Inheritance of A Few Quality Parameters of Rice Grain (*Oryza sativa* L.)

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**Abstract**

The experiment was carried out with a RIL (F7) population (100 lines out of 189 lines available) raised by crossing between Gobindabhog and Satabdi. They were grown in the Regional Research Sub-Station, Sekhampur, and Nadia. Out of eleven quality parameters, kernel breadth, ratio of length and breadth after cooking, elongation ratio, aroma and colour of kernel possess significant difference between two parents. Similar set of alleles for 100 SW is present in both parents as evidenced from the fact that 85% of RILs was within the narrow range of two parental values. Like Basmati, Kernel elongation ratio after cooking of Gobindabhog can be utilized as donor parent for improving this character in other rice too. Amylose contain (AC) in both the parental genotypes is 20-15% which may be called as intermediate category. However, instead of a similar major locus of chromosome 6 (amy6) for mylase present in both parents, RILs showed significant variation ranges from 17% to 22%. It is mainly due to contribution of favourable alleles of Gobindabhog present in another minor QTL, amy3, of chromosome 3. Betaine aldehyde dehydrogenase 2 (BAD2) gene is a major gene controlling aroma in Gobindabhog probably with one or two minor gene/s as evidenced from aroma analysis of RILs as well as their status of BAD2 gene. Among the hundred lines, RIL85 was selected as the best semidwarf high yielding line with higher kernel length, cooked kernel elongation ratio, low intermediate ASV, above 20% (by percentage of starch) amylose and aroma.

**Keywords**  
Rice, Inheritance, Quality parameters, Cooking quality, Grain characters.

**Introduction**

Rice is one of the most important cereal crops in the world, providing 21% of the food for the world population and up to 76% calorie intake for Southeast Asian. It has become a useful model crop, largely because it has a small genome size (400 mb) compared with other major crops. Not only for smaller genome size but also for the availability of high precision genome sequencing and saturated molecular markers, rice is the target crop for several map-based gene discoveries. It has been estimated that a 40% increase in rice production by 2030 will meet the demand of the predicted world population. Increase only in rice productivity will not be sufficient as consumers’ preference shifted towards specific quality parameters of rice. That’s why instead of availability of several high yielding rice varieties only a few of those are accepted by the farmers. Farmers accept only those which can fetch higher market price and meet consumers’ preference. So, now an ideal superior rice cultivar should have high grain-yield potential with improved grain quality, nutritional value, disease resistance and stress tolerance. Quality of rice depends on the

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**Introduction**

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consumers’ preference and it changes according to the region. Therefore, landraces which were adopted for a specific region by hundreds of years may be a source of alleles for quality parameters and can be utilized by the breeders for improvement of quality parameters specific for that niche. Although quality depends on the consumers’ preference but mainly grain shape and a few biochemical parameters are the major determinant. These two parameters primarily control the market value of rice grain. Over the past ten years, the development of DNA markers and genome sequencing technology have led to rapid development in the mapping and cloning of genes underlying grain quality parameters of rice. Four hundred QTLs have been assigned for explaining the variation in quality parameters of rice. Thirteen of them already cloned and their role in controlling quality parameters have been ascertained by reverse genetics approach. Not all of these genes or QTLs are responsible for controlling quality parameters of a particular rice variety. Rather, it depends on the genetic background of the specific variety and its growing environment. Thus, inheritance pattern of quality parameters is more complex than it was expected. In this study, a bi-parental RIL population (F7) comprising of one hundred heterogeneous lines and fifty rice genotypes were used for assessing the quality parameters when grown in RRSS, Sekhampur farm of our university. Two parents used for developing RIL population were famous for their quality parameters and they were Gobindabhog (an aromatic Bengal landrace) and Satabdi (released in WB, only based on consumer’s preference for its quality parameters). To understand the inheritance of quality parameters and role of some major QTLs controlling the parameters the present experiment was undertaken.

Materials and Methods

The experiment was carried out with a RIL (F7) population (100 lines out of 189 lines available) raised by crossing between Gobindabhog and Satabdi. They were grown in the Regional Research Sub-Station, Sekhampur, Nadia. The experimental soil was red laterite with good drainage facilities. The PH of soil was 5.8. Observations were recorded on different yield and quality parameters. Observations recorded from five individual plants of each lines (three replications of each line). Various primers used in the experiment were presented in table 1. Measurement of various quality parameters is given below.

**Cooked Kernel Elongation Ratio (CKE)**

It was measured by dividing the length of cooked rice kernel by the length of original (uncooked) kernel (Hussain et al., 1987).

