Research Article

Evaluation and Correlation Studies of Grain Quality Traits of Parental and Three-Gene Positive BC_3F_3 Genotypes (HKR-47 x IRBB-60)

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Abstract

This study was carried out in the molecular biology laboratory of the CCS HAU in Hisar to determine the grain quality traits of parental and three-gene positive BC_3F_3 genotypes (derived from the cross HKR-47 x IRBB-60) grown in fields of Kaul and in the net house at CCS HAU, Hisar. The major carbohydrate of rice is starch. Rice can be classified into three different types: long-grain, medium-grain and short-grain rice based upon their length as compared to their width. The hardiness and stickiness of cooked rice is important for eating quality and consumer acceptance. Amylose and amylopectin contents of rice are very important parameters in determining texture and eating quality of rice. Cooking quality of rice mainly depends on amylose content (AC) and gelatinization temperature (GT). Amylose determination was done using colorimetric assay and GT was measured with alkali spreading value (ASV). Long grain rice was found to have high amylose and low amylopectin content as compared to medium and short-grain rice. Also, the study indicated stickiness negatively correlated with amylose content and hardness, *i.e*., high-amylose rice is harder and less sticky.

Keywords: amylose, amylopectin, rice, alkali spreading value, gelatinization temperature

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Introduction

Rice (*Oryza* spp.) is the key cereal crop in India and many parts of the world, and is a predominant source of food for life, consumed by almost half of the world population. As consumers' incomes increase and markets become more liberalized, consumers' preferences for rice have been shown to shift from lower to higher quality [1].

The stickiness and texture of cooked rice is important for eating quality and it depends on the starch content of rice. The endosperm of the rice kernel is rich in starch (85-90%). Starch, the paramount constituent of rice, is a carbohydrate consisting of amylose and amylopectin. Cooking and receptive quality of the rice entirely depends on amylose and amylopectin proportion of rice kernel [2, 3]. Amylose, the crystallizable form of starch, is a long unbranched polysaccharide made of α-D-glucose units. Amylopectin is a water-soluble polysaccharide and highly branched polymer (occur every 24 to 30 glucose units). It is a soluble molecule that can be quickly degraded as it has many end points onto which enzymes can attach. Cooked rice with high amylose content contributes to non-stickiness and fluffiness properties of rice. Patindol *et al*. (2010) [4] suggested that the amylose-amylopectin ratio of the leached materials during rice cooking may be the main indicator of cooked rice hardiness and stickiness. Amylose content of the rice affects the Glycemic Index (GI). Usually, lower GI value was found in rice rich in amylose [5]. Glycemic index (GI) indicates the effect of carbohydrate on blood sugar levels by measuring how quickly food causes blood sugar levels to rise.

Cooking quality of rice mainly depends on amylose content and gelatinization temperature [6]. Amylose contents determine the texture of cooked rice, whereas gelatinization temperature determines the cooking time [7].

The most widely used method for amylose determination has been a colorimetric assay in which iodine binds with amylose to produce a blue color that is measured spectrophotometrically at one wavelength [8, 9]. Beer's Law forms the basis for the estimation of the concentration of a substance in a sample using spectrophotometry.

The peak temperature at which starch absorbs heat is the Gelatinization temperature (GT). Gelatinization is the irreversible melting process of starch.

Rice varieties with low GT represent huge potential savings in fuel consumption. GT can be measured either with heat or alkali, but the most common and routine method is alkali spreading value (ASV).

In the present study, we took high yielding BC_3F_3 genotypes pyramided with three Bacterial Blight (BB) resistance genes, *Xa21*, *xa5*, and *xa13* (selected by molecular markers) and the parental genotypes to evaluate grain quality traits; grain Length/Breadth (L/B) ratio, Alkali Spread Value (ASV) and Amylose Content (AC).

