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RESEARCH ARTICLE

Mapping QTLs for yield component traits using overwintering cultivated rice

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Abstract. The present study conducted QTL (quantitative trait locus) mapping using F_2 and $F_{2:3}$ generations derived from a cross between an overwintering cultivated rice Nuodao89-1 and Shuhui527, to identify potential yield component related QTLs. A total of 37 QTLs were detected across all chromosome except chromosome 7, with LOD values ranging from 3.10 to 12.67. Three QTLs including *qTGW3.1*, *qTGW6.1* and *qNEG9.1* were repeatedly detected in both generations. *qTGW3.1* was from Nuodao89-1, a total of 46 functional genes involved in 229 gene ontology terms were identified within its locus. Six QTL clusters were founded and corresponding agronomic traits of those QTLs showed highly significant correlation. Three types of epistatic interaction including 47 epistatic QTL pairs for eight yield component traits were detected, but the epistatic QTLs was not so important in controlling the genetic expression of the yield-related Nuodao89-1 as the additive QTLs. Overall, this research provides a theoretical basis for the mining of yield-related genes from overwintering cultivated rice.

Keywords. overwintering cultivated rice; yield; linkage map; epistatic interactions.

Introduction

Rice is one of the most important food crops in the world (Sakamoto and Matsuoka 2008; Xiao *et al*. 1996), and their yield traits have been receiving increased attention. Since the application of the quantitative trait locus (QTL) analysis strategy, many genes related to rice yield have been mapped and cloned, including *MOC1* (Lu *et al*. 2009), *IPA1* (Li *et al*. 2003), *PROG1* (Jin *et al*. 2008), *D3* (Ishikawa *et al*. 2005), *GIF1* (Wang *et al*. 2008), *TGW6* (Ishimaru *et al*. 2013), *GW2* (Song *et al*. 2007), *GS3* (Mao *et al*. 2010) and *GS5* (Li *et al*. 2011). These genes are involved in various factors related to production. In rice breeding and production, continuous exploration and innovation of the use of novel rice resources is an indispensable part of the cultivation of new high-yield rice varieties, and is the driving force behind the transformation and upgradation of the rice breeding industry (Yan *et al*. 2019). Continuous isolation of novel QTLs/genes from different genetic resources is important for the comprehensive evaluation of the genetic mechanisms underlying yield traits and the application of such information allows improved cultivar breeding.

Overwintering cultivated rice can survive through the cold winter and germinate in spring in the following year. A rice farmer needs to sow seeds only once to harvest multiple times. This considerably reduces the workload of farmers and is of practical significance to reduce the negative impact of large-scale land abandonment that has resulted from the sharp decline in the number of rice farmers. Presently, most studies on overwintering cultivated rice have focussed on the QTL mapping of cold tolerance (Deng *et al*. 2018). However, studying the inheritance of yield traits in overwintering cultivated rice is equally important to allow the production of novel variants that are both high-yielding and cold-

Conceptualization, ZZW and CJ; methodology, CJ; software, TLY and LL; validation, ZZW; formal analysis, XJ; investigation, CJ; resources, TLY; data curation, LL; original draft preparation, CJ; writing review and editing, CJ; supervision, ZZW; project administration, ZZW.

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tolerant. Nuodao89-1 is a typical overwintering cultivated rice variant, which can survive cold winter owing to the dormancy of its axillary buds. These buds sprout and regenerate in the following spring. Notably, the yield of this regeneration is equivalent to that of the normal season (Zhao *et al*. 2012). In the present study, we conducted QTL analysis of yield component traits using the two populations, and the F_2 and $F_{2:3}$ populations were constructed using Nuodao89-1 and Shuhui527 as parent strains.

Materials and methods

Plant materials

In the present study two sets of populations were involved, including 201 F_2 plants and 201 $F_{2:3}$ lines derived from a cross between Nuodao89-1 and Shuhui 527. Nuodao89-1 can survive cold winter and germinate in the spring of the following year (Zhao *et al*. 2006). Shuhui 527 has high combining ability, excellent grain quality, strong resilience and resistance to rice blast, and high recovery rates for hybridization (Zhao *et al*. 2012).

Phenotypic evaluation

All materials were planted at the experimental station of Chongqing Normal University, Chongqing (N29°32, E106°320) in China. The parents, F_2 and $F_{2:3}$ populations were planted in the summer of 2017 and 2018, respectively, and three plants of the parents and three effective panicles of individuals of F_2 and $F_{2:3}$ populations were harvested randomly to evaluate the phenotype each year. The field management was similar to that under normal rice production conditions. A total of nine yield traits including panicle length (PL), number of panicles (NP), number of filled grains (NFG), number of empty grains (NEG), spikelet per panicle (SPP), grain-setting density (GSD), seed-setting rate (SSR), grain yield plant^{-1} (GYP) and thousand-grain weight (TGW) were measured in a single plant in the F_2 population and in three plants in the middle of rows for each $F_{2:3}$ line at maturity stage. Phenotypic evaluation was performed following the method described by Shen (1995). For the F_2 population and the $F_{2:3}$ lines, the single plant trait value and the mean trait values for three individuals in the middle of rows were used as input data to identify QTLs, respectively. Data analysis and processing were performed using Microsoft excel 2003 and R statistics software (R Core Team 2017).

Molecular markers

To evaluate the polymorphisms that developed between the parent, we used 548 pairs of SSR primers and 31 pairs of STS markers developed by Tian *et al*. (2010). These markers covered all the 12 rice chromosomes. Polymorphic primers uniformly distributed across the 12 rice chromosomes were used to genotype the F_2 individual. All primers were synthesized by Sangon Biotech (Shanghai, China).

