DISTRIBUTION OF SEVEN GRAIN GENES AND EVALUATION OF THEIR GENETIC EFFECTS ON GRAIN TRAITS

YADONG ZHANG, QINGYONG ZHAO, CHUNFANG ZHAO, TAO HEN, ZHEN ZHU, LIHUI ZOU, SHU YAO, LING ZHAO AND CAILEN WANG*

Institute of Food Crops, Provincial Key Laboratory of Agrobiology, Jiangsu Academy of Agricultural Sciences, Jiangsu High Quality Rice R&D Center, Nanjing 210014, China Nanjing, Jiangsu, China.
*Corresponding author’s e-mail: clwang@jaas.ac.cn; Tel.:+86-25+84390307

Abstract

Grain size is one of the most important factors determining rice yield. Several major QTLs for grain size control have been molecularly characterized, and their roles in the regulation of grain size or weight have been explored but still remained obscure. In this study, we systematically examined the distributions of GW2, GS3, qSW5, qGL3, GS5, GW8, and TGW6 in 240 RIL of TD70 and Kasalath, compared the phenotypic differences between functional (additive) and non-functional alleles of the seven genes by monogenic lines and analyzed the interaction effect of gs3, gw2, and qgl3. The results showed that the 7 genes could be randomly combined, and an individual gene has its specific roles in grain length, grain width, and grain thickness, the seven functional genes regulating grain weight follows the order as qgl3 >gw2>gs3> GS5>qsw5> GW8>TGW6, and the combination effect among gs3, gw2, and qgl3 were revealed. These findings provide novel insight into grain size regulation in rice and are likely to be useful for marker assisted breeding in rice grain size.

Key words: Distribution, Genetic effect, QTL., Japonica.

Introduction

Rice is one of the most important cereal crops and a staple food in Asia (Rabbani et al., 2010). Grain weight is closely associated with rice yield. Grain shape, a typical complex quantitative trait, is a major determinant of grain weight and usually measured by grain length, grain width, and grain thickness. The identification of major QTLs for grain shape is an important objective of rice genetic research and breeding programs (Masoood et al., 2005; Bai et al., 2012; Huang et al., 2013). Several major QTLs for grain size, GS3 (Fan et al., 2006; Mao et al., 2010), GW2 (Song et al., 2007), qGL3(Hu et al., 2012; Qi et al., 2012; Zhang et al., 2012), GS5 (Li et al., 2011), GW3/qSW5 (Shomura et al., 2008; Weng et al., 2008), GW8 (Wang et al., 2012), TGW6 (Ishimaru et al., 2013), GW6a (Song et al., 2015), and GS6 (Sun et al., 2013) have been molecularly characterized by using various mapping populations. These genes were cloned from numerous varieties with different background, such as Zhenshan 97, Minghui 63, Nipponbare, 9311, WY3, HJX74, Basmati, and N411 (Zuo & Li, 2014). The role in the regulation of grain size or weight of one gene has been explored. As for GW2, a significant increase (+49.8%) in 1,000-grain weight in NIL (GW2) compared with the control of JAZ1 isogenic line was observed (Song et al., 2007). Filled grain of NIL-qgl3 showed 37.03% weight than those of 93-11 (Zhang et al., 2012). Additionally, seeds of over-expressed GS5 (NIL-ZS97) were 8.7% wider and 7.0% heavier than NIL-H94 (Li et al., 2011), while GW8 generated a 13.9% advantage for NIL-GW8 with respect to 1,000-grain weight (Wang et al., 2012). Comparing grain traits of independent homozygous transgenic lines of GS3 with Minghui 63, GS3 shows 23%-30 % reduction in 1,000-grain weight (Mao et al., 2010). All of these genes contributed significant variations to grain size, but it was difficult to directly sort effects of single gene under different genetic background. To precisely assess each gene’s function on grain size regulation and the potential interaction of these genes, we should characterize them under the same genetic background. Yan et al. (2011) studied the relationship between two grain size genes of rice, GS3 and GW2, via examining the gene expression based on GS3-RNAi and GW2-RNAi lines of rice variety Zhonghua 11, respectively. Lu et al. (2013) compared the rice (Oryza sativa) grain size among haplotypes of GW2, GS3, qSW5 and GS3 of in the genetic background of Zhanshan 97. These studies have advanced our understanding of grain size regulation in rice. However, it is much better if we can examine all grain size regulatory genes in a stable and identical or almost identical genetic background.

