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ORIGINAL RESEARCH

Genetic Relationships Among Panicle Characteristics of Rice (*Oryza sativa* L.) Using Unconditional and Conditional QTL Analyses

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Abstract Using mixed-model-based composite interval mapping and conditional statistical methods, we studied quantitative trait loci (QTLs) with epistatic effects and QTLs by environment interaction effects for rice seed set percent (SSP), filled grain number per plant (FGP), and panicle length (PL). A population of 241 recombinant inbred lines was used which was derived from a cross between "Zhenshan 97" and "Minghui 63." Its linkage map included 221 molecular markers. Our QTL analysis detected 28, 25, and 32 QTLs for SSP, FGP, and PL, respectively. Each QTL explained 1.37%~13.19% of the mean phenotypic variation. A comparison of conventional and conditional mapping provided information about the genetic control system involved in the synthetic process of SSP, FGP, and PL at the level of individual QTLs. Conditional QTLs with reduced (or increased) effects were identified for SSP, which were significantly influenced by FGP or PL. Some QTLs could express independently for the given traits, thereby providing possibilities for simultaneous improvement of SSR and PL, and SSR and FGP. Epistasis was more sensitive to environmental conditions than were additive effects.

Keywords Conditional mapping · Panicle characteristics · Quantitative trait locus (QTL) · Rice · Unconditional analysis

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The characteristics of rice panicles, such as seed set percent, filled grain number per plant, and panicle length, are generally considered important traits for improving grain yield. After mapping the quantitative trait locus (QTL) for yield and its components, Cao (2000) has proposed that the full grain number accounts for a larger contribution to yield than do panicle number or kilo-grain weight. Rao et al. (1997) have indicated that panicle length also directly and obviously affects yield. Therefore, it might be possible to obtain higher yields by improving some panicle characteristics. Panicle length is negatively correlated with seed set percent (Xing et al. 2001), and most rice panicle characteristics are quantitatively inherited. Because their performances are greatly associated with and easily affected by the growing environment, it is difficult to improve their traits through traditional breeding techniques. Therefore, understanding the underlying genetic control of such characteristics is of great importance to researchers.

Due to pleiotropy, gene linkage, and association at different expression levels, these quantitative traits are usually related. Independent studies have described the incidence of clustered QTLs for traits that are functionally related, including yield and yield components (Xiao et al. 1998; Moncada et al. 2001; Thomson et al. 2003), grain quality (Septiningsih et al. 2003), and grain weight (Li et al. 2004; Fan et al. 2006). To improve these correlated traits through breeding, research is necessary to investigate the use of favorable alleles for yield characteristics and their assembly through MAS (marker-assisted selection). Xiao et al. (1998) have reported QTLs controlling 1,000-grain weight, panicle length, and spikelet number per panicle linked to markers RZ422 and RG386, which define the same region as RM215. Thomson et al. (2003) have identified one QTL for yield, TGW, panicle length, spikelet

number per panicle, and grain number per panicle in association with RM242 and RM215.

Although complex relationships among rice panicle traits have been found by conventional statistical genetics analysis, those results have revealed only the correlations for pair-wise traits under the interference of other traits. Therefore, estimates of genotypic covariance through unconditional analysis methods cannot clarify the actual relationships among panicle traits. A protocol for conditional genetics analysis proposed by Zhu (1995) has been further developed to study closely related traits and to investigate the contribution of each trait to other related traits at the QTL level (Cao 2000; Guo et al. 2005; Zhao et al. 2006). For significantly correlated traits, a conditional QTL mapping method could be used to dissect the genetic interrelationship between traits at the level of individual QTLs, as well as reveal additional QTLs that are undetectable by unconditional mapping (Li et al. 2008).

Here, we employed conventional mapping to study the genetic basis of rice seed set percent (SSP), filled grain number per plant (FGP), and panicle length (PL), examining a population of 241 recombinant inbred lines (RIL) derived from a cross between "Zhenshan 97" and "Minghui 63." A conditional method was introduced to evaluate those QTLs with net additive and epistatic effects as well as *QE* interaction effects, based on mixed linear models that follow composite-interval mapping (Zhu 1999; Yang et al. 2007) and conditional statistical methods (Zhu 1995). By comparing unconditional and conditional QTLs for SSP, FGP, and PL, we could identify the genetic interdependencies between SSP and FGP and between SSP and PL, at the level of individual QTLs. Our objective was to provide valuable information for MAS that would improve SSP without negatively affecting FGP or PL.

Materials and Methods

Plant Materials

We used a population set of 241 RILs derived by single-seed descent from an elite hybrid cross of *Oryza sativa* SSP. *indica* "Zhenshan 97" and "Minghui 63."

Molecular Linkage Map

Preliminary RFLP mapping of the RIL population was performed by Yu et al. (1997). In all, 221 polymorphic markers, including 175 RFLPs, 45 SSRs, and one Waxy marker, were used to construct a linkage map with the Mapmaker program (Lander and Botsein 1989). This map covered 1,796 cM, averaging 8.7 cM between markers. Segregation ratios of the two parent genotypes in most loci fit the expected 1:1 Mendelian ratio. Segregation distortion at $P<0.001$ was detected for 38 marker loci located in 11 contiguous regions on ten chromosomes, except for chromosomes 1 and 10. We calculated the overall level of heterozygosity in the population as 0.81%, which was much higher than the expected 0.39% (1/28), based on eight generations of selfing. In map construction, the heterozygotes of individual loci were treated as missing data.

