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QTL mapping for Q-enzyme activity during grain development in rice

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Abstract

Q-enzyme is important for the synthesis of rice grain starch and vital for grain filling. In this study, QTL (Quantitative trait locus) analysis for Q-enzyme activity during grain filling was carried out using recombinant inbred line population derived from the super rice Xieyou 9308. The activity of Q-enzyme was greatest at 15 days after heading. In addition, the Q-enzyme activity in early stage (Q10 and Q15) showed significant correlation with the grain filling rate of each stage as well as the average grain filling rate. Nine main-effect QTLs for grain Q-enzyme activity were identified through QTL analysis, they were distributed on chromosome 2, 3, 5, 6 and 7, and the proportion of phenotypic variation explained (R^2) by individual QTLs ranged from 5.68% to 11.59%. Among them, *qQ10-3* and *qQ15-3-2* are located in the same region of RM282-RM6283 on chromosome 3. *qQ10-7-1* was detected in two years with an average R^2 of 8.86%, and significant epistasis effect with environment was also identified. These results enhance our understanding of the genetic bases of Q-enzyme activity in rice grain.

Key words: Q-enzyme, grain filling, quantitative trait locus, recombinant inbred line, rice

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world. In rice grains, starch is the main component that stores energy (Yoshida 1972). Thus, enzymes that are associated with synthesis and metabolism of starch during grain filling process are crucial for grain yield. There are mainly four types of important enzymes that are responsible for synthesis of grain starch, namely, ADP-Glc pyrophosphorylase (ADPase), starch synthase (SS), starch branching enzyme (Q-enzyme) and the starch de-branching enzyme (Keeling and Myers 2010). Among which, the Q-enzyme is a key component in the production of starch in endosperm (Nakamura et al. 1992). It has mainly two functions, catalyzes the formation of branch points by cleaving the α-1,4 linkage in polyglucans and reattaches the chain via an α-1,6-glucan linkage (Jeon et al. 2010; Tetlow and Emes 2014; Li and Gilbert 2016).

Some of the earlier studies had focused on physiological and biochemical aspects of enzyme activities related to starch synthesis. Yang et al. (2003) have reported that enhanced activities of Q-enzyme could increase the grain filling rate in rice plants subjected to water stress during grain filling stage. Tian et al. (2003) reported that decrease of starch synthase activities would lead to reduction of starch content. Increase of Q-enzyme was crucial for the ratio of amylose to total starch under weak light (Tian et al. 2006). Kato et al. (2007) found that improved activity of AGPase might increase the grain filling of extra-heavy panicle types. Wang et al. (2015a) discovered that abscisic acid is important for grain filling by regulating the activity of key enzymes that are associated with sucrose-to-starch transformation. In addition, some other studies have targeted on the effect of starch enzyme on grain weight and quality. Mohapatra et al. (2009) have reported that activities of starch synthase could be enhanced by manipulation of ethylene production, thus further leading to increase of grain weight of inferior spikelets.

During the last two decades, development of genomic and proteomic tools has greatly facilitated the understanding of genetic bases of rice for various agronomically important traits, such as grain weight (Li and Li 2014; Zuo and Li 2014), grain number (Bai et al. 2012), heading date (Hori et al. 2016), and grain

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quality (Fitzgerald et al. 2009). Recently, attention was also paid for rice starch enzyme, and a great progress has been achieved by genetic and proteomic analysis (Yamakawa 2011). It was reported that by up-regulating *OsSbe1* gene which encodes for the rice starch branching enzyme 1 could increase the eating quality of rice grain (Sun et al. 2013). Allelic variation in starch synthesis-related genes was responsible for variation of grain quality of Korean non-glutinous rice varieties (Mo et al. 2014). Thirty-four quantitative trait loci (QTLs) for grain quality traits were identified using recombinant inbred line (RIL) population derived from the cross Tainung 78/Taichung Sen 17 (Hsu et al. 2014). Starch biosynthetic genes played important roles for starch properties in a RIL population which was originated from the cross, IR64/Nipponbare (Luo et al. 2015). Allelic variation in the soluble starch synthase II gene family is important for grain quality and starch properties (Fan et al. 2017). In addition, genes that encode for rice starch branching enzyme IIb were identified through MutMapPlus (Nakata et al. 2017). However, despite of so much progress achieved in the genetic analyses of starch-related enzyme, only one report has focused on the Q-enzyme activity (Shen et al. 2005). However, there are no reports about the correlation between Q-enzyme activity and grain filling by QTL analysis.

