). High allelic variation is expected across the array of genotypes at the *Wx* locus and can be studied in relation to the glycaemic response of the genotypes.

The valley of Kashmir is located within the North-Western Himalayan region (34.5°N of latitude and 77.0°E of longitude), where rice forms a staple diet and is cultivated

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How to cite this article: Noor N., Naseer B., Naik H.R., Hussain S.Z., Zaffer S., Khan G.H., Sofi N.R. and Shikari A.B. 2025. Novel marker haplotypes and starch quality parameters in relation to glycaemic response of temperate rice landraces and germplasm. Indian J. Genet. Plant Breed., **85**(2): 188-197.

Source of support: DST, Ministry of Science, Govt. of India

Conflict of interest: None.

Received: Sept. 2024 **Revised:** March 2025 **Accepted:** April 2025

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within an altitude ranging from 1500-2200 msl. The stark variability exists in terms of rice quality of landraces and genetically improved varieties grown in the farmers' fields, which belong to *japonica* and indica sub-species (Sofi et al. 2020). Our previous study on 484 rice germplasm lines using 213 KASP markers contributed to a more nuanced understanding of the population sub-structure of temperate rice collections (Shikari et al. 2021). The characterization of the important germplasm accessions for genes governing essential traits is a reasonable step for value addition, informed conservation and utilization strategies. Previously performed the analysis for quality traits like GABA and other quality traits (Hussain et al. 2020). The present study aimed at the identification of genotypes with slow *in-vitro* starch digestibility, which indicates the low glycaemic response and the possibility of their utilization in breeding programs and preparation of value-added foods with key starch properties.

Materials and methods

The present study was carried out on a set of rice (*Oryza sativa* L.) accessions selected based on their origin, class, grain type and other relevant information (Shikari et al. 2021). The selected rice genotypes comprised local landraces and released varieties from groups basmati, *indica,* and *japonica*. Paddy samples were dehusked in Rice Husker Pearlest (Kett Electric Laboratory, Japan) and brown rice obtained thereof was polished in a Rice Polisher Pearlest (Kett Electric Laboratory, Japan). Polished head rice grains obtained from each variety were dried to a moisture content of 11-12 ± 0.5% in a cabinet drier (LSI-EC-STB), Lab Solutions, India, at a temperature of 40 ± 5^{o}C (Naseer et al. 2020). A portion of polished head rice grains was ground in a laboratory mill (Pertin, USA) to obtain rice flour. Both dried polished head rice gains and rice flour were stored in separate air-tight containers under ambient conditions for further analysis. All the post-harvest processing conditions were kept uniform for all the varieties and three independent samples from each variety were analyzed under uniform testing conditions.

Grain type, kernel characteristics and cooking quality

The genotypes were studied for grain type and kernel characteristics using the standard protocol of SES, IRRI, Philippines (2012). Observations were recorded on kernel traits such as kernel length before and after cooking (KLBC& KLAC), Kernel breadth before and after cooking (KBBC& KBAC) and Length/breadth ratio (LBR), Kernel elongation ratio (KER). Amylose content was determined using the method reported by Juliano et al. (1991). The method given by (Little et al. 1958) was used for the determination of alkali spreading value.

Estimated glycaemic index

The estimated glycaemic index was determined by Goñi et al. (1997). The rate of starch hydrolysis was worked out using a Megazyme kit (Megazyme, Bray, Ireland) as per the manufacturer's instructions. The starch digestion rate was expressed as the percentage of total starch (TS) hydrolyzed after 30-minute intervals from 0 to 180 min. eGI and hydrolysis index (HI) were calculated using the kinetics of starch digestion. eGI was calculated from the standard formula: $eGI = 39.71+ (0.549\times HI)$. Total starch content was measured using the total starch assay kit (K-TSTA, Megazyme, Bray, Ireland) by standard AACC (1976).

Pasting properties estimated through Rapid Visco-analyser

The pasting properties of eight selected genotypes were determined by RVA Starch^{TM} (New Port, Scientific Warrie Wood, Australia) following the methods described by Batey et al. (1997). A quantity of 3.24 g of rice flour was added to 25.08 mL of distilled water in aluminum canisters and cooked in a defined heating-cooling regime. Initially, the slurry was heated at a rate of 6°C per minute from 50 to 95°C, held at 95°C for 2.7 minutes, cooled at 6°C min to 50°C and then held for 2 minutes. The parameters such as peak viscosity, trough viscosity (hold), final viscosity, BDV and SBV, peak time and pasting temperature were recorded.

Differential scanning calorimetry

Thermal properties were determined by a differential scanning calorimeter (DSC) (Mettler Toledo) equipped with a refrigerated cooler using the method described by (Normand et al. 1989) with some modifications. A sample weight of 4 mg was taken in aluminum DSC pans (120 mL) and hermetically sealed. The DSC analysis was run by scanning from 20 to 150°C, ramping at 5°C/min. Nitrogen was used as a purging gas. The analysis of the resulting thermograms was done using Stare software (ver. 9.20, Mettler Toledo). Thermal transitions of samples for gelatinization were characterized by onset temperature (To), peak temperature (Tp), endset or conclusion (Tc) and gelatinization enthalpy (ΔHg) was evaluated based on the area of the main endothermic peak by the Universal Analysis Program.

Scanning Electronic Microscopy

Micrographs of the rice samples were generated through scanning electron microscopy (Hitachi, 3600, Tokyo, Japan). The samples were mounted on aluminum stubs using double-sided adhesive tape to which the samples were fixed and the visualization of the subject was carried out at five different magnifications.