**Procedure**

50ml graduated centrifuge tubes were taken and 15ml water (initial) added. And then 5gm rice sample was added, it was soaked for 10 mins. It was cooked in water bath for 20 mins. (for Gobindabhog 19 mins at 100°C). The cooked rice was put on blotting paper. 10 cooked grains (intact at both ends) were selected and measured the lengths of the kernel using mm paper.

\[
\text{Elongation Ratio} = \frac{\text{Kernel length after cooking}}{\text{Kernel length before cooking}}
\]

**Kernel Colour**

Kernel colour was observed visually.
Aroma

Aroma was detected by organoleptic panel test (IRRI, 1971). In the present study, aroma was detected from naked seed (brown rice).

Procedure

2gm of brown rice was taken in a test tube and then 10ml of distilled water was added. It was soaked for 10 min. The rice was boiled about 30-35 min. (till the rice properly cooked), with a cotton plug on the test tube. The test tubes were cooled by running tap water or putting the test tubes in beaker containing cold water. Aroma was detected by a panel of 3 judges.

Scoring: 0 = Absent, 1 = Mild, 2 = Medium, 3 = Strong

Amylose content

It was estimated as per (Sadasivam and Manickam, 2008). Weighed 100 mg of rice flour and added 1 ml of distilled ethanol. Then 10 ml of 1(N) NaOH was added and kept in a vigorously boiling water bath for 15 minutes. The volume was made up to 50 ml with distilled water. Then 5 ml of the extract in a 100 ml volumetric flask was taken and about 20 ml of distilled water is added. Two drops of phenolphthalein was also added. To neutralize it, 0.1N HCl was added drop by drop until the pink colour just disappeared. After then 1 ml of iodine reagent was added and make up the volume to 100 ml and read the colour at 590 nm after waiting 30 minutes. Diluted 1ml of iodine reagent to 100 ml with distilled water for a blank. The amount of amylose present in the sample was calculate using the standard graph.

Alkali spreading value

Duplicate sets of six whole milled kernels were selected without cracks and put them in plastic petridishes. Then 10 ml of 1.7% KOH was added. The kernels were spaced in such a way that enough space should present between kernels to allow for spreading. Kept the samples undisturbed at 27-300c for 24 hr. A standard variety was used as a check. The spreading and clearing of kernels noted on a 7 point scale (Table 2) was expressed as average of six values (Little et al., 1958).

Results and Discussion

The traits and parameters for measuring quality rice grain vary across country. Here a bi-parental recombinant inbred population (RIL) comprising of one hundred lines was considered for enumerating the nineteen parameters. Other than four yield attributing parameters (first four columns), fifteen parameters as shown in Appendix 1 are main determinant for assessing quality parameters of rice in India vis-a-vis West Bengal. RIL population used in this study was available at the department of Genetics and Plant breeding which was raised by crossing between Gobindabhog and Satabdi. Two cultivars used in this study are popular for their quality parameters. Gobindabhog is famous in Gangetic alluvial soil for its aroma, taste and texture whereas Satabdi, instead of less yield advantage it was released due to higher acceptance by the consumers, mainly for grain shape and texture.

Inheritance of quality and yield attributing parameters

Plant height

Mean plant height for Gobindabhog and Satabdi was observed to be 128.67 cm and 101.67 cm respectively. The most dwarf and tallest plant height among the RILs was recorded to be 83.33 cm and 134 cm for RIL-71 and RIL-53 respectively. Plant height below 110 cm was recorded for 53 RILs and
above 110 cm for 46 RILs and it is a good fit 1:1 ratio as per Chi-square test (p< 0.001). It is to mention that it had produced two discrete groups (Fig. 1). Height of any RILs was not in between 104cm to 109cm. Therefore, result is consistent with earlier observation that semi-dwarf trait of rice is controlled by a single gene, which is also known as SD1 (semi dwarf 1) gene. The SD1 gene has been mapped on chromosome 5 (Cho et al., 1994) functions as the α-subunit of GTP-binding protein (Fujisawa et al., 1999) which is insensitive to Gibberellins (GA), responsible for internode elongation. Although Plant height was neither contributing towards yield nor quality yet it was considered to check whether the population used for analysis was biased or not. As it had shown 1:1 segregation of semi dwarf and tall plant as expected for a monogenic trait, so, population used in analysis is a real unbiased population.

