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Grain yield responses to moisture regimes in a rice population: association among traits and genetic markers

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Abstract Drought is a major constraint to rice (*Oryza sativa* L.) production in rainfed and poorly irrigated environments. Identifying genomic regions influencing the response of yield and its components to water deficits will aid our understanding of the genetic mechanism of drought tolerance (DT) of rice and the development of DT varieties. Grain yield (GY) and its components of a recombinant inbred population developed from a lowland rice and an upland rice were investigated under different water levels in 2003 and 2004 in a rainout DT screening facility. Correlation and path analysis indicated that spikelet fertility (SF) was particularly important for grain yield with direct effect ($P = 0.60$) under drought stress, while spikelet number per panicle (SN) contributed the most to grain yield ($P = 0.41$) under well-watered condition. A total of 32 quantitative trait loci (QTLs) for grain yield and its components were identified. The phenotypic variation explained by individual QTLs varied from 1.29% to 14.76%. Several main effect QTLs affecting SF, 1,000-grain weight (TGW), panicle number (PN), and SN were mapped to the same regions on chromosome 4 and 8. These QTLs were detected consistently across 2 years and under both water levels in this study. Several digenic interactions among yield components were also detected. The identification of genomic regions associated with GY and its components under stress will be useful to improve drought tolerance of rice by marker-aided approaches.

Abbreviations *A*: Additive effect · *AA*: Additive × additive epistasis · *AE*: Additive × environment interaction · *AAE*: Epistasis × environment interaction · DT: Drought tolerance · E-QTL: Epistatic QTL · GY: Grain yield · M-QTL: Main effect QTL · PN: Panicle number · QTL: Quantitative trait locus · *Q*×*E*: QTL × environment interaction · RIL: Recombinant inbred line · SF: Spikelet fertility · SN: Spikelet number per panicle · SSR: Simple sequence repeats · TGW: 1,000-grain weight

Introduction

Rice is grown widely under rainfed conditions in Asia, and about 45% of the total rice area is estimated to have no irrigation input (Crosson 1995). Drought is a serious limiting factor to rice production and yield stability in these areas (Dey and Upadhyaya 1996). Drought-stress is an unpredictable event and its timing and intensity vary during different stages of crop growth.

Many studies have been undertaken to find genetic variation in traits that are expected to influence the response of rice to water deficit, including deeper and thicker roots (Yadav et al. 1997); root pulling resistance (Pantuwan et al. 2002); greater root penetration (Clark et al. 2000; Ali et al. 2000); osmotic adjustment (Lilley et al. 1996); membrane stability (Tripathy et al. 2000); leaf rolling score and leaf relative water content (Courtois et al. 2000). However, most of these traits have not been proven to be effective in increasing yield under drought conditions. Grain yield is determined as a result of many interacting processes, and increased drought resistance by enhancing a particular trait mentioned above does not necessarily result in a higher yield. In

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addition, a particular trait may be useful under a particular set of drought conditions but not under others. It is likely that different mechanisms are involved in responding to different types of drought. Blum et al. (1996) argued that the lack of concept, direction, and protocol had remained a significant obstacle to genetic improvement of drought tolerance.

In order to better understand the mechanism of drought tolerance, a standard assessment with a special facility used to screen drought tolerance was established recently in Shanghai, China (Liu et al. 2005). The facility enables the creation of a water gradient from well-watered condition to water stress in a block simultaneously and provides a controlled condition for DT screening.

To understand the response of yield and its components to water stress, a RI population was phenotyped in the facility in 2003 and 2004. Using a saturated SSR linkage map, main effect QTL, digenic epistasis and QTL × Environment (*Q*×*E*) interactions were investigated. The information obtained in this study will be helpful for us to understand the genetic control of yield and its components under well-watered and water stress conditions.

Materials and methods

Plant materials and field experiments

A set of 187 F_9 recombinant inbred lines developed from Zhenshan 97B (ZS97B, lowland indica rice, CMS maintainer with the largest production area in China) and IRAT109 (upland rice) and the two parents were planted in the drought screening facility in 2003 and 2004.

