Characterization of functional genes GS3 and GW2 and their effect on the grain size of various landraces of rice (Oryza sativa)

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Abstract

Background Grain size is an essential factor of grain quality and yield in rice. The genetic studies have substantially contributed to enhancing yield and maintaining a good quality of rice. The two major genes GS3 (a negative regulator of grain length) and GW2 (a negative regulator of grain width) with functional mutation play a significant role in controlling the grain size of rice.

Methods and Results In the study, 17 different widely grown Pakistani landraces of various genetic and geographic backgrounds were evaluated for grain phenotypic traits (1000-grain weight, length, width, and thickness) and also screened for genotypic mutation in GS3 and GW2 genes. Phenotypic data revealed the range for grain weight from 16.86 g (Lateefy) to 26.91 g (PS2), grain length ranged from 7.27 mm (JP-5) to 12.18 mm (PS2), grain width ranged from 2.01 mm (Lateefy) to 3.51 mm (JP5), and grain thickness ranged from 1.79 mm to 2.19. Correlation revealed a negative and significant correlation between grain width and length. There was no significant correlation between grain length and 1000-grain weight and grain width. LSD test displayed that the means of three variables grain length, grain width, and 1000-grain weight were statistically different from one another except grain width and grain breadth. Fifteen accessions carried the domesticated allele of GS3 while JP5 and Fakhr-e-Malakand carried the dominant allele. Similarly, fifteen accessions carried the dominant allele of GW2 while JP-5 and Fakhr-e-Malakand carried the mutant allele.

Conclusions The study shows that the mutant alleles of both genes are of significance to pyramid them in any breeding program. However, just incorporating favorable alleles is not the sole solution for improving the grain size. Therefore, further elucidation of GS3 and GW2 genes regulatory network, their interaction, trade-off, and pathways will better coordinate their marker-assisted selection in the future breeding program. Additionally, the study concluded that the selection of grain size was not dependent on 1000-grain weight in the selected germplasm.

Keywords Grain quality and yield · Genetic factors · Mutant alleles · Genotypic characterization

Introduction

Rice is used as essential staple food worldwide [1]. More than 90% of total rice is utilized and consumed in Asia, where 60% of the world population resides [2]. Rice is the staple food and second key cash crop in Pakistan, which produces a sufficient amount of basmati rice. Punjab and Sindh provinces are the major producers of rice in Pakistan. The fine long grain good quality Basmati is solely produced in Punjab which is famous for its tastes and aroma. Food Agriculture Organization 2019 placed Pakistan at ninth position in terms of rice production while International Trading Center ranked Pakistan the third largest exporter of rice in value as well as in volume, which accounted for 10% (4.59 million tons), worth $2.3 billion of the total world export of rice. What makes the demand for Pakistani rice is its fine grain quality including grain size, aroma, gelatinization temperature, and amylose content [3]. On the other hand, the yield of rice is predominantly represented by the 3 most important constituents, i.e.,
grain weight, grains number per panicle, and tillers quantity per plant [4]. The weight of grain is dependent upon grain size (grain length, grain width, and length to width ratio). Grain weight is frequently represented by 1000-grain weight that indirectly depends on grain length, width, and thickness as well [5]. Beyond the grain contribution to yield, the appropriate grain size of rice is also preferred among most consumers and in the worldwide market.

Rice grain quality comprises a few characters: cooking quality, aroma, grain appearance, milling, and dietary quality, etc. Among these, the appearance, eating, and cooking characteristics globally create critical commodity issues that adversely affect production. The appearance of the rice grain is chiefly indicated by the size of the grain as characterized by grain length (GL), grain width (GW) and the length vs. width ratio (LWR), and the whiteness of the endosperm [6]. However, the population is increasing day by day. On the other hand, various variables, such as water shortage, soil salinity, diseases, climate change, and the decreased fertile land zone, will increase inadequate food in the upcoming 50 years [6]. That is why grain size is also an essential agronomic attribute for artificial selection. Breeders will be likely to select plants with large seed estimates in rice breeding because grain size is firmly identified with the yield and superiority of rice [7]. However, it is hard for breeders to enhance grain size effectively by phenotypes since the characters of grain quality are quantitative [8, 9].