Field Experiments

Our RIL population and their two parents were grown at Hangzhou in 2004 and 2005, following a randomized complete block design with two replications. In both years, the germinated seeds were sown in a bed on 16 May, and the seedlings were transplanted to a paddy field on 13 June, with one plant per hill and a spacing of 17×26 cm. Each plot included one RIL with 60 plants. Fertility and cultivation regimes were consistent with optimum rice production for this region. We harvested 20 healthy plants from each plot for manual measurements of the average total seed number per plant, filled grain number per plant (FGP), and panicle length (PL). Values for SSP were calculated from the total number of seeds per plant and FGP.

$$\text{seed set percent } (\%) = \frac{\text{filled grain number per plant}}{\text{total seed number per plant}} \times 100$$

Statistical Analysis

Phenotypic data for SSP, FGP, and PL were analyzed for estimating variance and correlated coefficients by MINQUE methods (Zhu 1992). A conditional approach (Zhu 1995) was employed to evaluate the net genetic effects of SSP, which were independent of FGP or PL.

QTLs with additive and epistatic effects, as well as their environmental interaction effects, for these three traits, were mapped by QTLNETWORK 2.0 (Yang et al. 2007). The phenotypic values for SSP, FGP, and PL of the *k*-th RIL line in environment *h* were partitioned according to the following mixed linear model:

$$\begin{aligned} y_{hk} = {} & \mu + a_i x_{A_{ik}} + a_j x_{A_{jk}} + aa_{ij} x_{AA_{ijk}} + u_{E_{hk}} e_{E_h} \\ & + u_{A_i E_{hk}} e_{A_i E_h} + u_{AA_{ij} E_{hk}} e_{AA_{ij} E_h} + \sum_{f(h)} u_{M_{fk(h)}} e_{M_{f(h)}} \\ & + \sum_{l(h)} u_{MM_{lk(h)}} e_{MM_{l(h)}} + \varepsilon_{hk} \end{aligned} \quad (1)$$

where μ is the population mean; a_i and a_j are the additive main effects (fixed effects) of the two putative Q_i and Q_j, respectively; aa_{ij} is the additive × additive epistatic effect (fixed effect) between Q_i and Q_j; x_{Aik}, x_{Ajk}, and x_{AAijk} are coefficients of these genetic main effects; e_{E_h} is the random effect of environment h, with coefficient $u_{E_{hk}}$; $e_{A_iE_h}$ (or $e_{A_jE_h}$) is the random additive × environment interaction effect, with coefficient $u_{A_iE_{hk}}$ (or $u_{A_jE_{hk}}$) for Q_i (or Q_j); $e_{AA_{ij}E_h}$ is the random epistasis × environment interaction effect, with coefficient $u_{AA_{ij}E_{hk}}$; $e_{M_{f(h)}}$ is the random effect of marker f nested within the h-th environment, with coefficient $u_{M_{fk(h)}}$; $e_{MM_{l(h)}}$ is the random effect of the l-th marker × marker interaction nested within the h-th environment, with coefficient $u_{MM_{lk(h)}}$; and ε_{hk} is the random residual effect. Marker factors $e_{M_{f(h)}}$ and $e_{MM_{l(h)}}$ in the model were used to absorb additive and epistatic effects of background QTLs for controlling noise.

Conditional QTL analysis was conducted with the phenotypic value of SSP, given the phenotypic behavior of FGP or PL, using QTLNETWORK 2.0 (Yang et al. 2007). As with Eq. (1), the conditional value $y_{hk(T_1|T_2)}$ could be partitioned as:

$$\begin{aligned} y_{hk(T_1|T_2)} &= \mu_{(T_1|T_2)} + a_{i(T_1|T_2)}x_{A_{ik}} + a_{j(T_1|T_2)}x_{A_{jk}} \\ &+ aa_{ij(T_1|T_2)}x_{AA_{ijk}} + u_{E_{hk}}e_{E_h(T_1|T_2)} \\ &+ u_{A_iE_{hk}}e_{A_iE_h(T_1|T_2)} + u_{AA_{ij}E_{hk}}e_{AA_{ij}E_h(T_1|T_2)} \\ &+ \sum_{f(h)} u_{M_{fk(h)}}e_{M_{f(h)}(T_1|T_2)} \\ &+ \sum_{l(h)} u_{MM_{lk(h)}}e_{MM_{l(h)}(T_1|T_2)} + \varepsilon_{hk(T_1|T_2)} \end{aligned} \quad (2)$$

where $T_1|T_2$ denotes trait 1 conditioned on trait 2, i.e., "trait 1 given trait 2" (e.g., SSP|FGP = seed set percent conditioned on filled grain number per plant, or SSP|PL = seed set percent conditioned on panicle length), which meant excluding the phenotypic variation of trait 2. All parameters were defined as conditional effects as in Eq. (1). For example, $a_{i(T_1|T_2)}$ was the net additive main effect of putative Q_i, which contributed to the phenotypic value of trait 1, but was independent of that for trait 2.