Each genotype was directly seeded in two-row plots in random block design with three replications. There were two field blocks surrounded by 1.8 m drainage canals in the facility. In each field block, two 15-plant rows were arranged from canal to canal, and a drip line was placed in the middle between the two canals. Water was fully supplied by the sprinkler irrigation at the seedling stage. The irrigation was stopped on July 22, and water stress was allowed to develop. As the soil moisture decreased more rapidly at one end of the field close to the drainage canals, a gradient of soil moisture formed along each row of 15 rice plants. Drip irrigation was set along the space between two row plots to apply more water to about 4 or 5 plants in the part of the row farthest from the canals. These represented the non-stress plants. Drip irrigation was engaged from Aug 11 in 2003 or just after the stop of sprinkler irrigation in 2004 to apply about 4 tons of water per day in an area of about 900 m^2. The drip system enhanced the soil moisture gradient and helped the gradient to last for a longer period. Full irrigation was resumed on September 8 after severe leaf rolling was observed in the sensitive lines as described in our former report (Liu et al. 2005).

Yield data and its components were collected from two plants under well-watered and two plants under stress condition located next to both ends of each row-plot. At harvest, panicle number per hill (PN) was measured. Filled and unfilled spikelets were measured to determine the spikelet number per panicle (SN). Percentage of spikelet fertility (SF) was calculated from the filled grain numbers and SN. Grains were threshed from the sampled plants, dried in a oven at 80°C for 24 h, and then weighed to determine grain yield per plant (GY) and 1,000-grain weight (TGW).

Data analysis and QTL mapping

ANOVA was performed based on fixed effect model by using S-Plus for Windows V6.1 (Insightful Corporation 2001). Phenotypic correlation coefficients were calculated using the mean values. Path-coefficient analysis was conducted by multiple regressions to determine the factors contributing to GY. Broad sense heritability (H^2) was estimated as: $H^2 = V_G/(V_G + V_B + V_E)$, where V_G is the genetic variation between RILs, V_B is the variation among blocks, and V_E is the variation of error. As the drip irrigation was applied in different dates in 2003 and 2004, the heritability was estimated separately for 2 years.

A total of 213 SSR markers were used to genotype the population. A linkage map spanning 1825.0 cM of genome size with an average distance of 9.4 cM between adjacent markers was constructed by using MapMaker/Exp V3.0 (Lincoln et al. 1992).

QTL analysis was conducted by using QTLMapper V1.6 based on a mixed model approach (Wang et al. 1999). Means of three replications were used as input data while different years were regarded as environmental factors. Data collected from plants under well-watered condition and drought stress were analyzed separately during QTL mapping. A threshold of $P \leq 0.005$ was used to declare significant main effect QTLs (M-QTL), digenic epistatic QTLs (E-QTL), and *Q*×*E* interaction. Additive effect was estimated as the phenotypic difference when the IRAT109 allele was substituted by the ZS97B allele. Epistatic effect was estimated according to the definition by Mather and Jinks (1982). Contribution rate (h^2) was estimated as percentage of variance explained by each locus or epistatic pair in proportion to total phenotypic variance. M-QTLs were named following the popular nomenclature (McCouch et al. 1997), but using alphabetic order for QTLs on the same chromosome.

Results

Performance of yield and its components under well-watered and stress conditions

The mean values of grain yield and its components of the parents and the population under well-watered and stress conditions are shown in Table 1. The phenotypic values of the traits in the RI lines have continuous distribution, which approximately fitted normality with skewness less than 1.4, indicating that all measured traits were quantitatively inherited. Highly significant differences were observed among the lines for all the traits measured. ANOVA also indicated that the effect of water treatments was significant for yield and for its components in both years. And significant treatment-by-genotype interaction was observed for yield and its components, except for PN (Table 1).

The 2-year average yields of ZS97B and IRAT109 were 13.17 g and 10.13 g in well-watered condition, and 3.29 g and 8.64 g in water stress conditions (Table 1). Yield loss from water stress was 75% for ZS97B and 15% for IRAT109, respectively. ZS97B produced higher GY than IRAT109 under well-watered condition but much lower GY than IRAT109 under stress condition. The two-year average GY of RI lines in well-watered and water stress conditions was 8.10 g and 5.20 g, respectively, and was reduced by approximately 37% under water stress.

ZS97B produced a higher number of panicles than IRAT109 under different water levels in the two years (Table 1). Average values of PN of the two parents and the RI lines were significantly different across different water levels. This indicated that panicle initiation and development were affected by water deficit. PN was determined by the onset of drought stress.