Material and Methods

The experimental material comprised of rice grains of parental and three-gene positive BC_3F_3 genotypes (HKR-47 x IRBB-60). Various grain quality characteristics were measured for the traits, as follows:

Grain Length/Breadth (L/B) ratio

Physical characteristics were measured using Vernier Caliper for length and breadth of milled rice grains. The measurements recorded for an average of 5 grains per rice genotype. Ratio of length and breadth gave L/B ratio of milled rice [10].

Determination of Alkali Spread Value (ASV)

GT classification of rice varieties was done using an inexpensive alkali spreading value (ASV) test. Milled rice grains (five intact grains from each genotype) were incubated at room temperature (32±2^{o}C) for 23 hours in a Petri dish containing 20 ml dilute aqueous KOH (1.7% w/v) allowing the spreading of the grains [11] and then visualized for scoring the grain spreading. During the process of rice grain gelatinization, starch gets gelatinized, resulting in the degradation of the long linear and branched chains of amylose and amylopectin. Grains swollen to the extent of a cottony center and a cloudy collar were given an alkali spread value (ASV) score 4 and used as a check for scoring the rest of the samples in the population. Grains that were unaffected were given ASV of 2, and grains that dispersed and disappeared completely were given a score of 6.

Determination of Amylose Content (AC)

Amylose content was analyzed using the method described by Juliano et al. (1981) [12]. To 100 mg of rice flour, 1 ml of 95% ethanol and 9 ml of 1.0 N NaOH was added and was left for 18h. Distilled water was added to the mixture to make up the final volume to 100 ml. From this suspension, a sample of 5 ml was measured into a volumetric flask, 1 ml of 1M acetic acid and 1.5 ml iodine solution (0.2% iodine + 2% potassium iodide) were added into the solution and the volume made up to 100 ml with distilled water.

The contents were stirred and allowed to stand for 20 minutes before absorbance was measured at 620 nm with a UV-Spectrophotometer. As a control, NaOH solution was used. The AC of different varieties was calculated in comparison with standard graph [13].

Amylopectin content of the rice samples was calculated by the difference based on Juan et al. (2006) [14] using the following equation;

$$\text{Amylopectin (\%)} = 100\% - \text{amylose (\%)}$$

These values were used to classify the rice based on their amylose content [15] (**Table 1**). The textural and thermodynamic properties of starch were determined by the ratio of its constituents, *i.e.*, amylose and amylopectin.

Table 1 Amylose content (%) category

Amylose content (%)	Category
25 – 33	High
20 – 25	Intermediate
10 – 20	Low
2 – 9	Very low amylose
1 – 2	Waxy

Source: Juliano, 1971

Statistical Analysis

The test statistics, Pearson Product-Moment Correlation Coefficient (PPMCC) at 5% level of significance was used to measure the correlation between different traits. It measures the strength of a linear association between two variables and is denoted by *r*. The value of *r* ranges from +1 (positive correlation) to -1 (negative correlation). The analysis is important as it tells us how selection of a particular character results in the parallel change in some other characters. It also helps understand the genetic role through the pleiotropic effect of genes on correlation.

Results

Data was recorded for the grain quality traits of the positive BC_3F_3 genotypes and the parental genotypes. Significant variation was observed among the three-gene positive lines and parental rice genotypes for grain L/B ratio, alkali spread value and amylose content.

Grain L/B ratio

The length and breadth of a rice grain are important attributes that determine the class of the rice. The ratio of the length and the breadth is used internationally to describe the shape and class of the variety. The grain size of each genotype was measured by using Vernier caliper. Length/breadth ratio of grain in the parental genotypes HKR-47 and IRBB-60 was recorded to be 3.90 and 3.20, respectively. Length/breadth ratio of grain among positive BC_3F_3 plants varied between 3.40 and 3.90. Genotypes G1-8, G1-12, G1-13, G2-3, G2-6 and G5-17 grown in field at Kaul had L/B ratio of 3.90, 3.80, 3.60, 3.70, 3.40 and 3.40, respectively (**Table 2**). Genotypes G1-15, G3-11, G3-13 and G5-13 grown at net house had L/B ratio of 3.70, 3.30, 3.10 and 3.50, respectively (**Table 3**).

