Evaluation of Traits Associated With Seed Characteristics in Arkansas Restorer Lines

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Abstract

Rice Grain dimension and weight are two critical factors for marketing and increasing yield capacity. Seed shape is measured by its length, width, thickness, and ratio of length-width. In this study, an experiment was conducted in a controlled condition from fall 2017 to 2020 to identify QTL and candidate genes associated with seed dimension and weight using a bi-parental population resulting from two University of Arkansas developed genotypes: a restorer line 367R and an advanced breeding line RU1501139, in Stuttgart, Arkansas. Five seed dimension traits, including seed length, seed width, seed thickness, seed length-width ratio, and 100-seeds weight, were obtained for QTL detection. The study detected a total of 17 QTL. Four QTL associated with seed length were identified, in which two were positioned on chr. 3, one on chr. 7, and one on chr. 11. Two QTL related to seed length-width ratio were detected on chr. 3 and 7. Whereas a total of three QTL were identified for seed thickness, one each on chr. 5, 6, and 8. Eight QTL associated with seed weight were found, of which four QTL were detected on chr. 12, two each on chr. 1 and 10, and one on chr. 3. Of 17 QTL, four QTL originated from RU1501139, while the origin of the other 13 QTL was 367R. Since multiple genes could control the yield and seed physical characteristics, the detected QTL can play a role in introducing superior parental lines for developing conventional and hybrid rice production.

Introduction

Rice (*Oryza sativa* L.) is one of the major crops for food and income resources for almost half of the world's population. With the rapid increase in the world's population, rice production must continuously increase as well. To satisfy the demand, an increase of 30% in rice production by 2050 is necessary (World Bank, 2013; Feng et al., 2014). In order to speed up the improvement of rice yield, yield components must be improved. In particular, the number of seeds per panicle, panicle number, and seed weight should be further studied (Weng et al., 2008 & Wang et al., 2015). Seed width (SDWD hereafter) is controlled by multiple genes and several quantitative trait loci (QTLs) and has a great impact on improving yield (Weng et al., 2008; Huang et al., 2013 & Wang et al., 2015). Additionally, increasing seed dimensions are key breeding objectives for higher yield. Other Seed dimension characteristics that affect the yield potential include seed length (SDLG hereafter), seed width (SDWD hereafter), seed thickness (SDTH hereafter), and seed length-width ratio (L/W hereafter) (Huang et al., 2013; Qiu et al., 2012 & Wang et al., 2015). Based on L/W, rice is classified into three market classes long-grain, medium-grain, and short-grain (Hardke et al., 2018 & Qiu et al., 2017). Seed length and SDWD and their ratio determine the kernel size where the ratio is between 3.0 or greater in long-grain rice and 2.0 to 3.0 in medium-grain and 2.0 or smaller for short-grain rice.

Researchers have reported several QTLs related to yield and seed sizes (Fan et al., 2006 & Wang et al., 2015). Che et al. (2015) conducted a QTL study on an F₂ population created by crossing two *indica* rice lines (RW11 x BobaiB) that were significantly distinct (about 37%) from each other in terms of their SDLG s. They identified a QTL identified on chr. 2 as GL2 from the backcross with RW11. The GL2 improved the seed dimension around 24% for SDLG, 16% for SDWD, and about 27% more in 1000 seed weight. Qiu et al. (2020) conducted a two-year (2015–2016) genetic mapping study to clarify the QTLs associated with seed dimension. Qiu et al. (2020) used 1016 accessions in five populations: *indica*, *japonica*, *aus*, *basmati*, and *admixture* from the 3K Rice Genome Project (accessions collected from China, India, Philippines, Bangladesh, Japan, and other Asian countries). Seventy QTL were identified for seed dimension (SDLG, SDWD, L/W) on all 12 chromosomes. Twenty-four QTLs were identified on chr.s 1–7 and 9–11 for L/W, and the phenotypic effect was between 1–30%. Twenty-one QTLs were identified on all chromosomes, excluding chr.s 10 and 12 for SDWD, and the phenotypic variation changed between 1 and 42%. They detected 25 QTLs for L/W on chr.s 1–8, 11, and 12 with between about 1 and 28% phenotypic variation (Qiu et al., 2020).

Eizenga et al. (2018) identified a total of 27 QTLs for yield-related traits. A recombinant inbred line (RIL) population was developed using two *tropical japonica* lines, 'Estrela' and 'NSFTV199.' F₁ seeds were advanced to F₇, producing a final population size of 256 RILs. Seed characteristics studied include SDLG, SDWD, L/W, and 100-Seed weight (SWT100 hereafter). The research detected seven QTLs, including a major QTL '*qHULGRLG3*', associated with grain length explaining around 40% of the phenotypic variation on chr.3. Six QTL for SWT100 were identified with the major QTL '*qHULGRWD5*', explaining 38% of the phenotypic variation. Eight QTL were detected for L/W, which were at the same locations as QTL '*qHULGRLG3*' and '*qHULGRWD5*', explaining 32.6% and 38.9% phenotypic variations, respectively. Six QTL were identified for SWT100. University of Arkansas (UA hereafter) Rice Research and Extension Center rice breeding program located in Arkansas, US, has developed a number of rice cultivars used in most US Southeast rice-growing regions, some of which are used by the public rice breeding institutions for developing new rice cultivars. However, the knowledge of seed dimension traits in UA's rice germplasm is limited. Therefore, this study focuses on identifying QTLs associated with seed characteristics that can be found in the rice breeding program in the State of Arkansas and other rice breeding programs in the US. Therefore, the objective of this study was to identify QTL associated with seed characteristics, including SDLG, SDWD, SDTH, L/W, and SWT100. Results of this study could contribute to the improvement of the genetic background of yield-related QTLs through the introduction of each QTL themselves for the advancement of rice's yield potential.