QTLs were presented when genetic main effects (*a* and *aa*) and/or the *QE* interaction effect (*ae* and *aae*) were significantly different from zero ($P \leq 0.05$ or 0.01). All selecting probabilities were at the genome-wide level of significance.

Results

Phenotypic Variations

The phenotypic distribution of rice seed set percent (SSP), filled grain number per plant (FGP), and panicle length (PL) were analyzed over 2 years (Table 1). Both parents had higher SSP, FGP, and PL values in 2004 than in 2005 (except for FGP of "Zhenshan 97"). Transgressive segregation from lines greater than the higher parent to lines smaller than the lower parent was observed for all three traits in both years. The RIL population segregated continuously, with skewness and kurtosis values for all traits being <1.0, thereby suggesting that these data were suitable for QTL analysis.

Based on estimates of variance components and correlated coefficients for SSP, FGP, and PL (Table 2), SSP and PL were mainly controlled by genetic main effects, whereas a genotype × environment interaction effect was the main cause for FGP. Conditional variance of SSP|FGP had a smaller value than that of SSP|PL, implying that FGP gene expression had more common influence on SSP than that of PL. The genetic correlation coefficient was significantly positive and with a relatively high value between SSP and FGP but was opposite between SSP and PL or FGP and PL. No significant genotype × environment interaction correlation was detected between SSP and PL.

Table 1 Summary of phenotypic data for FGP, PL, and SSP in parents and RIL population evaluated over 2 years (mean of two replications)

Year	Trait	Parents		RIL population (n=241)					
		MH63	ZS97	Min	Max	Mean	SD	Kurtosis	Skewness
2004	SSP	68.05	70.45	27.45	87.80	67.90	10.50	−0.70	0.64
	FGP	542.50	508.00	380.50	980.00	689.62	131.37	0.17	−0.53
	PL	24.78	17.95	16.02	26.49	21.67	1.86	−0.30	0.23
	SSP	50.05	62.25	25.80	88.85	52.51	12.31	0.30	−0.29
2005	FGP	526.50	523.00	259.50	1053.00	599.83	157.72	0.52	−0.28
	PL	23.39	17.27	16.20	26.11	21.33	1.70	−0.01	0.25

Table 2 Variance proportion and covariance coefficient of FGP, PL, and SSP

Para.	FGP($\times 10^2$)	PL($\times 10^{-1}$)	SSP	SSP|FGP	SSP|PL	Para.	FGP and PL	FGP and SSP	PL and SSP
V_G	16.69*	39.04*	144.18*	69.85*	133.80*	r_G	−0.21	0.73**	−0.32**
V_{GE}	95.37*	6.52**	40.88**	15.4**	32.49*	r_{GE}	0.13*	0.82**	−0.19
$V\varepsilon$	14.97	9.62	54.11	39.65	61.80	r_P	−0.04	0.68**	−0.22**

*P=0.05, significant; **P=0.01, significant

Additive and Additive × Environment Interaction QTLs

QTLs with additive (*a*) and additive × environment interaction effects (*ae*) for SSP, FGP, and PL are shown in Fig. 1. These were named as suggested by McCouch et al. (1997).

In total, 14, 11, and 13 putative QTLs with additive and/or additive × environment effects were identified for SSP, FGP, and PL, respectively. Among them, five were located on chromosomes 2, 3, 7, 10, and 11 within marker intervals RM211–RG634, C1087–RZ403, RZ471–RM70, C153A–RM222, and CD0534–RG2; additive effects were detected for both SSP and FGP. The effects of QTLs on chromosomes 2, 7, and 10 had the same direction for both traits. Two QTLs on chromosomes 7 and 10 within marker intervals C1023–R1440 and C153A–RM222 had additive effects, with the opposite direction for SSP and PL. This meant that when QTL expression caused increasing (or decreasing) effects on SSP with alleles from "Zhenshan 97" (or "Minghui 63"), negative effects might appear for PL. Each single QTL explained 1.37%~13.19% of the mean phenotypic variation. The general contribution from these additive and/or additive × environment interaction effects by all putative QTLs was 22.31%, 11.17%, and 20.75% for SSP, FGP, and PL. A relatively lower contribution rate for FGP, which represented the explained ratio of phenotypic variation in the RIL population, was consistent with the small genetic main variance of FGP. Alleles with positive and negative effects (increasing or decreasing trait values) were dispersed between the two parents, explaining the occurrence of transgressive segregation in the RIL population. QTLs with additive × environment interaction effects were detected for all three traits. Unlike the QTLs with additive main effects, most *ae* QTLs were specific for three traits.