Spikelet number per panicle was significantly different between two parents. ZS97B produced more SN than IRAT109 under well-watered condition. The SN of ZS97B decreased dramatically while the SN of IRAT109 and mean value of the RI lines declined slower than ZS97B as water stress was imposed during panicle initiation (Table 1).

Under well-watered condition, the spikelet fertility of ZS97B and IRAT109 was not significantly different. The spikelet fertility of ZS97B and the RI lines decreased dramatically in 2003 and significantly but with less magnitude in 2004 under drought stress, while that of IRAT109 decreased more slightly in both years (Table 1). This result indicates that drought stress in 2003 was more severe than in 2004. The difference of spikelet fertility was large among the RI lines under different water levels.

TGW was also decreased significantly by the drought, but the loss was not as remarkable as that of other traits (Table 1). The TGW of ZS97B and IRAT109 was significantly different ($P < 0.01$) and both lost about 1–2 g under stress. In RI population, significant effects were observed from the water treatments, genotypes, and interaction between them.

The broad sense heritability (H^2) was estimated for each trait under well-watered and stress conditions for both years (Table 2). The H^2 values for GY were the lowest among all traits and varied from 0.43 to 0.59 across water stress and non-stress in two years (except $H^2 = 0.35$ for SN under stress in 2003). TGW had the highest H^2 values (from 0.61 to 0.86). For most cases, higher H^2 were observed under well-watered condition than under water stress. The H^2 values were also higher in 2004 than in 2003 caused by relatively better water supply in 2004 by earlier application of drip irrigation.

Phenotypic correlations

There was significant positive correlation between phenotypic values observed in the two water treatments for all traits. TGW showed the largest correlation coefficient ($r = 0.88^{**}$) while the GY showed the smallest ($r = 0.56^{**}$), suggesting TGW was more stable but GY was more easily affected by drought (Table 3). Even for GY, the coefficient was quite high, showing a positive effect of performance in favorable condition (i.e. potential values) on yield performance in drought condition.

Grain yield (GY) was positively correlated with all other traits under both water conditions. In well-watered condition, GY was highly correlated with SN ($r = 0.65$) and SF ($r = 0.51$), but less correlated with PN ($r = 0.19$) and TGW ($r = 0.17$). In water stress, the highest coefficient was observed between GY and SF ($r = 0.66$), followed by that between GY and PN ($r = 0.43$). Lower values were observed for GY and TGW ($r = 0.28$) and

Table 1 Performance of parents and 187 recombinant inbred lines (RILs) across two water levels in 2 years

Year	Traits	Control				Stress				Significant effects
		ZS97B [a]	IRAT109 [a]	RILs [a]	Skewness	ZS97B [a]	IRAT109 [a]	RILs [a]	Skewness	
2003	GY (g)	10.12 ± 0.93	7.60 ± 0.94	7.07 ± 4.05	1.07	1.92 ± 0.79	7.5 ± 1.16	4.36 ± 2.64	1.12	W** G** W×G**
	PN(panicle)	8.0 ± 1.0	6.0 ± 2.2	6.8 ± 2.2	1.32	6.2 ± 1.2	4.8 ± 0.9	5.5 ± 1.9	0.56	W** G**
	SN (spikelet)	131.4 ± 7.5	116.8 ± 22.3	106.2 ± 46.4	1.39	85.7 ± 23.8	115.6 ± 20.4	100.4 ± 44.1	0.92	W* G ** W×G**
	SF (%)	66.21 ± 9.69	75.59 ± 6.69	51.74 ± 15.96	0.12	19.53 ± 7.47	67.27 ± 8.63	39.16 ± 16.24	0.13	W** G** W×G**
	TGW (g)	23.34 ± 0.92	27.60 ± 0.23	22.77 ± 3.15	0.06	22.38 ± 0.480	25.13 ± 0.15	21.61 ± 3.52	0.25	W** G** W×G**
2004	GY (g)	16.96 ± 1.19	12.61 ± 4.28	9.12 ± 3.57	0.54	6.0 ± 0.79	9.00 ± 2.23	6.03 ± 2.73	0.44	W** G** W×G**
	PN (panicle)	7.8 ± 0.8	6.0 ± 1.8	6.7 ± 1.6	0.74	6.3 ± 0.5	4.9 ± 0.6	5.9 ± 1.5	0.34	W** G**
	SN (spikelet)	140.6 ± 11.2	127.2 ± 8.2	113.5 ± 38.1	1.19	109.9 ± 5.0	123.8 ± 7.4	110.7 ± 32.6	0.89	W** G** W×G**
	SF (%)	71.00 ± 6.95	72.79 ± 10.05	62.09 ± 14.68	0.24	60.1 ± 5.42	67.20 ± 5.91	55.53 ± 20.61	−0.39	W** G** W×G**
	TGW (g)	22.09 ± 0.10	27.86 ± 0.23	23.60 ± 2.83	0.33	21.14 ± 0.07	25.99 ± 0.10	22.00 ± 3.45	0.25	W** G** W×G**