Materials And Methods

Plant Materials

A bi-parental population resulting from a cross between the restorer line '367R' and a non-restorer line 'RU1501139' was developed for this study. Restorer line 367R is medium-grain rice and was developed at the UA's Rice Research and Extension Center (RREC), Stuttgart by Yan et al. (2012). It is derived from Katy/IR30//IR140 (PI-458443)/Jasmine-85(PI-595927) crosses. Non-restorer line RU1501139 is a long-grain advanced line developed by the RREC long-grain rice breeding program. A total of 300 F_2 plants from this population were grown in three replications in a greenhouse using a completely randomized design (CRD) to evaluate traits associated with seed characteristics. The $F_{2:3}$ seeds were harvested and used for phenotypic evaluation. Each replication consisted of three plants. Three panicles for each plant were randomly collected in the greenhouse. The panicles were dried (15% moisture) and threshed in Stuttgart, Arkansas. In order to analyze the seed dimension, 30 seeds from each line were randomly selected, cleaned, and evaluated via Mettler Toledo® balance and Winseedle® Pro to measure the seed dimension's significance level. According to the JMP Pro 14 software (SAS Institute Inc., Cary, NC), an ANOVA analysis followed by Student's T-test had significant results regarding SDLG, SDWD, L/W, SDTH, and SWT100

Phenotyping

The $F_{2:3}$ seeds from the 367R x RU1501139 population were harvested in the greenhouse and measured for L/W, SDWD, SDTH, L/W, and SWT100 in the Spring of 2018 at RREC in Stuttgart, Arkansas. One hundred seeds from each $F_{2:3}$ line were measured to evaluate seed dimension and SWT100 via Winseedle® Pro and the Mettler Toledo® balance, respectively. Three replications were obtained for each $F_{2:3}$ line. Each replication consisted of 100 seeds. The mean for each $F_{2:3}$ line was calculated from average value of three replications in an Excel® file.. One-way ANOVA analysis followed by Student's T-test significance results of the seed dimension (SDLG, SDWD, L/W, SDTH, and SWT100) (Table 1). In addition, multivariate analysis was run to understand the correlations between traits using JMP Pro 14 software.

Table 1
ANOVA analysis of Seed Dimensions in parental lines $F_{2:3}$ population

Trait	Parents		F2:3				
	367R	RU1501139	μ^*	Range†	SD§	SE	F
SDLG	10.24	9.06	9.73	8.2-11.03	0.480	0.03	118.0720**
SDWD	2.56	2.5	2.61	2.1–3.23	0.185	0.01	3.1865*
L/W	4	3.62	3.75	3.0-4.45	2.890	0.018	42.0621**
SDTH	1.96	1.93	2.12	1.9–2.3	0.070	0.004	0.8935
SWT100	2.5	2.36	1.03	0.1–1.8	0.478	0.02	32.000*
* μ , Average is estimated from $F_{2:3}$ population resulting cross between 367R x RU1501139							
† Effects with P-values < 0.01 given * and effects with P-values < 0.001 given ** between two parental lines of 367R and RU1501139							
‡ Range is the lowest and highest value of the trait under evaluation from the $F_{2:3}$ population							
§SD representing standard deviation estimated variation within $F_{2:3}$ population							
¶ SE, standard error measured from $F_{2:3}$ population							

Genotyping

Tissue samples were collected from both parental lines; 367R, 396R, Newbonnet, RU1501139, RU1501047, and each F_2 plant from the populations of 367R x RU1501139 at the V5 growth stage (Counce et al., 2015) for genotyping via single nucleotide polymorphism (SNP) markers. The parental line samples and the F_2 plant population samples were sent to the Illumina sequencing CO., located in River Falls, Wisconsin, to be genotyped with an Infinium Rice 7K Chip (Morales et al., 2020). The F_1 plants were genotyped using 7,000 SNP Infinium markers. Then, the $F_{2:3}$ seeds from each single plant were harvested in three separate replications. The linkage map was created via inclusive composite interval mapping (ICI) software using genotypic data from F_2 and phenotypic data from $F_{2:3}$ seeds while making QTLs related to seed dimension. The ICI Mapping was used with the Kosambi function for linkage mapping, and SNP markers were ordered for linkage mapping. Identifying and detecting the QTLs, 2.5 LOD score, was considered a threshold level for a major QTL. The Oryzabase database was used to detect any co-localized QTLs. The distribution of the seed dimension and detected QTLs were analyzed using JMP Pro 14 software (Fig. 1). Oryzabase, a comprehensive rice data source, was used to identify candidate genes.