The present study was therefore, carried out to understand the genetic relations of Q-enzyme activity in rice grain during filling period. QTL analysis for grain Q-enzyme activity was performed using RIL population derived from an *indica* rice cross. Correlation between Q-enzyme activity and grain filling was also analyzed.

Materials and methods

Plant materials

Xieyou 9308 is a three-line super hybrid rice widely cultivated in China. Its maintainer and restorer lines, Xieqingzao B and Zhonghui 9308, respectively, differ a lot in many important agronomic and physiologic traits such as grain Q-enzyme activity and grain quality. Xieqingzao B has superior grain Q-enzyme activity but inferior grain quality than Zhonghui 9308. To characterize the genetic bases of Q-enzyme activity of Xieyou 9308, a $F_{13:14}$ RIL population derived from the cross Xieqingzao B/Zhonghui 9308 was used in this study.

Field experiment and trait evaluation

The RIL and parental lines were planted in the experimental field of China National Rice Research Institute (30°36′N, 119°54′E) at Hangzhou and Zhejiang in 2010 and 2011 seasons. The numbers of lines used were 160 and 200 in 2010 and 2011, respectively. Because the Q-enzyme activity is closely correlated with its growth period, in order to synchronize the flowering of all the lines, different lines were sown at different dates according to the heading dates recorded from their previous performances. After 25 days, healthy and identical looking seedlings were field transplanted. Each line was planted in ten rows with each row having ten plants, thus totally 100 plants were transplanted for each line. The planting spacing was 20 cm x 20 cm. Normal field management was applied.

Trait evaluation

Panicles that flowered at the same date and having uniform size were tagged using plastic cards, and more than 100 panicles were tagged for each line. At 10 and 15 days after flowering in 2010, and 10, 15 and 20 days after flowering in 2011, ten tagged panicles from each line were randomly picked and immediately put in the ice box and taken back to laboratory as soon as possible. The ten panicles were equally divided into two groups as sub-samples. Then the five panicles of each sub-sample were bulked threshed and treated with liquid nitrogen for one minute, and freeze stored at –70°C for the measurement of grain filling rate and Q-enzyme activity. The sub-samples were handled separately for measurement and the average values between sub-samples were used for data analysis.

The extraction of Q-enzyme and measurement of its activity was done following the protocol given by Shen et al. (1997). The Q-enzyme activity measured at 10, 15 and 20 days after flowering was termed as Q10, Q15 and Q20, respectively. Twenty-five grains of each line were ground with 7 ml of 0.05 mol/L citric acid buffer (pH = 7.0) in precooled mortar. The crude homogenate was transferred to a 10 ml centrifuge tube and centrifuged at 18000 rpm for 20 min at 4°C. 1 ml supernatant was transferred to a 10 ml centrifuge tube, and 1 ml of 0.2 mol/L citric acid buffer (pH = 7.0), 0.5 ml of 0.1 mol/L EDTA and 0.5 ml of 0.75% soluble starch were added. The contents were immediately mixed and incubated at 37°C. After 40 min, 4 ml of 10% trichloroacetic acid was added to terminate the reaction. The mixed solution was then centrifuged at 2000 rpm for 10 min at room temperature. 1 ml of supernatant was added with 0.3 ml of 0.1% iodine solution, and were mixed and left for 10 min for color

development. For the control group, 1 ml of precooled 0.05 mol/L citric acid buffer (pH = 7.0) was substituted for the supernatant crude extract, and the following steps and reagent added were identical to the treatment group as described above. The extinction value was determined using a spectrophotometer as optical density (OD) at 660 nm. The activity of Q-enzyme was represented using the decrease of OD_{660} as described below:

$$\Delta OD_{660}\% = (OD_{660\ control} - OD_{660\ treatment})/OD_{660\ control} \times 100\%.$$

