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A large-effect QTL for grain weight in rice on chromosome 10

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Abstract

Grain yield of the rice can be enhanced through genetic manipulation of the yield components. Thousand grain weight is an important component which can be improved through conventional as well as molecular breeding approach. A major effect novel genomic region, TGW_{10}, for high grain weight was identified through a bulked segregant analysis approach in Azucena × IR64 population. Markers associated with grain weight in this region were RM25719 and RM5352. These markers were located on chromosome 10 at 19.8Mb and 21.1 Mb, respectively. This QTL explained phenotypic variance of more than 20%indicating this region to be a major effect locus. Markers linked with the QTL identified in present study should be used for enhancement in the grain yield potential of rice.

Keywords: Bulked segregant analysis; Grain weight; Grain yield; Quantitative trait loci; Rice.
Abbreviations: BSA_Bulked segregant analysis; QTL_Quantitative trait loci; RIL_Recombinant inbred lines; TGW_Thousand grain weight.

Introduction

Rice accounts for around 20% of the global calorie intake and is a staple food in South and Southeast Asia. The region shares more than 60% of the world's calorific intake (Fitzgerald et al., 2009; Miura et al., 2011). Rice production and productivity require a continuous increase for ensuring food security. The development and application of superior rice varieties is one of the most effective, sustainable, and economical approaches for improving rice yield (Rosegrant et al., 2003). An ideal superior rice cultivar should have high grain-yield potential with improved grain shape, nutritional value, disease resistance, and stress tolerance. Grain yield in rice is determined by three major components: number of panicles per plant, number of grains per panicle, and grain weight. Among these, the most important trait is grain weight, which is measured as the 1000-grain weight (*TGW*). Grain shape is characterized by the combination of grain length, grain width, grain length-to-width ratio, and grain thickness. These four parameters are positively correlated with grain weight (Tan et al., 2000). Most of these agronomically important traits are known to be genetically controlled by multiple genes (Tan et al., 2000) which are referred asquantitative trait loci (QTLs). Rice genetic improvement is currently being done by conventional and the molecular breeding approaches. Identification and deployment of the QTLs for *TGW* will improve the trait with minimum linkage drag in popular rice cultivar. A number of QTLs have been mapped for grain weight and grain shape on 12 chromosomes of rice but only GS_3, GW_2, and qSW_5 have been cloned till date. The GS_3 gene, which is located near the centromere of chromosome 3, was cloned by Fan et al. (2006). This gene is considered as a key gene for grain length because it existed in all long-grain varieties used by Fan et al., (2006). The GW_2 gene, controlling grain width, is located on chromosome 2 (Song et al., 2007).This gene encodes a protein with the function of ring-type E3 ubiquitin ligase. qSW_5gene, responsible for seed width, was mapped on chromosome 5 and cloned by Shomura et al. (2008). A 1.2 kb deletion was found in the coding sequence of qSW_5 (Shomura et al., 2008). Besides, QTLs $GW_{8.1}$ and $GW_{9.1}$ for grain width were fine-mapped in a 306.4 kb region on chromosome 8 and a 37.4 kb on chromosome 9 respectively (Xie et al., 2006; Xie et al., 2008). Two QTLs for grain weight, $GW_{1\text{-}1}$ and $GW_{1\text{-}2}$, have been mapped on chromosome 1 (Yu et al., 2008). Total number of fine-mapped QTL for grain weights are less than 10 in number (Huang et al., 2013). Majorly characterized QTLs are GW_3 and GW_6 on chromosomes 3 and 6 respectively (Guo et al. 2008). Other major QTLs characterized are on chromosome 8 ($GW_{8.1}$), chromosome 9 ($GW_{9.1}$), chromosome 11 (tgw_{11}) and chromosome 1 ($GW_{1\text{-}1}$, $GW_{1\text{-}2}$) (Xu et al., 2006; Xu et al., 2008; Oh et al., 2011; Yu et al., 2008). Not only the grain weight, but also other rice traits are quantitative in nature, governed by QTLs and show continuous genetic variation in natural populations and inbred lines. Characterization of QTLs is an