Genomic DNA extraction and genotyping

Total genomic DNA was extracted from young quadrifoliate leaves of parental and F_2 generations using the cetyltrimethyl ammonium bromide (CTAB) method. A total volume of 20 μL reaction mixture composed of 10 μL 2×*Taq* MasterMix, 1 μL template DNA (50 ng μL^{-1}), 1 μL of each primer (10 μM), and 7 μL ddH_2O was applied for polymerase chain reaction (PCR). The amplification procedure was performed according to the manufacturer's instructions (Genstar). The PCR products were separated using an 8% nondenatured polyacrylamide gel, followed by silver staining. Marker analysis was performed using the method described by Suh *et al*. (2009).

Construction of linkage maps and QTL analysis

Genetic linkage map construction and QTL mapping analysis were performed using the QTL ICIM Mapping v4.1 software, and all primers were added based on their physical positions on the chromosome. Using the BIP function, additive QTLs were identified by inclusive composite interval mapping (ICIM) at a threshold value of LOD $\geq$ 3.0, and pairwise epistatic interactions between all markers were tested at a threshold value of LOD $\geq$ 6.0. The mean trait values for each line in both populations were used for QTL analysis.

Results

Evaluations of yield component traits

A highly significant difference in NFG, TGW, GYP and SPP ($P<0.01$), and a significant difference in NEG and GSD ($P<0.05$), between the two parents Nuodao89-1 and Shuhui527 were observed. Each trait showed a pattern of continuous distribution and exhibited broad distribution in each population. Transgressive segregations were observed for all of the traits over the two population, studied (table 1; figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/).

Traits correlations

Correlation coefficient analysis was performed using regression effect values from one trait when applied to the

Table 1. Trait performance among parents, F_2 and $F_{2:3}$ population.

Traits	Population	Mean±SE	CV (%)	Range	Nuodao89-1	Shuhui527
PL	F_2	28.78±3.92	13.63	16.50–39.00	24.40±0.89	22.50±1.15
	$F_{2:3}$	27.36±3.32	12.13	17.8–34.93		
NP	F_2	4.65±2.24	48.13	1.00–16.00	11.00±0.26	13.00±0.85
	$F_{2:3}$	10.46±3.09	29.53	2.00–19.00		
NFG	F_2	95.12±39.23	41.24	3.50–237.00	102.90±12.56	78.30±7.62**
	$F_{2:3}$	138.37±57.43	41.50	7.33–291.33		
NEG	F_2	96.06±49.36	51.38	57.70–187.00	20.60±1.12	13.70±1.60*
	$F_{2:3}$	44.62±32.38	72.57	5.67–301.67		
SSR	F_2	0.51±0.17	33.21	0.04–0.94	0.83±0.06	0.85±0.04
	$F_{2:3}$	0.75±0.15	20.14	0.02–0.95		
TGW	F_2	25.44±2.86	11.24	18.13–34.19	23.90±1.23	31.30±1.31**
	$F_{2:3}$	25.05±3.11	12.43	17.53–34.97		
SPP	F_2	191.19±62.05	32.46	57.70–339.00	123.50±12.3	92.00±6.71**
	$F_{2:3}$	182.84±61.93	33.87	51.33–365.00		
GSD	F_2	6.56±1.68	25.66	2.86–11.29	5.10±1.12	4.10±0.78*
	$F_{2:3}$	6.63±2.00	30.17	2.18–13.24		
GYP	F_2	15.11±7.70	66.49	1.90–38.63	22.05±2.05	28.30±1.90**
	$F_{2:3}$	25.05±17.65	48.96	0.19–43.19		

*, ** Indicate a significance level at $P<0.05$ and $P<0.01$, respectively; CV, coefficient of variation (%); SE, standard error of mean.

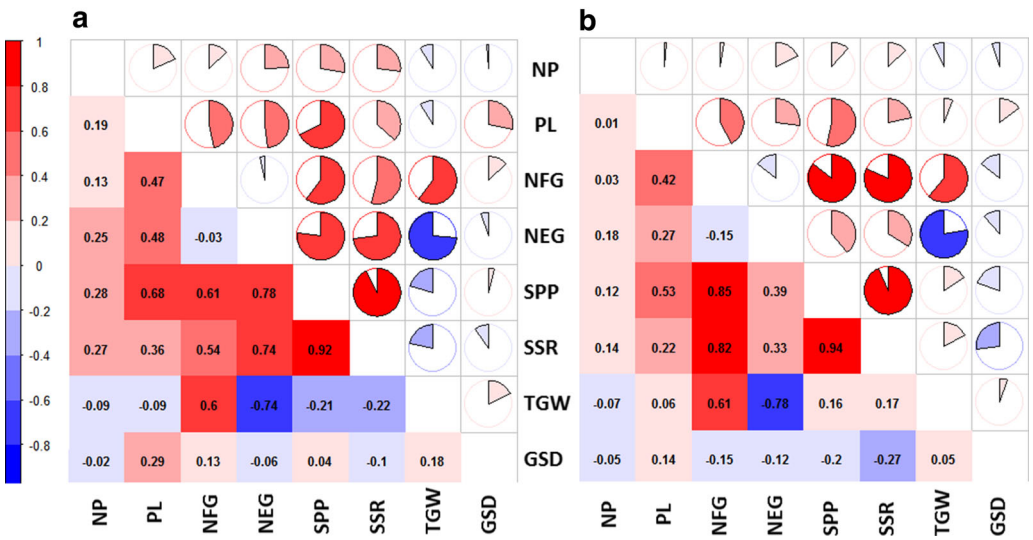

Figure 1. Correlation between yield component traits in the two populations. (a) F_2 generation; (b) $F_{2:3}$ generation. Red colour shows positive correlation and blue colour negative correlation ($P<0.05$).

others. Results showed that there is a significant correlation between most of the traits in these two populations (figure 1). Of the 36 possible correlations from each population, 26 (23 positive and three negative) were significant ($P<0.05$) in F_2, while 25 correlations (20 positive and five negative) were significant in $F_{2:3}$. Most of the correlations between the traits were similar in both populations. Panicle length was positively correlated with grain number per panicle and thousand-grain weight. The spikelet per panicle was positively correlated with seed-setting rate and grain-setting density. The GYP was significantly positive correlated with other seven traits except the number of empty grains. These results indicate the significance of other traits towards yield.