Molecular marker analysis and allelic profiling for loci controlling starch properties

DNA extraction and PCR

The genomic DNA was extracted following the protocol of Murray and Thompson (1980) with slight modifications. The germplasm lines were assayed for CT repeat length polymorphism at the *Wx* locus with the help of SSR marker RM190. PCR of 10 µL volume was set up using ~50 ng of template DNA, 5 pmol of each primer, 0.05 mM dNTPs (Thermo Fisher Scientific India Pvt. Limited, Mumbai, India), 10 × PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$) and 0.5 U of Taq DNA polymerase (Thermo Fisher Scientific India Pvt. Limited, Mumbai, India). The PCR program was as follows: initial denaturation at 94°C 5 minutes; denaturation in subsequent 35 cycles at 94°C for 30 s. Annealing at 55°C for 30 seconds, extension at 72 °C for 1-minute, and final extension at 72°C for 7 minutes.

Polyacrylamide gel electrophoresis (PAGE)

The amplification products were resolved using non-denaturing polyacrylamide gel electrophoresis. The standard protocol for PAGE was used with modifications (Laemmli 1967). About 10 µL of PCR product was loaded into each well of the polyacrylamide gel. A 50 bp size reference ladder (Thermo Fisher Scientific India Pvt. Limited, Mumbai, India) was also loaded alongside the samples. The amplicon was detected through the PAGE gel silver staining method to detect the allelic profile (Huang et al.2018).

Kompetitive allele-specific PCR (KASP) marker assay: Leaf samples were collected using a 96-well format 'Plant Sample Collection Kit' and the lines were subjected to genotyping as per the protocol devised by Biosearch Technologies, Hoddesdon, Herts., U.K. The KASP markers selected here were designed previously using an indica reference genome (Steele et al. 2018). Eight KASP loci near the *Wx* gene at chromosome 6 and one locus related to starch properties at chromosome 10 were utilized for haplotype analysis of rice germplasm.

Statistical analysis

A set of twenty rice genotypes was evaluated for cooking quality and physico-chemical traits using a completely randomized design with three replications. A model applied for CRD is given as: $Y_{ij} = m + ti + e_{ij}$; where m = overall mean effect, t_i= true effect of the ith treatment, e_{ij} = error term of the jth unit receiving ith treatment.

Results and discussion

Evaluation of genotypes for cooking quality

ANOVA revealed highly significant mean squares (at $p < 0.01$) for kernel traits. Pusa Basmati 1509 had significantly higher KLBC values (8.80 ± 0.07) compared to other genotypes (Table 1).KBBC of genotypes ranged from 1.4 ± 0.04 to 2.7 ± 0.08mm with maximum kernel breadth for Shalimar rice-5 and least for Zeera Rice and Pusa Basmati 1509. Maximum cooked rice length (KLAC) of 16.83 ± 0.10 recorded for Pusa Basmati 1509 was significantly higher ($p < 0.01$) than the rest of the genotypes and the minimum value of KLAC recorded in Zeera Rice (5.60 ± 0.12 mm). These two genotypes differed statistically ($p < 0.01$) in comparison to the next lowest and highest genotypes, respectively. The kernel elongation ratio showed the lowest value for Black Rice (1.28) to the highest value for Pusa Basmati 1509 (1.91) (Table 2; Fig. 1). Most genotypes had a chalkiness percentage of <10%, while others ranged between 11 to 20%. A high chalkiness of 95% was noted for NR-AB1(Fig. 2).

Evaluation of rice germplasm for various physicochemical properties

The study focuses on the importance of apparent amylose content (AAC), gel consistency (GC), and gelatinization temperature (GT) in determining rice eating and cooking quality. Rice genotypes are classified as waxy (1–2%), very low (2-9%), low (10–20%), intermediate (20–25%), and high (25–30%) amylose types. The lowest (12.42%) and highest amylose content (25.01%) were recorded for NR-AB 1 and Pusa Basmati 1509, respectively (Table 2). ASV, the indirect estimate of gelatinization temperature (GT), varied between 1-7. Pusa Basmati 1509, NR-AB 1, Kamad, Zeera Rice, Black Rice, Stejaree, K-332, and Shalimar Sugandh-1 showed a

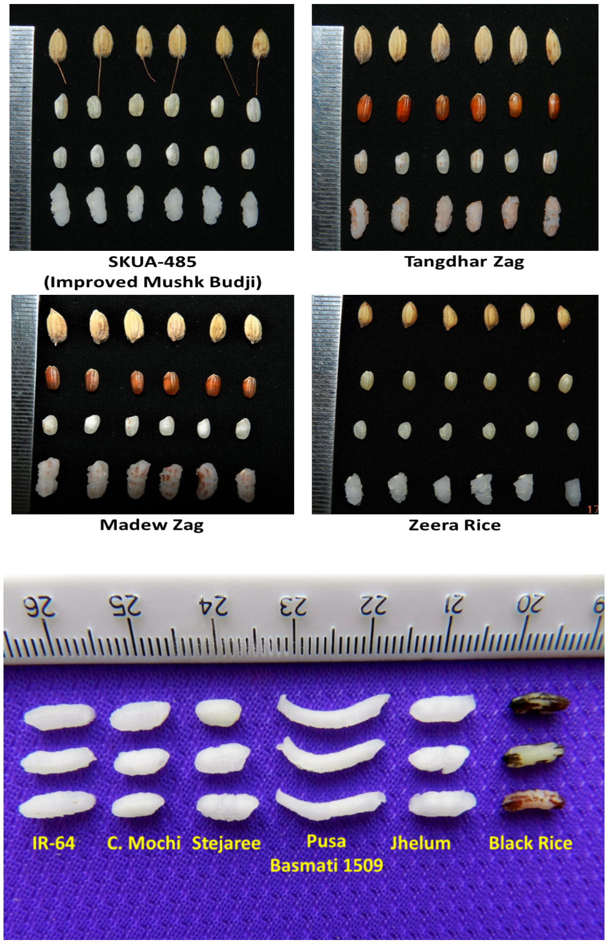

Fig. 1. Grain dimensions, kernel aspect and cooking quality of the rice germplasm