Results

Preliminary Study

The ANOVA study showed that there are significant differences between 367R and RU1501139 on SDLG, L/W (p-value < 0.001), and SWT100, SDWD (p-value < 0.05). However, there was no difference in SDTH between these two lines (Table 1).

Analysis of F_{2:3} Population

SEED LENGTH: The distribution of F_{2:3} for SDLG followed a normal distribution (Fig. 1). SDLG had a mean of 9.7 mm with a range from 8.2 to 11 mm. The trait had a standard deviation (SD) of 0.48 and a standard error (SE) of 0.03. These two values explained the significance of seed length with a p-value < 0.001 for the population (Table 1).

SEED WIDTH: The distribution of F_{2:3} for SDWD followed a normal distribution (Fig. 1). There was no difference in seed width with a mean of 2.5mm and ranges from 2.3 to 2.7mm. The seed width had a 0.15 SD and SE of 0.06. While the trait was not significant at a p-value of 0.001, it had significance with a p-value < 0.05 (Table 1).

SEED LENGTH-WIDTH RATIO: The distribution of F_{2:3} for L/W followed a normal distribution (Fig. 1). There was no difference for seed width; however, the seed length-width ratio had a significant difference with a mean of 3.74 mm and a range from 3 to 4.5 mm and an SD of 0.29 and an SE of 0.018. In addition, a significant difference between parents 367R and RU1501139 was expressed with a p-value < 0.001 for the population (Table 1).

Seed Thickness: The distribution of F_{2:3} for SDTH followed a normal distribution (Fig. 1). For SDTH, the mean of SDTH in the population was 2.11 mm and ranged from 1.7 to 2.11 mm. The SD for SDTH was 0.09, and SE was 0.012. The difference between parents 367R and RU1501139 was not significant with a value p-value > 0.05 (Table 1).

100-seed weight: The distribution showed the majority of the F_{2:3} lines ranged between 1.25 to 1.5gr (Fig. 1). For SWT100, the mean was 2.5gr, ranging from 2.3 to 2.7gr. The trait had a 0.15 SD and SE of 0.06 in the population. The difference between parents in the population expressed a p-value < 0.05 (Table 1).

Multivariate analysis showed that a positive significant correlation (p-value > 0.001) between SDLG and L/W ($r = 0.44$) and SDTH ($r = 0.23$), and SDWD ($r = 0.166$, p-value > 0.01). The results revealed that L/W has a strong negative correlation with SDWD ($r = 0.622$, p-value > 0.001) but positive with SWT100 (0.213, p-value > 0.01). The analysis showed that SDTH has a positive correlation with SWT100 (0.48, p-value > 0.001) (Fig. 2).

Genotypic study

A total of 17 major QTL were identified in the bi-parental population of 367R x RU1501139. For SDLG, four QTL were detected, including two QTL, *qSDLG 3 - 1* and *qSDLG 3 - 2* on chr. 3, and one QTL *qSDLG 7 - 1* *qSDLG 11 - 1* each on chr.s 7 and 11, respectively (Fig. 3). The detected QTL were linked to RU1501139, inferred increasing seed yield, and explained 5.1 to 8.4% of phenotypic variation (PVE) on the population (Table 2). No major QTL for SDWD was detected; however, 12 minor QTL were found, including eight minor QTL with ($2 < \text{LOD} < 3$): two QTL on chr. 2, and three QTL each on chr.s 7 and 10, respectively. Two major QTL, *qL/W3-1*, *qL/W7-1* were detected on chr.s 3 and 7 for L/W. These two QTL were co-localized with the QTL, *qSDLG - 2* and *qSDLG 7 - 1*, identified for SDLG. The detected QTLs were linked to RU1501139 and explained 5.5 to 11.1% of phenotypic variation (PVE) in the population. The *qL/W3-1* and *qL/W7-1* were co-localized with other detected QTL, *qSDLG 3 - 2*, and *qSDLG 7 - 1*, respectively (Table 3). Eight QTL were identified for SWT100, including two QTL on each of chr. 1, 2, 10, and 12. Seven of these QTLs were co-localized with previously reported QTLs, AQEI043, AQBA011, AQAP004, AQCI003, AQCS003, AQAE008, and AQF014, respectively (Table 3). Furthermore, all eight QTL originated from 367R. Three QTLs were identified on chr.s 5, 6, and 8 associated with SDTH. The QTL *qSDTH5-1* on chr. 5 co-localized with a previously reported QTL AQFU013 (Table 3) for seed thickness. The detected QTLs linked to the 367R had a range of 4.6 to 7.5% phenotypic variation.