In this study, TD70, a large grain rice carrying with additive (functional) genes of GW2, GS3, qGL3, GS5, qSW5, and GW8 (Zhang et al., 2015), and Kasalath, a small grain Indica rice carrying with a functional gene of TGW6 (Ishimaru et al., 2013), and 240 recombinant inbred lines (RIL) deriving from these two parents at F5 and F10 generation were used to examine the distribution of seven genes and evaluate effect of seven genes on seed size and their interactions. Our findings have critical reference value for the understanding of grain size regulation and for molecular designed breeding in grain size of rice.

Materials and Methods

Plant materials and growth condition: Japonica variety TD70 (an large grain derived from Tian-e-gu//9520/ (72-496/Yu-nuo)), Indica variety Kasalath (a small size grain) and 240 recombinated inbred lines (RILs) developing from a these two parents at F5 and F10 generation population were used as research materials (Zhang et al., 2013). They were grown with two replicated plots in the experimental field of Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, China, during the natural growing season in 2012 and 2013, respectively. All plants were transplanted at the 30th days after sowing, and each line was planted into a plot of four rows with ten plants. The
planting density was 16.5 cm between plants in a row, and the rows were 26 cm apart. Field management, including irrigation, fertilizer application and pest control, was followed with the normal agricultural practice. Seeds were harvested immediately once they developed to maturation, then they were dried by wind for 30 days to maintain the water content of them around 14%.

**Phenotype characterization:** Length, width, thickness, and 1 000-grain weight of rice grain were investigated in this study. Ten full seeds of each single plant from parents and 240 lines were selected randomly and measured for length, width and thickness by vernier caliper (to 0.01 mm). The mean value of each trait from five plants was defined as its phenotypic value. Total weight of one hundred grains from each plant was measured and converted to its 1000-grain weight using electronic balance (to 1/10000 gram).

**Genotype determination:** Three derived cleaved amplified polymorphic sequences (dCAPS) markers for GW2, qGL3, and GW8, two derived cleaved amplified polymorphic sequences (CAPS) markers for GS3 and TGW6, a SSR marker for GS5 genes, and an Indel marker for qSW5(GW5) were used to identify the differences of the seven genes sequences among TD70, Kasalath and their RILs. Products generated by PCR or endonuclease digestion are listed in Supplemental Table 1. The method to identify functional and non-functional grain size gene was following the reported procedure (Zhang et al., 2015).

**Results**

**Distribution of grain genes in RILs:** TD70 was accumulated 6 additive functional genes of GW2, GS3, qGL3, GS5, qSW5, and GW8, and Kasalath was accumulated a functional gene of TGW6 in previous work (Zhang et al., 2015). We developed some molecular markers to identify these functional polymorphisms of seven genes and used these markers to test the distribution of seven grain genes in RILs. In theory, seven additive genes were classified eight kinds of combinations with the feature of numbers ranging from 0 to 7 of additive genes and assembled 128 kinds of combinations with the randomly separation of some functional genes, and the corresponding statistically segregation ratios were estimated to be 1: 7: 21: 35: 35: 21: 7: 1. Our results showed that the number of lines in these combinations is 4, 22, 69, 54, 53, 30, 5, 3 (Fig. 1A), which converts into the actual segregation ratio of 1: 7: 17: 24: 23: 15: 3: 1 (Fig. 1B), respectively. Compared with estimated segregation ratios, the types of combinations carrying with three and four additive genes missed more than other combinations, which had the number of 11 and 12, respectively. Both of them stand for more than 50% in total number of loss combinations. Loss of genotype and disparity in the number of different genotypic lines might result from the limitation of population size. However, the result had indicated that GW2_TD70 (gw2), GS3_TD70 (gs3), qGL3_TD70 (qgl3), GS5_TD70 (GS5), GW8_TD70 (GW8), qSW5_TD70 (qsw5), and TGW6_Kasalath (TGW6) were randomly separated and assembled in RILs.