For evaluation of grain filling rate, the dehulled grains were dried at 105°C for 30 min and at 80°C to constant weight for 24 h, and weighed after cooling in dryer for 2 h. Two types of grain filling rate were estimated. The first type was estimated according to the dynamic process of grain filling which was fitted using the logistic growth equation as described by Mo (1992),

$$Y = K/(1 + ae^{-bt})$$

Where, Y is the dry grain weight at the sampling day, K is the dry final grain weight, t is the day from flowering to sampling, a and b are coefficients determined by regression. When the logistic growth equation for grain filling was fitted, the first derivative of the logistic growth equation $d_Y/dt = Kabe^{-bt}/(1+ae^{-bt})^2$ was calculated as the grain filling rate. Grain filling rate at 10, 15 and 20 d after flowering were estimated, which were termed as GFR_{10d}, GFR_{15d} and GFR_{20d}, respectively. The second type represents the grain filling rate between two sampling stages which is calculated as follows: GFR = the margin of grain weight for two sampling times/number of days between two sampling times.

Data analysis

The dynamic process of grain filling was fitted using Curve-expert 1.3. The genetic linkage map of RILs used in this study was developed by Zhai et al. (2012). It contained 281 lines, a total of 198 SSR markers covered 1814.5 cM of the whole genome, with an average genetic distance of 9.2 cM. QTL analysis was performed with Windows QTL Cartographer 2.5 (Wang et al. 2006) using composite interval mapping. In the analysis, the model 6, standard model, was used to control background, and forward and backward method was chosen as the regression method. The walk speed was 1.0 cM. Threshold for claiming the presence of putative main-effect QTL was determined by 1000 permutation test. The logarithm of odds (*LOD*) at genome-wise significance level of $P < 0.05$ ranged from 2.12 to 2.46. Thus *LOD* = 2.5 was used as the threshold for detection of a significant QTL effect. The additive effect and percentage of phenotypic variation explained by each QTL (R^2) were estimated. The mixed-model-based composite interval mapping (MCIM) was used to detect QTL by environment interaction using QTLNetwork 2.0 (Yang et al. 2008). QTL nomenclature was according to McCouch et al. (1997).

Results

The variation of Q-enzyme activity of RILs and parental lines

The Q-enzyme activity of RILs and parental lines has been presented in Table 1. The activity of Q-enzyme for parental lines and RILs reached to the highest at Q15 but decreased to a lower level at Q20. Xieqingzao B had higher activity than that of Zhonghui 9308 at all stages investigated in this study. The Q-enzyme activity of RILs at each stage showed continuous variation and transgressive segregation was observed as well (Fig. 1); the kurtosis and skewness were all less than 1.00. These results suggest that the activity of Q-enzyme was controlled by multiple genes with small effect.

Table 1. Phenotypic performance and distributions of Q-enzyme activity of the Xieyou 9308 recombinant inbred line (RIL) population and its parents

Year	Trait	Parent		RIL		Kurtosis	Skewness
		Xieqingzao B	Zhonghui 9308	Mean ± Sd	Range		
FY2010	Q10	32.11 ± 0.94	28.74 ± 1.13	35.31 ± 10.88	15.12 ~ 56.28	-0.81	0.11
	Q15	51.71 ± 0.86	39.52 ± 1.98	44.71 ± 7.46	21.42 ~ 58.86	-0.40	-0.01
FY2011	Q10	48.01 ± 1.23	30.57 ± 0.93	40.75 ± 7.73	23.81 ~ 56.32	-0.91	-0.15
	Q15	50.23 ± 1.07	37.58 ± 1.15	43.42 ± 5.63	32.18 ~ 57.72	-0.32	0.28
	Q20	36.73 ± 0.88	31.38 ± 0.93	38.19 ± 7.51	23.54 ~ 45.16	0.12	-0.15