Table 2
Correlation is a measure of the linear association between two traits

Trials*	SDLG	SDWD	L/W	SDTH	SWT100
SDLG	1	0.1663	0.441*	0.23838	0.27978
SDWD	0.1663	1	0.6222**	0.095	0.074
L/W	0.441*	0.6222**	1	-0.0038 ^{ns}	0.2134
SDTH	0.23838	0.095	0.0038 ^{ns}	1	0.48068
SWT100	0.2797	-0.074	0.2134	0.4806**	1
* SDLG, Seed Length, SDWD, Seed Width; SWT100, 100 Seed Weight; L/W, Seed Length/ Width ratio; SDTH, Seed Thickness					

Table 3
List of QTL detected and parental origin of the positive allele for major QTL

QTL*	Origin†	Chr.	Left Marker	Right Marker	Position (bp)	LOD	PVE(%)	Add.	Dom.
<i>qSDLG3-1</i>	367R	3	2624847	2641058	6.22×10 ⁶ –6.99×10 ⁶	3.4262	5.2782	0.0334	0.2151
<i>qSDLG3-2</i>	367R	3	id3014217	3417192	30.12×10 ⁶ –30.45×10 ⁶	3.8808	5.9111	0.1625	0.0063
<i>qSDLG7-1</i>	<i>RU1501139</i>	7	7818489	7869914	23.68×10 ⁶ – 25.52×10 ⁶	4.8256	8.3937	-0.199	0.0649
<i>qSDLG11-1</i>	<i>RU1501139</i>	11	11465340	c11p17119245	17.11×10 ⁶ – 17.23×10 ⁶	3.4371	5.148	0.1511	0.0813
<i>qL/W3-1</i>	367R	1	id3014217	3417192	30.12×10 ⁶ – 30.45 x10 ⁶	3.8508	5.4423	0.0958	0.0525
<i>qL/W7-1</i>	<i>RU1501139</i>	1	id7004041	SNP- 7.23491886.	23.08 x10 ⁶ – 23.49×10 ⁶	7.4073	11.0463	-0.145	0.0299
<i>qSWT100-1-1</i>	367R	1	255699	312212	8.29×10 ⁶ –10.34×10 ⁶	14.259	2.4703	0.0571	0.8289
<i>qSWT100-1-2</i>	367R	1	312212	id1007778	10.34×10 ⁶ – 10.80×10 ⁶	19.082	2.5001	0.4698	0.3372
<i>qSWT100-3-1</i>	367R	3	2650075	3399945	7.3×10 ⁶ – 29.75×10 ⁶	18.405	2.4567	-0.435	0.4044
<i>qSWT100-3-2</i>	367R	3	id3014217	3417192	30.12×10 ⁶ – 30.45×10 ⁶	4.0224	0.2401	0.1734	-0.002
<i>qSWT100-10-1</i>	367R	10	SNP- 10.8934622.	10348161	9.00×10 ⁶ –9.2×10 ⁶	14.595	2.4698	-0.011	-0.835
<i>qSW T100-10-2</i>	367R	10	10348161	SNP- 10.9220148.	9.2×10 ⁶ –9.3×10 ⁶	14.613	2.471	-0.015	-0.835
<i>qSWT100-12-1</i>	367R	12	12661368	SNP- 12.20165789.	15.9×10 ⁶ – 20.19×10 ⁶ –	13.833	2.4302	0.008	-0.821
<i>qSWT100-12-2</i>	367R	12	SNP- 12.20165789.	SNP- 12.21730645.	20.19×10 ⁶ – 21.76×10 ⁶	21.584	2.5226	-0.477	0.3241
<i>qSDTH5-1</i>	367R	1	5604007	5612073	22.29×10 ⁶ – 22.59×10 ⁶	4.3585	7.5423	0.0254	-0.009
<i>qSDTH6-1</i>	367R	1	6642523	6684382	21.53×10 ⁶ – 22.48×10 ⁶	2.7006	4.6149	0.022	-0.001
<i>qSDTH8-1</i>	<i>RU1501139</i>	1	8757429	8764880	18.74×10 ⁶ – 18.91×10 ⁶	3.4779	5.8963	-0.02	-0.016
* <i>qSDLG3</i> , QTL associated with seed length; <i>qL/W</i> , QTL associated with Seed Length/ Width ratio; <i>qSWT100</i> , QTL associated with 100 seed weight; <i>qSDTH</i> , QTL associated with seed thickness									
† Origin; Parental origin of the positive allele									

