



Molecular Characterization of Diverse Rice (*Oryza sativa* L.) Genotypes for Glycemic Index

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Authors' contributions

This work was carried out in collaboration among all authors. Author HM wrote the protocol and the first draft of the manuscript. Author PPT designed the study and performed the statistical analyses. Authors NJ, AH and PB managed the study analyses and conducted the literature search. All authors read and approved the final manuscript.

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ABSTRACT

The Glycemic Index (GI) of food is a measure of the rate at which glucose is released after consuming a specific amount and is important in diabetes management. The GI and digestibility of rice starch depends upon its amylose content and structure. The aim of current study was to assess GI of diverse genotypes and validate their association with GI linked marker. Considering the availability of diverse rice genotypes and their genetic variability, sixteen different rice

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genotypes/lines which includes six inbred lines and four improved varieties developed by ICAR-Indian Institute of Rice Research Hyderabad and six genotypes from local market were screened for biochemical quality trait viz., amylose, carbohydrate, resistant starch along with glycemic index. The amylose content observed over the varieties ranged from 10.12 % to 62.58 %. Carbohydrate content ranged from 83.08 % to 86.33 % with the mean of 84.87 and resistant starch content varied from 1.75% to 5.34 % with the mean of 3.52. Glycemic index of selected rice varied from 52.40 to 137.06 with general mean of 83.33. In our study significant variation was observed among all varieties except carbohydrates. Among all varieties, CSR23 has significantly higher GI (137.06) and rice inbred lines serially numbered as 84, 87, 97, 6-5 and 128 have shown significantly lower GI. Glucose uptake capacity by yeast cells was evaluated in presence of all rice genotypes/lines extract and it was observed that all the varieties except Jaya, Komal and 97 shows increase in % glucose uptake as compared to control sample. Allelic variations of marker RM190 for the waxy gene have also shown variations for tested rice genotypes/lines. Rice varieties such as inbred lines 84, 87, 97, 6-5 and 128 which have lower GI are better for diabetes management.

Keywords: *Glycemic index; Oryza sativa L.; resistant starch; amylose content; carbohydrate.*

1. INTRODUCTION

Rice is the most common cereal grain consumed in India. It is a staple diet for 65% of the Indian population, particularly in the country's eastern and southern regions (Joshi and Srivastava, 2020). Rice accounts 44.7 percent of total cultivated acreage in India and accounts for 70.3 percent of total food grain production (Madhubabu et al., 2020). Consumers are increasingly concerned about the quality of the rice varieties they consume. As a result, along with rice production, it is critical to focus on quality attributes.

Rice has a higher glycemic index than other cereal grains, ranging from 54 to 121 %. The identification of low GI (55 or less) rice types would require research. The rice consumer's potential health benefits make research a priority. As a result, rice with a lower GI and increased nutritional quality may aid in the prevention of diet-related disorders. Rice, while having a higher glycemic index (GI) ranging from 54 to 121, is nonetheless used as a staple diet in comparison to other starchy foods. As a result, rice with a low GI should aid in the prevention of diet-related disorders.

Like most "white" foods, it causes blood sugar spikes due to its high glycemic index (GI) value (Nagdeve, 2019). White rice causes a rather significant glycemic response and is consequently linked to worsening impaired glucose tolerance. Because of the enormous amount consumed and the higher GI, it provides a significant glycemic load (GL) to the diets of people living in countries where it is the main staple.

The glycemic response to meals, which influences the insulin response, is determined by the rate of carbohydrate breakdown and absorption from the small intestine (Jenkins et al., 1981). GI is influenced primarily by the structure and composition of the starch. The GI of rice depends on the genotypic variation. Varietal effects on GI appear to be mainly mediated by its amylose content. The apparent amylose concentration of boiled rice correlates with water absorption and volume expansion during cooking, as well as the hardness of the rice (Juliano, 2003). As a result, the amylose content influences the textural properties of cooked rice and is an important factor to consider when selecting rice for specific uses.

The hydrolysis of starch has an important role in regulating the glycemic index. SDS and resistant starch (RS) have been shown in studies to cause slower glucose release and a slower glycemic response. Resistant starch, which exists as an indigestible starch fraction in the stomach and small intestine, can provide effective GI regulation (AP, 2005).