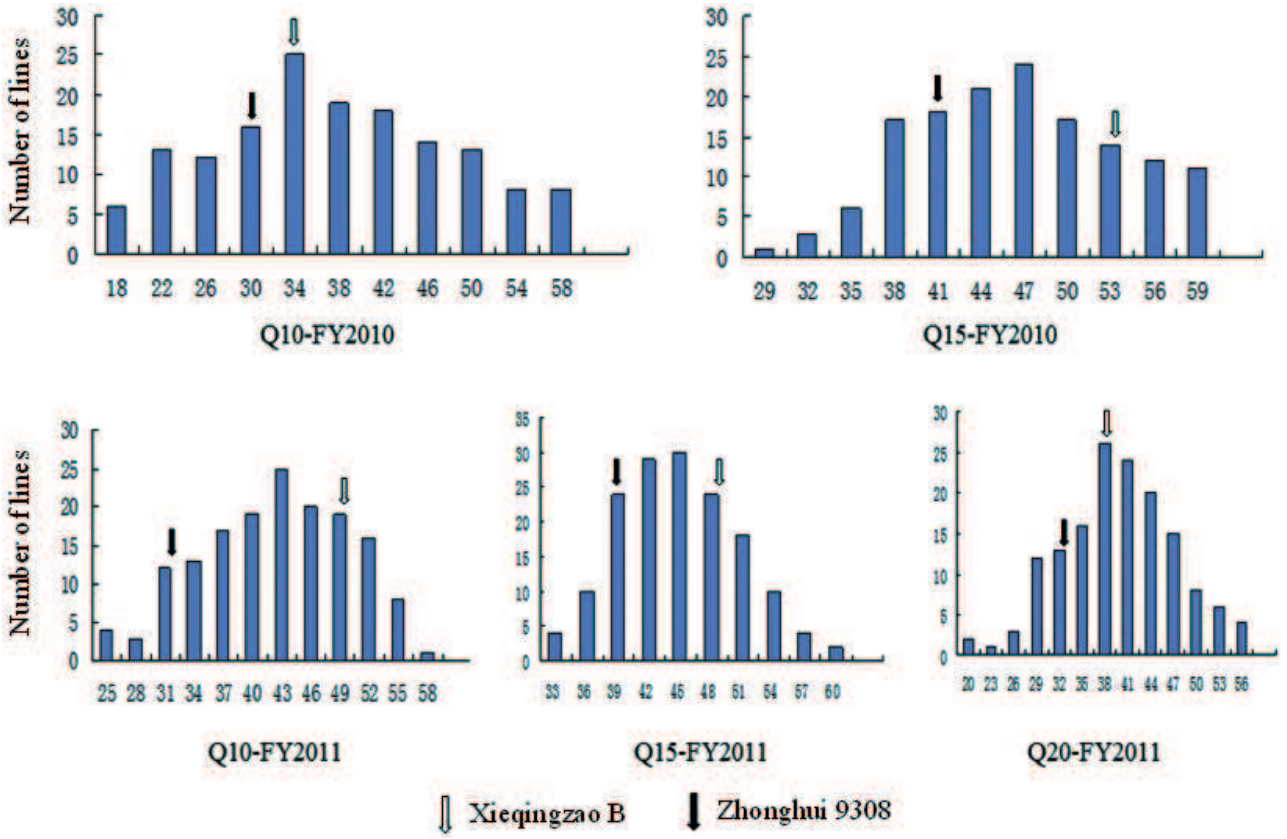

Fig. 1. Phenotypic distribution of Q-enzyme activity in Xieyou 9308 RILs in two years

Correlation analysis for Q-enzyme activity and grain filling rate

Significant positive correlation coefficient ($P < 0.05$) was detected between Q10 and GFR_{10d}, $GFR_{1\text{-}10d}$, GFR_{mean}, and GFR_{max} in two years, as well as between Q15 and GFR_{15d} and $GFR_{11\text{-}15d}$ (Tables 2 and 3), suggesting a positive correlation between the activity of Q-enzyme and grain filling rate in earlier stage. Significant negative correlation coefficients were observed between Q10 and GFR_{15d}, Q10 and GFR_{20d}, Q10 and $GFR_{1\text{-}15d}$, Q10 and $GFR_{16\text{-}20d}$ (Table 3), suggesting a negative correlation between Q-enzyme activity in earlier stage and grain filling rate in later stage.