![Fig. 1. Distribution of additive grain size genes, gw2, gs3, qgl3, GS5, qsw5, GW8, and TGW6, and their combinations in RIL.](image)

(A) The number of lines with zero to seven additive grain size gene, respectively. (B) The comparison of combination types number of lines with zero to seven additive grain size gene in theory and in practical in RIL, respectively. Zero to seven on the abscissa represents the number of additive grain size gene.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Marker type</th>
<th>Restriction endonuclease</th>
<th>Size of fragment</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>TD70</td>
<td>Kasalath</td>
</tr>
<tr>
<td>GS3</td>
<td>CAPs</td>
<td>PstI</td>
<td>168bp</td>
</tr>
<tr>
<td>qGL3</td>
<td>dCAPS</td>
<td>AccI</td>
<td>105bp</td>
</tr>
<tr>
<td>GW2</td>
<td>dCAPS</td>
<td>ApoI</td>
<td>180bp,21bp</td>
</tr>
<tr>
<td>GW8</td>
<td>dCAPS</td>
<td>SpeI</td>
<td>128bp</td>
</tr>
<tr>
<td>TGW6</td>
<td>CAPs</td>
<td>BssHII</td>
<td>589bp</td>
</tr>
<tr>
<td>GS5</td>
<td>SSR</td>
<td>-</td>
<td>216bp</td>
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<tr>
<td>GW5</td>
<td>Indel</td>
<td>-</td>
<td>759bp</td>
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**Supplemental table 1. Restriction endonuclease, marker type, and size of fragment used in this work.**
Phenotypes of monogenic lines: We obtained seven types of monogenic lines containing each functional gene and one type of lines without these functional additive genes in combinations with zero or one additive gene. In detail, the number of gw2, gs3, qgl3, GS5, qsw5, GW8, and TGW6 plants were 3, 2, 1, 1, 1, 7, 1, 6, respectively. Typical grain size of each gene is shown in Figure 2. Compared with control (CON), additive (functional) gs3 increases grain length, width and thickness of 1.36mm, 0.03mm and 0.2mm, and it increased 3.26g weight in 1000-grain weight in 2013. For gw2, it considerably increased grain width and thickness of 0.65mm and 0.28mm, which resulted in an enhanced 1000-grain weight of 10.27g. Furthermore, the average grain length of qgl3 monogenic lines was 10.47mm, which longer than the seeds of gs3 plants. At the meantime, it increased grain thickness by0.43mm, which immediately resulted in an increase of 1000-grain weight by 12.54g in 2013. It contributes more on grain length than gs3. GS5 was a gene mainly controlling grain width and increases grain width and length by 0.37mm and 0.35mm, respectively, resulting in an increase of 1000-grain weight by 2.26g. qsw5 only increased grain width by 0.1mm, but increases grain length by 0.37mm which increased the 1000-grain weight by 1.94g. The additive role of single GW8 and TGW6 was weaker than other genes in 1000 grain weight. GW8 increased grain weight by 1g and TGW6 reduced grain weight by 1g as to control line in 2013.

Every gene has its special function, resulting variations in the corresponding grain trait(s). The qgl3 and gs3 were major genes that increased grain length, and gw2, GS5 and qgl3 were major genes that increased grain width. All genes had slightly increases on grain thickness and significant differences on increasing 1000-grain weight (Fig. 3). The influence effect of gene on grain length was illustrated as qgl3 >gs3 > gw2>TGW6>qsw5>GS5>GW8. For grain width, the gene order was the following as gw2> GS5>qgl3 >qsw5>GW8>TGW6> gs3, and it was the order of qgl3 >gw2>gs3>GW8>GS5> qsw5>TGW6 for grain thickness. For the grain weight, we found that qgl3 and gw2 were the two major genes with most significant effects. The order of the seven genes for their effect on grain weight was followed as qgl3 >gw2>gs3>GS5>qsw5>GW8>TGW6. A similar trend could also be observed from the data in 2012 (Fig. 3).
Genetic interaction effects of gs3, gw2, and qgl3: Comparing genetic effects of monogenic, we found gs3, gw2, and qgl3 were major genes on improving grain size gene. To study the interaction among these three genes, 240 RIL were divided into eight groups, a control group carrying without anyone of the three functional genes, three groups carrying with one of the three additive genes, three groups carrying with two of the three genes and one group carrying with all the three additive genes. The number of lines in each group was listed in Figure 4. The mean value of grain length, grain width, and 1000-grain weight of each group was used for comparison. Substantial differences were observed among different groups (Fig. 5A-D). The 1000-grain weight effects of these three genes were in following order: qgl3 > gw2 > gs3. In addition, the three genes demonstrated additive effect in all the four tested traits. The more functional genes a plant carried, the higher grain weight, grain length, grain width, and grain thickness the plant generated. Further, the interaction effects of these three genes were also revealed. The RIL lines carrying with the qgl3 (gs3) and gw2 possessed higher weight than those of qgl3 and gs3 (Fig. 5D).

