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QTLs for drought escape and tolerance identified in a set of random introgression lines of rice

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Abstract A large set of 254 introgression lines in an elite indica genetic background were evaluated for grain yield (GY) and related traits under the irrigated (control) and drought (stress) conditions in two consecutive years for genetic dissection of adaptive strategies of rice to water stress. A total of 36 quantitative trait loci (QTLs) affecting heading date (HD), plant height (PH), GY and yield components were identified and most QTLs showed pronounced differential expression either qualitatively or quantitatively in response to drought. These QTLs could be grouped into three major types based on their behaviors under control and stress conditions. Type I included 12 QTLs that expressed under both the stress and non-stress conditions. Type II comprised 17 QTLs that expressed under irrigation but not under stress. Type III included seven QTLs that were apparently induced by stress. The observation that the Lemont (japonica) alleles at all HD QTLs except *QHd5* resulted in early heading under stress appeared to be responsible for the putative adaptation of Lemont to drought by escaping, whereas the Teqing (indica) alleles at most PH/GY QTLs were consistently associated with increased yield potential and trait stability and thus contributed to DT. Our result that most DT QTLs were non-allelic with QTLs for drought escaping suggests that the two adaptive strategies in the parental lines are under possible negative regulation of two largely non-overlapping genetic systems.

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Introduction

Rice is particularly sensitive to drought during the reproductive stage, when it can lead to various degrees of sterility (Widawsky and O'Toole 1990). Crop tolerance to drought is complex both genetically and physiologically (Blum 2002). Many morpho-physiological traits putatively contribute to drought tolerance (DT), and each of these traits is typically controlled by multiple genes or quantitative trait loci (QTLs), and is influenced by environment to a great extent. Developing robust DT rice varieties has not been very successful despite the efforts made by breeders, because in practical breeding programs, populations are typically segregating for maturity, making it difficult to accurately and repeatedly time and manage a uniform and relevant water stress level for selection. To overcome this problem, it was proposed to improve DT by marker-assisted selection (MAS) for secondary traits if genes/QTLs affecting the secondary traits contributing to DT could be accurately mapped and characterized (Lafitte and Courtois 2000). Over the past decade, several DT component traits of rice, such as root traits and osmotic adjustment have been genetically dissected through QTL mapping (Champoux et al. 1995; Ray et al. 1996; Price and Tomos 1997; Zhang et al. 2001), and improving rice DT by MAS has been practiced (Shen et al. 2001). However, it remains unclear how to apply QTL information from mapping populations to genetic improvement of DT in breeding populations unrelated to the reference mapping populations because of possible epistasis and QTL-by-environment interactions, uncertain relationships between secondary traits and grain yield (GY) under drought, and unknown allelic diversity at identified DT QTLs in breeding materials (Li et al. 2000). Recently, Tanksley and Nelson (1996) proposed a promising strategy of using advanced backcross (BC) QTL, or AB-QTL, analyses for simultaneous introgression and detection of QTLs in elite genetic backgrounds. Because of its obvious advantages, this approach has been widely used in genetic dissection of quantitative trait variation (Bernacchi et al. 1998; Thomson et al. 2003; Huang et al. 2003).

We report here a genetic dissection of DT using the AB-QTL approach. The primary objective of this work was to use a large set of introgression lines (ILs) for identifying and characterizing main-effect QTL (M-QTL) and epistatic QTL (E-QTL) associated with GY and related traits under both irrigation and water stress around anthesis.