Epistasis and Epistasis × Environment Interaction QTLs

Altogether 14, 14, and 19 pairs of QTLs with an epistatic main effect (*aa*) and/or epistasis by environment interaction effect (*aae*) were detected that were associated with SSP, FGP, and PL, respectively (Fig. 1). Among these pairings, six (qSSP1-2 and qSSP3-1, qSSP3-2 and qSSP10-3, qSSP4-1 and qSSP9-3, qSSP5-1 and qSSP12-2, qSSP7-2 and qSSP10-1, and qSSP8-1 and qSSP12-3), four (qFGP1-3 and qFGP5-2, qFGP4 and qFGP12-2, qFGP5-1 and qFGP9-3, and qFGP11-1 and qFGP11-2), and five (qPL1-4 and qPL3-1, qPL3-2 and qPL9-1, qPL4 and qPL9-4, qPL5 and qPL11-2, and qPL9-3 and qPL10-1) had *aa* effects, while only one for SSP (qSSP6-4 and qSSP10-2) and one for PL (qPL1-1 and qPL6-4) had both *aa* and *aae* effects. The fact that more QTL pairs had epistasis × environment interaction effects than epistasis main effects indicated that the latter was more easily subjected to environmental influence. All detected pairs explained 17.96%, 35.93%, and 49.05% of the phenotypic variation for SSP, FGP, and PL in our RIL population, respectively. Most QTLs with additive effects were engaged in digenic interactions, but the detected epistasis largely involved loci without detectable QTL additive effects. Of all the pairings, five , seven, and eight had epistasis involving one locus with a QTL additive effect and one locus without, while six, seven and six had epistasis that resulted entirely from QTLs without additive effects. This might suggest that epistatic effects were largely due to the induction of the loci without detectable QTL additive effects, which might serve as modifying agents (Cao et al. 2001). Some loci were involved in more than one distinct interaction (i.e., qSSP1-4, qSSP1-5, qSSP3-2, qSSP7-1, and qSSP7-2 for SSP; qFGP5-1, qFGP3-3, qFGP4, qFGP8-2, and qFGP11-2 for FGP; and qPL3-2, qPL4, qPL6-1, qPL8-1, qPL5, qPL9-1, qPL8-2, and qPL10-1 for PL), indicating the possibility of multilocus associations for the genetic processing of a trait. Our study did not consistently detect any pairings of interaction QTLs for three traits; only six QTLs (qSSP1-1, qSSP3-2, qSSP6-4, qSSP7-2, qSSP8-1, and qSSP11-1) were concerned with epistatic interactions for all three traits. Furthermore, three (qSSP4-2, qSSP5-3, and qSSP12-3) and four (qSSP3-6, qSSP10-4, qSSP6-6, and qSSP9-3) QTLs were identical or linked for SSP and FGP or SSP and PL, respectively.

Genetic Links and Decomposition of QTLs

Seed Set Percent Conditioned on Filled Grain Number Per Plant

When SSP was conditioned on FGP, ten QTLs with conditional additive main effects and/or conditional