Fig. 4. The number of lines with the grain gene(s) of con, gs3, gw2, qgl3, qgl3 + gs3, qgl3 + gw2, 2 + gs3, and qgl3 + gw2 + gs3, respectively. Con stands for Control lines with no functional genes, gw2, gs3, gw2 represent that only one itself is a functional gene and the other two are non-functional genes. qgl3+gs3, qgl3 + gw2 and gw2+gs3 represent that only two genes themselves are two functional gene and another is non-functional genes. qgl3+gw2+gs3 represents that three genes themselves are functional genes assembled.

Discussion

Randomly separation of functional genes: TD70, a variety gathering six functional genes and a normal TGW6, and Kasalath, a small size grain and only having a positive gene TGW6 were two suitable materials to study the genetic effect of grain size genes (Zhang et al., 2015). In this study, we tested distribution of 7 grain genes in RILs, and found the randomly separation of these functional genes. Theoretically, seven functional genes could be assembled into 128 kinds of combinations, but only 91 kinds of combinations were identified by molecular markers. The combinations with three and four additive genes of seven genes missed more than other combinations, which stand for the total loss combinations number by 23. However, we had successfully identified that 22 lines were only accumulated with one single additive gene, 4 lines were found to accumulate without anyone of seven additive genes and 3 lines were assembled with all seven genes, which may explain the loss of genotype and disparity in the number of different genotypic lines may result from the limitation of population size. Seven genes were located on chromosome 2, 3, 5, 6, 8, respectively, and our work show that these genes did not have the characteristics of being closely linked genetically. Except for TD70, we obtained some stable materials with many grain size genes, which also indicated that donor materials of functional genes could be gathered into one genetic material. Therefore, seven genes of TD70 and Kasalath could be independently pyramided. This was a good example for pyramiding many grain genes.

Evaluating genetic effect of additive grain size genes by monogenic lines: The research on the genetic effect of grain size genes always limit on explaining the genetic effect between functional and non-functional genes. Seven known genes were cloned from various genetic materials and controls, so it was difficult to directly compare the difference effect of seven genes. During the process of evaluating the genetic effect of seven genes, the expected gene resources should come from common parents being accumulated by functional and non-functional genes, which could ensure the genetic effect not being interrupted by the haplotypes of genes. Only this could exactly evaluate the differences of genetic effect on functional and non-functional alleles of grain genes. Lots of agronomic mutants exist in natural, like dwarf genes D1 (Ashikari et al., 1999; Ishikawa et al., 1995), D2 (Hong et al., 2003) and D11 (Tanabe et al., 2005), and they can influence grain size in an indirect pathway, but the grain size of normal agronomic plants is mainly regulated by grain size genes. TD70 and Kasalath were two stable germplasm with normal agronomic traits, so their grain size should be completely dependent on their own genotype of grain gene. In this study, we selected some monogenic lines with one additive gene to analyze the genetic effect generated by the corresponding gene in their RILs. Though we all knew that some novel genes not being cloned in rice germplasm might cause some variations on grain traits, we still can preliminary evaluate the genetic effects of existing seven genes in a single genetic background, at least our results had counteracted the effects of known genes. It is a viable method to evaluate and compare the genetic effects of one gene by some monogenic lines.
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Fig. 5. Comparison of grain traits among eight combinations came from three grain size gene. gw2, gs3, gw2 represent that only one itself is a functional gene and the other two are non-functional genes. qgl3+gs3, qgl3+gw2 and gw2+gs3 represent that only two genes themselves are two functional gene and another is non-functional genes. qgl3+gw2+gs3 represents that three genes themselves are functional genes assembled. (A-D) mean comparison of grain length, grain width, grain thickness and 1,000-seed weight among gw2, gs3, gw2, qgl3+gs3, qgl3+gw2, gw2+gs3, qgl3+gw2+gs3 and control (con), respectively. Con represents that three genes themselves are non-functional genes.