Resistant starch refers to residual fractions of starch that are resistant to enzyme hydrolysis and enter the large intestine with dietary fibre. Despite accounting for a modest part of total calorie consumption, resistant starch has an effect like that of other fibre components. The glycemic index (GI) and resistant starch (RS) concentration are key measures of starch digestion (Singh et al., 2013). However, resistant starch (RS), which acts as a non-glycemic starch fraction in rice, affects its digestion. Type-5 resistant starch is often generated in rice by the interaction of amylose and lipids. These

complexes are made up of hydrophobic polyglucan chains and are responsible for decreasing GI due to their resistance to enzymatic hydrolysis (Fuentes-Zaragoza et al., 2011).

At the DNA level, molecular markers can reveal significant differences between genotypes. They provide a more direct, dependable, and efficient method for characterization, screening, and evaluation of germplasm. Micro-satellites are plentiful and widely distributed in the genome (Akagi et al., 1996). They are useful as genetic markers because they are co-dominant in nature, detect large levels of allelic diversity, and are easily evaluated using the PCR technique (McCouch, 2002). Therefore, the current study aims to estimate carbohydrate and amylose content of the 16 different genotypes along with their characterization of the genetic diversity using SSR markers. Simple sequence repeats (SSRs) are nucleotide sequence motifs that are tandemly repeated and flanked by distinct sequences. SSR markers, which can be detected by PCR, are utilized to detect a high level of allelic diversity, and genotyping of these codominant markers can be automated (McCouch, 2002).

Amylose content of rice is regulated by waxy (Wx) gene that codes for Granule Bound Starch Synthase (GBSS) protein. The simple sequence repeat (SSR) marker RM190, which is associated with the Wx phenotype, has been shown to explain around 60% of the diversity in amylose concentration in rice (Wan et al., 2007). The RM190 is based on a dinucleotide repeat CT that ranges from (CT)₈ to (CT)₂₀ and is situated in the 5' Untranslated Region (UTR) of the Waxy exon (Bligh et al., 1995).

2. MATERIALS AND METHODS

2.1 Plant Materials

Seeds of rice (*Oryza sativa*) genotypes viz., Jaya, Ratna, Gujarat Kolam, Komal, Zini Kolam and Swarna were collected from the Regional Agricultural Research Station, Karjat, Maharashtra state while six recombinant GI trait specific lines (99, 97, 87, 84, 128 and 6-5) and four improved varieties (Samba Masuri, CSR23, DRR Dhan 58, FL-478) were procured from ICAR-Indian Institute of Rice Research, Hyderabad, Telangana state, India.

2.2 Determination of GI Related Traits and Statistical Analysis

Seeds of all the genotypes/recombinant inbred lines were ground in mixer and grinder to obtain fine powder (rice flour) and were evaluated for different biochemical parameters viz., amylose content (Juliano, 1971), Carbohydrate content (DuBois et al., 1956) and resistant starch (Goni et al., 1996). The total amylose content was categorized into waxy (0-2 %), very low (3-9 %), low (10-19%), intermediate (20-25 %) and high (> 25%) (Juliano, 1971). Analysis of variance (ANOVA) for biochemical traits (Glycemic index, resistant starch, carbohydrates content) was carried out based on mean value per entry per replication.

2.3 Estimation of Glycemic Index

Glycemic index was estimated using a slightly modified version of starch hydrolysis method (Goni et al., 1997). To 100 mg of rice sample, 10 mL of HCl-KCl buffer pH=1.5 was added followed by addition of 0.2 mL of a solution containing 1 g of Pepsin in 10 mL of HCl-KCl buffer and incubation at 40°C for 1 hour in a shaking water bath at 100 rpm. The volume of the sample was made to 25 ml using Tris-Maleate buffer (pH 6.9). Reaction was started by adding α-amylase (2.6 units in 5 ml of buffer pH 6.9) followed by incubation at 37 °C in a shaking water bath. To the 1 ml of the above solution, 80 ml of 0.2 M HCl and 10 µl amylo-glucohydrolase were added to hydrolyze the digested starch to glucose and was incubated at 60 °C for 45 min at 100 rpm. To the 25 µl aliquot of this solution, 4ml distilled water and 40 µl GOPOD was added. After 20 minutes of incubation at 37 °C, absorbance was recorded at 510 nm. The starch hydrolysis (%) of the sample was determined with reference to Goni et al., (1997). Also, the hydrolysis index (HI) and the estimated glycemic index (eGI) of the sample were determined using the below equations (Goni et al., 1997):