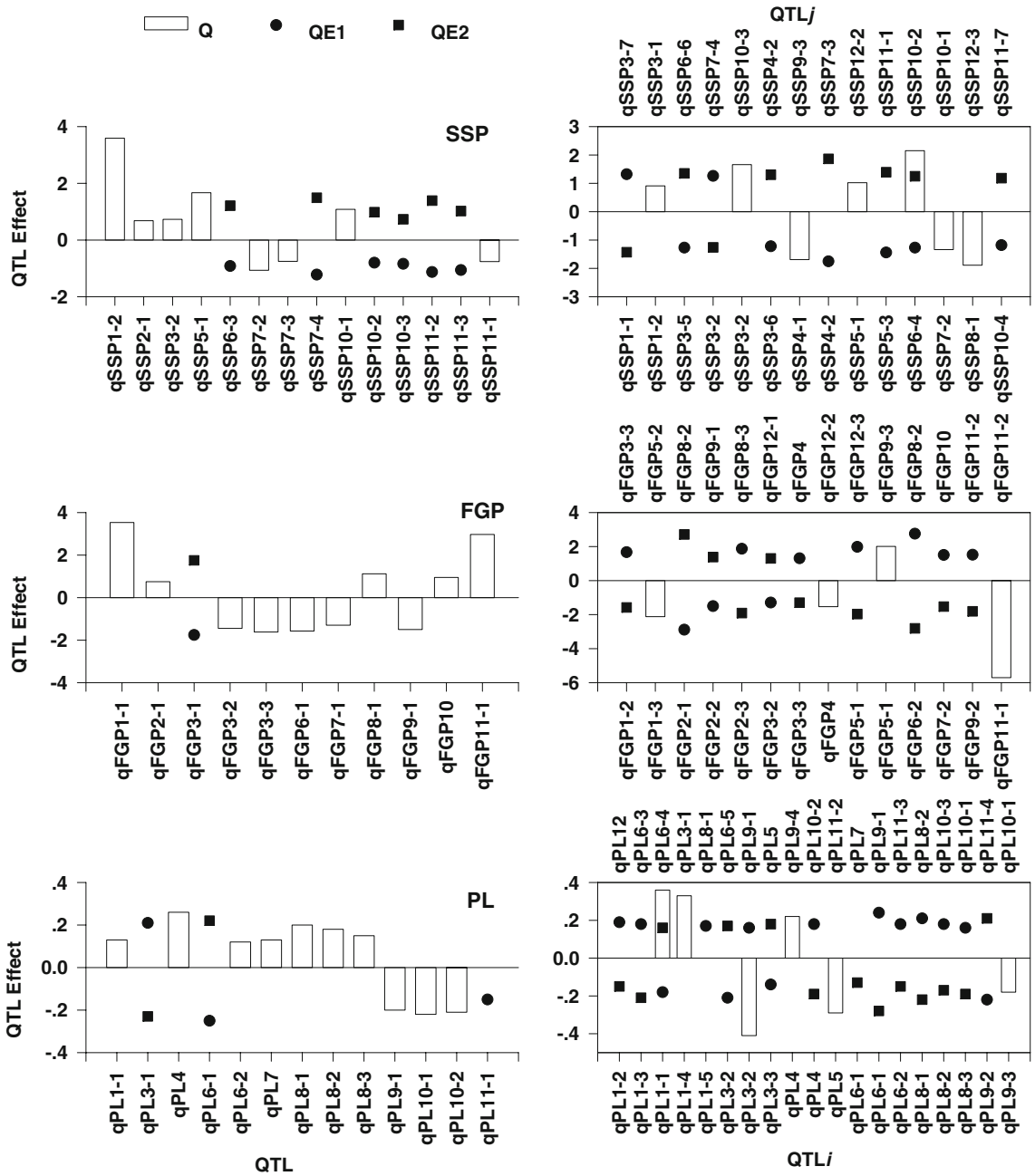

Fig. 1 QTL additive and epistatic effects for seed set percent, filled grain number per plant (×10), and panicle length

additive × environment interaction effects were detected (Table 3). Compared with unconditional QTLs for SSP with *a* effects, five unconditional QTLs (qSSP2-1, qSSP3-2, qSSP7-3, qSSP10-1, and qSSP11-1) failed to be detected, i.e., the common QTLs between unconditional SSP and FGP. This indicated that the additive effects of these five loci contributed simultaneously to the genetic behavior of SSP and FGP. Three QTLs (qSSP1-2, qSSP5-1, and qSSP7-2) had reduced additive effects (on magnitude), showing that the genetic effects of FGP could stimulate the expression of these three loci with alleles from "Zhenshan 97." When the FGP effect was excluded, five new QTLs (qSSP1-1, qSSP2-2, qSSP6-2, qSSP7-1, and qSSP9-1) were identified. Of six unconditional *ae*-related loci (qSSP6-3, qSSP7-4, qSSP10-2, qSSP10-3, qSSP11-2, and qSSP11-3), only qSSP11-3, within marker interval RM254–G4001, showed a significantly reduced conditional effect (on magnitude) when we took a conditional mapping approach. One new QTL (qSSP5-2) exhibited additive × environment interaction effects in both years for SSP given FGP. We inferred from the behavior of conditional qSSP1-2 that this additive effect

Table 3 Unconditional and conditional additive (*a*) and additive × environment (*ae*) interaction effects

QTL	Marker interval	Position (cM)	SSP	*a* SSP|FGP	SSP|PL	QTL	Marker interval	Position (cM)	SSP	*ae* in 2004 SSP|FGP	SSP|PL	SSP	*ae* in 2005 SSP|FGP	SSP|PL
qSSP1-1	R753-G359	21.7		−0.84**		qSSP1-3	RM81A-G1128b	99.9			1.08**			−1.17**
qSSP1-2	C2340-RG236	192.2	3.59**	0.73*	2.75**	qSSP1-2	C2340-C86	192.2		1.00**			−1.02**	
qSSP2-1	RM211-RG634	13.4	0.68*			qSSP3-4	C1087-RZ403	64.9			−1.77**			1.74**
qSSP2-2	RM208-RM207	45.9		−0.86**		qSSP5-2	R830-R3166	5.9		−0.92**	−1.17**		0.97**	1.23**
qSSP3-1	RG393-C1087	68.1			1.74**	qSSP6-3	C688-R1952a	17.1	−0.92**			1.21**		
qSSP3-2	C1087-RZ403	86.0	0.73*			qSSP7-4	RM70-R1245	94.9	−1.22**			1.49**		
qSSP3-3	R321-RM55	114.2			−1.26**	qSSP10-2	RM239-C1633	41.1	−0.80*			0.98**		
qSSP5-1	R0360-RM42	30.1	1.67**	0.92*	2.01*	qSSP10-3	RM258-RG561	97.7	−0.84**			0.73*		
qSSP6-1	C474-R3139	6.2			0.74**	qSSP11-2	clone1-Y6854L	9.9	−1.13**			1.39**		
qSSP6-2	R2549-C962	128.5		0.67**		qSSP11-3	RM254-G4001	51.3	−1.06**	−0.63*		1.02**	0.62*	
qSSP7-1	RG528-RG128	2.6		0.75**										
qSSP7-2	C1023-R1440	46.0	−1.07**	−0.64*	−0.55*									
qSSP7-3	RZ471-RM70	73.0	−0.75*		−0.72**									
qSSP9-1	R2638-RM257	67.8		−0.69**										
qSSP10-1	C153A-RM222	4.0	1.08**											
qSSP11-1	CD0534-RG2	83.6	−0.76*											

* P=0.05, significant; ** P=0.01, significant

of the locus could increase SSP with an allele from "Zhenshan 97" under normal growing conditions. In fact, when climate conditions were similar to those in 2005, this effect also worked with the allele from "Minghui 63."