[a] Performances of parents and RI lines were represented by mean value followed by standard deviation (mean ± sd); $^{*}P < 0.05$; $^{**}P < 0.01$

Table 2 Broad sense heritability of grain yield and its components estimated in ZS97B/IRAT109 RI population for 2 years under different water conditions

Years	Traits	Well-watered	Water stress
2003	GY	0.43	0.48
	TGW	0.67	0.61
	PN	0.64	0.62
	SF	0.58	0.50
	SN	0.53	0.35
2004	GY	0.59	0.50
	TGW	0.86	0.74
	PN	0.53	0.42
	SF	0.59	0.65
	SN	0.75	0.53

GY and SN ($r = 0.27$). These results suggested that water stress influenced the magnitude of associations of yield components with grain yield in rice. SN was the highest contributor to GY in well-watered condition, but SF was more important under drought stress.

Path analysis was used to partition the correlation coefficients into direct and indirect effects (Table 3). The results indicated that all yield components had positive direct effects on grain yield in both water levels. In well-watered condition, SN had the highest direct effect on GY followed by SF, PN and TGW. SN also had the highest indirect effect on GY. All traits had positive indirect effects on GY except for PN. The negative indirect effect from PN was probably caused by the significant negative correlation between PN and SN ($r = -0.33$). Larger PN caused smaller panicle (lower SN), resulting in lower yield. In water stress condition, SF had the highest direct effect on GY followed by SN, PN and TGW. PN and SN had the negative indirect effect on GY. The negative indirect effect from SN was quite high and probably caused by the significant negative correlation between SN and PN ($r = -0.35$) and between SN and SF ($r = -0.19$).

Main QTLs for yield and its components

Thirty-two main effect QTLs, distributed at 26 genetic regions, were detected for the traits studied (Table 4). Among them, 18 and 14 QTLs for GY and its components were found in well-watered condition and in water stress condition, respectively.

Under well-watered condition, three QTLs were detected for GY including *qGY-3* and *qGY-10* having positive effect (i.e. positive allele came from ZS97B), *qGY-4* with negative additive effect (i.e. negative allele came from ZS97B). No significant $Q \times E$ interaction was detected. Two QTLs (*qPN-2* and *qPN-4b*) were found to influence PN by 0.29 and 0.44. Two QTLs had negative effect (*qSN-4a*) or positive effect (*qSN-8b*) on SN. Three QTLs were detected for SF (*qSF-3*, *qSF-8a*, and *qSF-8b*) that had the positive additive effect and the other two (*qSF-2a*, *qSF-7b*) had negative additive effects. Six QTLs for TGW were identified on chromosome 1, 2, 3, 4, and 12 with negative additive effects. The contribution rate of single QTL varied from 1.94% to 14.76% for additive effect among all traits.

Under water stress, two QTLs were associated with GY (*qGY-2a* and *qGY-2b*) with negative additive effects. Two QTLs were located on chromosome 4 and 8 for PN designated as *qPN-4a* and *qPN-8* having small positive effect. Three QTLs were detected for SN, including *qSN-8a* and *qSN-9* with positive effect, and *qSN-4b* with negative effect. Four QTLs were declared for SF, including *qSF-7a*, *qSF-8a* and *qSF-8b* having positive effect, *qSF-2b* with negative effect. Three QTLs for TGW were identified on chromosome 2, 3, and 4 with negative additive effects. The additive effect of single QTLs could only explain 1.29% to 12.85% of total variance.

In this study, only two loci were detected with significant QTL$\times E$ interaction. One was for SF (*qSF-7b*) showing $Q \times E$ interaction ($h^2 = 7.36\%$) under well-watered condition. The other was for SN (*qSN-9*), which can explained 9.17% of total variance for SN under water stress condition.