Genetic linkage maps

We used 579 markers (31 STS makers and 548 SSR markers) to determine the polymorphism between the two parent strains (Nuodao89-1 and Shuhui527). However, most of the markers were monomorphic. In total, 157 polymorphic

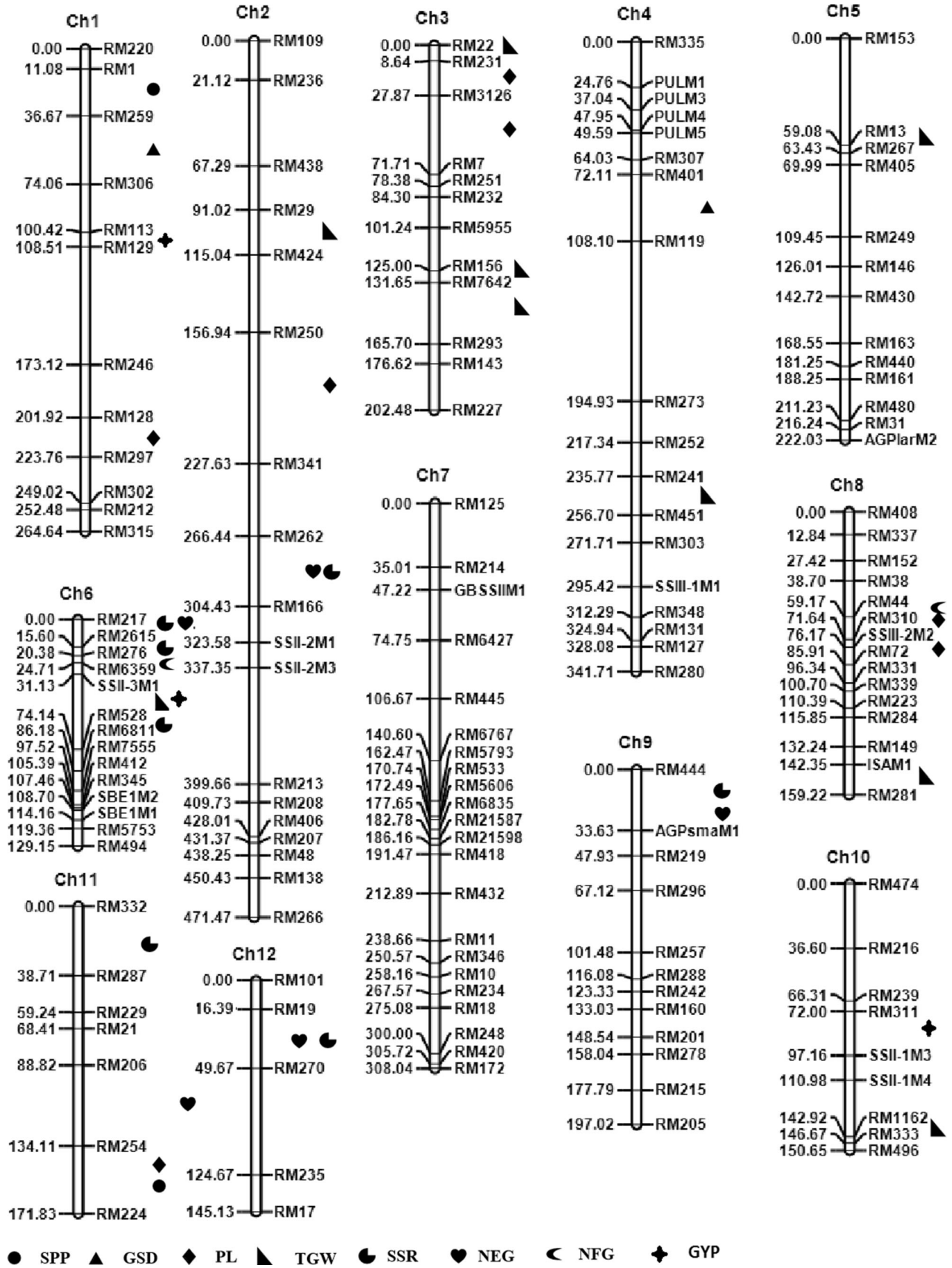

Figure 2. Linkage map constructed based on the F_2 population of Nuodao89-1× Shuhui527.

Table 2. QTLs for yield component traits in two generations.