Compared with additive effects, QTLs with conditional epistatic effects varied widely (Table 4). Six of seven unconditional QTLs with the *aa* effect exhibited insignificant effects after conditional mapping. Similar results were found for QTLs with the *aae* effect. Only the interaction between qSSP6-4 and qSSP10-2 had reduced *aa* effects. Ten new pairs of conditional *aa* loci also were identified, including qSSP1-4 and qSSP2-6, qSSP1-4 and qSSP11-4, qSSP1-4 and qSSP2-4, qSSP1-5 and qSSP6-5, qSSP2-3 and qSSP6-4, qSSP3-1 and qSSP6-5, qSSP3-3 and qSSP10-5, qSSP7-1 and qSSP8-2, qSSP7-1 and qSSP8-3, and qSSP7-2 and qSSP9-1.

Seed Set Percent Conditioned on Panicle Length

QTLs with additive and/or additive × environment interaction effects were also compared between the unconditional mapping of SSP and the conditional mapping of SSP given PL (Table 3). Four of eight unconditional QTLs with *a* effects (qSSP2-1, qSSP3-2, qSSP10-1, and qSSP11-1) were undetectable; three (qSSP1-2, qSSP7-2, and qSSP7-3) had a reduced magnitude of additive effects; and three new ones (qSSP3-1, qSSP3-3, and qSSP6-1) were detected. When given PL, qSSP5-1 exhibited an enhanced positive *a* effect, indicating that its genetic effects could depress the expression of this locus. All six unconditional *ae*-related loci (qSSP6-3, qSSP7-4, qSSP10-2, qSSP10-3, qSSP11-2, and qSSP11-3) failed to be detected by the conditional mapping approach, whereas three new conditional loci were found on chromosomes 1, 3, and 5, within the marker intervals of RM81A–G1128b, C1087–RZ403, and R830–R3166. Ten pairs of epistatic QTLs were identified for SSP conditioned on PL (Table 4). Six pairs of unconditional *aa* QTLs (qSSP1-5 and qSSP3-1, qSSP3-2 and qSSP10-3, qSSP4-1 and qSSP9-3, qSSP5-1 and qSSP12-2, qSSP7-2 and qSSP10-1, and qSSP8-1 and qSSP12-3) disappeared. Only one pair (qSSP6-4 and qSSP10-2) maintained a similar epistatic main effect, demonstrating that their interaction might increase (or decrease) SSP with either parental digenic allele (or a combination of alleles from different parents) independent of PL.

Discussion

Many methods have been proposed for evaluating correlations among related genetic traits. A superior means for analyzing phenotypic covariance or partitioning phenotypic covariance into components is the conditional method

Table 4 Unconditional and conditional epistatic effects (*aa*=additive × additive epistatic effect; *aae*=epistasis × environment interaction effect)

QTL*i*			QTL*j*			SSP			SSP|FGP	SSP|PL
QTL name	Marker interval	Position (cM)	QTL name	Marker interval	Position (cM)	*aa*	*aae* in 2004	*aae* in 2005	*aa*	*aa*
qSSP1-1	R753-G359	21.7	qSSP3-7	C1176-C316	0.0		1.32**	−1.43**		
qSSP1-3	RM81A-G1128b	99.9	qSSP12-1	R643-C87	107.6					−1.75**
qSSP1-4	C922-RG101	154.0	qSSP2-6	RM42-RG520	51.8				1.08**	
			qSSP11-4	L1044-Y2668LA	17.9				1.51**	
			qSSP2-4	RZ386-G1314a	94.3				−0.96**	
qSSP1-2	C2340-C86	192.2	qSSP3-9	C312-C63	6.9					−2.24*
			qSSP3-1	RG393-C1087	68.1	0.91**				
			qSSP6-5	RG653-G342	163.6				−0.74**	
			qSSP6-2	R2549-C962	128.5					−1.81**
qSSP2-3	RZ324-RM29	73.0	qSSP6-4	RG424-R2549	110.7				−0.92**	
qSSP2-4	RZ386-G1314a	94.3	qSSP7-5	R1440-RG678	48.3					1.01**
qSSP2-5	RM207-RM48	43.9	qSSP11-1	CD0534-RG2	83.6					−1.09**
qSSP3-5	C63-RM232	14.1	qSSP6-6	Waxy-C1496	15.7		−1.27**	1.35**		
qSSP3-1	RG393-C1087	68.1	qSSP6-5	RG653-G342	163.6				−1.07**	
qSSP3-2	C1087-RZ403	86.0	qSSP7-4	RM70-R1245	94.9		−1.26**	1.26**		
			qSSP10-3	RM258-RG561	97.7	1.66**				
qSSP3-3	R321-RM55	114.2	qSSP10-5	R2174-C909A	14.6				1.03**	
qSSP3-6	RM200-RM227	158.2	qSSP4-2	RM255-G235	56.8		1.30**	−1.22**		
qSSP4-1	C56-C820	8.0	qSSP9-3	C734-R1164	11.1	−1.69**				
qSSP4-2	RM255-G235	56.8	qSSP7-3	RZ471-RM70	73.0		1.86**	−1.75**		
qSSP5-1	R0360-RM42	30.1	qSSP12-2	R2672-C996	31.6	1.02**				
qSSP5-3	C1447-RM31	136.7	qSSP11-1	CD0534-RG2	83.6		−1.44**	1.39**		
qSSP6-1	C474-R3139	6.2	qSSP11-5	C794-R2918	153.5					1.57**
qSSP6-4	RG424-R2549	110.7	qSSP10-2	RM239-C1633	41.1	2.15*	1.25**	−1.27**	1.65*	2.14**
qSSP7-1	RG528-RG128	2.6	qSSP8-2	R1629-C483	29.4				−2.84**	
			qSSP8-3	C483-C347	41.4				0.98**	
qSSP7-2	C1023-R1440	46.2	qSSP10-1	C153A-RM222	4.0	−1.34*				−1.71*
			qSSP9-1	R2638-RM257	67.8				−0.90**	
			qSSP11-6	G4001-C1003B	59.6					−1.50**
qSSP8-1	G1149-R2272	102.9	qSSP12-2	G1314b-R643	85.1	−1.89**				
qSSP9-2	C472-R2638	44.5	qSSP11-6	G4001-C1003B	59.6					−1.44**
qSSP10-4	RM239-C1633	22.1	qSSP11-7	R3203-RM20a	168.7		1.18**	−1.18**		