Table 2 Grain dimensions of parental and BC_3F_3 genotypes grown in Kaul

Serial No.	Plant no.	Grain length (mm)	Grain breadth (mm)	L/B ratio
1	MEAN	6.70	1.88	3.61
2	RANGE	6.20-7.10	1.78-2.12	3.20-3.90
3	IRBB60	6.80±.07	2.12±.03	3.20
4	HKR47	6.62±.24	1.80±.01	3.90
5	G1-8	7.00±.05	1.81±.03	3.90
6	G1-12	7.10±.20	1.86±.04	3.80
7	G1-13	6.91±.07	1.91±.02	3.60
8	G2-3	6.59±.15	1.78±.05	3.70
9	G2-6	6.20±.10	1.82±.01	3.40
10	G5-17	6.80±.07	2.00±.05	3.40

Table 3 Grain dimensions of parental and BC_3F_3 genotypes grown in the net house

S. No.	Plant No.	Grain length(mm)	Grain breadth (mm)	L/B ratio
1	MEAN	6.34	2.14	3.45
2	RANGE	5.90-6.80	1.72-2.12	3.10-3.90
3	IRBB60	6.80±.07	2.12±.06	3.20
4	HKR47	6.62±.24	1.80±.04	3.90
5	G1-15	6.40±.04	1.72±.04	3.70
6	G3-11	6.11±.11	1.84±.03	3.30
7	G3-13	5.90±.05	1.90±.02	3.10
8	G5-13	6.22±.16	1.77±.01	3.50

Alkali spread value (ASV)

The indexing of Gelatinization temperature (GT) was done by alkali spread value (ASV) test. The parental genotypes HKR-47 and IRBB-60 scored 4 (intermediate gelatinization 70-74◦C) and 6 (low gelatinization 55-60◦C) in ASV test, respectively. The three three-gene pyramided BC_3F_3 genotypes G1-8, G1-12 and G1-13 having the maximum recovery of recurrent parent genome showed the ASV score of 4 corresponding to intermediate gelatinization temperature (**Table 4**).

Amylose content

Based on amylose content, rice is classified as waxy (1-2% amylose), very low amylose content (2-9% amylose), low amylose content (10-20% amylose), intermediate amylose content (20-25% amylose) and high amylose content (25-33% amylose). The amylose percent for the parental genotypes, IRBB-60 and HKR-47 was found 29.1% and

23.7%, respectively. The percent amylose for the positive BC_3F_3 genotypes varied from 24% to 35.5%. The three three-gene positive BC_3F_3 genotypes G1-8, G1-12 and G1-13 having the maximum recovery of recurrent parent genome were found to have amylose and amylopectin percentage close to that of recurrent parent HKR-47 *i.e.*, 24.1%, 24.9%, and 24.0%, and 74.5%, 73.09%, and 74.5%, respectively and amylose to amylopectin ratio was found to be 0.34 (**Table 5**).

Figure 1 BC_3F_3 genotypes and parental genotypes were scored based on alkali spread value (ASV) test

Table 4 Scoring based on alkali spread value (ASV) test

Genotype	ASV
IRBB-60	6
HKR-47	4
G1-8	4
G1-12	4
G1-13	4
G1-15	4
G2-3	2
G2-6	4
G3-11	6
G3-13	6
G5-13	2
G5-17	6

Table 5 Amylose%, Amylopectin% and Amylose: Amylopectin of the parental genotypes and three-gene positive BC_3F_3 genotypes (HKR-47 x IRBB-60)