Several genes controlling grain size has been cloned including GS3, GW2, GW5/qSW5, GS5, GW8/OsSPL16 while other genes such as TGW6, Small Grain 1(SMG1), BG2, OsFBK12, SAMS1, OsPPKL1, and qGL3 has been identified [10, 11]. Among these genes, GS3 and GW2 are the major genes controlling the grain size of rice. GS3 is a negative regulator of grain size and contains an Organ Size Regulation (OSR) domain, and itself interacts as γ subunit with G-protein. The mutant allele of gs3 results in a large grain size. GS3 also enhance nitrogen use efficiency outcome in large grain size [12]. GW2 is also negatively regulating grain cell expansion by encoding RING-type E3 ubiquitin ligase [13]. It is recently reported that GW2 in interaction with other genes are involved in carbohydrate metabolism [14], while also playing a partial function with others genes in ubiquitination and de-ubiquitination [15]. The present study was carried out to characterize the functional alleles of the two major grain size regulating genes GS3 and GW2 and their effect on controlling the grain size in selected landraces.

Materials and methods

The current study was conducted at the Department of Botany, University of Science and Technology Bannu in 2018. For this purpose, 17 different widely grown landraces, i.e., JP-5, Bas-370, GA-5015, PS-2, Swat-1, Swat-2, DR-2, Fakhr-e-Malakand, Dilrosh, Malhar-346, Kashmir Basmati, Rachna Basmati, KS-282, KSK-133, KSK-434, MG-Basmati, and Lateefy in Pakistan were chosen among Pakistani rice Germplasm that showed highly significant variation for grain size, including grain length and breadth. The two accessions JP5 and Bas370 were used as control. To get fresh seeds, the seeds of each sample were grown in a greenhouse under a controlled environment as temperature-controlled at an optimal growth condition of 26 ± 0.2 °C 20.8 ± 0.4 °C (day/night) 12 h each, relative humidity was 67.2 ± 3.2% while the light intensity of 400 µmol PAR/m/s. Twenty days old seedlings were transplanted to pots each sample in three replications. Mature paddy seeds were collected from each accession.

Morphological characterization

Measurements of grain traits

Ten randomly and filled paddy rice was phenotyped for grain length, width, and thickness. Then the values of 10 seeds were averaged. For grain weight, 200 grains were weighted and converted to 1000-grain weight for simplicity of comparison with other studies [16].

Molecular characterization

DNA extraction was performed from young leaf tissue using a modified CTAB method [17]. The two primers GS3 and GW2 (Supplementary Table 1) were used to detect the existence of corresponding grain length and width genes on chromosomes 3 and 2, respectively. PCR was carried for each accession for GS3 and GW2. The respective amplicons of the GS3 and GW2 were digested with PSTI and APOI respectively. Polymorphic banding patterns of each amplicon of the corresponding gene were separated and examined with gel electrophoresis. Both the primers were scored (+) for the presence and (−) for the absence of respective genes.

Statistical analysis

Statistical analysis was carried out using the statistical package Statistics 8.1 for Pearson correlation. Later the data were subjected to Analysis of variance and LSD test to report whether the means of two variables were statistically significant or not.

Results

Morphological results

Morphological evaluation of selected germplasm revealed the highest 1000-grain weight of (26.74 g) for PS2, while the
lowest (20.06 g) were recorded for Rachna Basmati. Similarly, the highest grain length (12.18 mm) was recorded for PS2. Although JP-5 showed the shortest grain of (7.27 mm) but had the widest grain (3.51 mm) among the Germplasm. The narrowest grains of (2.21 mm) were recorded for Rachna Basmati. We also measured the grain thickness, and the JP-5 (2.19 mm) showed the highest value, while Rachna Basmati showed the lowest (1.90 mm) measure for grain thickness.

The narrowest grains of (2.21 mm) were recorded for Rachna Basmati. We also measured the grain thickness, and the JP-5 (2.19 mm) showed the highest value, while Rachna Basmati showed the lowest (1.90 mm) measure for grain thickness.

Grand mean of 22.87 g, 9.59 mm, 2.47 mm, and 1.94 mm were recorded for grain weight, respectively. The highest standard deviation was recorded for grain weight while the lowest was recorded for grain thickness. However, the highest coefficient of variation was recorded for grain width, while the lowest was recorded for grain thickness. Table 1.