important step for utilizing them in rice breeding programs. The QTL mapping basically relies on detecting correlations between genetic markers and phenotypic traits in a segregating population (Weir et al., 1987; Tanksley et al., 1993; Falconer et al., 1996; Lynch et al., 1998). An efficient QTL mapping requires a large segregating population (bi-parental mapping population) such as an F_2 population or Recombinant Inbred Lines (or RILs).It is a well-known fact that maximum variation is observed in the F_2 generation followed by F_3.Chances of identifying major QTLs is more in these initial segregating generations compared to advance RILs.Bulk segregant analysis (BSA) has been proven to be an efficient method of detecting large effect QTLs for grain yields (Vikram et al., 2012). This approach involves genotyping of the pooled DNA samples of the phenotypic extremes to identify the linked markers. Further, linked markers are run on whole population to determine QTL effects (Vikram et al., 2012). Major QTLs can be detected reliably following BSA in early segregating generations. Present study aims to identify a large effect QTL for *TGW* using a F_2 derived F_3 population through BSA.

Results

Phenotypic variation in the population

Evaluation of the Azucena × IR64 population RILs for TGW at two environments (New Delhi, India: Environment-1 and Faizabad, India: Environment-2) revealed a normal distribution pattern (Fig 1).At environment-1 number of entries ranging 20 – 25, 26 – 30, 31 – 35, 36 – 40 and <2 grams were 3, 75, 148, 10 and 2 respectively. Similarly, at environment-2 number of entries ranging 20 – 25, 26 – 30, 31 – 35 and 36 – 40 grams were 4, 96, 125 and 13 respectively (Table 1).Mean TGW of Azucena × IR64 population at environment-1 and environment-2 were 31.19 and 30.60 grams respectively.Both parents differed significantly for the TGW and transgressive segregants having grain weight more than the high grain weight parent Azucena were also observed (Table 3).

BSA for grain weight analysis

Two markers showed association with grain weight in the BSA. These SSR markers were RM25719 and RM5352. RM25719 and RM5352 were located on chromosome 10 at 19.8Mb and 21.1 Mb respectively. These markers were polymorphic in parents as well as two bulks as revealed in Fig 2. The band pattern of bulks (bulk high and bulk low) for both markers was similar. Band of bulk high correspond to Azucena whereas the band of bulk low correspond to IR64 for both the markers. Further, polymorphic markers adjacent to RM25719 and RM5352 were run on whole population to define the QTL boundary.

Analysis of the QTL region identified through BSA

Markers linked withTGWas well as adjacent polymorphic markers were analyzed to determine QTL position and effect. Both linked markers identified in BSA were found to be significantly associated with grain weight. Two QTL peaks were identified in the analysis using two environment data. These two loci were named-$TGW_{10.1}$ and $TGW_{10.2}$.

Table 1. Distribution of genotypes in population for different grain weight ranges.

Grain weight range (g)	Environment-1	Environment-2
20-25	3	4
26-30	75	96
31-35	148	125
36-40	10	13
>40	2	0

Table 2. Phenotypic variation of the trait.

Source of variation	DF	Mean sum of square	F Ratio
Varieties	71	14.32	12.15**
Replication	2	3.37	2.86*
Error	142	1.17	1
Total	215	5.54	

Flanking marker of $TGW_{10.1}$ were RM5352 and RM5666. Flanking marker of $TGW_{10.2}$ were RM25719 and RM8202. The phenotypic variance explained by $TGW_{10.1}$ and $TGW_{10.2}$were 21.67 and 24.49.Additive effects contributed by $TGW_{10.1}$ and $TGW_{10.2}$ were 2.09 and 1.99. F-values confirming significance of QTL were 27.76 and 37.06 respectively for $TGW_{10.1}$ and $TGW_{10.2}$ (Table 4). These two loci shared common markers indicating towards a common region (Fig 3).