100 seeds weight

No significant difference of 100-seed weight was observed between Gobindabhog (2.15g) and Satabdi (2.0g). 100-seed weight of Gobindabhog, popularly known as khaschal which is readily available in Burdwan, Midnapore and other districts of West Bengal, is approximately 18-19g. Therefore, it is conclusively prove that Gobindabhog used in this study probably originated by field mutant. As grain shape is also longer than that of original Gobindabhog, it may be concluded that studied genotypes probably originated from the mutation of a gene which controlling both grain shape and 100 seed weight. It has been evidence by mapping analysis of earlier author that a major QTL for 100 seed weight (GW3) also located on the same place where a major locus for kernel shape GS3 is situated (Guo et al., 2009). Average 100 seeds weight of 100 RILs was recorded to be 2.21g. 100 seeds weight ranges from 1.23g to 3.57g among the RILs (Appendix table). 100 seed weight of more than 85% of RILs was within the range of two parental values. Approximately 10% of RILs showed higher 100 seed weight than that of heavier parent, Gobindabhog (Fig. 2). Based on frequency distribution pattern (Fig. 5), it can be concluded that same set of alleles for 100 SW is present in both parents.100 SW of rice to be controlled by several loci located on chromosome 1, 3, 6, 8 and 9. A locus present in chromosome 3 is same for both the parents (Fig-2). So, transgressive segregation may be due to allelic difference of one or two loci other than GS3 or GW3 located on chromosome 3.

Kernel Length after Cooking (KLAC)

No significant difference was observed for KLAC or KBAC between Gobindabhog and Satabdi. Average KLAC of 100 RILs was recorded to be 7.70 mm and ranges from 6.81 mm to 7.41 mm. Majority of RILs were within the range between Gobindabhog and Satabdi. Surprisingly, significant difference was observed for L/B ratio after cooking. Grain L/B ratio was higher in Satabdi but KL/KB after cooking was higher in Gobindabhog. So like Basmati type aromatic rice, Kernel elongation ratio (KLAC/LBAC) of Gobindabhog can be utilized as donor parent for improving this character in other rice too. To understand the possible chromosomal loci responsible for elongation ratio, two markers were considered for validation using two extreme groups of RILs. These two markers were GS3 derived markers of chromosome 3 and RM339 of chromosome 8 (Ramkumar et al., 2010). GS3 marker did not ampliy any polymorphic fragments between Gobindabhog and Satabdi. Thus GS3, a major determinant for kernel elongation is same for both the genotype. Probably, that’s why elongation in most of the RILs is within the range of their parental value (Fig. 3).
RM339 when used for genotyping the RILs of two extreme groups, it amplified Satabdi like alleles in four RILs with higher elongation ratio whereas reverse in RIL with lower elongation ratio (Table 3). So, locus near RM339 is not sufficient for explaining higher elongation ration in Gobindabhog (Fig. 4). Other loci present in chromosome 2, 6 and 11 (Tian et al., 2005) which were not included in this study may be responsible for higher elongation in Gobindabhog. So, RM339 is not a suitable marker for MAS when Gobindabhog will be used as a parent even it show polymorphism with other recipient parent.

**Amylose Content (AC)**

Higher amylose containing cultivars (>25%) are prevalent in most of the land races (Chattopadhyay et al., 2008) of Bengal which correlates with dry, firm and separate grains of cooked rice, usually become hard after cooling. Intermediate amylose (20–25%) rice is soft but not sticky and generally prevalent in most semi-dwarf indica cultivars. Low amylose cultivars (15–20%) are tender, cohesive, glossy and contain nearly all temperate japonica cultivars whereas very low (less than 10%) and waxy rice (<2%) grains are sticky. Here, both the parental genotypes are in the category of intermediate category. They contain above 20% of amylose. It is to note that percentage of amylose presented in table 4 was evaluated based on dry weight of rice flour not based on the total starch contain. As rice grain contain almost 90% or percentage of starch so percentage of amylose will be above 20% but not above 25%. However, instead of little variation exists between two parents, RILs showed wide variation ranges from 17% to 22%.