Materials and methods

Materials, field experiment and data collection

Teqing, a high-yielding semidwarf indica rice variety from China was used as the female parent to cross with Lemont, a commercial semidwarf japonica rice variety from the southern US. The F_1 plants were simultaneously backcrossed to Teqing to develop a BC_1F_1 population with 100 plants. The BC_1F_1 plants were used as the male parent to BC with Teqing to produce the BC_2F_1 population. Consecutive backcrossing was carried out in the same way until BC_3F_1 and BC_4F_1 populations, resulting in a set of Teqing near-isogenic ILs, consisting of 254 lines (133 BC_2F_5, 96 BC_3F_4 and 25 BC_4F_3), as described previously (J. L. Xu et al., submitted). Most near-isogenic ILs showed similar heading date (HD) in irrigated condition and those with significant early or late HD were excluded in this study. The ILs were evaluated in two replicated experiments under fully irrigated (non-stress) and water stress conditions in the experimental farm of the International Rice Research Institute (IRRI) in 2003 dry season (December – April). A subsample of lines from the population had also been evaluated under similar conditions in 2001 dry season. This subset, while providing much less power in QTL detection than the full population of 254 lines, represents a largely independent confirmation of results across years. Seeds of the ILs were sown in the seedbed and 30-day seedlings were transplanted into three-row plots (36 plants per plot or entry) with a spacing of $25\times20\ \mathrm{cm}^2$ in a randomized block design with an incomplete block arrangement in three replications in 2003. The field was managed according to standard experiment station procedures, with a basal fertilization rate of 30 kg of each N, P, and K ha^{-1} and two additional 30 kg ha^{-1} N applications, made at 44 and 66 days after sowing. Three applications of insecticide were applied to control brown planthoppers. Weeds were controlled by a combination of chemical and manual methods. For the stress treatment, the field was drained at 60 days after transplanting and no further irrigation was applied. This treatment resulted in leaf rolling by 15 days after the field was drained. By the date of heading in the control plots, soil moisture in the stress plots reached 100 kPa at 15 cm depth.

Heading date (days) was recorded when 50% plants in each plot were flowering. Data of plant height (PH, cm) were collected by measuring 15 plants in each plot at maturity. Grain yield (g m^{-2}) was recorded by harvesting all plants in each plot. In 2001, data on HD (days), GY (g m^{-2}) and its component traits including panicle number (PN, m^{-2}) and 1,000-grain weight (GW, g) were collected in a subset of 100 lines randomly selected from the same population evaluated under the conditions similar to 2003.

Linkage map construction and data analyses

The lines were assayed with 160 well distributed polymorphic SSR markers and genotypic data from 133 BC_2F_5 ILs were used to construct a complete linkage map for the ILs as described previously (J. L. Xu et al., submitted). This map covers all 12 rice chromosomes with a total genome size of 1,677 cM and an average distance of 10.5 cM between adjacent markers. Analysis of variance was performed to evaluate differences among the ILs, between the parents and the conditions (water irrigation and water stress) using the SAS PROC GLM (SAS Institute 1996). A mixed model was used in which the water conditions were treated as the fixed variable, and genotypes were treated as random variable (to get general information about the total variation in the population). Correlation between the three traits in each of the conditions and between lines for the same traits across the conditions was determined using the SAS PROC CORR (SAS Institute 1996).

Phenotypic data of the ILs for each year, obtained from both non-stress and stress conditions, were used as input data to identify M-QTL affecting PH, HD and GY by one-way ANOVA using SAS PROC GLM (SAS Institute 1996). In addition, trait differences (stress–non-stress) of the ILs between the stress and non-stress conditions were used to identify QTLs showing differential expression between the water conditions. The probability level of $P<0.005$ was used for claiming a significant M-QTL. When a QTL was detected by two or more linked markers, the one with the highest F value was presented. A mixed linear model was used to detect digenic interactions (E-QTL) using QTLMapper 1.0 (Wang et al. 1999) and the threshold to claim a statistically significant interaction was $P\leq 0.001$ and LOD≥3.0 (Li et al. 2001). All identified E-QTL pairs were also confirmed by two-way analysis of variance using SAS PROC GLM (SAS Institute 1996). The maximum likelihood estimates of individual interaction effects, τ_{ij} associated with each pair of interacting alleles in a significant interaction were obtained using the mean trait values of the four digenic genotypes (Graybill 1976), and t tests were performed to test the null hypotheses Ho: $\tau_{ij}=0$ using the method described previously (Li et al. 1997).