				F_2			$F_{2:3}$		
Trait name	QTL	Chr.	Marker interval	LOD	PVE (%)	Add[a]	LOD	PVE (%)	Add [a]
NEG	*qNEG12.1*	12	RM19-RM270				6.75	26.60	84.12
	qNEG6.1	6	RM217-RM2615				3.41	18.40	− 2.85
	qNEG2.1	2	RM262-RM166				5.25	35.18	41.58
	qNEG9.1	9	RM444-AGPsmaM1	3.64	23.24	2.24	12.67	36.02	1.53
	qNEG6.2	6	RM6811-RM7555	3.78	12.66	− 2.33			
NFG	*qNFG6.1*	6	RM276-RM6359				5.15	12.76	− 10.44
	qNFG8.1	8	RM44-RM310	3.23	7.81	− 7.76			
PL	*qPL3.1*	3	RM231-RM3126				7.10	13.78	− 1.51
	qPL2.1	2	RM250-RM341				4.04	10.46	− 1.28
	qPL11.1	11	RM254-RM224	4.04	7.93	− 1.15			
	qPL1.1	1	RM297-RM302				3.10	5.24	0.84
	qPL8.1	8	RM310-SSIII-2M2	3.59	8.87	− 1.08			
	qPL3.2	3	RM3126-RM7	4.69	13.11	− 1.68			
	qPL8.2	8	SSIII-2M2-RM72				3.05	7.45	− 1.66
SPP	*qSPP1.1*	1	RM1-RM259				3.44	8.71	− 24.11
	qSPP11.1	11	RM254-RM224	3.46	7.56	− 13.83			
GSD	*qGSD1.1*	1	RM259-RM306				6.50	12.28	− 0.94
	qGSD4.1	4	RM401-RM119				3.41	8.92	− 0.79
SSR	*qSSR12.1*	12	RM19-RM270				3.87	21.25	− 0.35
	qSSR6.1	6	RM217-RM2615				11.06	43.16	0.02
	qSSR6.2	6	RM2615-RM276	3.67	9.24	0.01			
	qSSR2.1	2	RM262-RM166				5.11	26.68	− 0.14
	qSSR11.1	11	RM332-RM287				3.19	6.26	− 0.05
	qSSR9.1	9	RM444-AGPsmaM1				9.13	21.6	− 0.01
	qSSR6.3	6	RM528-RM6811	3.86	9.64	− 0.01			
TGW	*qTGW8.1*	8	ISAM1-RM281	3.53	7.31	0.01			
	qTGW10.1	10	RM1162-RM333				5.10	7.41	0.92
	qTGW5.1	5	RM13-RM267				3.18	4.83	0.87
	qTGW3.1	3	RM156-RM7642	9.21	18.59	1.54	6.63	12.10	1.53
	qTGW3.2	3	RM22-RM231				4.17	6.57	− 1.04
	qTGW4.1	4	RM241-RM451				3.88	6.65	− 0.88
	qTGW2.1	2	RM29-RM424	3.60	11.95	− 0.21			
	qTGW3.3	3	RM7642-RM293				4.63	11.24	− 1.41
	qTGW6.1	6	SSII-3M1-RM528	3.35	6.16	0.71	3.74	8.23	1.19
GYP	*qGYP1.1*	1	RM113-RM129	4.53	12.61	− 1.68			
	qGYP6.1	6	SSII-3M1-RM528	4.54	36.49	5.82			
	qGYP10.1	10	RM216-SSII-1M3	3.72	43.59	− 6.07			

[a]Positive values indicate that the positive alleles come from Nuodao89-1. Negative values indicate that the positive alleles come from Shuhui527.

markers (table 1 in electronic supplementary material) including 15 STS makers and 142 SSR markers were scored and used to construct the genetic linkage map (figure 2). Among these, chromosome 7 had the most markers (22) and chromosome 12 the least (5). The full map covered a genetic length of 2763.37 cM with a mean intermarker distance of 17.6 cM. The highest marker density was found on chromosome 8, with an average interval of 10.6 cM.

QTL analysis

ICIM mapping was employed to identify putative QTLs and revealed a total of 37 QTLs (table 2; figure 2) were identified. The 37 QTLs for yield component traits were distributed across all chromosomes, with the exception of chromosome 7, with LOD values ranging from 3.10 to 12.67 and an explained PVE ranging from 4.83% to 43.59%. Of these, 16 QTLs were identified in F_2 and 24 in $F_{2:3}$. Three QTLs including *qTGW3.1*, *qTGW6.1* and *qNEG9.1* were detected in both populations. The details of the QTLs are presented in table 2. Four QTLs (*qNEG12.1*, *qNEG6.1*, *qNEG2.1* and *qNEG6.2*) for number of empty grains mapped to chromosomes 2, 6 and 12, explained 2.55%, 1.76%, 3.37% and 2.29% of the phenotypic variation, respectively. Two SPP QTLs (*qSPP1.1* and *qSPP11.1*) with significant LOD values (3.44 and 3.46) were detected on chromosomes 1 and 11 in F_2 and $F_{2:3}$, respectively. ICIM-ADD detected five significant QTLs (*qSSR12.1*, *qSSR6.1*, *qSSR2.1*, *qSSR11.1* and *qSSR9.1*) with LOD values ranging from 3.19

to 11.06 for SSR on chromosomes 12, 6, 2, 11 and 9 in $F_{2:3}$, which explained the PVE range from 1.4% to 9.63%. Two SSR QTLs (*qSSR6.2* and *qSSR6.3*) with significant LOD values (3.67 and 3.86) were detected on chromosome 6 in F_2, these two QTLs had a PVE of 9.24% and 9.64%, respectively. A total of nine QTLs for thousand-grain weight were detected in both populations and mapped to chromosomes 1, 2, 3, 6 and 9, with LOD values ranging from 3.18 to 9.21, PVE ranged from 3.85% to 13.95%. Of these, *qTGW3.1* and *qTGW6.1* were identified in both populations with an average LOD value of 7.92 and 3.55, and accounted for an average PVE of 15.35% and 7.20%, respectively. The synergistic alleles, *qTGW3.1* and *qTGW6.1*, originated from Nuodao89-1. Two grain density QTLs were detected in $F_{2:3}$ and were mapped to the positive traits from Shuhui 527. Three QTLs (*qGYP1.1*, *qGYP6.1* and *qGYP10.1*) for grain yield per plant mapped to chromosomes 1, 6 and 10, explained 12.61%, 36.49% and 43.59% of the phenotypic variation, respectively.