Fig. 2. Image analysis for estimation of kernel chalkiness

Fig. 3. Alkali spreading test carried across genotypes

score of 6 to 7, while C. Mochi, Egyptian Rice, Madew Zag, Tangdhar Zag, MushkBudji, Dular, Heera, and IR-64 showed a score of 4 to 5 (Fig.3). Most of the genotypes recorded soft GC ranging (80–148 mm) whereas for SS-1 and Pusa Basmati the gel front length was 34 and 30 mm respectively.

A high AAC (25.01%) was recorded for Pusa Basmati 1509, while Koshikari recorded low amylose (17.8%), confirmed with the results of (Ohtsubo et al. 2016). NR-AB1 had a low amylose content of 12.42%. AAC is controlled by the *Wx* gene, which encodes an enzyme GBSS (granule-bound starch synthase) and plays a major role in the synthesis of amylose fraction of starch. GT refers to the temperature range at which the crystalline portion of the starch structure melts irreversibly (Chen et al. 2008). The gelatinization behavior of starch granules in rice is associated predominantly with amylopectin structure (Umemoto et al. 2002), and the *SSIIa* gene is responsible for the difference in amylopectin structure between *indica* and *japonica* rice varieties. The KASP marker associated with this locus was found to differentiate between *indica* and *japonica* rice germplasm lines in the study. Numerous intrinsic and extrinsic parameters, including genetic composition, starch composition, and the amount of starch–protein interaction, impact starch digestibility. Contrary to low amylose lines, which exhibit higher starch digestibility, high amylose (Badoni et al. 2024) genotypes are frequently marked by lower glycemic food load and higher RSD (Emide et al. 2023). Grain weight, physical appearance, and general quality are further impacted by the intricate process of starch biosynthesis, which is controlled by many genes as well as a large number of transcription factors and mediators (Badoni et al. 2024). The AAC content is the primary factor that determines the eating and cooking quality of rice types, which in turn is the primary factor that influences consumer preference and market value within the rice market. As a result, one of the most important factors in rice development programs is the manipulation of the starch content (Dixit et al. 2024).

Pasting properties

The viscosity parameters of eight selected genotypes were estimated through RVA (Rapid Visco Analysis) (Table 3; Fig. 4). Madew Zag had the highest peak viscosity, followed by NR-AB 1, C. Mochi, Jhelum, K-332, Koshihikari, Heera, and Pusa Basmati 1509. Madew Zag had the highest Trough Viscosity (TV), followed by C. Mochi, NR-AB1, Jhelum, K-332, Heera, Koshihikari, and Pusa Basmati 1509. Several genotypes had low Breakdown Viscosity (BDV), but Jhelum and Madew Zag had the highest Final Viscosity (FV). K-332 had the highest Setback Viscosity (SBV), while Jhelum, Madew Zag, C. Mochi, Pusa Basmati 1509, Koshihikari, and Heera had moderate values. All genotypes had a peak time of seven seconds, and Pusa Basmati 1509 had the highest pasting temperature. Our results from RVA indicate the difference in peak viscosities among low AAC genotypes, which may be explained in terms of the varying crystalline structure of amylopectin and the organization of the starch granules (Gallant et al. 1997). Variations in a fine structure are responsible for variations in starch functional properties between species, tissues and genetic backgrounds. In order to acquire deeper insight into the nature and properties of starch, the sub-set of eight genotypes was selected based on varied GI range (56.4–82.9). Different starch types have different viscosity parameters, with larger molecules producing higher viscosity (Han et al. 2001; Noda et al.2003). Peak Time Viscosity (PV) was lowest for NR-AB1 and was uniform for the other seven genotypes indicating less time taken to gelatinize. The highest BDV was noted for Heera, Madew Zag, and Jhelum, indicating the enhanced ability of starch to swell due to poor cross-bonding and subsequent breakdown of highly swollen granules. NR-AB1 had the lowest BDV, indicating delayed cooking. Jhelum and Madew Zag had the highest FV and lowest SBV, confirming high amylose content after attaining a high peak coupled with low TV, high amylose starch retrogrades during the cool phase and results in increased FV. Pusa Basmati 1509 showed low PV and higher FV and SBV, indicating higher re-association between starch molecules, confirming its high amylose property.