*P=0.05, significant; **P=0.01, significant

described by Zhu (1995), in which the net genetic effects and variance components are examined by excluding variations in given traits. This technique has been used to investigate causal genetic effects for correlated traits (Mei et al. 2007) and developmental traits (Ye et al. 2003). To probe the genetic control system of correlated traits on individual loci, we took this conditional approach, combined with mixed model-based interval mapping (Zhu 1999), to decompose the genetic links between correlated traits at the level of an individual QTL. This philosophy has previously been followed to map conditional QTLs for molecular dissection of the development of traits such as rice plant height and tiller number (Yan et al. 1998a, b; Cao et al. 2001) as well as to evaluate the genetic contribution of yield components to yield (Li et al. 2008). Here, we adopted this methodology to analyze the interrelationship between SSP and FGP and between SSP and PL.

We determined that four types of QTLs control SSP. First, common or linked QTLs for pair traits that could be mapped by an unconditional approach were undetectable via conditional mapping. For example, unconditional QTLs qSSP7-3 and qFGP7-1, located within interval RZ471–RM70, were mapped for SSP and FGP. For these, we explored the publicly available QTL database (http://www.gramene.org) to search for the alignment of QTLs previously identified in the same region. Identical QTLs have been reported for filled grain number (Zhuang et al. 2002; Xing et al. 2002; Brondani et al. 2002), seed set percentage (Zhuang et al. 2002), and spikelet number (Zhuang et al. 2002; Xing et al. 2002; Jiang et al. 2004). These results showed pleiotropy of the locus within interval RZ471–RM70, which impacted both SSP and FGP. Our conclusion was confirmed by the failed detection of a conditional QTL for SSP on FGP. Other similar QTLs were mapped, e.g., within interval RM211–RG634 for SSP, and within interval C153A–RM222 for FGP.

Second, some QTLs showed similar effects, whether by conventional or conditional mapping, including qSSP7-3 for SSP and SSP conditioned on PL. Differing from that for SSP and FGP, this QTL failed to be mapped for panicle length in our study. Because qSSP7-3 affected the behavior of SSP independent of PL, it provided the possibility for improving the former but having no impact on the latter. Recent progress in plant genome analysis has enabled researchers to examine the molecular basis for naturally occurring allelic variations that account for complex traits. Map-based or positional cloning has successfully isolated genes underlying QTLs in several plant species, including rice (Yano et al. 2000; Ashikari et al. 2005; Song et al. 2007) and tomato (Frary et al. 2000; Liu et al. 2002). Fan et al. (2006) have elucidated the molecular mechanism for the gene underlying GS3 for grain length and width. A newly discovered quantitative trait locus, GW2, which encodes a new RING-type E3 ubiquitin ligase, regulates rice grain width and weight (Song et al. 2007). Therefore, it is possible to find and clone the gene that underlies SSP but has a relatively small or no relationship with PL.

The third type of QTL was one with reduced (or increased) effects after conditional mapping. For example, the QTL located within interval R0360–RM42 exhibited reduced effects for SSP given FGP but had an increased effect for SSP given PL. They were identified for SSP but were significantly affected by FGP or PL. Although the unconditional genetic effects of these loci were relatively small and insignificant for FGP and PL, their information that would improve SSP might be disadvantageous to FGP or PL.

Finally, new QTLs were detected only by conditional mapping, e.g., qSSP6-2 for SSP conditioned on FGP, and qSSP3-1 for SSP given PL. The QTL effects on SSP were suppressed by these two given traits. Although if one could keep those traits from changing, these QTLs could be improved , it is too difficult to do so when modifying a correlated trait.

QTLs with epistatic effects were also included in our classification of types. However, the positioning and effects of most epistatic QTLs changed largely after conditional mapping, which suggested that interactions between loci depended largely on the internal condition of the plant system (e.g., synthetic genetic control for SSP). Once that background changed, the behavior of epistasis involving different non-allelic genes shifted correspondingly. Moreover, it was fairly common for one locus to interact with more than one non-allelic locus, perhaps indicating the possibility of multilocus associations for correlated traits. Because interactions were greatly affected by environment, the contribution of any locus to a particular trait should also have varied according to growing conditions. This implied, due to some epistatic interactions having only *aae* effects, that gene expression of these epistatic interactions could be mainly induced by environment. We detected more pairs with epistatic interactions (7, 10, and 14 pairs for SSP, FGP, and PL, respectively), and fewer QTLs with additive effects (only six, one, and three QTLs for SSP, FGP, and PL); these were mainly induced by specific environmental conditions. Therefore, this might indicate that epistasis was more sensitive than the additive effect to such conditions.