Gene ontology annotation of the QTL- qTGW3.1

IM was conducted to confirm the validity of the three QTLs (*qNEG9.1*, *qTGW3.1* and *qTGW6.1*) that were repeatedly detected in both populations during ICIM mapping. The result showed that *qTGW3.1* and *qNEG9.1* could also be detected by IM (figure 3, a&b). Based on the linked marker information, *qTGW3.1* was located at 916.87 kb region between RM156 and RM7642 on chromosome 3. Gene ontology (GO) annotation was performed via the rice genome annotation project (http://rice.plantbiology.msu.edu) and revealed that this QTL locus contained 124 candidate genes. A total of 229 GO terms under 46 functional genes (table 2 in electronic supplementary material) were found and they were divided into three major GO types, namely, 'cellular component', 'biological process', and 'molecular function'. Within these categories, the four most represented GO terms were 'biological process', 'metabolic process', 'cellular process', and 'cellular component' (figure 3c).

Genomic regions of QTL clusters

Six regions with several significant QTLs for yield-related traits were detected on chromosome 2 (RM262-RM166), 6 (RM217-RM2615 and SSII-3M1-RM528), 9 (RM444-AGPsmaM1), 11 (RM254-RM224), 12 (RM19-RM270), and four of six genomic regions were detected in the $F_{2:3}$ population. The QTL (*qPL11.1* and *qSPP11.1*) genomic region was flanked repeatedly by RM254 and RM224 on chromosome 11 affecting panicle length and spikelet per panicle. Two QTLs including thousand-grain weight and grain yield per plant were detected at the region flanking SSII-3M1 and RM528 on chromosome 6. The other four regions contained a QTL for number of empty grains and a QTL for seed setting rate, respectively. These QTL clusters with large effects on yield traits lay good foundation for the future genetic mechanism elucidation.

Epistatic interactions

Epistasis is a phenomenon where the function of one gene is influenced by one or more other genes (Mo *et al*. 2018). In the present study, 47 epistatic interactions (table 3) were detected for yield component traits, distributed across all chromosomes. The proportions of PVE explained by these epistatic interactions ranged from 2.53% to 16.94% in F_2 and 1.07% to 18.54% in $F_{2:3}$. Of the 47 digenic interactions, three types of digenic interactions were identified: (i) four interactions between QTLs, including marker interval RM2615-RM276 for SSR on chromosome 6 and marker interval ISAM1-RM281 for TGW on chromosome 8. (ii) 14 interactions between QTLs and background loci including marker interval RM119-RM273 for TGW and background loci RM332-RM287 with LOD of 6.21 for SSR. (iii) 29 interactions between complementary loci, including marker interval RM153-RM13 on chromosome 5 with marker interval RM445-RM6767 or RM206-RM254. There were four digenic interactions with large effects (>10%) for GSD, SSR, TGW and PL. For PL, the interactions of loci on chromosomes 2 and 9 accounted for 18.54% PVE while for SSR, the interactions of loci on chromosomes 4 and 11 accounted for 16.94% PVE. But the contribution rate and effect values for most of the epistasis interactions were limited.

Discussion

QTL mapping analysis is a useful tool to identify genomic regions related to yield component traits (Jia *et al*. 2019). A total of 37 yield component trait QTLs were detected in this study. Among them, 22 had a contribution rate greater than 10%, and thus were classified as major QTLs. However, only three QTLs could be repeatedly detected in both populations, and the locations of the remaining QTLs in the F_2 population significantly differed in the $F_{2:3}$ population. This could be due to agronomical trait in QTL mapping population that were easily affected by multi environmental factors including water, temperature, gas, light and fertilizer (Liang *et al*. 2019). The synergistic genes of multiple QTLs were from Shuhui527, which were consistent with the results of multiple traits which are significantly better in Shuhui527.

We found 2060 records related to yield-related QTLs on the gramene website (http://www.gramene.org). Among the 37 QTLs for nine yield-related traits, 19 QTLs have been previously located and, in some cases, cloned. The region where *qPL1.1* is located contains cloned gene *FLR2*, which is involved in tillering of rice, and regulate the yield of rice (Li *et al*. 2016). The *OsMCA1* (Liu *et al*. 2015) and *PAD*