Thermal properties

Differential Scanning Calorimetry (DSC) is a form of thermal analysis that is used to predict and analyze conformational and phase transitions due to a change in thermal energy, monitored as a function of temperature or time at a certain

Table 1. Mean sum of squares for cooking quality traits, amylose content and estimated glycaemic index

Source of variation	d.f.	Mean sum of squares						
		KLBC	KBBC	LBR	KLAC	KER	AC	pGI
Genotypes	19	3.957**	0.420**	3.246**	10.514**	0.070**	28.192**	834.010**
Error	38	0.023	0.011	0.021	0.030	0.003	0.192	3.598

** indicates *p-value* <0.01

Table 2. Evaluation of germplasm lines for traits related to cooking quality

S. No.	Rice genotypes	Type	KLBC (mm)	KBBC (mm)	KLAC (mm)	LBR	KER	Grain type	Amylose (%)	Amylose class
1	K-332	Tej	4.70 ± 0.09	2.6 ± 0.02	7.23 ± 0.03	1.83 ± 0.03	1.49 ± 0.02	Bold	19.66 ± 0.03	Low
2	Shalimar Rice-5	Tej	4.17 ± 0.05	2.7 ± 0.08	7.47 ± 0.02	1.52 ± 0.03	1.79 ± 0.03	Bold	18.69 ± 0.05	Low
3	MushkBudji	Tej	4.68 ± 0.05	2.5 ± 0.02	7.37 ± 0.05	1.87 ± 0.01	1.57 ± 0.02	Bold	17.63 ± 0.06	Low
4	SKUA-485 (Improved MushkBudji)	Der	4.63 ± 0.05	2.6 ± 0.02	7.37 ± 0.05	1.78 ± 0.03	1.59 ± 0.03	Bold	21.04 ± 0.05	Intermediate
5	Kamad	Tej	4.70 ± 0.03	2.5 ± 0.05	8.62 ± 0.04	1.91 ± 0.05	1.83 ± 0.01	Bold	18.35 ± 0.08	Low
6	Koshihikari	Tej	4.97 ± 0.02	2.5 ± 0.04	7.27 ± 0.08	1.99 ± 0.01	1.46 ± 0.02	Bold	16.38 ± 0.04	Low
7	Stejaree	Tej	4.47 ± 0.02	2.2 ± 0.05	8.00 ± 0.03	2.06 ± 0.05	1.79 ± 0.01	Bold	15.69 ± 0.05	Low
8	C. Mochi	Tej	4.40 ± 0.06	2.1 ± 0.02	7.67 ± 0.05	2.06 ± 0.04	1.74 ± 0.01	Bold	16.08 ± 0.05	Low
9	Tangdhar Zag	Tej	5.10 ± 0.03	2.4 ± 0.02	7.63 ± 0.05	2.15 ± 0.03	1.50 ± 0.02	Medium	19.15 ± 0.04	Low
10	Madew Zag	Tej	5.13 ± 0.02	2.2 ± 0.02	7.91 ± 0.03	2.37 ± 0.02	1.54 ± 0.01	Medium	23.02 ± 0.04	Intermediate
11	Egyptian Rice	-	4.97 ± 0.02	2.2 ± 0.03	8.12 ± 0.04	2.26 ± 0.04	1.63 ± 0.01	Medium	21.92 ± 0.07	Intermediate
12	IR- 64	Ind	5.50 ± 0.03	1.9 ± 0.03	7.92 ± 0.03	2.89 ± 0.07	1.44 ± 0.01	Medium	24.73 ± 0.06	Intermediate
13	Heera	Ind	5.80 ± 0.10	2.2 ± 0.03	7.95 ± 0.02	2.64 ± 0.01	1.37 ± 0.03	Medium	24.08 ± 0.06	Intermediate
14	NR-AB1	Ind	5.47 ± 0.05	2.3 ± 0.03	7.14 ± 0.03	2.38 ± 0.05	1.28 ± 0.02	Medium	12.42 ± 0.03	Low
15	Zeera Rice	-	3.53 ± 0.02	1.4 ± 0.04	5.65 ± 0.12	2.59 ± 0.08	1.58 ± 0.04	Medium	23.07 ± 0.05	Intermediate
16	Jhelum	Ind	5.10 ± 0.03	2.1 ± 0.03	8.42 ± 0.03	2.43 ± 0.02	1.65 ± 0.01	Medium	21.83 ± 0.06	Intermediate
17	Dular	Trj	5.93 ± 0.02	1.7 ± 0.02	8.23 ± 0.1	3.56 ± 0.04	1.39 ± 0.01	Slender	24.55 ± 0.05	Intermediate
18	Black Rice	Aus	6.37 ± 0.02	2.1 ± 0.02	9.10 ± 0.03	2.98 ± 0.04	1.41 ± 0.01	Fine-grained	18.04 ± 0.04	Low
19	Shalimar Sugandh 3	Der	7.10 ± 0.09	1.7 ± 0.02	11.14 ± 0.03	4.10 ± 0.04	1.56 ± 0.02	Fine-grained	21.92 ± 0.05	Intermediate
20	Pusa Basmati 1509	Bas	8.80 ± 0.07	1.4 ± 0.04	16.83 ± 0.1	6.15 ± 0.13	1.91 ± 0.02	Basmati	25.01 ± 0.05	High

heating rate. The study found that Koshihikari recorded the highest values for To, Tp, and Tc (Onset, Peak and Conclusion temperatures) at 47.12, 50.08, and 98.18°C, followed by IR-64 at 46.81, 51.59, and 92.90°C. The lowest values were found in Jhelum at 20.55, 48.78, and 88.20°C. The highest enthalpy of gelatinization (*ΔHG*) was recorded in K-332, followed by Pusa Basmati 1509, Jhelum, Zeera Rice, and IR-64. Gelatinization temperature range (R) = 2(Tp-To) was highest in K-332, Pusa Basmati 1509, Dular, and Zeera Rice, with the lowest values in Koshihikari and IR-64. Peak Height Index (PHI)=ΔHg/Tp-To. The highest value of 36.08 J/g/°C for PHI was observed in IR-64, followed by PHI values of 10.3, 7.4 and 6.98 J/g/°C in Koshihikari, Jhelum and Zeera Rice, respectively. The lowest value PHI was observed in SKUA-485 (Improved Mushk Budji), Shalimar Sugandh 1 and Stejaree, respectively. (Table 4; Fig. 5).