Digenic interactions

In this study, epistatic interactions between QTLs were identified for all measured traits under different water levels (Table 5). In well-watered condition, a total of

Table 3 Phenotypic correlations between well-watered and water stress conditions, and correlation/path analysis between grain yield and its components in either well-watered condition or water stress condition estimated in 187 ZS97B/IRAT109 RILs

Trait	Well-watered versus water stress	Yield components with GY					
		Well-watered			Water stress		
		Correlation coefficient	Direct effect	Indirect effect	Correlation coefficient	Direct effect	Indirect effect
GY	0.56**						
PN	0.69**	0.19**	0.27	−0.08	0.43**	0.49	−0.06
SN	0.84**	0.65**	0.41	0.24	0.27**	0.54	−0.27
SF	0.69**	0.51**	0.34	0.17	0.66**	0.60	0.06
TGW	0.88**	0.17*	0.14	0.03	0.28**	0.28	0.00

*$P < 0.05$; **$P < 0.01$

Table 4 Main effect QTLs influencing grain yield and its components under well-watered and water stress conditions in the rice RI population

Trait		Interval	LOD	*Ai*	*AEi*	$h^2(Ai)$	$h^2(AEi)$	$h^2(A)$	$h^2(AEi)$
QTLs detected under well-watered condition									
qGY-3	3	RM132-RM22	2.89	0.53		3.37		7.45	
qGY-4	4	RM451-RM317	4.06	−0.58		4.08			
qGY-10	10	RM294A-RM228	2.38	0.42					
qPN-2	2	RM318-RM6	3.53	0.29		3.99		13.16	
qPN-4b	4	RM317-RM255	7.48	0.44		9.17			
qSN-4a	4	RM317-RM255	12.09	−10.76		13.81		18.93	
qSN-8	8	RM80-RM149	4.26	6.54		5.11			
qSF-2a	2	RM526-RM525	3.14	−2.18		2.55		15.38	
qSF-3	3	RM132-RM22	2.77	1.90		1.94			
qSF-7b	7	RM134-RM420	4.2	−1.36	−2.62		7.36		7.36
qSF-8b	8	RM404-RM339	5.14	3.05		4.99			
qSF-8c	8	RM342-RM515	3.08	2.48		3.31			
qTGW-1	1	RM476B-RM315	5.32	−0.05		5.55		34.85	
qTGW-2a	2	RM526-RM525	9.02	−0.07		9.66			
qTGW-3a	3	RM426-RM203	3.67	−0.05		4.69			
qTGW-4	4	RM119-RM273	5.4	−0.06		7.21			
qTGW-12a	12	RM415-RM4A	3.8	−0.04		3.37			
qTGW-12b	12	RM463-RM235	2.35	−0.04		3.73			
QTLs detected under water stress condition									
qGY-2a	2	RM561-RM341	4.26	−0.49		4.91		12.37	
qGY-2b	2	RM526-RM525	4.23	−0.50		5.05			
qPN-4a	4	RM451-RM317	4.61	0.30		5.73		9.5	
qPN-8	8	RM404-RM339	3.62	0.25		3.77			
qSN-4b	4	RM255-RM349	13.2	−10.82		12.85		26.26	
qSN-8	8	RM80-RM149	6.66	6.87		5.18			
qSN-9	9	RM215-RM245	12.88	8.66	6.46	8.23	9.17		9.17
qSF-2b	2	RM525-RM318	3.55	−2.75		2.55		10.66	
qSF-7a	7	RM478-RM134	2.46	1.96		1.29			
qSF-8a	8	RM544-RM547	3.51	2.75		2.54			
qSF-8b	8	RM404-RM339	2.51	2.50		2.1			
qTGW-2b	2	RM525-RM318	3.07	−0.05		3.28		20.95	
qTGW-3b	3	RM520-RM571	6.39	−0.07		8.48			
qTGW-4	4	RM119-RM273	6.22	−0.08		9.18			

nine epistatic interactions were detected for GY and its components. One significant QTL pair for each trait was detected in GY, PN, SN, and SF, respectively. The epistatic effect (*AAij*) explained 1.93% to 8.30% of total variance. Five QTL pairs were detected for TGW, contributing from 1.44 to 2.01% of the variance. Most epistatic QTLs involved one interval having a significant main effect. Only the last QTL pair in TGW did not involve any main effect locus, but had significant AAE interaction. In water stress condition, 12 epistatic QTL pairs were detected. The contribution rates of a single QTL pair varied from 1.29 to 8.18%. One pair of epistatic loci involved two intervals both having significant main effects while the other 11 QTL pairs involved one main effect locus. No epistasis × environment interaction was detected in the stress condition.