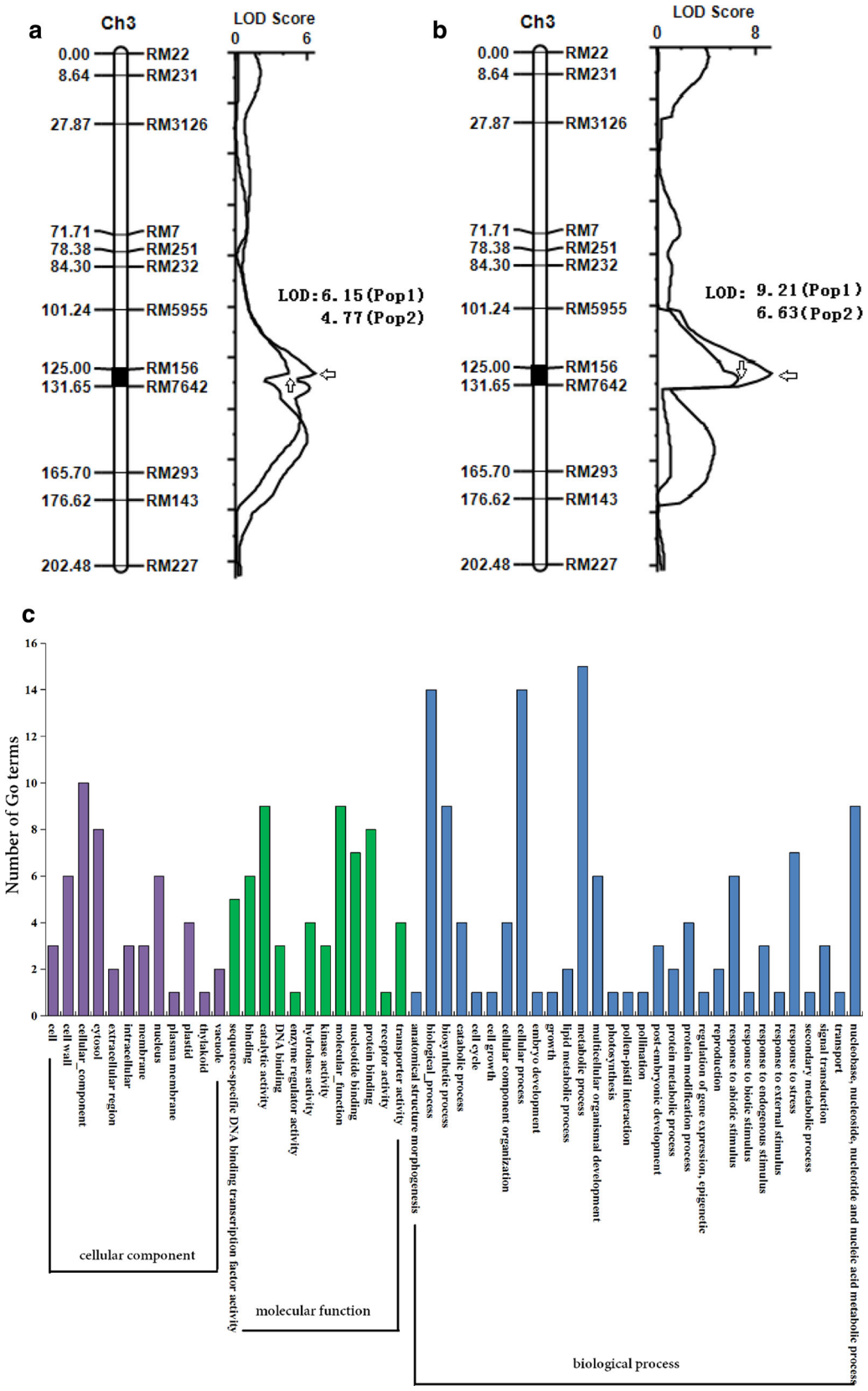

Figure 3. GO annotation of QTL-*qTGW3.1*. (a) IM of *qTGW3.1* on rice chromosome 3, (b) ICIM of *qTGW3.1* on rice chromosome 3, and (c) distribution of GO terms.

Table 3. Epistasis for partial yield component traits in two generations.