Table 4
List of Previously reported co-localized QTL

QTL*	Chr	Candidate Genes			Previously Reported QTL			Reference
		Name †	Triat	Position(bp)	Name	Triat	Position (bp)	
<i>qSDLG7-1</i>	7	<i>GL7</i>	Grain size	24,682,874... 24,682,428	AQE0012	seed length	24.08×10 ⁶ -25.99×10 ⁶	Redona et al., 1998
		<i>OsGASR9</i>	Gibberellin-regulated					
<i>qSDLG11-1</i>	11	<i>RBG1 - FLA</i>	Grain shape	17694857... 17696042	<i>GL11</i>	seed length	9.95×10 ⁶ -17.34×10 ⁶	Xing et al., 2001
			Grain shape	16,316,873... 163,213,29				
<i>qSWT100-1-1</i>	1				AQE1043	leaf senescence	10.07×10 ⁶ -10.07×10 ⁶	Jiang 2004
<i>qSWT100-3-1</i>	3				AQBA011	brown planthopper resistance	5.72 ×10 ⁶ -7.35 ×10 ⁶	Alam et al., 1998
					Pdw3-1	panicle weight	5.18 ×10 ⁶ -5.73 ×10 ⁶	He et al., 2000
<i>qSWT100-3-2</i>	3				AQCU183	heading date	30.31×10 ⁶ -30.31×10 ⁶	Xu et al., 2002
							29.92×10 ⁶ -30.31×10 ⁶	Mei et al., 2003
<i>qSWT100-10-1</i>	10	<i>HAP5L</i>	Grain shape	6,404,227... 6,405,891	AQCI003	Abiotic stress	7.69 ×10 ⁶ - 14.27×10 ⁶	Wissuwa et al., 1998
<i>qSWT100-10-2</i>	10				AQCS003	Abiotic stress	3.77×10 ⁶ - 12.68×10 ⁶	Sato et al., 2003
<i>qSWT100-12-1</i>	12				<i>qLS12-1</i>	flag-leaf length	11.91×10 ⁶ - 26.10×10 ⁶	Cui et al., 2002
<i>qSWT100-12-2</i>	12				AQCF014	Yield	15.12×10 ⁶ - 23.77×10 ⁶	Zuang et al., 2001
<i>qSDTH5-1</i>	1	<i>MR5</i>	Grain quality	22,671,210...27,342,124	AQFU013	Grain quality	22.67×10 ⁶ - 27.34×10 ⁶	Aluka et al., 2004
* <i>qSDLG3</i> , QTL associated with seed length; <i>qL/W</i> , QTL associated with Seed Length/ Width ratio; <i>qSWT100</i> , QTL associated with 100 seed weight; <i>qSDTH</i> , QTL associated with seed thickness								
† The list of genes and QTL and information regarding them can be found in the website Gramene at https://archive.gramene.org/								

Detection of Candidate Genes for Major QTL

A total of five candidate genes were identified via rice genomic annotation using the online rice database of Oryzabase (<https://shigen.nig.ac.jp/rice/oryzabase/>), including four for SDLG and two candidate genes for SWT100 (Table 3). Two candidate genes of the *GL-7* and *OsGASR9* are identified within a detected QTL *qSDLG 7 – 1* (2.3×10⁶..2.3×10⁶) associated with SDLG. *GL-7* is a previously reported gene regulating seed length by increasing the length and starch structure in the endosperm (Wang et al., 2015). *OsGASR9* is a gene associated with plant growth that can be detected in all parts of plant, specially panicle. The *OsGASR9* is associated with plant growth and development and increases SDLG and SWT100 by increasing the efficiency of gibberellic acid (Li et al., 2019). It is worth noting that *qSDLG 7 – 1* is co-localized with another detected QTL *qL/W7-1* associated with L/W.

Two candidate genes were identified on the detected QTL *qSDLG 11 – 1* (16.28 ×10⁶.. 17.69 ×10⁶) associated with SDLG on chr. 11, including Rice Big Seed-1 (*RBG1*) and Flower and Leaf Color Aberrant (*FLA*). The *RBG1* gene is responsible for seed development, abiotic stress tolerance, and the gene improves root development by enhancing the plant's auxin level (Lo et al., 2020). The *RBG1* is 948 bp, and its four allelic genes are located near the *RBG1* gene, 5 kb to M37341, ~ 27 kb to M37342, and M825941, 46 kb to M44256 (Lo et al., 2020). The *FLA*

gene is a ubiquitously expressed gene and a key factor for flower and chloroplast development. The *FLA* improves seed length and rice yield. The *FLA* is located between the marker *M11-3* and *S6* with 56 kb on the long arm of chr. 11 (Ma et al., 2019). One gene (*HAP5L*) is located within a detected QTL, *q*SWT100-10-1 (6.64×10^6 .. 9.26×10^6) associated with SWT100. The *HAP5L* is an endosperm-specific gene-regulating starch accumulation and protein concentration (Xiong et al., 2019). The accumulation of starch increases the width, but any decrease in *HAP5L* causes sharp reductions in seed weight (Xiong et al., 2019).

Discussion

In this study, we aimed to identify the genetic sources associated with seed characteristics in rice. The preliminary study on two genotypes (367R and RU1501139) determined significant differences between the two genotypes for four seed characteristics of SDLG, SDWD, L/W, and SWT100. Restorer line 367R is a medium-grain rice cultivar, while RU1501139 is a long-grain breeding line. The seed length-width ratio is an essential measurement for the classification of rice cultivars. The results showed a positive correlation between L/W and SDLG, But a negative correlation between L/W with SDWD. In addition, the data showed a positive correlation between SWT100 with SDLG. Although there was no significant correlation between SWT100 and SDWD, the data showed a weak negative correlation between these two traits. Furthermore, results revealed that there was a positive correlation between SDWD and SDTH. Therefore, it can be assumed that longer and thicker seeds are heavier than shorter and wider seeds.