Discussion

DT screening facility and effects of water stress

In this study, the experiment was conducted in a rainproof DT screening facility with an accurate water control system. The facility can create a water gradient from the middle to the edge of blocks, thus allowing the assessment of different levels of water stress at specific growth stages (Liu et al. 2005). The effect of water treatments was significant for yield and for its components in both years (Table 1). Compared to well-watered condition, drought stress reduced the grain yield of the sensitive parent by 75% and the tolerant parent by 15%, while grain yield of RILs were decreased by 37% on average. The yield loss varied from 15 to 100% for different lines. The accurate application of water treatments in this study allowed the population to express genetic differences for DT, not only for some physiological behavior like leaf water potential and relative water content (Liu et al. 2005); but also for grain yield and its components.

The components affecting GY under water stress and well-watered conditions

Highly significant correlations were observed between performances under well-watered and drought conditions for GY and all yield components in this study. On

Table 5 Digenic epistasis and $Q \times E$ interaction influencing grain yield and its components under well-watered and water stress condition in rice

Trait	Ch-Int *i*	Interval *i*	Ch-Int *j*	Interval *j*	LOD	*Ai*	*Aj*	*AAij*	*AAEij*	$h^2(Ai)$	$h^2(Aj)$	$h^2(AAij)$	$h^2(AAEij)$
Digenic epistasis detected under well-watered condition													
GY	1-12	RM9-RM294B	10-9	RM294A-RM228	4.09		0.486	0.445			2.30	1.93	
PN	4-12	RM317-RM255	6-5	RM225-RM204	12.09	0.455		0.479		7.49		8.30	
SN	1-2	RM495-RM476A	4-9	RM241-RM470	5.75		−5.593	−5.973			2.22	2.53	
SF	5-4	RM13-RM548	6-17	RM275-RM528	6.33	1.921		2.51		1.46		2.50	
TGW	2-21	RM213-RM535	4-6	RM119-RM273	8.63		−0.069	0.039			6.30	2.01	
	3-2	RM22-RM231	12-11	RM463-RM235	10.12		−0.047	0.061			2.92	4.92	
	5-11	RM509-RM430	12-1	RM415-RM4A	5.64		−0.041	0.033			2.22	1.44	
	6-2	RM435-RM587	12-11	RM463-RM235	4.55		−0.045	−0.042			2.68	2.33	
	9-13	RM278-RM553	11-9	RM21-RM206	4.55			0.035	−0.043			1.62	4.89
Digenic epistasis detected under water stress condition													
GY	2-14	RM526-RM525	3-17	RM130-RM514	6.07	−0.426		−0.31		2.60		1.38	
	2-15	RM525-RM318	7-4	RM500-RM432	5.9	−0.382		0.53		2.09		4.02	
PN	2-19	RM250-RM166	10-4	RM467-RM596	8.05	−0.214		0.392		2.44		8.18	
SN	4-10	RM470-RM451	8-17	RM149-RM477	10.48	−3.632	4.858	−7.111		1.00	1.80	3.85	
	4-13	RM255-RM349	5-14	RM163-RM459	14.14	−10.488		−4.516		8.37		1.55	
	4-16	RM131-RM559	12-1	RM415-RM4A	4.65		4.721	−5.398			1.70	2.22	
	6-19	RM30-RM340	8-15	RM531-RM80	8.51		5.435	−7.64			2.25	4.44	
	7-8	RM346-RM336	8-17	RM149-RM477	8.54		8.234	−4.156			5.16	1.31	
SF	3-9	RM282-RM156	8-7	RM544-RM547	5.7		2.704	2.055			2.23	1.29	
	9-1	RM285-RM444	10-3	RM311-RM467	4.5		1.745	−2.651			0.93	2.14	
TGW	1-13	RM294B-RM486	3-14	RM520-RM571	16.03		−0.069	0.082			5.17	7.30	
	2-2	RM211-RM279	3-17	RM130-RM514	6.27		−0.039	0.069			1.65	5.17	