Trait name	Population	Chr.	Position1	Interval	Chr.	Position2	Interval	LOD	PVE (%)	Add1	Add2	Add by Add	TI[a]
GSD	F_2	5	50	RM153-RM13	7	120	RM445-RM6767	6.79	8.91	− 0.47	0.81	− 0.32	C
GSD	F_2	5	35	RM153-RM13	11	95	RM206-RM254	6.13	9.97	0.62	− 0.95	− 1.17	C
GSD	F_2	2	400	RM213-RM208	10	100	RM216-SSII-1M3	6.50	7.43	− 0.47	0.11	0.34	C
GSD	$F_{2:3}$	1	140	RM129-RM246	2	20	RM109-RM236	6.04	13.76	0.41	0.44	1.28	C
NEG	F_2	4	150	RM119-RM273	7	0	RM125-RM214	7.55	2.81	− 20.83	15.25	− 18.43	C
NEG	F_2	7	10	RM125-RM214	11	10	RM332-RM287	7.53	2.53	− 10.57	1.03	− 27.83	C
NEG	$F_{2:3}$	6	10	RM217-RM2615	3	60	RM3126-RM7	17.83	1.39	− 4.82	6.52	2.43	B
NEG	$F_{2:3}$	2	295	RM262-RM166	3	55	RM3126-RM7	16.54	1.31	62.92	− 59.04	− 57.37	C
NFG	F_2	10	10	RM239-RM474	10	35	RM474-RM216	7.45	6.06	36.68	− 26.03	− 31.75	C
NFG	F_2	6	20	RM276-RM6359	8	145	RM44-RM310	6.38	2.90	9.29	− 11.13	0.99	A
NFG	F_2	6	20	RM276-RM6359	12	95	RM270-RM235	6.09	6.51	4.33	− 23.81	− 1.68	B
NFG	F_2	2	365	SSII-2M3-RM213	5	100	RM405-RM249	6.12	6.66	− 8.72	− 11.59	4.81	C
NFG	F_2	2	360	SSII-2M3-RM213	7	195	RM418-RM432	6.24	5.81	8.14	− 15.79	− 21.50	C
NFG	F_2	2	370	SSII-2M3-RM213	9	45	AGPsmaM1-RM219	6.37	5.21	− 11.60	− 3.65	− 8.37	C
NFG	F_2	2	365	SSII-2M3-RM213	8	145	RM44-RM310	6.47	7.67	28.35	− 16.27	− 20.83	B
PL	F_2	1	190	RM246-RM128	4	335	RM127-RM280	6.26	9.67	0.11	− 1.68	− 0.94	C
PL	$F_{2:3}$	2	5	RM109-RM236	9	95	RM296-RM257	6.07	18.54	− 0.79	0.00	0.99	C
NP	$F_{2:3}$	1	130	RM129-RM246	10	65	RM216-SSII-1M3	6.14	5.10	− 0.61	1.12	0.22	C
NP	$F_{2:3}$	8	90	RM72-RM331	10	70	RM216-SSII-1M3	6.35	4.93	− 0.21	1.57	0.88	C
NP	$F_{2:3}$	2	360	SSII-2M3-RM213	10	60	RM216-SSII-1M3	6.44	5.25	2.41	0.02	− 0.77	C
SPP	F_2	4	165	RM119-RM273	4	280	RM303-SSIII-1M1	6.38	4.17	− 45.91	20.11	− 21.46	C
SPP	F_2	4	145	RM119-RM273	5	90	RM405-RM249	6.60	4.94	− 29.45	− 20.64	16.14	C
SPP	F_2	5	35	RM153-RM13	5	150	RM430-RM163	6.86	3.67	6.63	− 32.53	− 42.29	C
SPP	F_2	5	50	RM153-RM13	7	120	RM445-RM6767	7.76	3.54	− 22.55	24.62	− 18.24	C
SPP	F_2	5	35	RM153-RM13	11	90	RM206-RM254	6.44	3.65	14.62	− 33.22	− 36.00	C
SPP	F_2	1	50	RM1-RM259	11	140	RM254-RM224	6.22	5.16	− 50.20	− 47.39	− 11.59	A
SPP	F_2	1	50	RM1-RM259	5	35	RM153-RM13	6.94	4.57	− 18.99	− 22.64	− 64.00	B
SPP	F_2	11	55	RM287-RM229	11	100	RM206-RM254	6.24	2.57	28.68	− 30.07	− 23.31	C
SPP	F_2	9	85	RM296-RM257	11	140	RM254-RM224	7.06	4.66	6.97	40.88	− 26.33	B
SPP	F_2	3	160	RM411-RM293	4	175	RM119-RM273	6.04	3.69	13.77	− 40.20	− 33.42	C
SPP	$F_{2:3}$	1	110	RM129-RM246	2	120	RM424-RM250	6.27	3.38	0.15	3.04	18.84	C
SPP	$F_{2:3}$	4	325	RM131-RM127	11	140	RM254-RM224	7.00	5.17	− 27.42	− 6.99	− 15.24	B
SPP	$F_{2:3}$	5	140	RM146-RM430	5	180	RM163-RM440	7.43	4.65	21.50	− 33.35	− 9.05	C
SPP	$F_{2:3}$	5	190	RM161-RM480	12	15	RM101-RM19	7.50	3.22	− 14.25	12.46	5.69	C
SPP	$F_{2:3}$	7	275	RM234-RM18	12	75	RM270-RM235	6.17	7.54	12.00	− 4.85	25.75	C
SPP	$F_{2:3}$	10	0	RM311-RM239	10	40	RM474-RM216	6.04	6.65	26.30	− 8.76	− 18.85	C
SPP	$F_{2:3}$	4	320	RM348-RM131	7	90	RM6427-RM445	6.39	5.70	22.03	19.35	20.98	C
SPP	$F_{2:3}$	5	185	RM440-RM161	7	245	RM11-RM346	6.86	3.48	− 15.44	12.40	0.41	C
SSR	F_2	4	135	RM119-RM273	11	35	RM332-RM287	6.21	16.94	0.03	0.07	− 0.01	B
SSR	$F_{2:3}$	4	190	RM119-RM273	4	200	RM273-RM252	11.35	1.15	− 0.06	0.05	0.03	C
SSR	$F_{2:3}$	1	115	RM129-RM246	3	155	RM411-RM293	8.36	1.07	− 0.06	− 0.04	− 0.03	C
TGW	F_2	4	150	RM119-RM273	9	150	RM201-RM278	6.05	6.47	− 1.05	0.21	− 2.71	C
TGW	F_2	1	115	RM129-RM246	8	155	ISAM1-RM281	6.01	2.90	0.51	0.40	0.31	B
TGW	F_2	8	100	RM331-RM339	12	95	RM270-RM235	6.21	8.01	1.07	0.90	1.85	C

Table 3 (contd)

Trait name	Population	Chr.	Position1	Interval	Chr.	Position2	Interval	LOD	PVE (%)	Add1	Add2	Add by Add	TI[a]
TGW	F_2	9	30	RM444-AGPsmaM1	12	105	RM270-RM235	7.76	8.18	− 1.27	− 0.69	0.55	C
TGW	$F_{2:3}$	7	20	RM125-RM214	8	155	ISAM1-RM281	8.46	11.06	0.91	− 0.46	1.17	B
TGW	$F_{2:3}$	1	110	RM129-RM246	8	155	ISAM1-RM281	6.47	4.61	0.56	0.79	0.70	B

[a]TI, type of interaction; A, interaction between QTLs; B, interaction between QTLs and background loci; C, interaction between complementary loci.