Discussion

There is a need to double the food production in worldto meet the growing food demands of human population.Increase in genetic yield potential of rice is merely 1%inspite of expected 2.4% to meet the global food demands (Rays et al., 2013). Significant efforts have been made in past to increase grain yield of rice and further increase requires focus on grain yield parameters. TGW is the most important grain parameter associated with grain yield potential of rice. Identification and characterization of major QTLs for grain weight in rice will contribute significantly towards enhancing genetic yield potential of rice. Our present analysis was performed using a RIL population developed with the cross of a japonica cultivar 'Azucena' known for high grain weight and well known indica rice variety 'IR64'. TGW was scored from experiments conducted at two different environments. Distribution of genotypes for TGW was more or less normal rendering population to a fit candidate for genetic analysis (Fig 1). Genotyping of the population was carried out through BSA method. Earliernumerous QTLs have been identified through this approach (Sandhu et al., 2014, Venuprasad et al., 2009). In this approach extreme/tail lines are used for analysis due to which smaller effect QTL regions are ignored which makes it cost effective. The cost effective genotyping strategy enables breeders to analyze multiple populations simultaneously to identify major effect genomic regions associated with trait of interests (Vikram et al., 2012). We have identified two different markers on chromosome 10 associated with TGW (Table 4, Fig 2). These markers were more than 1 Mb apart. These two loci may or may not belong to the same QTL region (Fig 3). Further analysis with population of recombinants will provide a clear picture of grain weight genomics associated with this region.

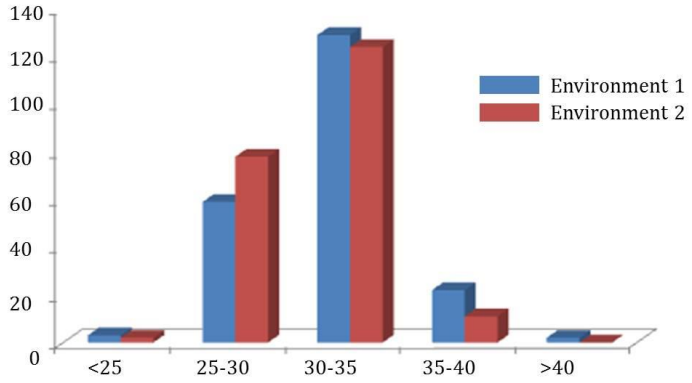

Fig 1. Distribution of population lines for 1000-grain weight. On 'Y' and 'X' axes number of genotypes and TGW were shown respectively. Figure also shows a normal distribution of population for TGW.

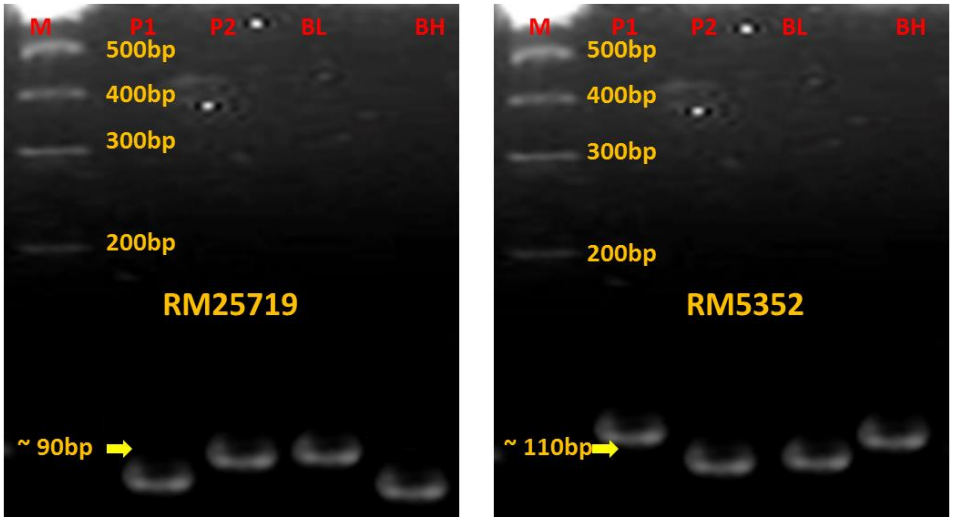

Fig 2. BSA results for grain weight in the Azucena × IR64 population. P1 = Azucena, P2 = IR64, BL = Bulk low & BH = Bulk high. BSA results with the two markers (RM25719 and RJ5352) depicted a pattern. Band pattern of P1 and P2 correspond BH and BL, respectively.