Enhancing seed yield, milling, and eating quality of rice can be achieved by developing superior cultivars by incorporating several agronomic traits, such as seed dimension and seed weight. The majority of these traits are classified as quantitative traits and are controlled by several QTL located in different parts of the rice genome. Each QTL has a different impact on the phenotypic variation. A breeder considers only those QTL that have the more significant impact on the phenotypic variations in a breeding program. In this study, we identified 17 major QTL and several minor QTL associated with seed characteristics. Annotation analysis revealed that five detected QTL contain genes associated with seed characteristics, and 11 were co-localized with previously reported QTLs (Huang et al., 1997; Redona et al., 1998; Xing et al., 2001; Jiang 2004; Alam et al., 1998; Zhu et al., 2000; Xu et al., 2002; Mei et al., 2003; Wissuwa et al., 1998; Sato et al., 2003; Cui et al., 2002; Zuang et al., 2001; Aluka et al., 2004). It can be concluded that 1) the annotation analysis of the QTL validates our finding via previously reported genes/QTLs associated with traits, and 2) these QTLs can be incorporated into the genomes of new superior genotypes.

For example, one important detected QTL is *q*SDLG 7 – 1 on chr. 7 associated with SDLG. This QTL is co-localized with *q*L/W-1 associated with L/W. Further investigation identified two candidate genes, *GL7* and *OsGASR9*, in this genomic region. Another important detected QTL *q*SDLG 3 – 2 on chr. 3 is associated with SDLG and co-localized with *q*L/W3-1, associated with L/W.

On chr. 11, one QTL *q*SDLG 11 – 1 was detected for SDLG. Two candidate genes, *RBG1* and *FLA* were identified on chr. 11 for SDLG. The *RBG1* gene is associated with seed, root development, and stress tolerance by enhancing cell division and auxin levels; thus, it helps to improve root development and stress tolerance, which are essential factors for having a greater yield. (Lo et al., 2020). The second candidate gene, *FLA*, is a cell membrane protein that belongs to the Ubiquitin-specific proteases. The *FLA* is a common amino acid for eukaryotic cells. The *FLA* improves seed length and yield by regulating chloroplast and flower development (Ma et al., 2019). Thus, we can summarize that the QTLs *q*SDLG 7 – 1 and *q*SDLG 11 – 1 contain several candidate genes associated with seed length and significantly impact the phenotypic variations; thus, these two QTL can be integrated into a new generation of long-seed rice cultivars.

Although the ANOVA analysis showed the significance of SDWD in this population, no major QTL were identified on chr.s. However, a total of 12 minor QTL were detected with an LOD from 2 to 3 LOD scores. Therefore, it can be assumed that SDWD is controlled by several minor QTLs that, overall, significantly enhance SDWD.

The ANOVA analysis showed there was no difference between 367R and RU1501139 for the SDTH trait, but the genotypic analysis identified three major QTLs associated with the SDTH trait. Furthermore, the genotypic analysis showed that the two QTL of *q*SDTH5-1 and *q*SDTH6-1 originated from 367R, while *q*SDTH8-1 originated from RU1501139. Therefore, there is a biological significance between these two genotypes despite no statistical significance due to these detected QTLs.

Rice is one of the major crops in the world, with vast marketing all around the world. The rice breeders' goals are to address farmers' and consumers' expectations by improving seed yield and seed characteristics. Further study is needed to identify major genes associated with these characteristics and developing molecular markers via a multi-location/year study. The results of this study can be used for marker-assisted selection in breeding programs.

Abbreviations

QTL, quantitative trait locus; SDLG, Seed Length, SDWD, Seed Width; SWT100, 100 Seed Weight; L/W, Seed Length/ Width ratio; SDTH, Seed Thickness

Declarations

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare:

- 1) This material is the authors' own original work, which has not been previously published elsewhere.
- 2) The paper is not currently being considered for publication elsewhere.
- 3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
- 4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
- 5) The results are appropriately placed in the context of prior and existing research.
- 6) All sources used are properly disclosed (correct citation).
- 7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.
- 8) We have no conflicts of interest to disclose.
- 9) We did not conduct any harmful activities on humans or animals, nor used any blood product or microorganisms.