The present study explored endothermic peaks between 20.55 and 115.46°C, which indicate starch gelatinization and the conversion of semi-crystalline starch to an amorphous form. Dular had the lowest gelatinization onset values, whilst Koshihikari had the highest. Tangdhar Zag had the lowest peak temperature, whereas K-332 and Pusa Basmati 1509 had the highest. SKUA-485 had the lowest conclusion temperature, but K-332 needed a higher temperature to complete gelatinization. K-332 had the highest gelatinization enthalpy, whereas Koshihikari had the lowest. The difference in ΔHg indicates the melting of amylopectin crystallites. IR-64 had the highest peak height index, indicating more

Table 3. Pasting properties of different genotypes

Rice genotypes	Peak Viscosity (cP)^{1}	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)	Peak time (s)	Pasting temperature (°C)
Madew Zag	2703	1094	1609	3466	2372	7	79.70
K-332	2084	715	1369	3465	2750	7	84.90
C. Mochi	2587	1016	1571	3364	2348	7	78.00
Heera	1039	485	554	1891	1406	7	87.00
NR-AB 1	509	487	22	591	104	5	69.95
Koshihikari	1131	383	748	2285	1902	7	87.85
Jhelum	2582	974	1608	3629	2655	7	80.35
Pusa Basmati 1509	654	240	414	2365	2125	7	92.40

(cP)^{1}= (centi Poise)

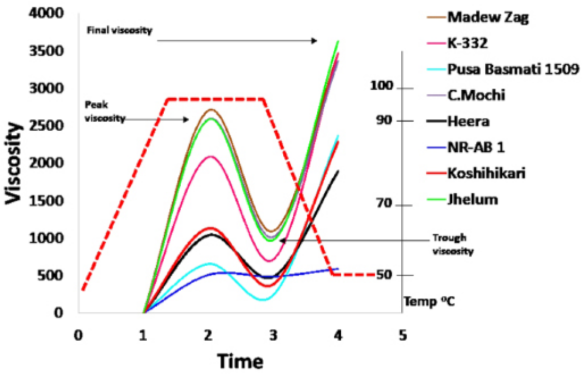

Fig. 4. Viscosity parameters of selected germplasm lines

gelatinization homogeneity. K-332 had the greatest R-value, and Koshihikari had the lowest.

Scanning Electron Microscopy (SEM)

The study used SEM analysis of eight different genotypes to investigate the starch structure of polished rice. A comparison was drawn over a set of eight rice genotypes at a magnification of 1.0k, which covers a kernel dimension of 50µm. Angular to rough-surfaced granular structures were found in Pusa Basmati 1509, Heera, and Jhelum. Jhelum granules were smaller and compact, indicating a crystalline structure. NR-AB1 had tiny granules of low density, indicating amorphous regions. C. Mochi and Madew Zag grains have various patterns and forms with sparse packing. Koshihikari, a well-known cultivar, had a robust, granular structure and moderate density(Fig. 6).

The surface morphology of selected rice genotypes from *indica, basmati,* and *japonica* classes showed varying sizes of starch granules with similar shapes (Fig. 6). The genotypes had a granulated matrix of polyhedral and oval-shaped starch granules with angular to rough surfaces. A coarse surface suggests multicellular structures, such as lipoprotein matrix, which might influence gelatinization and starch digestion behavior (Farooq et al. 2018). *Indica* had larger starch granules than *japonica*. Genotypes with higher amylose showed compound and semi-compound starch granules with dense aggregates (Zhu et al. 2011). Low amylose *japonica* types had polygonal granules, while intermediate amylose *indica* genotypes had elongated and irregular shapes. The accessions Pusa Basmati 1509, Heera,

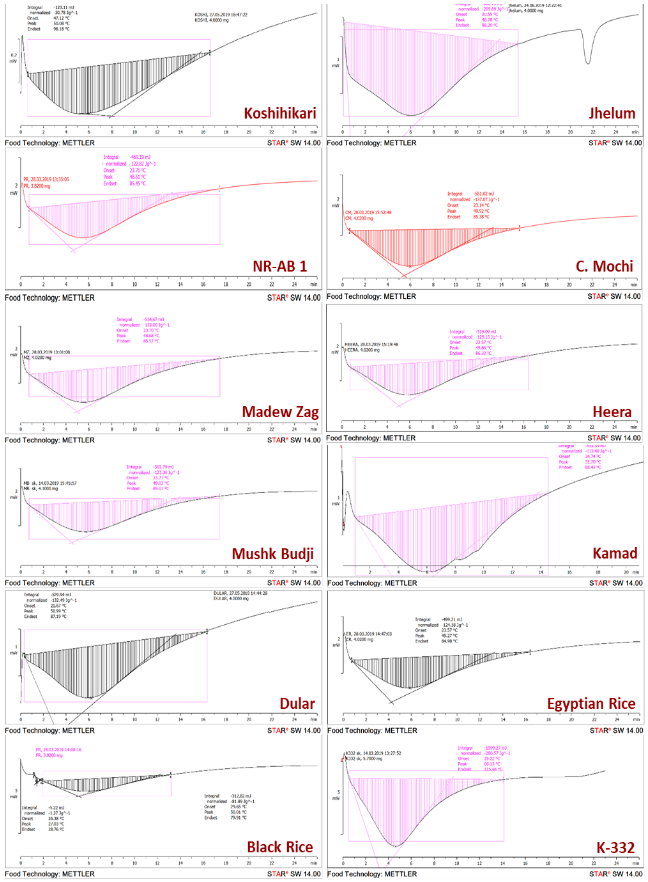

Fig. 5. DSC graphs of selected germplasm lines

Table 4. Thermal analysis through differential scanning calorimetry, estimated glycaemic index and associated parameters of twenty rice genotypes