We realize that the use of a single arbitrary threshold in QTL mapping could easily detect a QTL in one environment but not in another. To examine the extent to which inconsistent QTL detection across the two conditions actually arose from type-II errors, all identified M-QTLs and E-QTLs in one condition were reexamined using the data from the other condition under the minimum threshold of $P < 0.05$. In other words, when a QTL was identified using the data from the irrigated experiment, this QTL was also tested by the data from the stress condition and vice versa, and the test statistics and QTL parameters associated with the QTL are also reported as long as the QTL reached the minimum threshold. In addition, QTLs from the subset of lines collected in 2001 were compared with the 2003 results.

Results

Phenotypic variation of the ILs

Table 1 shows summary statistics of the phenotypic performance of the ILs and parents for the measured traits under the two conditions across years. ANOVA results indicated that the differences between the stress conditions for PH and GY, among the ILs for HD, PH and GY, and variances due to the stress by IL interaction, for HD and GY were all highly significant in 2003. The three variance components accounted for 0.01, 46.01 and 21.68% for HD; 66.32, 9.95 and 8.35% for PH; and 63.52, 14.48 and 9.66% for GY, respectively. Under irrigated conditions, the parents had a similar PH in 2003 and a similar HD but differed significantly for GY across years. Under water stress, significant differences between the parents were detected for all three traits in the two years. Compared to the performance under irrigation, water stress, on average, caused a 4 days earlier heading, 12.8 cm height reduction and a GY reduction of 754 g m^{-2} (94.%) for Lemont in 2003. In contrast, the stress resulted in a 6 day heading delay, 11 cm height reduction and 784 g m^{-2} (90%) GY reduction for Teqing. Water stress had a similar effect on HD and GY of the parents as compared to the performance under irrigation in 2001 (Table 1).

The ILs showed transgressive segregation for the three traits, especially for GY in the two conditions across years (Table 1). As compared with the control, the stress in 2003 and 2001 caused significant heading delay in 62 ILs (ranging from 3.5 to 16.0 days) and 45 ILs (ranging from 3.0 to 15.0 days), but significantly earlier heading in 83 lines (ranging from 3.5 to 22.0 days) and 11 ILs (ranging from 3.0 to 9.5 days), and no HD change in 103 lines (2003) and 44 lines (2001), respectively. The stress caused significant reductions in height and yield in most lines (>97%) by an average of 20 cm (ranging from 6 to 42 cm) and 798 g m^{-2} (ranging from 64 to 1,820 g m^{-2}) in 2003, respectively. The stress also caused significant reductions in yield by an average of 165 g m^{-2} (ranging from 13 to 370 g m^{-2}) in 2001.

Identification of QTLs associated with differentiated responses to water stress

QTLs for HD

Twelve M-QTLs for HD were identified in 2003 and mapped to ten rice chromosomes except chromosomes 2 and 11, including eight detected under irrigated

Table 1 Phenotypic performance for heading date (HD in days), plant height (PH) and grain yield (GY) of the Teqing introgression lines (ILs) and parents, Lemont (donor) and Teqing (recipient) evaluated under the normal irrigated (control) and lowland drought (stress) conditions in 2001 and 2003