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References

1. Alam, S. and M. Cohen. 1998. Detection and analysis of QTLs for resistance to the brown planthopper, *Nilaparvata lugens*, in a doubled-haploid rice population. *Theoretical and Applied Genetics* 97: 1370-1379.
2. Aluko, G., C. Martinez, J. Tohme, C. Castano, C. Bergman and J. Oard. 2004. QTL mapping of grain quality traits from the interspecific cross *Oryza sativa* × *O. glaberrima*. *Theoretical and Applied Genetics* 109: 630-639.
3. Cui, K., Peng, S., Xing, Y., Xu, C., Yu, S., and Zhang, Q. 2002. Molecular dissection of seedling-vigor and associated physiological traits in rice. *Theoretical and Applied genetics*, 105(5), 745-753.
4. Che, R., Tong, H., Shi, B., Liu, Y., Fang, S., Liu, D., Xiao, Y., Liu, L., Wang, H., and Zhao, M. 2015. Control of seed size and rice yield by GL2-mediated brassinosteroid responses. *Nature plants*, 2(1), 1-8.
5. Counce, P. A., Siebenmorgen, T. J., and Ambardekar, A. A. (2015). Rice reproductive development stage thermal time and calendar day intervals for six US rice cultivars in the Grand Prairie, Arkansas, over 4 years. *Annals of Applied Biology*, 167(2), 262-276.
6. Eizenga, G.C., Jia, M.H., Jackson, A.K., Boykin, D.L., Ali, M.L., Shakiba, E., Tran, N.T., McCouch, S.R., and Edwards, J.D. 2019. Validation of Yield Component Traits Identified by Genome-Wide Association Mapping in a tropical japonica × tropical japonica Rice Biparental Mapping Population. *The Plant Genome*, 12(1), 1-18.
7. Fan, C., Xing, Y., and Mao, H. *et al.* 2006. *GS3*, a major QTL for seed length and weight and minor QTL for seed width and thickness in rice, encodes a putative transmembrane protein. *Theor Appl Genet* 112, 1164–1171. <https://doi.org/10.1007/s00122-006-0218-1>.
8. Feng, C., Rotich, F., and Correll, J.C. 2014. Screening for Rice Blast Resistance of Uniform Rice Regional Nursery Varieties. B.R. Wells Arkansas Rice Research Studies. Series: 626. Page: 50-55.
9. Hardke, J., K. Moldenhauer, and X. Sha. 2018. Rice Cultivars and Seed Production. In: J. Hardke and L. Goforth, editors, *Rice Production Handbook*. University of Arkansas Cooperative Extension Service Print Media Center, Little Rock, Arkansas. P. 21-28.
10. He, C., J. Zhu, J. Yan, M. Benmoussa and P. Wu. 2000. QTL mapping for developmental behaviour of panicle dry weight in rice. *Scientia Agricultura Sinica* 33: 24-32.
11. Huang, M., Jiang, L., Xia, B., Zou, Y., Jiang, P., and A, H. 2013. Yield gap analysis of super hybrid rice between two subtropical environments. *Australian Journal of Crop Science*. Series: 7(5). Page: 600-608. ISSN: 1835-2707.