average, the phenotypic value under normal condition explained a large proportion of the variation for performance under drought. This result had two implications in breeding for drought tolerance: (1) Yield potential, expressed as the yield in a favorable environment, should be emphasized (Rajatasereekul et al. 1997; Fukai et al. 1999); (2) Selection in well-watered condition was generally effective to improve grain yield and its components (e. g. spikelet number per panicle, 1,000-grain weight) under drought. As the drought occurs intermittently among seasons and years, selection for yield potential in well-watered conditions and yield under drought in turn is appropriate for a DT breeding program.

It was indicated by correlation and path analysis that SF was the highest contributing factor to grain yield under water stress while SN was more important for grain yield than other yield components in the well-watered condition (Table 3). This result suggested that more emphasis should be put on SF under stress for a DT breeding program. A similar result has been reported by Boonjung and Fukai (1996), in which 40% of the yield reduction was due to the increment of spikelet sterility when drought occurred during the grain filling period.

In drought condition, lower correlation ($r = 0.27$) was observed between SN and GY (Table 3). But path analysis showed a high direct effect together with high indirect effects in opposite directions (Table 3). The negative indirect effect probably came from the negative effect from PN to SN ($P = -0.19$), and SN to SF ($P = -0.11$; data not shown). In this case, it could be concluded that genotypes with more panicles (PN) would offer a moderate panicle size (SN) and then give higher SF and GY under water stress.

Co-location of QTLs for grain yield and its components

QTL mapping indicated that many main effect QTLs and all epistatic QTL pairs were detected only in stress or non-stress condition, implying that some QTLs controlled GY and its components only under one water condition. However, it was also found that the yield components share a similar genetic basis under different water conditions. Several main effect QTLs (*qPN-4a/b*, *qSN-4a/b*, *qSN-8*, *qSF-2a/b*, *qSF-7a/b*, *qSF-8b*, *qTGW-2a/b*, and *qTGW-4*) were detected consistently under both water treatments in this study. Furthermore, the directions of additive effects of these loci were coincident between well-watered and stress conditions, except *qSF-7a/b* that had an opposite additive direction and $Q \times E$ interaction in well-watered conditions.

Only two cases of QTL-by-environment interactions and one case of epistasis-by-environment interaction were detected. This indicated that a highly coherent detection of QTLs was achieved between experiments conducted in two years. Compared to the higher proportion of QTL-by-year interaction detected in field experiments (Cooper et al 1999; Wade et al 1999), especially the conflicting results caused by varied stress between seasons, it was confirmed that the environmental conditions and the water stress treatments were