**Table.1** Primer sequences of used SSR and functional markers

| SSR Marker | Forward primer | Reverse primer | Chromosome No. | cM |
|------------|----------------|----------------|----------------|----|
| RM7        | TTCGCCATGAAGTCTCTCG | CCTCCCATCATTTCGTTT | 3              | 64 |
| RM190      | CTTTGTCTATCTCAAGACAC | TTGCAGATGTTTCTCTGTATG | 6              | 7.4 |
| RM253      | TCCCTCAAGAGTGCAAACC | GCATTGTCATGTCGAAAGCC | 6              | 37 |
| RM339      | GTAATCGATGCTGTGGGAAG | GAGTCATGATGACCCGATATG | 8              | 72.2 |
| EFP        | AGGCTAAACACATGCCCACATC | CCCCCATCATAGTTTGGTTT | 3              |    |
| ERP        | CCCCAGTGTCAGAAAATTTAAAAATGTGCTG | GATATGACCCGATATG | 8              | 72.2 |
| IRSP       | ACGCTGCTCCAGATGCTGA | CCCCCATCATAGTTTGGTTT | 3              |    |
| IFLP       | ACGCTGCTCCAGATGCTGA | GAGTCATGATGACCCGATATG | 8              | 72.2 |

**Table.2** A 7 point scale of spreading and clearing of kernels

| Clarification | Rating | GT |
|---------------|--------|----|
| 1-2           | Low    | High>740C |
| 3-4           | Low intermediate | High, intermediate (71-740C) |
| 5-6           | Intermediate | Intermediate (700C-740C) |
| 7             | High   | Low<700C |

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Table 3 Genotyping by RM339 and phenotyping of cooked kernel elongation ratio in two parents and members of two extreme RILs

| Genotype     | Kernel elongation ratio | Allele of RM339 |
|--------------|-------------------------|-----------------|
| RIL 7        | 1.3                     | S               |
| RIL 22       | 1.28                    | S               |
| RIL 28       | 1.25                    | S               |
| RIL 6        | 1.24                    | G               |
| RIL 13       | 1.23                    | S               |
| RIL 12       | 1.22                    | S               |
| Gobindabhog  | 1.17                    | G               |
| Satabdi      | 1.05                    | S               |
| RIL 49       | 0.98                    | S               |
| RIL 37       | 0.98                    | S               |
| RIL 59       | 1.03                    | S               |
| RIL 53       | 1.04                    | S               |
| RIL 30       | 1.04                    | S               |
| RIL 27       | 1.04                    | G               |
| RIL 29       | 1.05                    | S               |

Table 4 Genotyping by RM7 and phenotyping of amylose content in two parents and RILs

| GENOTYPE   | AMYLOSE CONTENT (%) | GENOTYPING (RM-7) |
|------------|----------------------|-------------------|
| RIL 100    | 21.94                | G                 |
| RIL 82     | 21.16                | S                 |
| RIL 88     | 21.16                | G                 |
| RIL 41     | 21.14                | G                 |
| RIL 74     | 21.14                | G                 |
| RIL 65     | 21.1                 | G                 |
| RIL 55     | 21.06                | G                 |
| Gobindabhog| 20.19                |                  |
| Satabdi    | 19.82                |                  |
| RIL 31     | 17.03                | G                 |
| RIL 1      | 17.07                | G                 |
| RIL 97     | 17.49                | S                 |
| RIL 80     | 17.68                | S                 |
| RIL 76     | 17.72                | G                 |

Table 5 Single point ANOVA analysis for amylose content

| Groups | Count | Sum  | Average | Variance  |
|--------|-------|------|---------|-----------|
| Column 1 | 7     | 144.57 | 20.65286 | 2.646557 |
| Column 2 | 5     | 91.12 | 18.224 | 2.76013  |

ANOVA

| Source of Variation | SS       | df | MS     | F        | P-value  | F crit     |
|---------------------|----------|----|--------|----------|----------|------------|
| Between Groups      | 17.20643 | 1  | 17.20643 | 6.391722 | 0.029964 | 4.964603   |
| Within Groups       | 26.91986 | 10 | 2.691986 |          |          |            |
| Total               | 44.12629 | 11 |         |          |          |            |
**Fig. 1** Frequency curve for plant height

![Graph showing frequency curve for plant height]

**Fig. 2** Frequency curve 100 seed weight

![Graph showing frequency curve for 100 seed weight]

**Fig. 3** Frequency curve for cooked kernel elongation ratio

![Graph showing frequency curve for cooked kernel elongation ratio]
**Fig. 4** Agarose gel picture showing different alleles of RM339 in two parents and members of two extreme RILs (G- Gobindabhog, S- Satabdi, No. shows RIL No.)

**Fig. 5** Frequency curve for amylose content

**Fig. 6** Agarose gel picture showing different alleles of RM7 in two parents and RILs (G- Gobindabhog, S- Satabdi, No. shows RIL No.)
Fig. 7 Frequency curve for alkali spreading value

![Frequency curve for alkali spreading value]

Fig. 8 Frequency curve for aroma

![Frequency curve for aroma]

Fig. 9 Agarose gel picture showing different alleles of multiplex marker (EAP, ESP, IFAP, INSP) in two parents and RILs. (G- Gobindabhog, S- Satabdi, (Parent blue colour) No. shows RIL No.)