Table 2. Correlation between Q-enzyme activity and grain filling rate (GFR) in Xieyou 9308 RIL population in FY2010

Trait	Q10	Q15	10d-GFR	15d-GFR	1~10d GFR	11~15d GFR	GFR_{mean}	GFR_{max}
Q10	-							
Q15	0.31**	-						
10d-GFR	0.24**	0.19*	-					
15d-GFR	-0.14	0.22*	-0.15	-				
1~10d GFR	0.23**	0.09	0.48**	-0.66**	-			
11~15d GFR	-0.15	0.26**	0.43**	0.89**	-0.42**	-		
GFR_{mean}	0.18*	0.11	0.64**	-0.42**	0.91**	-0.15	-	
GFR_{max}	0.20*	0.06	0.70**	-0.33**	0.78**	-0.06	0.95**	-

Note: *Significant at 0.05 level, ** Significant at 0.01 level

Table 3. Correlation between Q-enzyme activity and grain filling rate in Xieyou 9308 RIL population in FY2011

Trait	Q10	Q15	Q20	10d-GFR	15d-GFR	20d-GFR	1-10d GFR	11-15d GFR	16-20d GFR	GFR_{mean}	GFR_{max}
Q10	-										
Q15	-0.12	-									
Q20	0.13	0.34**	-								
10d-GFR	0.45**	0.01	0.14	-							
15d-GFR	-0.55**	0.16*	0.08	-0.45**	-						
20d-GFR	-0.60**	0.12	0.05	-0.69**	0.84**	-					
1-10d GFR	0.50**	0.02	0.13	0.84**	-0.59**	-0.68**	-				
11-15d GFR	-0.25**	0.18**	0.16	0.23**	0.74**	0.35**	-0.09	-			
16-20d GFR	-0.60**	0.14	0.06	-0.64**	0.94**	0.98**	-0.68**	0.51**	-		
GFR_{mean}	0.32**	0.01	0.19*	0.94**	-0.23**	-0.51**	0.72**	0.41**	-0.43**	-	
GFR_{max}	0.35**	0.01	0.19**	0.95**	-0.26**	-0.53**	0.72**	0.39**	-0.46**	0.99**	-

Note: *Significant at 0.05 level, ** Significant at 0.01 level

QTL analysis for Q-enzyme activity

Nine main-effect QTLs responsible for Q-enzyme activity were detected in two years (Table 4), which were distributed on chromosome 2, 3, 5, 6, and 7 (Fig. 2). The R^2 for individual QTLs ranged from 5.68% to 11.59%. For *qQ10-3*, *qQ10-5*, *qQ10-7-1*, *qQ15-3-2*, and *qQ20-2*, alleles increasing enzyme activity were from Xieqingzao B, while for the remaining QTLs, enhancing alleles were from Zhonghui 9308. The enhancing alleles of different QTLs were from different parents indicating that the potential of improving Q-enzyme activity could be achieved through pyramiding superior alleles from the two parents.

For Q10, seven main-effect QTLs were detected. Among which, *qQ10-7-1* had the largest *LOD* value of 4.54, explaining 11.59% of the phenotypic variation in 2011, and was consistently detected in both years with an average R^2 of 8.86%. For Q15, two QTLs were detected. Of which, *qQ15-3-2* had an *LOD* of 3.73, with the Xieqingzao B allele increasing enzyme activity by 2.33 explaining 9.52% of phenotypic variation. It was located between SSR markers RM282 and RM6283, which was coincided with the *qQ10-3* in location. For Q20, only one QTL, *qQ20-2*, was identified. Its *LOD* was 3.24, the Xieqingzao B allele increased enzyme activity by 2.34, with an R^2 of 8.69%.

Significant QTL by environment interactions were detected for two QTLs, including *qQ10-6-2* and *qQ10-7-1*, and the effect of interactions were 2.16 and 2.61, which explained 4.72% and 6.34% of phenotypic variation, respectively.

Discussion

The yield potential and grain quality of rice are closely correlated with the starch synthesis during grain filling. There are mainly four types of enzymes that determining the starch synthesis, among which the Q-enzyme is important in the synthesis of starch during grain filling (Nakamura et al. 1992). QTL analysis for Q-enzyme activity using RIL population derived from Xieqingzao B x Zhonghui 9308 was performed in this study. Nine main-effect QTLs were detected, which were distributed on chromosome 2, 3, 5, 6, and 7, with the R^2 ranging from 5.68% to 11.59%. The *qQ10-3* and *qQ15-3-2* were located in the same interval of RM282-RM6283 on chromosome 3. The *qQ10-7-1* was detected in two years, with an average R^2 of 8.86%.