$$HI = (\text{Absorbance of Sample}) / (\text{Absorbance of Reference}) \times 100$$

$$GI = 39.71 + (0.803 \times HI)$$

Where,

Absorbance of reference- 100 mg/ml glucose

In vitro evaluation of glucose uptake by yeast cells

Evaluation of glucose uptake by yeast cells was carried out in accordance with Cirillo's well-defined technique (Cirillo, 1962). To make a 1% suspension, commercial baker's yeast was dissolved in distilled water. The suspension was left at room temperature (25°C) overnight. On the following days, the yeast cell culture was centrifuged for 5 minutes at 4200 rpm. By adding distilled water to the pellet, the process was repeated until a clear supernatant was achieved. To obtain a 10% v/v yeast cell suspension, 10 parts clear supernatant fluids were combined with 90 parts distilled water.

About 10mg of rice flour of each genotype/line was individually mixed with dimethyl sulfoxide (DMSO) till dissolution. The mixture was then incubated for 10 minutes at 37°C with varied concentrations (5mM and 15mM) of 1 mL of glucose solution. To begin the reaction, 100 ml of yeast suspension was added to the above mixture, vortexed, and incubated at 37°C for an additional 60 minutes. Following incubation, the tubes were spun for 5 minutes at 3800 rpm, and glucose was measured using a Glucometer.

$$\% \text{ increase in glucose uptake} = \frac{(\text{glucose level of Control} - \text{glucose level of Sample})}{(\text{glucose level of Control})} \times 100$$

Where control is the solution having all reagents except the test sample. Metronidazole was used as standard drug.

2.4 PCR Assay for Glycemic Index Trait

For molecular studies, seeds of rice genotypes were germinated in pots under greenhouse conditions. Total genomic DNA was extracted using modified cetyl trimethyl ammonium bromide (CTAB) procedure (Rogers and Bendich, 1994). Rice microsatellite marker RM190 (RM190-F: 5'-CTTTGTCTATCTCAAGACAC-3' and RM190-R:

5'-TTGCAGATGTTCTTCCTGATG-3') was used to amplify isolated DNA to find out polymorphism among the rice varieties. PCR reactions were carried out in 0.2 ml polypropylene PCR tubes (Bangalore Genei, India) using Thermal Cycler (Eppendorf). Each 15 µl reaction mixture contained 1 x Taq buffer, 2.5 mM MgCl₂, 0.2 mM dNTPs (Bangalore Genei, India), 5 ppm primers (Sigma Genosys, India), 1U Taq DNA polymerase (Bangalore Genei, India) and 25 ng template DNA. The reactions were subjected to initial denaturation at 94°C for 4 min followed by 35 amplification cycles, each consisting of 1 min at 94°C (denaturation step), 1 min at 55°C (annealing step) and 1 min at 72°C (extension step) with a final extension of 7 min at 72°C. The amplification products were separated on non-denaturing 10% polyacrylamide gel (BioRad power PAC 1000). DNA ladder 1 Kb (Bangalore Genei, India) was used as molecular weight marker for comparison of amplified products and the variation in banding pattern of GI specific marker was observed in different rice genotypes and low GI produced in improved recombinant inbreed lines.

3. RESULTS AND DISCUSSION

3.1 Genotypic Variation Based on Different Biochemical Traits Related to Glycemic Index

The different varieties of rice and the procured recombinant inbreed lines related to low glycemic index were differed in terms of their glycemic index. For this, different biochemical characteristics like amylose and amylopectin, starch resistant, carbohydrate content and glycemic content were recorded. The Analysis of variance showed significant difference between the genotypes and there was no significant difference found in between the replications of each trait (Table 1).