S.No.	Rice genotypes	To (C)	Tp (°C)	Tc (°C)	ΔHg (J/g)	Gelatinization temp range (R)	Peak height index (PHI) (J/g/° C)	HI	eGI	GI Class
1	K-332	25.22	66.13	115.46	280.57	81.82	6.85	77.0 ± 0.7	82.0 ± 0.7	High
2	Shalimar Rice-5	22.60	49.60	85.59	133.69	54	4.95	77.2 ± 0.7	82.1 ± 0.7	High
3	MushkBudji	23.71	49.03	89.01	123.36	50.64	4.87	54.3 ± 0.1	69.5 ± 0.8	Intermediate
4	SKUA-485 (Improved MushkBudji)	27.70	49.30	69.32	58.76	43.2	2.72	63.8 ± 1.1	74.7 ± 0.7	High
5	Kamad	24.74	51.70	88.45	113.40	53.92	4.20	60.5 ± 0.7	72.9 ± 0.6	High
6	Koshihikari	47.12	50.08	98.18	30.78	5.92	10.3	36.7 ± 0.7	59.8 ± 0.6	Intermediate
7	Stejaree	22.19	47.70	77.09	93.59	51.02	3.66	60.3 ± 0.3	72.8 ± 0.7	High
8	C. Mochi	23.14	49.92	85.38	137.07	53.56	5.11	76.8 ± 1.0	81.8 ± 0.7	High
9	Tangdhar Zag	22.88	45.19	81.40	120.31	44.62	5.39	58.8 ± 0.7	72.0 ± 0.6	High
10	Madew Zag	23.70	48.68	85.52	133.00	49.96	5.32	36.5 ± 0.5	59.8 ± 0.6	Intermediate
11	Egyptian Rice	23.57	49.27	84.98	124.18	51.4	4.83	70.3 ± 0.2	78.3 ± 0.7	High
12	IR-64	46.81	51.59	92.90	172.49	9.56	36.08	54.2 ± 0.4	69.4 ± 0.7	Intermediate
13	Heera	23.57	49.86	86.32	129.13	52.58	4.91	41.6 ± 0.3	62.5 ± 0.8	Intermediate
14	NR-AB1	23.71	48.61	85.45	122.82	49.8	4.93	68.5 ± 0.7	77.3 ± 0.7	High
15	Zeera Rice	22.32	51.51	92.69	204.03	58.38	6.98	55.3 ± 0.9	70.0 ± 0.7	Intermediate
16	Jhelum	20.55	48.78	88.20	209.69	56.46	7.42	70.8 ± 0.7	78.5 ± 0.5	High
17	Dular	21.67	50.99	87.19	129.13	58.64	4.40	78.7 ± 0.7	82.9 ± 0.7	High
18	Black Rice	29.40	50.08	83.59	96.90	41.36	4.68	58.7 ± 0.4	71.9 ± 0.7	High
19	Shalimar Sugandh 1	28.65	54.60	92.45	94.70	51.9	3.64	52.5 ± 0.4	68.5 ± 1.1	Intermediate
20	Pusa Basmati 1509	23.69	54.66	95.65	211.53	61.94	6.83	30.4 ± 0.7	56.4 ± 0.8	Low-Int
21		-	-	-	-	-	-	100 ± 0.4 *	94.2 ± 0.5	High