(Kurusu *et al*. 2012) gene were cloned at the *qPL3.1* locus. The cloned gene of *UAP1* (Wang *et al*. 2015) was located at genetic locus of *qPL8.2* and regulated panicle length, seed setting rate, spikelet per panicle and thousand-grain weight. For GSD, two intervals (RM259-RM306 and RM401-RM119) containing seven cloned genes, including *IPI1* (Wang *et al*. 2003) and *Gnp4* (*LAX2*) (Zhang *et al*. 2011; Hiroaki *et al*. 2011). The cloned gene of *TGW6* was located at genetic locus of *qTGW6.1*, the loss of function of the IAA-glucose hydrolase gene *TGW6* enhances rice grain weight and increases yield (Ishimaru *et al*. 2013). *qNEG2.1* and *qSSR2.1* were located at the same genomic region of *qSPP2* reported by Zhang *et al*. (2009) on chromosome 2. The region where *qTGW3.3* was located contains the cloned gene *GL3.1*, which controls rice grain size and yield by regulating Cyclin-T1;3 (Qi *et al*. 2012). *qGSD1.1*, *qNEG6.1* and *qNEG6.2* were in the same interval as *qGSD1*, *qNEG6a*, and *qNEG6b* reported by Yan *et al*. (2019) separately, and they may even refer to the same site. The cloned gene of *OsCNGC13* was located at genetic locus of *qSSR6.1* and regulated seed setting rate by facilitating pollen tube growth in stylar tissues (Xu *et al*. 2017). These QTLs have shown large and consistent effects under varying environmental and across a wide range of genetic backgrounds, may be genetically independent and could be used for MAS (Swamy *et al*. 2016). Eighteen QTLs including 11 major QTLs are reported for the first time and are considered novel. Among them, six QTLs were from overwintering rice, which may be a unique genetic pattern for overwintering cultivated rice. *qTGW3.1*, explained 43.59% of the phenotypic variation, is a positive QTL for yield improvement, could be repeatedly detected in two populations, and originated from Nuodao89-1. Further, IM also detected *qTGW3.1* at the interval of RM156 (17715030 bp) and RM7642 (17789026 bp). A total of 46 functional genes involved in 229 GO terms were found at this location, and these candidate genes might affect thousand-grain weight in Nuodao89-1. But none of them were previously reported genes related to grain weight directly. It was still difficult to accurately predict a single candidate gene from this large region. The synergistic allele for *qTGW3.1* was from Nuodao89-1, fine mapping and gene cloning of *qTGW3.1* may be important for increasing yields of overwintering cultivated rice in molecular breeding. Thus, fine mapping at the *qTGW3.1* region and the related near-isogenic lines should be developed to identify candidate genes in future research.

Some of the genomic regions can be associated with more than one trait based on multi-trait QTL mapping (Zuo *et al*. 2019). The cluster distribution was generally founded in yield-related traits. For instance, Brondani *et al*. (2002) detected that specific marker regions strongly associated with multiple traits including panicle number, 100-grain weight, spikelets per panicle, percentage of filled grains per panicle, grain yield per panicle, filled grain number per panicle and grain yield per plant. Zhao *et al*. (2013) found that the same chromosome segment is often associated with

the expression of several traits, which may be the result of the pleiotropic effects of a single gene or the effect of tightly linked genes. The present study detected six regions associated with more than one trait. The QTLs (*qPL11.1* and *qSPP11.1*) were flanked repeatedly by RM3484 and RM6776 on chromosome 11, simultaneously affecting the genetic expression of panicle length and spikelet per panicle in the F_2 population. QTLs (*qNEG6.1*, *qNEG12.1*, *qNEG2.1* and *qNEG9.1*) for NEG always shared the same location with the SSR QTLs (*qSSR6.1*, *qSSR12.1*, *qSSR2.1* and *qSSR9.1*). *qGYP6.1* and *qTGW6.1* were detected at the region flanking SSII-3M1 and RM528 on chromosome 6. Notably, there was a significant correlation between PL and SPP, NEG and SSR, GYP and TGW. It is consistent with the findings that QTL for significantly correlated traits usually had same chromosome location reported by Hittalmani *et al*. (2003) and Tian *et al*. (2006). In addition, the cloning and functional studies of QTL also indicate the pleiotropic effects of various QTLs. For instance, *Ghd7* (Xue *et al*. 2008) simultaneously controls the number of grains per panicle, heading date and plant height. *DEP1* (Huang *et al*. 2009) affects many traits including panicle length, grain-setting density, grain number per panicle, grain length and grain weight. These results provide an explanation for the correlation between traits.

Epistasis is an important factor underlying quantitative traits (Caicedo *et al*. 2004). A considerable body of classical evidence has strongly suggested the prevalence of epistatic effects on quantitative traits in genetic populations (Malmberg *et al*. 2005; Fei *et al*. 2011; Ku *et al*. 2012). In the present study, 47 epistatic interactions were detected for yield component traits, however, the contribution rate and effect value of most of these interactions were limited, which may show that the epistatic interactions have some impact on the inheritance of yield component traits of Nuodao89-1, but that this is relatively small. It should be noted that mean distance of markers is quite wide in the population under study, thus we cannot exclude the small epistatic effects were also could be due to low resolution of linkage map or low polymorphic markers. Seven intervals were involved in more than one distinct interaction. This might indicate that there are multi-locus associations in the development of yield component traits. Many main-effect QTLs were involved in the epistatic interactions. This indicates that epistasis, in the form of additive by additive interactions, plays an important role in controlling the expression of yield-component traits. The usual estimates of main effect of a QTL can be confounded by interactions, which may change according to genetic backgrounds, environments, and other factors (Li *et al*. 1997). Three types of digenic interactions were identified in this study. Corresponding to the genetic mode of action of phenotypic traits are the regulatory network of genes, the three patterns of epistatic interactions between the QTLs may reflect the positive or negative feedback inhibition mechanism between the QTL loci.

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