Similar to our findings two different genomic regions have been identified on chromosome 1 for grain weight (Yu et al., 2008). Similarly multiple QTLs were identified on same chromosome arms for grain weight and many other traits.As an example for grain yield under drought stress in rice sub-QTLs were reported with a large effect QTL region (Dixit et al., 2012). On chromosome 10, QTLs associated with grain weight have been reported on different arms (Ishimaru et al., 2003). The nearest marker to QTL in this study was R1629 which was positioned around 12.6 Mb. However the $TGW_{10\text{-}1}$ and $TGW_{10\text{-}2}$ markers which we identified in thestudy are more than 6Mb apart from R1629. The two QTL locus$TGW_{10\text{-}1}$ and$TGW_{10\text{-}2}$ explained phenotypic variance of 21.67 and 24.49 % respectively.Positive alleles were contributed by the high grain weight parent 'Azucena' ruling out any possibility of epistatic effect associated with these regions and strengthening the view that they are additive. For an additive trait like TGW, QTL explaining phenotypic variance of more than 10% will be good enough to be utilized in pyramiding. Further the identified QTL alleles were characterized in a set of 50 random varieties with an aim of identifying multiple alleles, if available. Two markers RM5352 and RM25719 were run on all of the 50 genotypes and revealed that only two alleles were prominent. These two alleles were observed in the population analysis too (data not presented). Fine-mapping of these two QTL regions through high density mapping in a recombinant population can provide more insights. From breeding point of view, identified QTLs- $TGW_{10\text{-}1}$ and $TGW_{10\text{-}2}$ if pyramided with other large effect TGW and/or grain yield QTLs will significantly increase genetic yield potential of rice. Markers linked with high TGW for effect of $TGW_{10\text{-}1}$ and $TGW_{10\text{-}2}$, are suitable for enhancing the rice genetic yield improvement through pyramiding approach.

Materials and Methods

Plant material

The $F_{2:3}$ RIL population was developed from a cross of a *japonica* cultivar 'Azucena'and an *indica* variety 'IR64'. F_1 seeds were selfed to get F_2 seeds. The F_2 plants were grown and

Table 3. Mean and range of grain weight in Azucena × IR64 population at two environments.

	Mean		Range	
	Environment-1	Environment-2	Environment-1	Environment-2
Population	31.19	30.6	23.11 - 41.35	22.62 - 37.84
Azucena	35.08	36.11	-	-
IR64	27.68	25.64	-	-

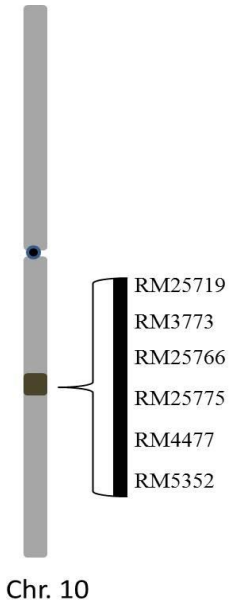

Fig 3. QTL region on chromosome 10 associated with the thousand grain weight. This region was located between RM25719 and RM5352.

harvested individually to obtain $F_{2:3}$ seeds. $F_{2:3}$ recombinant inbred lines (RILs) of both populations were phenotyped for TGW in kharif season 2012 (July 2012 – November 2012) at two environments, one at Indian Agricultural Research Institute, Pusa, New Delhi, India (Environment-1) and other at Narendra Dev University of Agriculture and Technology, Faizabad, India (Environment-2).

Phenotyping

A total of 215 $F_{2:3}$ were evaluated in randomized block design with two replications. Seeds were sown in a nursery and 21-day-old seedlings were transplanted. Single seedling per hill was planted at 20 × 20-cm spacing in 2.0-meter 3-row plots. Middle row was harvested to measure the 1000 grain weight. NPK fertilizers were applied at the rate of 90-30-30 kg ha^{-1}. Hand weeding was done after 25-30 days of transplantation to control weeds. Furadan (carbofuran 1 kg a.i. ha^{-1}) was applied after 5 days of transplantation to control stem borer and other insects. Irrigation was provided through maintaining a 5-cm water level until maturity.