Condition	Trait	Lemont	Teqing	TQ-ILs		
				Mean ± SD	CV%	Range
2003						
Control	HD (days)	89.5	90.7	92.8 ± 5.0	5.4	73.8–104.5
	PH (cm)	81.2	84.3	90.9 ± 5.2	5.7	69.5–103.0
	GY (g m^{-2})	800.2	872.4	867.9 ± 288.8	33.3	188.1–1895.8
Stress	HD (days)	85.5	96.9	92.9 ± 4.8	5.2	70.9–105.9
	PH (cm)	68.4	73.5	70.9 ± 5.2	7.3	56.5–89.1
	GY (g m^{-2})	46.6	88.3	71.7 ± 65.6	91.5	0.0–359.9
Difference[a]	HD (days)	−4.0	6.2	0.1 ± 5.4	–	−19.9–16.2
	PH (cm)	−12.8	−10.8	−20.0 ± 7.1	35.5	−41.7–2.8
	GY (g m^{-2})	−753.6	−784.1	−797.9 ± 285.4	35.8	−1819.5–23.8
2001						
Control	HD (days)	87.5	88.9	90 ± 4.8	5.4	78.0–100.0
	GY (g m^{-2})	610.3	772.4	615.1 ± 76.9	35.7	56.8–1459.5
Stress	HD (days)	81.5	94.1	92 ± 7.9	8.5	75.0–111.0
	GY (g m^{-2})	40.2	78.5	53.3 ± 29.0	54.5	1.7–132.3
Difference[a]	HD (days)	−6.0	5.2	3.1 ± 4.4	–	−6.0–15.0
	GY (g m^{-2})	−570.1	−693.9	−165.3 ± 72.6	43.9	−370.4–13.6

Plant height was not recorded in 2001

[a] Difference = Stress − Control for all measured traits of the individual ILs

conditions, ten detected under stress, and four by the trait differences between the stress and control conditions (Table 2, Fig. 1). Eight (67%) of these QTLs were also identified in the subset of ILs evaluated in 2001. Based on their differential behaviors, these QTLs could be classified into three types. Type I included six QTLs,

Table 2 Twenty-six QTLs affecting heading date (HD, in days) and plant height (PH, in cm) detected by one-way ANOVA in the Teqing ILs evaluated under irrigated and water-stress conditions in 2001 and 2003

Trait	QTL	Ch.	Marker interval[a]	Parameters	2003			2001[b]			Population[c]
					Control	Stress	Dif.	HD-c	HD-s	HD-d	
HD	*QHd1*	1	RM9 – RM246	*F* value		12.05					3
				Effect		−1.8					
	QHd3	3	RM132 – RM22	*F* value	20.55	28.07		10.98	9.84	4.23	1,2,3,4
				Effect	−2.4	−2.9		−8.8	−6.9	−5.2	
	QHd4a	4	RM261 – RM307	*F* value	13.78	13.09		4.42	12.35		
				Effect	−1.5	−1.5		−3.9	−3.6		
	QHd4b	4	Ph – RM280	*F* value		6.9	8.33			4.19	
				Effect		−1.2	−2.1			−2.6	
	QHd5	5	RM163 – RM161	*F* value	9.93		7.44				3
				Effect	1.5		−1.6				
	QHd6	6	RM439 – RM340	*F* value	6.51	11.45		4.31			2,3
				Effect	−1.2	−1.9		−3.5			
	QHd7	7	OSR4 – RM505	*F* value	7.78	12.57		3.91		4.6	
				Effect	−1.7	−2.1		−4.7		2.4	
	QHd8	8	RM72 – RM339	*F* value	12.8		7.29	4.34	9.85	8.24	2
				Effect	−2.0		1.7	5.5	5.4	4.7	
	QHd9	9	RM219 – RM105	*F* value		14.03	6.61			6.62	
				Effect		−2.6	−1.4			2.3	
	QHd10a	10	RM216 – RM311	*F* value		13.67					
				Effect		−1.6					
	QHd10b	10	RM228 – RM333	*F* value	6.22	21.14		4.4	9.5	7.33	
				Effect	−1.3	−2.3		−4.4	−3.8	−3.1	
	QHd12	12	RM235 – RM17	*F* value	9.68	5.91					2
				Effect	−2.5	−1.9					
PH	*QPh1*	1	RM246 – OSR27	*F* value	13.16	6.64	11.68				
				Effect	−1.9	0.7	2.6				
	QPh2	2	RM250 – RM48	*F* value	20.12		6.95				2
				Effect	−2.3		2				
	QPh3	3	RM22 – RM231	*F* value	15.85		6.74				
				Effect	−2.7		2.6				
	QPh4a	4	RM261 – RM307	*F* value	13.38	8.27	21.51				4
				Effect	−1.5	1.27	2.8				
	QPh4b	4	*Ph* – RM280	*F* value		19.63	6.45				4
				Effect		2.2	1.8				
	QPh5	5	RM592 – RM13	*F* value	14.96		5.65				
				Effect	−2.0		1.8				
	QPh6a	6	RM50 – RM276	*F* value	10.57						
				Effect	2.4						
	QPh6b	6	RM30 – RM439	*F* value	13.69		12.29				3
				Effect	−2.1		2.9				
	QPh7	7	RM214 – RM445	*F* value		9.2	11.82				
				Effect		2	2.9				
	QPh8	8	RM339 – RM223	*F* value	8.04	7.36	13.17				1
				Effect	−1.6	1.7	3.1				
	QPh9	9	RM219 – RM105	*F* value	22.96		9.77				
				Effect	−3.3		3.2				
	QPh10	10	RM228 – RM333	*F* value	11.81						2
				Effect	−1.7						
	QPh11	11	RM123 – RM224	*F* value	11.49		5.12				
				Effect	−2.0		1.9				
	QPh12	12	RM235 – RM17	*F* value	17.86		10.69				
				Effect	−3.2		3.7				