Information based on an association of various traits is essential to gather them for yield and quality improvement in rice. To understand the nature and magnitude of the association between the four-grain traits, i.e., 1000-grain weight, grain length, grain width, and grain thickness were calculated in Table 2.

Pearson correlation revealed positive and highly significant associations between grain width and grain thickness followed by 1000-grain weight and grain thickness with significant correlation. However, the highest negative correlation was observed between grain length and width, which were statistically significant, followed by grain length and grain thickness, which were not statistically significant. The correlation between 1000-grain weight and grain length was not statistically significant. Further, the overall correlation revealed that the contribution of grain traits in the selected Germplasm to 1000-grain weight—one of the main constituents of yield, was in order TGW=GT>GW>GL (Table 2).

The LSD test which uses a t-test to compare the means, revealed, that the traits 1000-grain weight, grain length,

Table 1 Grain evaluation for 1000-grain weight, grain length, grain width, and grain thickness

| S. No. | Landraces         | TGW ± SD          | GL ± SD           | GW ± SD          | GT ± SD          |
|-------|------------------|------------------|------------------|------------------|------------------|
| 1     | JP-5             | 24.78 ± 1.165    | 7.27 ± 0.514     | 3.51 ± 0.052     | 2.19 ± 0.115     |
| 2     | Fakhr-e-Malakand  | 25.69 ± 0.582    | 8.06 ± 0.240     | 3.10 ± 0.116     | 2.01 ± 0.144     |
| 3     | GA-5015          | 25.44 ± 0.101    | 11.15 ± 0.906    | 2.30 ± 0.041     | 1.91 ± 0.076     |
| 4     | PS-2             | 26.74 ± 0.901    | 12.18 ± 0.119    | 2.44 ± 0.037     | 1.85 ± 0.062     |
| 5     | Swat-1           | 18.35 ± 0.205    | 7.99 ± 0.39      | 2.81 ± 0.383     | 1.93 ± 0.080     |
| 6     | Swat-2           | 25.42 ± 0.134    | 9.83 ± 0.257     | 2.45 ± 0.255     | 1.99 ± 0.055     |
| 7     | DR-2             | 24.67 ± 0.240    | 9.45 ± 0.172     | 2.49 ± 0.110     | 1.88 ± 0.096     |
| 8     | Dilrosh          | 24.91 ± 0.254    | 10.55 ± 0.026    | 2.45 ± 0.062     | 1.96 ± 0.052     |
| 9     | Malhar-346       | 19.75 ± 0.490    | 8.80 ± 0.196     | 2.22 ± 0.105     | 1.9 ± 0.0529     |
| 10    | Kashmir Basmati  | 20.13 ± 0.351    | 9.023 ± 0.492    | 2.21 ± 0.025     | 1.92 ± 0.055     |
| 11    | Rachna Basmati   | 20.06 ± 1.001    | 9.11 ± 0.262     | 2.21 ± 0.105     | 1.90 ± 0.023     |
| 12    | KS-282           | 26.00 ± 0.474    | 10.43 ± 0.438    | 2.44 ± 0.180     | 2.02 ± 0.1       |
| 13    | Basmati-370      | 19.02 ± 0.414    | 9.33 ± 0.170     | 2.17 ± 0.100     | 1.84 ± 0.020     |
| 14    | KSK-133          | 26.91 ± 0.472    | 9.82 ± 0.310     | 2.64 ± 0.111     | 2.09 ± 0.064     |
| 15    | KSK-434          | 23.70 ± 0.352    | 10.09 ± 0.21     | 2.37 ± 0.063     | 1.98 ± 0.081     |
| 16    | MG-Basmati       | 20.38 ± 0.335    | 10.78 ± 0.286    | 2.14 ± 0.104     | 1.79 ± 0.115     |
| 17    | Lateefy          | 16.86 ± 1.320    | 9.13 ± 0.340     | 2.01 ± 0.045     | 1.9 ± 0.04       |
|      | Grand mean ± SD  | 22.87 ± 3.32     | 9.59 ± 1.23      | 2.47 ± 0.37      | 1.94 ± 0.09      |
|      | CV               | 14.52            | 12.85            | 15.22            | 5.02             |
|      | MAX              | 26.91            | 12.18            | 3.51             | 2.19             |
|      | MIN              | 16.86            | 7.27             | 2.01             | 1.79             |