It was reported that Q-enzyme activity was

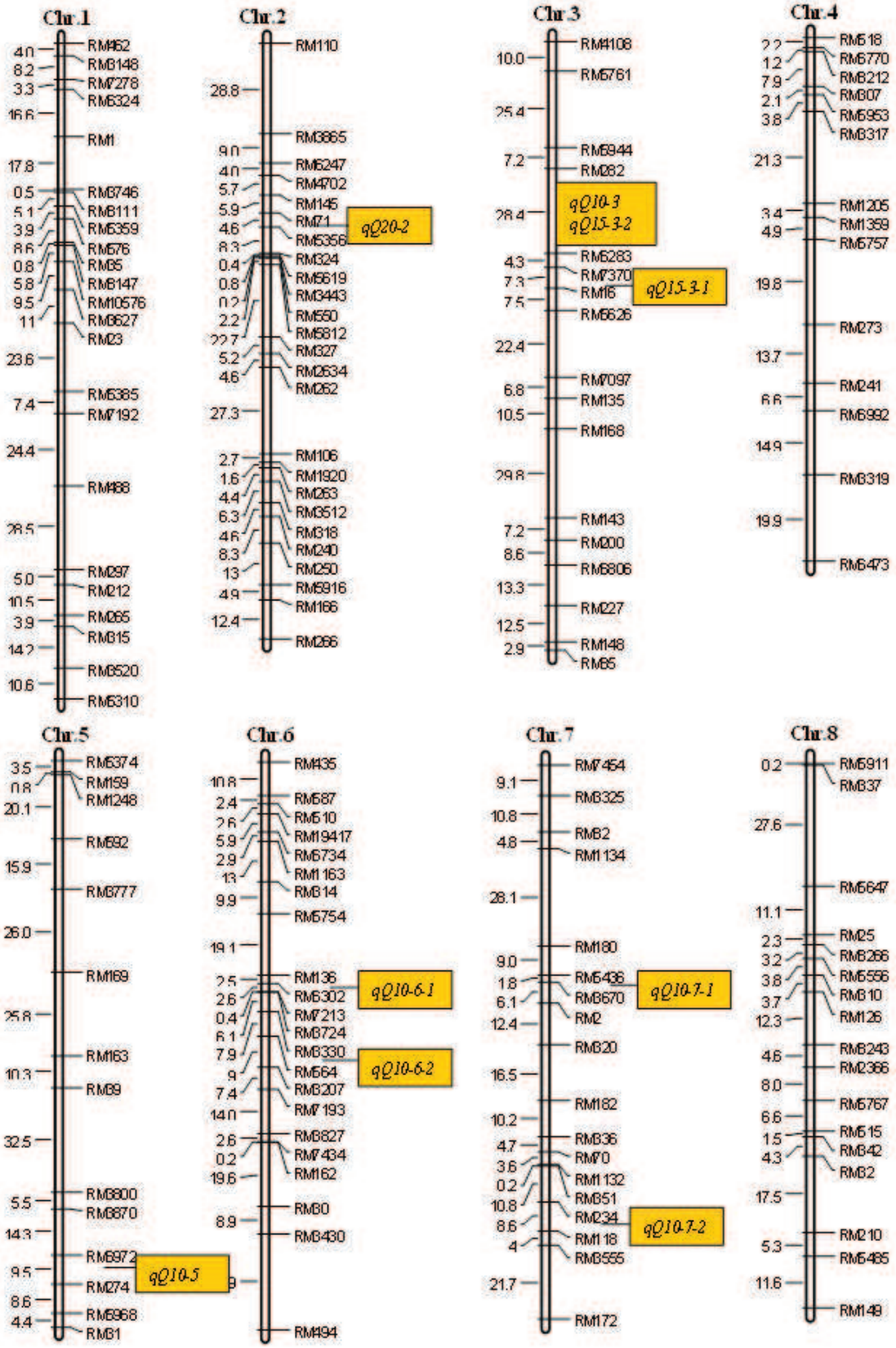

Fig. 2. Distribution of main-effect QTLs for Q-enzyme activity on the molecular linkage map based on Xieyou 9308 RIL population