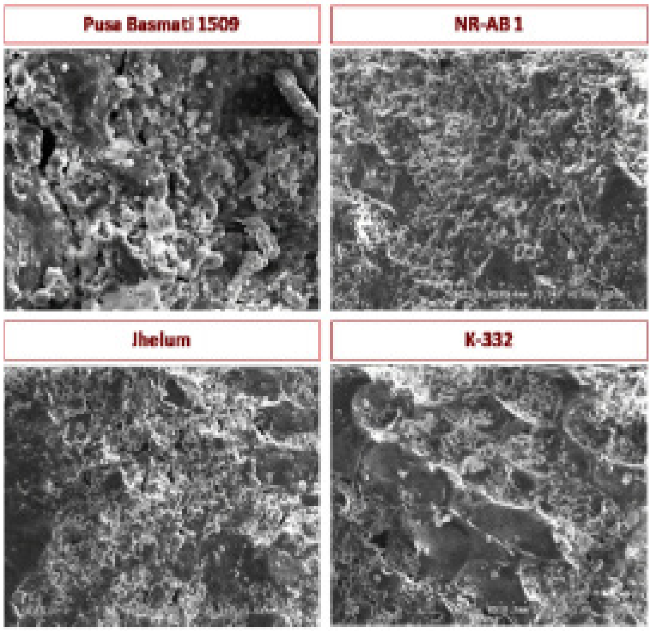

Fig. 6. SEM images depicting starch microstructure in milled rice

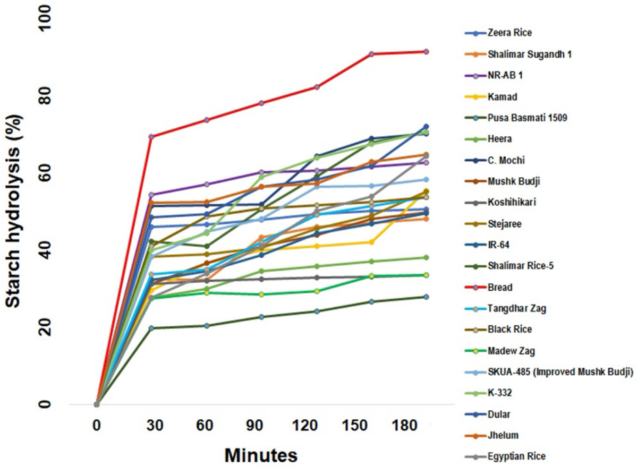

Fig. 7. *In-vitro* Starch digestibilityfor the estimation of glycaemic index

and Jhelum had rough, angular granules and crystalline structures, while NR-AB1 had fine granules with low density, indicating considerable amorphous regions. The structure of starch granules, including size, shape, and surface area,

Table 5. KASP marker-based haplotype information generated at genomic loci involved in starch synthesis

Genotype	AC%	AC class	CT repeat	CT class	Wx	SNP#ID								
						Chr 6								Chr 10
						1	2	3	4	5	6	7	8	9
						1.2	1.2	1.6	1.6	1.6	2.1	6.8	9.7	17.4
MushkBudji	17.63	Low	17	Low-Int	b	C	C	T	A	C	**A**	**G**	**C**	**A**
SKUA-485	21.04	Int	14	Int	a	C	C	T	A	C	**A**	**G**	**C**	**A**
Kamad	18.35	Low	17	Low-Int	b	C	C	T	A	C	G	G	C	G
K-332	19.66	Low	19	Low	b	C	C	T	A	C	G	G	T	A
Stejaree	15.69	Low	18	Low	b	C	C	T	A	C	G	T	C	A
Tangdhar Zag	19.15	Low	18	Low	b	C	C	T	A	C	**A**	**G**	**C**	**G**
Madew Zag	23.02	Int	17	Low-Int	a	C	C	T	A	C	**A**	**G**	**C**	**G**
IR-64	24.73	Int	11	High	a	C	C	T	A	C	G	G	C	A
Heera	24.08	Int	10	High	a	C	C	T	A	C	G	G	C	A
Egyptian Rice	21.92	Int	14	Int	a	C	C	T	A	A	G	T	C	G
Black Rice	18.04	Low	19	Low-Int	a	C	C	T	A	A	A	T	C	A
NR-AB 1	12.42	Low	14	Int	a	C	C	T	A	A	G	G	C	A
Kohsar	16.71	Low	18	Low-Int	a	C	C	T	A	A	G	G	C	G
Shalimar Rice-5	18.69	Low	19	Low-Int	b	C	C	T	A	A	A	T	C	G
C. Mochi	16.08	Low	17	Low-Int	a	C	C	T	A	A	G	G	C	A
Zeera Rice	23.07	Int	19	Low-Int	b	T	G	T	A	C	G	G	C	A
Shalimar Sugandh 1	21.92	Int	17	Low-Int	b	T	G	T	A	C	G	T	C	A
Pusa Basmati 1509	25.01	High	11	High	b	T	G	T	A	C	G	T	C	A
Koshihikari	16.38	Low	17	Low-Int	b	C	C	C	G	A	G	T	C	A
Jhelum	21.83	Int	14	Low	a	C	C	C	G	A	G	G	C	A
Dular	24.55	Int	8	High	a	T	G	C	A	A	G	G	C	A

SNP#IDs: 1: OsR4 SNP_ff_3; 2: RM589_SNP_4; 3: Waxy_SNP; 4: Amy_W2_R_1;
5: Amy_RM190 fun; 6: SNP_ff_1; 7: ALK_SNP_ff_1; 8: RM527_SNP1; 9: SSII_1_SNP_ff_1

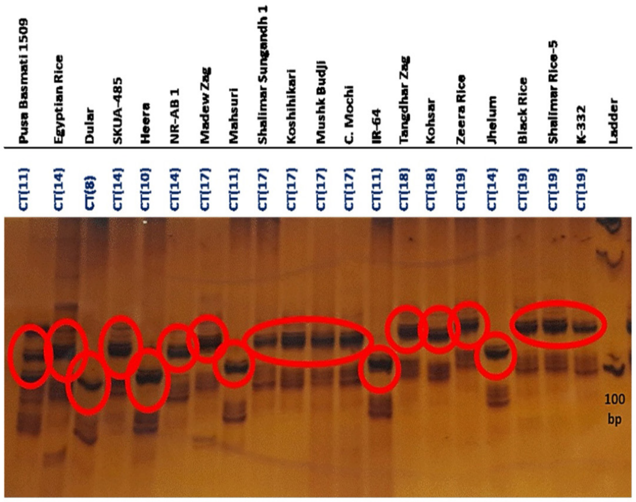

Fig. 8. Allelic polymorphism for $(CT)_n$ repeat-based marker RM190 at Wx locus

significantly impacts starch digestion. Larger granules have lower enzyme susceptibility compared to smaller fractions. Rice genotypes with altered starch granule structures resist enzymatic digestion and nano-clustering into large granules decreases digestibility.