Table 2 Pearson correlation among the grain phenotype

|        | TGW | GL  | GW  | GT  |
|--------|-----|-----|-----|-----|
| GL     | 0.3818 |     |     |     |
| GW     | 0.4545 | -0.532** |     |     |
| GT     | 0.4908* | -0.4581 | 0.7748*** |     |

TGW thousand grain weight, GL grain length, GW grain width, GT grain thickness

LSD test revealed that the three traits mean compared were statistically different from one another except grain width and grain thickness at P=0.05 (Supplementary Table 2)

*Significance at 0.05 whereas, **significant at 0.01 levels, respectively

Thousands grain weight (TGW) (g); Grain length (GL) (mm); Grain width (GW) (mm); Grain thickness (GT) (mm)
SD standard deviation, CV coefficient of variance, MAX maximum, MIN minimum
and width were statistically different from one another. Pearson correlation also showed a negative correlation of grain length with grain width, and form (Fig. 1), it was also evident that profoundly meaningful Germplasm was selected to distinguish the individual effect of GS3 and GW2 on their respective traits.

**Molecular genotyping**

**GS3 C-A allele genotyping**

The extracted DNA was amplified for SF28 respective primer for GS3, which amplified ~ 140 bp fragment. After the amplification, the product was digested with a PSTI restriction enzyme. Those accessions which carried wild type C allele at splicing site CTGCAG for PstI which cleaved the wild type (JP-5 as positive control) PCR product into (~ 110 bp) and (~ 30 bp) fragment size while did not digest the domesticated allele carry C–A substitute i.e., CTGAAG (Fig. 2).

The accessions with a non-digested fragment of (~ 140 bp) (Bas-370 as negative control) fragments were represented with (+) for presence and those with digested fragments of (~ 110 bp) and (~ 30 bp) were represented with (−) for absence. Out of 17 accessions, Germlasm the two accessions JP-5 and Fakhr-e-Malakand carried dominant allele for while the remaining fifteen accessions i.e., GA-5015, PS-2, PS-2, Swat-1, Swat-2, DR-2, Dilrosh, Malhar-346, Kashmir Basmati, Rachna Basmati, KS-282, Basmati-370, KSK-133, KSK-434, MG-Basmati, and Lateefy carried the domesticated allele Table 3.

**GW2 allele genotyping**

The GW2 marker, when amplified, also revealed a monomeric fragment size of 51 bp for each accession. The amplified product digest with APO1 exhibited two different banding patterns, i.e., one a 51 bp, which did not carry the restriction site for APO1, and the other cleaved of 30 bp and 21 bp [18] as shown in (Fig. 3). The two accessions JP5 and Fakhr-e-Malakand carries the domesticated allele while Bas-370, GA-5015, PS-2, PS-2, Swat-1, Swat-2, DR-2, Dilrosh, Malhar-346, Kashmir Basmati, Rachna Basmati, KS-282, KSK-133, KSK-434, MG-Basmati, and Lateefy carried the dominant allele for GW2 as listed in Table 3.

**Discussion**

Grain size has a vital role in the domestication of cereal crops [19, 20], because grain size, besides its role in grain quality, indirectly regulates grain yield by increasing grain weight [21, 22]. Rice domestication at various geographical origins created diverse germlasm of variable grain size around the world. Grain size is a complex trait that shows various fundamental molecular and biological processes. So many genes and processes that occur in multiple regulatory
networks modulate grain size at various levels such as epigenetic changes, transcription regulation, translational modification, protein activities, metabolites accumulation, transportation and supply and cell division and cell differentiation [11]. The two genes \textit{GS3} and \textit{GW2} are the major genes that play a pivotal role in grain size regulation. Therefore, the current study was conducted to characterize the functional alleles of these two major grain sizes regulating genes and their effect on controlling the grain size in selected landraces of rice.