![Agarose gel picture]
Variation is continuous and skewed towards low amylose content. So, more than one loci is influencing the amylose content. As it showed a large amount of transgressive segregants, so two parents probably contain diverse set of alleles or some epistatic interaction is controlling the amylose content. A major QTL for amylose content, amy6, which explain more than 70% of the total variation (Aluko et al., 2004; He et al., 1999; Tan et al., 1999; Lanceras et al., 2000; Septinsons et al., 2003) is located in between RM190 and RM253. This locus is also assigned as waxy (wx) locus of rice. Both the markers, RM190 and RM253 did not show any polymorphism between two parents. Probably that is one of the reasons that both parents contain approximately same amount of amylose.

So another marker, RM7, linked with the other locus, amy3, of chromosome 3 (He et al., 1999; and Lanceras et al., 2000) was considered and found polymorphism. This polymorphic SSR was considered for genotyping of two extreme group members comprising of twelve RILs. All seven higher amylose containing RILs amplified Satabdi allele except RIL31. As per single point ANOVA analysis, mean amylose contents of RILs with gobindabhog and Satabdi allele was 20.65% and 18.22% respectively. This difference in amylose contents between two groups due to presence or absence of RM7 alleles is statistically significant (Table 5). Therefore, it can be concluded that amy3 locus of Gobindabhog has significant positive contribution than that of Satabdi and RM7 can be used for marker assisted breeding for improvement of amylose in rice.

**Alkali Spreading Value (ASV)**

Alkali spreading value of whole kernel milled rice in contact with dilute alkali is a measure of gelatinization temperature and partly associated with amylose content of starch. Low intermediate alkali-spreadin score was predominant as 80 of the RIL which scored between 3-4. RIL-63, RIL-30, RIL-46, RIL-26, RIL-27, RIL-60, RIL-61, RIL-62, RIL-31 and RIL-29 have shown 5-6 ASV scale which indicates intermediate gelatinization temperature (700C-740C). It is evident from the figure 7 that a common major gene in both the parents is responsible for ASV with some modifier gene action since two major groups
were formed but a few transgressive segregants were also observed.

**Aroma**

Small grain aromatic rice that has long been preferred in eastern India now become popular in middle east, Australia, Europe. Among the several chemical constituents that are important for aroma of cooked rice, 2-acetyl 1-pyroline (AP) has been shown to be the most important component in small or large grain aromatic rice (Lorieux et al., 1996). It has now been established that single recessive gene (fgr) is responsible for aroma as determined by the level of AP. Accumulation of AP increases in rice due to loss of an enzyme betaine aldehyde dehydrogenase 2 (BAD2). AP is a substrate for the enzyme BAD2. Thus loss of function of BAD2 gene causes increase deposition of AP vis-à-vis aroma. In this study, four categories of RILs were observed as per degree of aroma those were 0,1,2,3 (Fig. 8). Two parents showed either zero or three. Therefore it can be concluded that more than one gene is responsible for aroma in Gobindabhog. Probably, in addition of BAD2 gene, one or two minor genes are also responsible. When, a multiplex marker considering the eight base pair deletion region of BAD2 gene was used for validation of a twenty random RILs it was clearly established the presence of a minor gene other than BAD2 gene. RIL19, 23, 25 did not contain the defective BAD2 gene (Fig. 9) but aroma was found in their grain although much less than that of Gobindabhog. Out of one hundred RILs, aroma was absent in 25 RILs, 49 RILs observed to have mild aroma, 23 RILs showed medium aroma and only two RILs showed strong aroma like Gobindabhog. It is also to prove that several lines with BAD2 gene did not show stronger aroma like Gobindabhog. So, BAD2 gene is a major gene controlling aroma in Gobindabhog probably with one or two minor gene/s.

**Kernel colour**

The outer bran layer of the grain and embryo (germ) is what gives rice its color and can vary from light yellow to red to dark purplish black. In this study, four categories of Kernel color appeared in RILs and they were white, light brown, brown and red, although colour for Gobindabhog and Satabdi was observed to be brown and light brown respectively. Two extreme and additional colors like white (in 15 RILs) and red (in 14 RILs) were appeared in RILs almost in equal ratio (Fig. 10). Therefore, more than three loci with epistatic gene actions are probably controlling the kernel colour of two parents included in this study.

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