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References

Ashikari M, Sakakibara H, Lin S et al (2005) Cytokinin oxidase regulates rice grain production. Science 309:741–745

Brondani C, Rangel P, Brondani R et al (2002) QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. Theor Appl Genet 104:1192–1203

Cao GQ (2000) QTL analysis of epistatic and conditional effects for some agronomic traits in rice (*Oryza sativa* L.). Ph.D. Dissertation, Zhejiang University, Hangzhou, China

Cao G, Zhu J, He C et al (2001) Impact of epistasis and QTL × environment interaction on the developmental behavior of plant height in rice (*Oryza sativa* L.). Theor Appl Genet 103:153–160

Fan C, Xing Y, Mao H et al (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112:1164–1171

Frary A, Nesbitt TC, Grandillo S et al (2000) Cloning and transgenic expression of fw 2.2: A quantitative traits locus key to the evolution of tomato fruits. Science 289:85–87

Guo LB, Xing YZ, Mei HW et al (2005) Dissection of component QTL expression in yield formation in rice. Plant Breed 124 (2):127–132

Jiang GH, Xu CG, Li XH et al (2004) Characterization of the genetic basis for yield and its component traits of rice revealed by doubled haploid population. Acta Genet Sin 31:63–72

Lander ES, Botsein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Li JM, Thomson M, McCouch SR (2004) Fine mapping of a grain-weight quantitative trait locus in pericentromeric region of rice chromosome 3. Genetics 168:2187–2195

Li YL, Dong YB, Cui DQ et al (2008) The genetic relationship between popping expansion volume and two yield components in popcorn using unconditional and conditional QTL analysis. Euphytica 162(3):345–351

Liu JP, van Eck J, Cong B et al (2002) A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci U S A 99(20):13302–13306

McCouch SR, Cho YG, Yano M et al (1997) Report on QTL nomenclature. Rice Genet Newslett 14:11–13

Mei YJ, Ye ZH, Xu Z (2007) Genetic impacts of fiber sugar content on fiber characters in Island cotton (*Gossypium barbadense* L.). Euphytica 154:29–39

Moncada P, Martinez CP, Borrero J et al (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment. Theor Appl Genet 102:41–52

Rao SA, Khan MA, McNeilly T et al (1997) Cause and effect relations of yield and yield component in rice (*Oryza sativa* L.). J Genet Breed 51:1–5

Septiningsih EM, Prasetiyono J, Lubis E et al (2003) Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. Theor Appl Genet 107:1419–1432

Song XJ, Huang W, Shi M et al (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nature Genet 39(5):623–630

Thomson MJ, Tai TH, McClung AM et al (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theor Appl Genet 107:479–493

Xiao J, Li J, Yuan L et al (1998) Identification of QTL affecting traits of agronomic importance in a recombinant inbred population from a subspecific rice cross. Theor Appl Genet 92:230–244

Xing YZ, Xu CG, Hua JP et al (2001) Analysis of QTL × environment interaction for rice panicle characteristics. Acta Genet Sin 28(5):439–446

Xing YZ, Tan YF, Hun JP et al (2002) Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. Theor Appl Genet 105:248–257

Yan JQ, Zhu J, He CX, Benmoussa M et al (1998b) Quantitative trait loci analysis for the developmental behavior of tiller number in rice (*Oryza sativa* L.). Theor Appl Genet 97:267–274

Yan JQ, Zhu J, He CX, Benmoussa M et al (1998a) Molecular dissection of developmental behavior of plant height in rice (*Oryza sativa* L.). Genetics 150:1257–1265

Yang J, Zhu J, Williams RW (2007) Mapping the genetic architecture of complex traits in experimental populations. Bioinformatics 23:1527–1536

Yano M, Katayose Y, Ashikari M et al (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell 12:2473–2484

Ye ZH, Lu ZZ, Zhu J (2003) Genetic analysis for developmental behavior of some seed quality traits in upland cotton (*Gossypium hirsutum* L.). Euphytica 129(2):183–191

Yu SB, Li JX, Xu CG et al (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci U S A 94:9226–9231

Zhao JY, Becher HC, Zhang DQ et al (2006) Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. Theor Appl Genet 113:33–38

Zhu J (1992) Mixed model approaches for estimating genetic variances and covariances. J Biomath 7:1–11

Zhu J (1995) Analysis of conditional genetic effects and variance components in developmental genetics. Genetics 141:1633–1639

Zhu J (1999) Mixed model approaches of mapping genes for complex quantitative traits. J Zhejiang Univ (Natural Sci) 33 (3):327–335

Zhuang JY, Fan YY, Rao ZM et al (2002) Analysis on additive effects and additive-by-additive epistatic effects of QTLs for yield traits in a recombinant inbred line population of rice. Theor Appl Genet 105:1137–1145