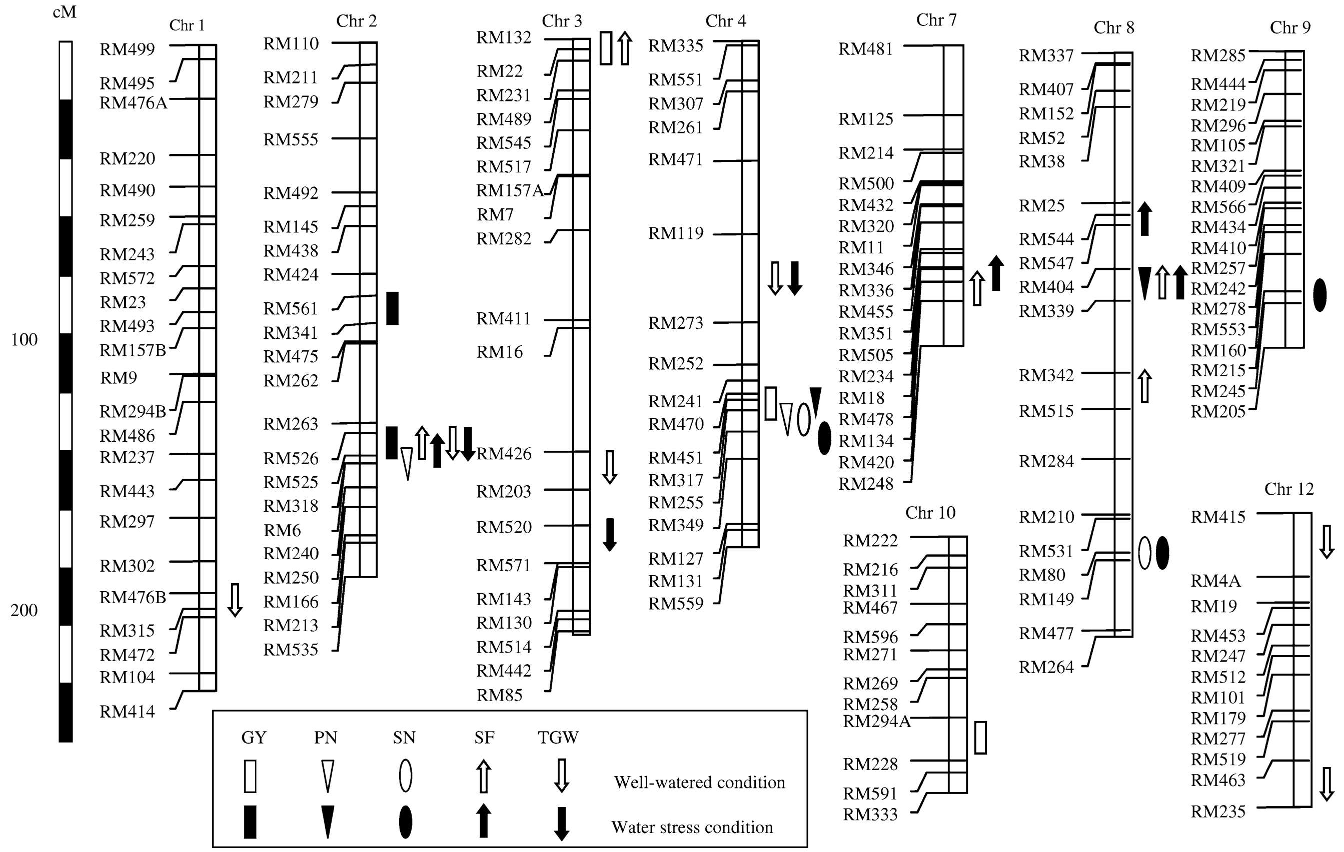

Fig. 1 Chromosomal locations of QTLs influencing grain yield and its components identified in the Zhenshan97B×IRAT109 RI population under water stress and well-watered conditions

well controlled in our DT screening facility (Liu et al. 2005).

Improving drought tolerance using QTL information

Phenotypically correlated traits are known to be mapped together (Lebreton et al. 1995; Shashidhar et al. 1999; Hittalmani et al. 2002). It was also observed that several QTLs for GY overlapped or were linked with several QTLs for its components in this study. For example, the RM526-RM525-RM318-RM6 interval on chromosome 2 contained the QTLs *qGY-2b*, *qPN-2*, *qSF-2a*, *qSF-2b*, *qTGW-2a*, and *qTGW-2b*. The allele from IRAT109 at this locus increased SF and TGW under both conditions, and increased the GY under stress. This region was reported to be associated with DT traits, such as root number (Hemamalini et al. 2000), root thickness, root depth and root pulling force (Kamoshita et al. 2002a, b), and root length and root number (Price et al. 2002a). Another region on chromosome 4 flanked by the markers RM241and RM349 contained the QTLs *qGY-4*, *qPN-4a*, *qPN-4b*, *qSN-4a*, *qSN-4b* (Fig. 1). IRAT109 contributed the favorable alleles for all QTLs except PN. QTLs were also reported in this region for root to shoot ratio (Price et al. 2002b), root length (Hemamalini et al. 2000), PN, SN and biomass (Lanceras et al. 2004), and canopy temperature (Liu et al. 2005). This result showed that closely-linked genes or genes with pleiotropic effect controlled yield and its components, and explained the significant positive correlation between GY and its components. It remains to be tested whether these genomic regions have pleiotropic effects or there are clusters of tightly linked genes for many drought-related traits in these regions. Detailed characterization of these genomic regions through the development and evaluation of near-isogenic lines will definitely lead to an improved understanding of drought tolerance and might set the stage for the positional cloning of drought tolerance genes. Marker assisted BC procedures has been initiated in our group to transfer IRAT109 alleles of chromosomal regions mentioned above into CMS maintainer lines (Zhenshan 97B, Huhan 1B) and restorer lines (Minghui 63, 752).

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