Table 1. Analysis of variance for different biochemical traits associated with glycemic index

S.V.	D.F.	Carbohydrate	Starch resistance	Glycemic index
Rep.	2	0.28646	0.0018	4.26
Treat.	15	5.13617	2.59245	803.76
Error	30	1.06738	0.00458	3.23
SE (m)		1.033	0.03	1.03
CD @ 1%		2.91	0.15	4.22
CD @ 5%		2.17	0.116	3.08
C.V.		1.21	1.91	2.70
F test	-	S	S	S

Table 2. Mean performance of different biochemical traits related to glycemic index of different rice varieties

Name of Genotypes/line	Amylose content (%)	Carbohydrate (%)	Resistant starch (%)	Glycemic index (%)
99	23.59	84.09	4.82	50.67
84	10.12	81.49	4.01	53.00
87	43.74	84.36	5.34	47.19
97	31.49	87.22	4.08	46.31
6_5	59.31	88.09	2.18	49.67
128	38.33	84.91	1.75	52.67
Jaya	22.58	85.78	3.06	67.33
Ratna	27.84	86.05	4.03	84.00
Gujarat kolam	24.60	85.23	3.85	66.67
Komal	20.22	84.45	2.83	71.33
Zini kolam	23.88	86.42	2.87	64.33
Suvarna	16.90	83.23	3.71	76.33
Samba Masuri	21.25	84.30	2.88	65.33
CSR 23	26.57	85.00	3.87	97.83
DRR Dhan 58	21.79	83.49	3.12	95.17
FL 478	25.18	84.22	4.06	76.20
Range	10.12-59.31	81.49-88.09	1.75-4.82	46.31 - 97.83
Mean		84.89	3.52	66.5
CD @ 5%		2.17	0.116	3.08
CV		1.21	1.91	2.7

Correct expression: ($P = .05$). Wrong Expression: ($P < .05$), unless $P < .001$

3.1.1 Amylose content

Amylose determines the starch content of rice and this influences the physical appearance after cooking. The amylose content observed over the rice genotypes/lines ranged 10.12 % to 59.31 %. Based on the amylose composition, rice varieties were classified as waxy (1-2 % amylose), very low (2-9 %), low (10-20), intermediate (20-25) and high (>25) (Juliano, 1992). In the present study, the genotype CSR23, FL478, Ratna and lines 87, 97, 6-5, 128 recorded higher amylose content with value 26.57, 25.18, 27.84 and 43.74, 31.49, 59.31, 38.33 respectively. Frei et al., (2003) report that starchy foods with high amylose levels are associated with lower glycemic index in human that help formulation of diet in diabetic patient. This high amylose rice decreases breakdown of starch molecule by increasing its stability in a body. Higher amylose starch has less soluble components such as crystallites of longer chain lengths that need higher heat energy for solubilization and destruction of their crystal-line structure.

3.1.2 Carbohydrate content

The carbohydrate content of all the varieties was ranged 81.49 % to 88.09 %. The statistical analysis for carbohydrate content showed significant difference between the genotypes of

both CD value of 5% and 1%. The CD @ 5% and CV value for this trait was 2.17 and 1.22 respectively. Considering the 5% CD value, the lines 97, 6-5 and the genotypes, Ratna and Zini kolam recorded higher carbohydrate content with value 87.22, 88.09 and 86.05, 86.42 respectively. However developed GI trait specific line 84 and the genotypes Suvarna, DRR Dhan 58 recorded lower carbohydrate content with value, 81.49 and 83.23, 83.49 respectively. According to Chandel et al., (2016) four white (Swarna, CGMD-55, HMT, CGZRI) and one brown (BR check rice) grains of rice (*Oryza sativa*) varieties were screened to determine predicted GI together with influencing traits which includes carbohydrate content, it was observed that carbohydrate content varied from 83.3% to 89% and results showed that Swarna had the highest carbohydrate content (89%), whereas BR check rice had the lowest carbohydrate level (83.3%), for a total of 85.7%.

3.1.3 Resistant starch

Resistant Starch content of all 16 rice genotypes/lines was estimated and the recorded range for resistant starch content was 1.75 % to 5.34 %. The statistical analysis for glycemic index showed significant difference between the genotypes of both CD value of 5% and 1%. The 5 % CD and CV value for this trait was 0.12 and

1.92 respectively. Considering the 5% CD value, the GI trait specific line 87 recorded higher resistant starch content (5.34 %). However, the developed GI trait specific lines, 128 recorded lower resistant starch content with value 1.75 % respectively. Agasimani, (2011) conducted a study on rice to quantify variability for resistant starch (RS) content in a collection of germplasm accessions. The RS content was estimated in all the 50 accessions based on the method suggested by Goni et al. (1996) using the Megazyme kit, and it was reported that Kattanur has the highest mean RS value of 9.53 percent, followed by Norungan (9.45 percent) and others. Kallurundaikar has a 9.33 percent chance of winning. The germplasm accession Jegatsal (JGL) had the lowest mean value of RS (2.09%), followed by ASD 18 and Chinna Ponni, which had values of 2.57 and 2.81 percent, respectively. The ADT 43 cultivar has a mean RS value of 4.08 percent. For all germplasm lines, the mean and range were 5.46 and 7.44 percent, respectively.