A great amount of research progress is made for every gene, especially genetic effect of different haplotypes of GS3, qSW5, GW2, GS3, GS5 and TGW6 were been revealed, and all experiments had been tested that only functional polymorphic sites contributes more significantly to grain traits than other haplotypes (Dixit et al., 2013; Lu et al., 2013; Mao et al., 2010; Xu et al., 2015; Yan et al., 2011; Zhang et al., 2012). The additive alleles of GS3, qSW5/GW5, GS5 and GW8 for grain size are common in modern rice varieties, but the beneficial allele qGL3 was rare, which was similar to GW2 (Zuo & Li, 2014). We confirmed qgl3 and gw2 were two genes with the most significant effect, which are followed with gs3 and GS5. The order of the seven genes controlling grain weight was as follows qgl3>gw2>gs3>GS5>qsw5>GW8>TGW6 in RILs.

Comparing the effect of grain genes: Many genes associated with rice grain development have been cloned in recent years and will be cloned in the near future. However, the successful cloning of a locus was not the end of the quest for that genetic element, but the start of a new journey to determine how the gene works (Huang et al., 2013). Clarifying the genetic effect of GS3, GW2, GS3, qSW5 (GW5), qGL3, GW8, TGW6 in a single genetic background is the theoretical basis for the molecular design breeding for rice yield. Therefore, only on the basis of understanding genetic information about background material and owning genes sources of the same donor functional genes, we can accurately evaluate the effect of different genes.

The gene function change of gs3, gw2, and qgl3, is determined by a single base substitution or deletion in gene encoding region, which leads to different protein of three major genes. In this study, we used 240 RIL lines to analyze the genetic interaction pattern of the gs3, gw2 and qgl3. We found that the combination carrying the qgl3 (gs3) and gw2 possessed higher weight than those of qgl3 and gs3.

Guiding significance for rice breeding: The aim of rice grain size study was to improve grain related trait and apply them on breeding with high efficiency (Bai et al., 2012; Huang et al., 2013). Before the application on breeding, we need to have the knowledge on the effect of each grain size gene. We found that grain size genes in RIL could combine randomly, so we could pyramid different combinations of grain genes to obtain different grain weights. Cloned grain gene GS3 controlling grain length in most of Indica, has been proved to have relatively great breeding value (Takano-Kai et al., 2011; Takano-Kai et al., 2009; Wang et al., 2011). GW2 and qGL3 did not exist in varieties under natural selection, whereas they had stronger effect on increasing grain
weight (Ding et al., 2014; Zhang et al., 2014). We compared the combination effect of qgl3, gs3, and gw2, which will provide a guiding significance for rice breeding. Once the interactions among these genes are clearly identified, we believe that the ultimate goal of integrating multiple favorable genes in one rice variety will become possible.

Conclusions

To compare the phenotypic effect of the seven grain size genes qgl3, gw2, gs3, GS5, gw5 (qsw5), GW8, and TGW6, some monogenic lines were used to evaluate the genetic effect. The seven functional genes regulating grain weight followed the order as qgl3 > gw2 > gs3 > GS5 > qsw5 > GW8 > TGW6. Analyzing the combination effect of the gs3, gw2 and qgl3, we found that the single rare allele of gw2 and qgl3 had stronger effects than gs3 had on grain size, and the combination carrying the qgl3 (gs3) and gw2 possessed higher weight than those of qgl3 and gs3. Our results would provide a meaningful guide toward the molecular design for yield improvement.

Acknowledgments

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