Table 4. Main-effect QTL for Q-enzyme activity detected in Xieyou 9308 RIL population in two years

Trait	Environment	QTL	Marker interval	*LOD* score	Additive effect	R^2 (%)
Q10	FY2010	*qQ10-3*	RM282-RM6283	2.53	2.22	5.68
		qQ10-6-1	RM136-RM6302	2.62	-2.30	6.15
		qQ10-6-2	RM3330-RM564	2.61	-2.35	6.54
		qQ10-7-1	RM5436-RM3670	2.59	2.24	6.12
	FY2011	*qQ10-5*	RM6972-RM274	2.52	1.92	6.19
		qQ10-7-1	RM5436-RM3670	4.54	2.82	11.59
		qQ10-7-2	RM234-RM118	2.51	-1.97	6.13
Q15	FY2010	*qQ15-3-1*	RM7370-RM16	2.51	-1.91	6.78
	FY2011	*qQ15-3-2*	RM282-RM6283	3.73	2.33	9.52
Q20	FY2011	*qQ20-2*	RM71-RM5356	3.24	2.34	8.69

relatively higher at earlier stage during grain filling (Shen et al. 2005). In the present study, seven QTLs were detected at Q10, while only two and one were detected at Q15 and Q20, respectively. This result indicates that QTL detection for Q-enzyme activity is period specific, and the earlier stage that has peak activity for Q-enzyme activity is more suitable for QTL detection.

Many studies have reported the correlation between the starch-related enzyme and grain filling. The ADPase, Q-enzyme and SS have important influence on the synthesis of starch (Du et al. 2012); they were significantly correlated with the average and maximum grain filling (Zhou et al. 2015). In addition, the activity of Q-enzyme and SS were significantly correlated with the accumulation rate of total starch and amylopectin (Wang et al. 2010). In this study, the Q-enzyme showed the greatest activity at Q15, and decreased afterward. The Q-enzyme activity at Q10 was significantly positive correlated with the average and maximum grain filling rate, which corresponds well with the previous studies. On the other hand, significantly negative correlation between Q10 and 15d-GFR, as well as Q10 and 20d-GFR were observed. Similar results were also reported by Yang et al. (2001). The underlying reasons causing this negative correlation is unclear to date, and it remains to be studied in later research.

During the last decade, a large number of QTLs for grain weight have been cloned in rice (Bai et al. 2012; Zuo and Li 2014). Among which several QTLs have been showed to have pleiotropism on grain filling, such as *GW2* (Song et al. 2007), *GS2* (Hu et al. 2015), *qGL3/GL3.1* (Qi et al. 2012; Zhang et al. 2012), *GS5* (Li et al. 2011), *GW6a* (Song et al. 2015), *TGW6* (Ishimaru et al. 2013) and *GW8* (Wang et al. 2012). Using RILs derived from the Zhenshan 97/Milyang 46, QTLs responsible for the Q-enzyme activity at Q10 were identified on chromosome 1, 6 and 7 (Shen et al. 2005). In the present study, nine main-effect QTLs were detected. Even though great progress has been achieved in QTL cloning for grain weight, and attention was also paid for Q-enzyme activity, the relationships between these two factors were unclear. However, some QTLs for grain weight located in the same region as Q-enzyme activity indicating that these two trait were closely related. The *qQ20-2* was mapped between markers RM71 and RM5356, which is coincidence with the location of *GW2* (Song et al. 2007). The *qQ10-3* and *qQ15-3-2* were resided in the region of RM282-RM6283, which covers the *GS3* (Fan et al. 2006). The *qQ10-7-2* was located in the interval of RM234-RM118; the upstream of this region contains the *qGL7* (Wang et al. 2015b). These coincident loci for Q-enzyme activity and grain weight and grain filling may be due to pleiotropism or gene linkage, but it remains to be validated by fine-mapping because the QTL region in this study were relatively large.

Authors' contribution

Conceptualization of research (LYC, ZLY); Designing of the experiments (LYC, ZLY); Contribution of experimental materials (XDZ); Execution of field/lab experiments and data collection (ZLY, ZQG); Analysis of data and interpretation (ZLY, ZQG); Preparation of manuscript (ZLY, LYC, HWZ).

Declaration

The authors declare no conflict of interest.

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