3.1.4 Glycemic index

After estimation of glycemic index, the recorded range for glycemic index was 52.40 to 137.06. The statistical analysis for glycemic index showed significant difference between the genotypes of both CD value of 5% and 1%. The CD and CV value for this trait was 12.39 and 8.67 respectively. Considering the 5% CD value, the genotype CSR23 recorded higher GI value (137.06). However, the developed GI trait specific lines, 87, 97, and 6-5 recorded lower GI value, 52.40, 49.42, and 54.87 respectively. Kumari et al., (2021) has studied twenty popular rice varieties developed by ANGRAU obtained from Regional Agricultural Research Station (RARS) for glycemic index and the glycemic index ranged from 56.72 to 66.43, with a mean of 60.67. Furthermore, all of the varieties were classified as medium GI (56-69). Pushyami has the lowest glycemic index rating (56.72) of all the types. Purwani et al., (2007) reported glycemic index in the rice varieties of IR36, Taj Mahal, Trunk Piaman, and Mekongga and it was recorded as 45 %, 60 %, 86 %, and 96 %, respectively, in this IR36 was considered as low glycemic index variety that is helpful for dietary people.

3.2 Effect of Rice Extract on Glucose Uptake by Yeast Cells

Glucose uptake capacity by yeast cells was evaluated in presence of all rice genotypes/lines

extract. The rice extract promoted the uptake of glucose across the plasma membrane of yeast cells. The glucose uptake at an initial concentration of 5 mM and 15 mM by the rice extract was comparable to that of known drug metronidazole. In this study, the genotypes Jaya, Ratna, Gujarat, Kolam, Suvarna and GI trait specific line 128 has recorded increase in % of glucose uptake in both the concentrations 5mM and 15mM, whereas genotypes Samba Masuri, CSR23, DRR Dhan 58 and line 99 has recorded decrease in % of glucose uptake in both concentrations mentioned above. Among the genotypes, the highest % glucose uptake recorded by Gujrat kolam (16.04) and Zinni kolam (16.04) and in the developed GI specific inbred line, 87 (15.81) showed highest glucose uptake in the yeast cell.

An inverse relationship to the molar concentration of glucose was observed, when glucose uptake by yeast cells was compared among 5 mM and 15 mM for the same amount of rice extracts for all 16 genotypes/lines. So, looking into the availability of different rice genotypes and checking their effect on glucose uptake on yeast cells it was observed, that all the varieties except Jaya, Komal and 97 shows increase in % uptake at lower concentration (5mm) of glucose and gradual decrease at higher concentration (15mm). From the results it is concluded that the lower the concentration of glucose in the solution, the higher the uptake by yeast cells.

In a study conducted by Rehman et al., (2018), The antidiabetic potential of ethanolic extract of *Cassia nemophila* pod (EECNP) was evaluated yeast glucose uptake assay. The EECNP absorption of glucose at starting concentrations of 5 mM and 10 mM was comparable to that of the recognized medication metronidazole. However, metronidazole had a little greater effect on glucose uptake by yeast cells at 25 mM glucose concentration than EECNP. Furthermore, the glucose absorption capacity at 1 mg/mL EECNP was greater than 60%, reaching nearly 80% when 5 mg/mL EECNP was employed. This indicates that increasing the concentration of EECNP will allow yeast cells to take in more glucose from the environment. The results showed a linear rise in glucose uptake by yeast cells as the concentration of the test material increased. When glucose absorption by yeast cells was tested at 5 mM, 10 mM, and 25 mM for the same quantity of EECNP, an inverse connection to the molar concentration of glucose was detected.