Serial No.	Genotypes	Amylose%	Amylopectin%	Amylose: Amylopectin
1	IRBB-60	29.10±.01	70.80±.01	0.41±0.0002
2	HKR-47	23.70±.02	76.20±.02	0.31±0.0003
3	G1-8*	24.10±.02	74.50±.02	0.34±0.0004
4	G1-12*	24.90±.03	73.09±.03	0.34±0.0000
5	G1-13*	24.00±.02	74.50±.02	0.34±0.0002
6	G1-15	27.80±.03	72.10±.03	0.38±0.0008
7	G2-3	30.60±.01	69.30±.01	0.44±0.0001
8	G2-6	24.40±.06	73.50±.06	0.33±0.0060
9	G3-11	27.50±.06	72.40±.06	0.37±0.0002
10	G3-13	26.20±.14	70.70±.14	0.37±0.0003
11	G5-13	35.50±.01	64.40±.01	0.55±0.0004
12	G5-17	25.60±.08	74.30±.08	0.34±0.0006

* indicates three-gene positive genotypes with maximum recurrent parent genome recovery

Correlation coefficient analysis of the three-gene positive BC_3F_3 genotypes for grain quality traits

Correlation studies were made on data to find out association between grain quality traits (**Table 6**). Correlation coefficient analysis of the three-gene positive BC_3F_3 genotypes revealed a positive correlation (0.972, p=0.01) between grain length/breadth ratio and grain length. Grain length/breadth ratio showed a significant negative correlation with grain breadth (-0.375, p=0.01). Amylose percent exhibited a significant negative correlation (-0.594, p=0.01) with alkali spread value (ASV).

Table 6 Correlation coefficients analysis for grain quality traits of parental genotypes and three-gene positive BC_3F_3 genotypes

	Grain length	Grain breadth	L/B ratio	Amylose %	Amylopectin %	Ratio of amylose/ amylopectin	ASV
Grain length	1.000						
Grain breadth	0.415	1.000					
L/B ratio	0.972**	-0.375**	1.000				
Amylose %	-0.014	-0.396	-0.221	1.000			
Amylopectin %	0.288	-0.105	0.084	0.953**	1.000		
Ratio of amylose/ amylopectin	-0.038	-0.385	-0.210	0.957**	0.004	1.000	
ASV	0.059	-0.318	-0.142	-0.594**	0.969**	0.968**	1.000

Discussion

Seed length and seed breadth are the two important quantitative traits closely related to the exterior quality of the rice [16]. Length breadth ratio is the major genetic variation of rice grain shape and highly associated with the quantitative traits parameters and can be used in the breeding program for the improvement of the rice varieties [17].

Seeds harvested from positive plants in the BC_3F_3 generation were analyzed for physicochemical characters such as grain L/B ratio, alkali spread value (ASV) and amylose content.

Parental genotypes HKR-47 and IRBB-60 showed a grain length/breadth ratio of 3.90 and 3.20, respectively. The Length/breadth ratio of grain among positive BC_3F_3 genotypes ranged between 3.40 and 3.90. The maximum grain Length/breadth ratio of 3.90 was found in the genotype G1-8. The two most important parameters of rice quality are alkali spread value (ASV) and amylose content (AC). The amylose percentage for the positive genotypes ranged from 24.0% to 35.5%. The maximum recovery of recurrent parent genome found in three-gene positive genotypes G1-8, G1-12, and G1-13, have amylose percentage similar to that of the recurrent parent HKR-47 (23.7%), *i.e.*, 24.1%, 24.9%, and 24.0%, respectively.

Cooked rice becomes moist and sticky due to low AC. Amylose and amylopectin in kernels determine the texture of cooked rice, and consumers prefer rice with intermediate AC. Amylose is more resistant to gelatinization on cooking than amylopectin and reviewed as a vital factor in rice texture. Because of the structure (complexes formed with lipids) formed during heating, digestion is relatively slower as the enzymes become inaccessible [18].

All the positive genotypes except G2-3 and G5-13 (ASV-2, high gelatinization, 75-70◦C) showed an ASV of 4 or 6. The ASV for HKR-47 was 4 (intermediate gelatinization, 70-74◦C). The intermediate ASV indicated intermediate GT meaning medium disintegration, which is highly desirable for quality grain [19]. Our studies agree with the various related studies that stickiness is always negatively correlated with amylose content and hardiness, *i.e.*, high-amylose rice is usually harder and less sticky while low-amylose rice is softer and sticky [20-22].

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