In the present study, the grain phenotypic characters for the accessions showed a negative but strong correlation between grain length and width. Similarly, [23] also reported a highly significant and negative correlation between grain length and width in 113 various Pakistani Germplasm of rice. However, in the Indian Germplasm, Ngangkham U and his correspondence also reported a negative but weak correlation between these two traits [24]. On the other hand, the correlation studies also revealed a highly significant correlation between grain length and 1000-grain weight [24]. Similarly, Bibi and his coworkers reported no correlation between grain length and 1000-grain weight [23]. Our results correspond with the latter study. Further, also reported that 1000-grain weight is strongly dependent upon grain width. reported no significant correlation between these two traits [23]. However, our results also revealed that these two traits were non-significant. These some contradictions in the relationship between the traits of grain size in various studies might be due to the variation in the selection of Germplasm, domesticated variably [24]. However, recent studies also concluded that traits and yield are ecotype-dependent [11, 25]. In Pakistan, frequently long-grain varieties are grown where people usually prefer long grain. The evidence suggested the phenomena, preference, and devotion of the plateau ancient conventional farmer to grain quality that domesticated long-grain rice independent of grain weight.

The present investigation also showed that both genes \textit{GS3} and \textit{GW2} were widely present in the selected genotypes and also in high correlation to the phenotype. We reported a high frequency of CTG\textsubscript{A} AG allele than CTG\textsubscript{C} AG allele for \textit{GS3} in long-grain rice. Many studies also reported the widespread of \textit{GS3} in the global collection of rice [19, 26]. Ngangkham and his colleagues also found 37.07% frequency of C–A mutation in 89 Indian origins Germplasm. In the present study, we even found the domesticated allele for a Swat-I, and Malhar-346 which have grain lengths of 7.99 mm, and 8.80 mm, respectively. Previous studies also reported that many genes are responsible for the regulation of grain length [24]. Additionally, Shao and his teammates also reported that the \textit{GS3} C–A allele activates another grain size regulator gene \textit{GS7}, while \textit{GS7} is ineffective in the presence of the C allele of \textit{GS3}. Besides \textit{GS3} importance for the presence in the current Germplasm, grain length is a quantitative trait [27]. Based on the germplasm in the present study and reported by others it reveals that C–A mutation has an ancient origin that played a vital role in diversity and cultivated rice domestication. On the other hand, \textit{GW2} is responsible for grain width and enhances

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
S. No. & Landraces & GS3 & GW2 & S. No. & Landraces & GS3 & GW2 \\
\hline
1 & JP-5 & + & – & 10 & Kashmir Basmati & – & + \\
2 & Fakhr-e-Malakand & + & – & 11 & Rachna Basmati & – & + \\
3 & GA-5015 & – & + & 12 & KS-282 & – & + \\
4 & PS-2 & – & + & 13 & Basmati-370 & – & + \\
5 & Swat-1 & – & + & 14 & KSK-133 & – & + \\
6 & Swat-2 & – & + & 15 & KSK-434 & – & + \\
7 & DR-2 & – & + & 16 & MG-Basmati & – & + \\
8 & Dilrosh & – & + & 17 & Lateefy & – & + \\
9 & Malhar-346 & – & + & & & & \\
\hline
\end{tabular}
\caption{List of accessions carrying \textit{GS3} and \textit{GW2}}
\end{table}
grain yield, grain filling, and grain weight in rice [13, 28–30]. Another study used STS marker (WOO4) for GW2 allele genotyping in 89 accessions and reported two alleles with a frequency of 73.03% and 29.94% respectively. However, they did not find any significant allelic variation for the grain width and recommended that the WOO4 marker should not be used further in any breeding program [30]. Ya-dong and his co-workers used the same primer as in the study and reported meaningful results [18]. Furthermore, they also testified the GW2 protein expression in Kasalath (of grain size 2.48 mm) having a dominant allele, and in TD70 carries the mutant allele (of grain size 4.42 mm). However, the present primer for GW2 also showed significant results for grain width in the current study [18].

Conclusions

Understanding the genes regulating grain size, along with its profound influence on phenotype is indeed important to increase grain size and to incorporate such genes in breeding programs. However just pyramiding favorable alleles is not just a sole solution for improving the grain size. The further elucidation of GS3 and GW2 genes regulatory network, their interaction, trade-off, and pathways will better coordinate their marker-assisted selection in the future breeding program. From the study, it is further concluded that the selection of grain size was not dependent on 1000-grain weight in the selected Germplasm.

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Declarations

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