3.3 Molecular Characterization of Rice Genotypes/Lines for Glycemic Index Trait

The analysis of genetic variation is a very important factor for the development of rice lines, which can be achieved through DNA profiling techniques that displays high amount of loci for large variability. The sample of rice cultivars collected from various origins were analyzed for GI trait encoding SSR marker, RM 190. This RM190 shows allelic variation within waxy gene among the rice genotypes.

With reference to the Naseer et al., (2021) study, whenever there is maximum number of bands per variety found, it is due to the presence of short repeats (CT)_n and high amylose composition. The CT repeat unit 10 to 14 shows low to intermediate amylose composition and high amylose content in rice elicit low GI. In the present study, GI developed recombinant line, 6_5 and 87 recorded higher amylose content, 59.31 and 43.74 respectively, which is

correspondingly shows low GI value, 54.87 and 52.40 respectively. However, the amplification profile of RM190 on this rice varieties show low repeats unit in line 6_5 and 87 therefore 2 or more than two bands were observed in this varieties. The line 97, shows single band with high glycemic index, this allelic variation was found due to presence of maximum (CT)_n repeat units.

Rice amylose content is controlled by the waxy (Wx) gene, which codes for the Granule Bound Starch Synthase (GBSS) protein. The simple sequence repeat (SSR) marker RM190, which is associated to the Wx phenotype, has been reported to explain around 60% of the diversity in amylose concentration in rice (Wan et al., 2007). Waxy gene specific simple sequence repeat (SSR) marker RM 190 (CT)_n (known to have a large effect on amylose content) at the 'waxy' locus was utilized to determine the presence of genetic diversity among the selected rice genotypes.

Table 3. Glucose uptake at different concentration of rice extract on yeast cell

Sr. No.	Name of the variety/ Lines	5 mM (%) uptake	15 mM (%) uptake
1	99	5.42	4.68
2	84	14.46	-2.00
3	87	7.83	15.81
4	97	3.01	6.01
5	6_5	12.05	4.90
6	128	10.24	9.80
7	Jaya	8.43	9.35
8	Ratna	15.06	8.24
9	Gujarat kolam	16.27	16.04
10	Komal	4.82	12.03
11	Zini kolam	3.01	16.04
12	Suvarna	15.66	12.25
13	Samba Masuri	4.22	2.67
14	CSR 23	3.61	1.11
15	DRR Dhan 58	1.20	-0.67
16	FL-478	12.65	4.23

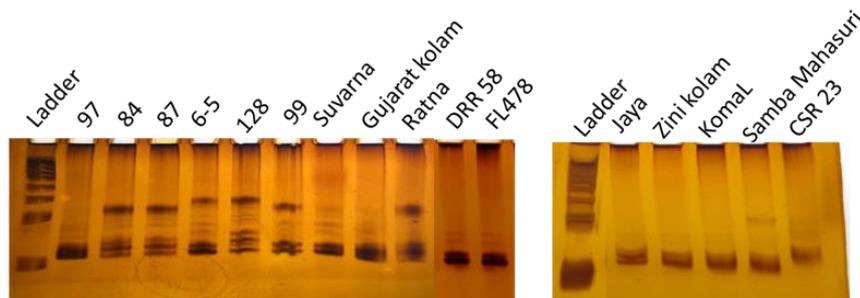


Fig. 1. PCR amplification profile of different rice (*Oryza sativa* L.) genotypes and recombinant lines for Glycemic index trait encoded to SSR marker RM190

4. CONCLUSION

The detection of reproducible molecular markers for low GI is very difficult, because RM 190 is a SSR marker encoding the waxy gene. Though it is a genic marker, but due to maximum number of (CT)_n repeats unit, more allelic variation is found. So, the characterization of low GI traits with their related biochemical traits co-relation with molecular markers difficult. In the present study developed low GI specific recombinant lines were also studied, among the 6 line, 5 recombinant lines showed multiple banding pattern due to present of short repeat units and which were specific to the low GI content, so further evaluation for these developed lines is to be needed. Similarly phenotypic variations in the different locations should be evaluated. The evaluated low GI (glycemic index) trait specific genotypes (Zini kolam and Samba Masuri) and recombinant line (6_5 and 87) and cloning and sequencing of unique polymorphic band could be used to develop low GI specific SCAR/CAPS marker for further molecular breeding program.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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