In-vitro starch digestibility, hydrolysis index and estimated glycaemic index

The glycaemic index (GI) is a method used to classify dietary carbohydrates based on their impact on lowering the blood glucose response, usually 2 hours after meals (Wolever. 2006). We examined starch digestibility across genotypes in comparison to white bread (reference). The genotypes were classified as low (<55), medium (56–69), and high (>70) based on their glycaemic index (GI). The reference showed a digestion value of around 90% at 180 minutes after treatment. Pusa Basmati 1509 showed a slower starch digestion rate, peaking at 25%. Other genotypes, such as Madew Zag, Koshihikari, Heera, and IR-64, had slower rates, with a peak of 30, 30, 35, and 43%. Pusa Basmati 1509 and Madew Zag had near-low GI values of 56.4 and 59.8, respectively. Dular, Shalimar Rice-5, K-332, C. Mochi, Jhelum, and Egyptian Rice showed a rapid hydrolysis rate and were Categorized as high GI types (Fig. 7).

Allelic characterization for loci controlling important starch properties

KASP marker haplotype analysis of rice germplasm and CT repeat length polymorphism at wx locus. The *wx* gene associated with the amylose phenotype carries a relation with SSR marker RM190, which amplifies $(CT)_n$ repeats within Intron 1 at this locus. PAGE helped us to resolve allelic polymorphism linked to amylose content. Repeat lengths of $(CT)_8$, $(CT)_{10}$, and $(CT)_{11}$ were observed in genotypes Dular, Heera, and Pusa Basmati 1509, respectively, while $(CT)_{14}$ alleles were found in Egyptian Rice, SKUA-485 (Improved MushkBudji), NR-AB1 and Jhelum. $(CT)_{17}$ repeat was depicted in Madew Zag, Shalimar Sugandh 1, Koshihikari, Mushk Budji, Kamad, C. Mochi, Tangdhar Zag, and Stejaree. $(CT)_{19}$ amplified in Zeera rice, SR-5, and K-332.CT polymorphism correlated with amylose phenotype at an R^2 value of 65%.

KASP (KompetetiveAllele Specific PCR) marker haplotype analysis of rice germplasm characterized novel SNPs within the waxy region associated with quality traits like Amylose content, ASV, and GT (Table 5). The *wx* locus was associated with SNPs OsR4 SNP_ff_3, RM589_SNP_4, Waxy SNP, AMY_W2_R_1, Amy_RM 190_fun, and SNP_f_1 located on chr-6. The TGTAC haplotype differentiated between fine-grained Pusa Basmati 1509 and Shalimar Sugandh 1, with two non-basmati showing a different haplotype CGA than other genotypes carrying TAC haplotype. Koshihikari and Jhelum carried CCCGA, while Egyptian Rice, Black Rice, NR-AB 1, Shalimar Rice 5 and C. Mochi had CCTAA haplotype. Dular carried a distinct TGCAA sequence at the corresponding site. Amy RM190_fun revealed A/C polymorphism across the set of germplasm, with eight genotypes carrying A against C. Low amylose rice carried A at the *wx* locus and was linked to $(CT)_{17}$, $(CT)_{18}$, and $(CT)_{19}$ at RM190 locus (Fig. 8).

Alk (alkali degeneration locus)

The *Alk* gene, which controls gelatinization temperature, lies distal to the *wx* locus located at 6.8 Mb on chromosome 6 and has two SNPs, ALK_SNP_ff_1 and RM527_SNP. High ASV genotypes carried T at the corresponding locus. The genotypes Stejaree, Shalimar Sugandh 1, Pusa Basmati 1509, Egyptian Rice, NR-AB 1, Shalimar Rice-5, and Koshihikari were conserved for T and high ASV against G for the rest of the genotypes. SSII SNP (Starch synthase II_SNP_ff_1) related to starch synthase, controls gelatinization temperature, thermal properties, and amylopectin branch chain length. Egyptian Rice, Shalimar Rice 5, Tangdhar Zag, and Madew Zag possessed G against A for the rest of the genotypes and scored high ASV. The distinction between *indica* and *japonica* sub-species in terms of rice quality, especially with traits like waxyness, grain type, and pericarp color, is explained based on the theory of domestication in rice. Many loci have shown complete drift into one or the alternate groups, with important examples being *GS3* for grain type(Fan et al. 2006), *wx* for amylose content, and *Rc* for pericarp color(Huang et al. 2012). Currently, the haplotype information at SSR and SNP marker loci explains the distribution and contrasting phenotypes between *indica* and *japonica* ecotypes. Taken together, genotyping using markers for important loci, like wx and ALK, helped us to characterize the alleles. Utilizing the unrealized genetic potential of traditional landraces of rice, especially those adapted to particular ecologies like the North-Eastern Himalayan region, is essential for improving grain quality, yield potential, and stress tolerance through focused breeding initiatives (Verma et al. 2024). We provided a detailed discussion on the allelic variation and biochemical properties that condition starch synthesis in rice endosperm and explained the genetic diversity (Zaffer et al. 2025). The *japonica* accessions identified for specific rice quality related traits carry commercial importance and can be further scaled up for area increase and product development.

Authors' contribution

Conceptualization of research (ABS, SZH); Designing of the experiments (ABS, SZH, BN); Contribution of experimental materials (NR, GK); Execution of field/lab experiments and data collection (NN, BN, SZH, ABS); Analysis of data and interpretation (BHS, HR, SZ); Preparation of the manuscript (NN, BN, ABS, SZ, SZH).

Acknowledgments

The authors acknowledge the support from Innovate U.K.-funded project on "KASP genomic selection: improving farmers' livelihoods through better rice varieties"; DST PURSE Project, SKUAST-K, DST, MoS, Govt. of India (Sanction No. SR/PURSE/2022/124).

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