12. Huang, J., and Rozelle, S. 1996. Technological change: Rediscovering the engine of productivity growth in China's rural economy. *Journal of Development Economics*, 49(2), 337-369.
13. Huang R, Jiang L, Zheng J, et al. Genetic bases of rice seed shape: so many genes, so little known. *Trends Plant Sci.* 2013;18(4):218-226. doi:10.1016/j.tplants.2012.11.001.
14. Jiang, G.-H., Y.-Q. He, C.-G. Xu, X.-H. Li and Q. Zhang. 2004. The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an indica by japonica cross. *Theoretical and Applied Genetics* 108: 688-698.
15. Li, X., Shi, S., Tao, Q., Tao, Y., Miao, J., Peng, X., Li, C., Yang, Z., Zhou, Y., Liang, G. 2019. OsGASR9 positively regulates seed size and yield in rice (*Oryza sativa*). *Plant Science*, 286, 17-27.
16. Lo, S.F., Cheng, M.L., Hsing, Y.I.C., Chen, Y.S., Lee, K.W., Hong, Y.F., Hsiao, Y., Hsiao, A.S., Chen, P.J., Wong, L.I., Chen, N.C., Reuzeau, C., Ho, T.H., and Yu, S.M. 2020. Rice Big Seed 1 promotes cell division to enhance organ development, stress tolerance and seed yield. *Plant Biotechnology Journal*. Volume: 18, Issue: 9 Page: 1969-1983.
17. Li, J., Xin, Y., and Yuan, L. 2009. Hybrid rice technology development: Ensuring China food security. 2020 Vision Initiative. Page: 1-21.
18. Ma, X., Zhang, J., Han, B., Tang, J., Cui, D., and Han, L. 2019. FLA, which encodes a homolog of UBP, is required for chlorophyll accumulation and development of lemma and palea in rice. *Plant cell reports*, 38(3), 321-331.
19. Mei, H.W., Luo, L.J., Ying, C.S., Wang, Y.P., Yu, X.Q., Guo, L.B., and Li, ZK (2003). Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two testcross populations. *Theoretical and Applied Genetics*, 107(1), 89-101.
20. McBride, W.D., Skoriansky, S.R., and Childs, N. 2018. *US Rice Production in the New Millennium: Changes in Structure, Practices, and Costs*, EIB-202, US Department of Agriculture, Economic Research Service, December 2018.
21. Morales KY, Singh N, Perez F.A, Ignacio J.C, Thapa R, Arbelaez J.D., Tabien, E.T., Famaso, A., Wang D.R., Septiningsih, E.M., Shi, Y., Kretschmar, T., McCouch, S.R., and Thomson, M.J. 2020. An improved 7K SNP array, the C7AIR, provides a wealth of validated SNP markers for rice breeding and genetics studies. *Plos ONE* 15(5): e0232479. <https://doi.org/10.1371/journal.pone.0232479>.
22. Oryzabase, Online Rice Database. <https://shigen.nig.ac.jp/rice/oryzabase/>
23. Redoña, E.D., Hipolito, L.R., Ocampo, T.D., & Sebastian, L.S. 1998. Molecular Polymorphism of Rice Cytoplasmic-Genetic Male-Sterile (CMS) Lines.
24. Sato, T., T. Ueda, Y. Fukuta, T. Kumagai and M. Yano. 2003. Mapping of quantitative trait loci associated with ultraviolet-B resistance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 107: 1003-1008.
25. Sato, H., Suzuki, Y., Sakai, M., and Imbe, T. 2002. Molecular characterization of Wx-mq, a novel mutant gene for low-amylose content in endosperm of rice (*Oryza sativa* L.). *Breeding Science*, 52(2), 131-135.
26. Qiu, X., Gong, R., Tan, Y., and Yu, S. 2012. Mapping and characterization of the major quantitative trait locus qSS7 associated with increased length and decreased width of rice seeds. *Theoretical and Applied Genetics*, 125(8), 1717-26. doi: <http://dx.doi.org/10.1007/s00122-012-1948-x>.
27. Qiu, X., Chen, K., Lv, W., Ou, X., Zhu, Y., Xing, D., Yang, L., Fan, F., Yang, J., Xu, J., Zheng, T., and Li, Z. 2017. Examining two sets of introgression lines reveals background-independent and stably expressed QTL that improve seed appearance quality in rice (*Oryza sativa* L.). *Theor Appl Genet* 130, 951–967. <https://doi.org/10.1007/s00122-017-2862-z>.
28. Qiu, X., Zhu, S., Hu, H., Wang, C., Lv, W., He, L., Xu, J., Li, Z., Xing, D., Yang, L.W., and Xu, J.L. 2020. Genome-wide association mapping for seed shape in rice accessions. *International Journal of Agriculture and Biology*, 23(3), 582-588.
29. Wan, X.Y., Wan, J.M., Jiang, L., Wang, J.K., Zhai, H.Q., Weng, J.F., Wang, H.L., Lei, C.L., Wang, J.L., Zhang, X., Cheng Z.J., and Guo, X.P. 2006. QTL analysis for rice seed length and fine mapping of an identified QTL with stable and major effects. *Theor Appl Genet* 112, 1258–1270. <https://doi.org/10.1007/s00122-006-0227-0>.
30. Wang, L., Lu, Q., Wen, X., and Lu, C. 2015. Enhanced sucrose loading improves rice yield by increasing seed size. *Plant Physiology*, 169(4), 2848-2862.
31. Weng, J., Gu, S., Wan, X., Gao, T., Guo, T., Su, N., Lei, C., Zhang, X., Cheng, Z., Guo, X., Wang, J., Jiang, L., Zhai, H., and Wan, J. 2008. Isolation and initial characterization of GW5, a major QTL associated with rice seed width and weight. *Cell Res* 18, 1199–1209. <https://doi.org/10.1038/cr.2008.307>.
32. Wissuwa, M., Yano, M., and Ae, N. 1998. Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 97(5-6), 777-783.
33. World Bank. 2013. Global food crisis response program. Retrieved from, World Bank, Washington, DC. <http://www.worldbank.org/en/results/2013/04/11/global-food-crisis-response-program-results-profile>.
34. Xiong, Y., Ren, Y., Li, W., Wu, F., Yang, W., Huang, X., and Yao, J. 2019. NF-YC12 is a key multi-functional regulator of accumulation of seed storage substances in rice. *Journal of experimental botany*, 70(15), 3765-3780.

35. Yan, Z.B., Deren, C.W., and Yan, W.G. 2012. Hybrid rice breeding. In: R.J. Norman and KAK. Moldenhauer (eds.). B.R. Wells Arkansas Rice Research Studies 2011. University of Arkansas Agricultural Experiment Station Research Series. 561:86-88. Fayetteville, AR.
36. Yong-Zhong, X., T. Yi-Fang, X. Cai-Guo, H. Jin-Ping and S. Xin-Li. 2001. Mapping quantitative trait loci for grain appearance traits of rice using a recombinant inbred line population. *Journal of Integrative Plant Biology* 43: 840.
37. Zhu, G. Y., Kinet, J. M., and Lutts, S. 2004. Characterization of rice (*Oryza sativa*) F3 populations selected for salt resistance. 2. Relationships between yield-related parameters and physiological properties. *Australian Journal of Experimental Agriculture*, 44(3), 333-342.
38. Zhuang, J., Fan, Y., Wu, J., Xia, Y., and Zheng, K. 2001. Importance of over-dominance as the genetic basis of heterosis in rice. *Science in China Series C: Life Sciences*, 44(3), 327-336.

Figure

Figures 2 and 3 are not available with this version.

Figures

Figure 1

Distribution of Seed Dimensions * SDLG, Seed Length, SDWD, Seed Width; L/W, Seed Length/ Width ratio; SDTH, Seed Thickness; SWT100, 100 Seed Weight