Genotyping

Leaf samples from F_2 plants were collected and DNA was extracted using a modified CTAB protocol (Murray and Thompson 1980). PCR reaction was performed using a 15 µl reaction mixture containing 1 µl of 25 ng DNA template, 2 µl of 10X PCR buffer, 2 µl of 1mMdNTPs, 0.6 µl of $MgCl_2$, 1 µl each of 5 µM forward and reverse primer, 1 µl of5 U/µl Taq DNA polymerase, and 6.4 µl sterile H_2O. The PCR amplification profile included initial denaturation at 94^oC for 5 minutes; then 35 cycles of denaturation at 94^oC for 45 seconds, annealing at 58^oC for 45 seconds, and extension at 72^oC for 45 seconds; and final extension of 72^oC for 7 minutes and storage at 4^oC. PCR products were resolved in 4% metaphor agrose gel system (Sambrook et al., 1989).

Bulk segregant analysis

Parental polymorphism was carried with a total of 600 simple sequence repeat (SSRs) markers distributed among all 12 chromosomes of rice from available rice genetic maps (Tenmykh et al., 2001; McCouch et al., 2002; IRGSP, 2005). Bulks were prepared using phenotypic data of the two experiments. DNA of each sample was isolated and two bulks (bulk high and bulk low) were prepared. Bulk high constituted the bulk of DNA of 10 highest grain weight lines. Equal amount of each sample was mixed to prepare the bulk so that there was equal representation of each sample in the bulk. Similarly bulk low was prepared. Further 140 polymorphic SSR markers distributed on all 12 chromosomes were run on four DNA samples- (1) Azucena (2) IR64 (3) Bulk high and (4) Bulk low. Two markers identified as polymorphic between high and low tails were run on the whole population. Two additional polymorphic markers were further run on the whole population for determination of the confidence interval.

Statistical and QTL analysis

Statistical analysis was performed using CROPSTAT software v7.2.3 (available at www.irri.org). To calculate entry means within a year, replications were taken as random while entries were taken as fixed. During estimation of the entry means across years, season effects were also taken as random. Single-marker regression analysis was carried out with QTL cartographer v 2.5. Marker orders were taken from published physical map of rice (IRGSP, 2005). An assumption of one million bases ~ 4 cM was made while estimating genetic distances (IRGSP, 2005). QTL analysis was carried out using QTL Network v2.1 software (Yang et al., 2008). Composite interval mapping was performed. A total of 1000 permutations were used to calculate the F-value and to control the genome-wide type I error. For QTL detection as well as QTL effects an experiment-wise significance of P = <0.01 was followed. The window size and walk speed were fixed as 1 and 0.1 cM, respectively for genome scan.

Conclusions

TGW is one of the most important grain yield related parameter which contributes to improve the grain yield potential of rice. A major effect genomic region associated with high grain weight was identified on chromosome 10. This region explained phenotypic variation up to 24%.QTLs identified in this region if pyramided with other major grain yield related QTLs should help in enhancing rice yield potential.

Table 4. QTLs identified in the Azucena × IR64 population for grain weight in rice.

Trait	Environment	QTL	Marker interval	Position interval (Mb)	R^2 (%)	*F-value*	Additive effect
GY	Environment 1	TGW_{10}	RM5352-RM4477	21.11 – 21.12	21.67	27.76	2.09
	Environment 2	TGW_{10}	RM25719-RM3773	19.82 – 19.7	24.49	36.06	1.99

Table 5. List of the foreground markers used for QTL characterization.

S.No.	Marker name	Chromosome	Position	Sequence of forward primer (5'-3')	Sequence of reverse primer (5'-3')
1	RM25719	10	19.82	ACCAGATCACATGAAGAGGA	ACCAGATCACATGAAGAGGA
2	RM3773	10	19.89	ACCAGATCACATGAAGAGGA	ACCAGATCACATGAAGAGGA
3	RM25766	10	20.54	ACCAGATCACATGAAGAGGA	ACCAGATCACATGAAGAGGA
4	RM25775	10	20.72	ACCAGATCACATGAAGAGGA	ACCAGATCACATGAAGAGGA
5	RM4477	10	21.11	ACCAGATCACATGAAGAGGA	ACCAGATCACATGAAGAGGA
6	RM5352	10	21.12	ACCAGATCACATGAAGAGGA	ACCAGATCACATGAAGAGGA

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