Plant height was not recorded in 2001

[a] The underlined markers are those closer to the true QTL positions and the underlined numbers indicate that these marker-trait associations were detected at the subthresholds of $0.005 < P < 0.05$

[b] c, s and d represent the control, drought stress and difference between the stress and control

[c] 1, 2, 3 and 4 indicated that the QTLs were previously detected in the populations of CT9993/IR62266 DH (Lanceras et al. 2004), IR64/Azucena DH (Lafitte and Courtois 2000), Azucena/Bala RILs (Lafitte et al. 2004) and CT9993/IR62266 DH (Babu et al. 2002), respectively

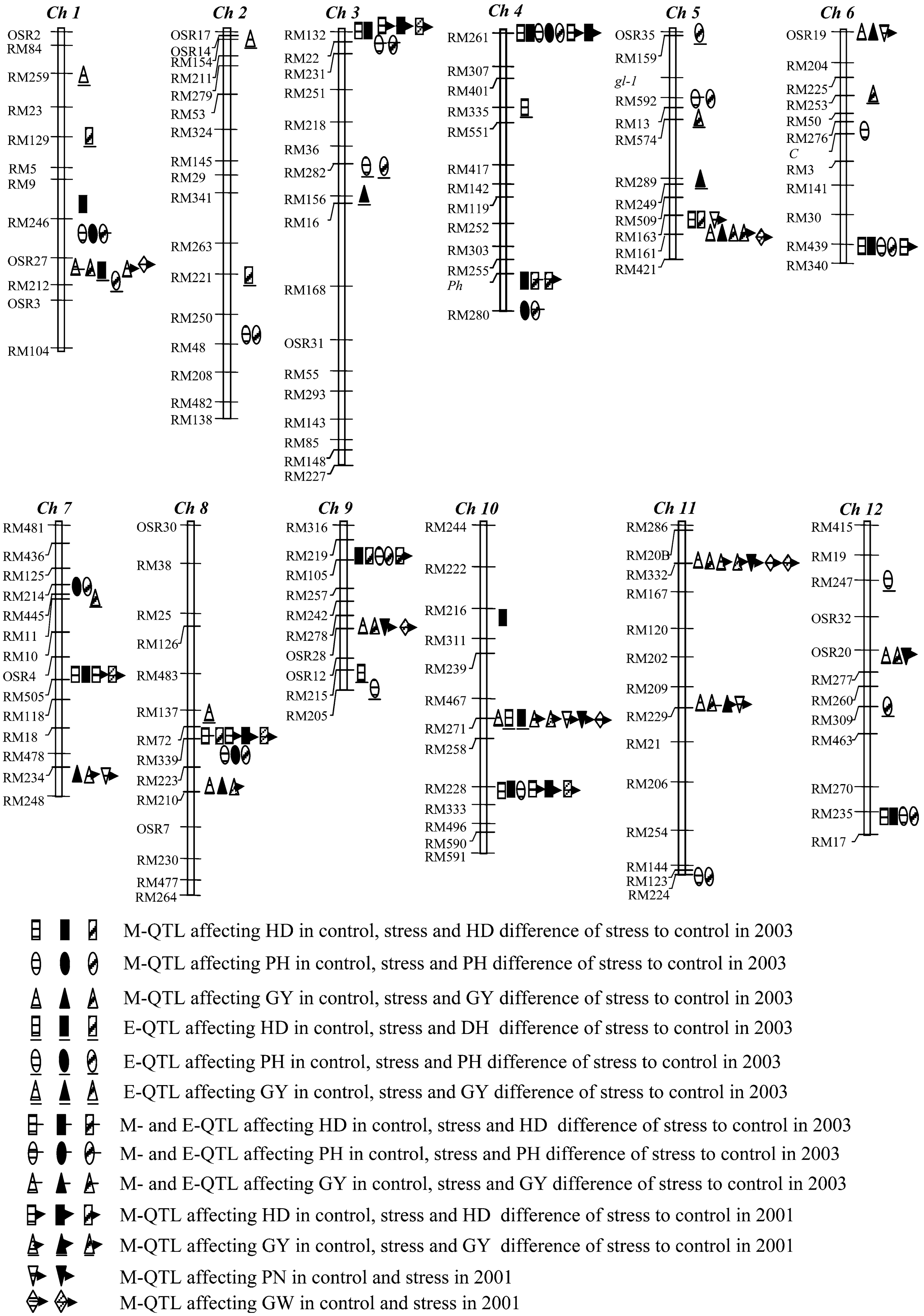

Fig. 1 QTLs for heading date (*HD*), plant height (*PH*) and grain yield (*GY*) detected in 254 Teqing ILs under the irrigated and water stress conditions in 2003 and QTLs for HD, GY and its component traits, panicle number (*PN*) and 1,000-grain weight (*GW*) identified in 100 ILs from the same population under the similar conditions in 2001

QHd3, *QHd4a*, *QHd6*, *QHd7*, *QHd10b* and *QHd12* which expressed under both conditions, and the Lemont alleles at all these loci resulted in early heading. The additive effects of the Lemont alleles at *QHd6* and *QHd10b* were significantly enhanced by the stress. Type II QTLs included *QHd5* and *QHd8* which expressed only in the control but not under stress. The Lemont allele at *QHd5* delayed heading, but its allele at *QHd8* resulted in early heading. Type III QTLs included *QHd1*, *QHd4b*, *QHd9* and *QHd10a*, which were detectable only under drought, suggesting they were apparently induced by stress. The Lemont alleles at all four loci caused early heading. Four QTLs (*QHd4b*, *QHd5*, *QHd9* and *QHd8*) also contributed to HD differences of the ILs between non-stress and stress conditions, and the Lemont alleles at the former three loci reduced HD difference while the Teqing allele *QHd8* was associated with reduced HD difference. Eight HD QTLs (*QHd3*, *QHd4a*, *QHd4b, QHd6, QHd7, QHd8*, *QHd9* and *QHd10b*) located in the same regions in different water stress in 2001 were identified (Table 2).

In addition, five pairs of E-QTLs affecting HD were identified, including two pairs detected under the irrigated condition, one pair under stress, and two pairs by the HD differences across water levels (Table 4). Under the non-stress condition and by the trait differences, the epistatic effects of the recombinant type (1L/2T and 1T/2L) were all associated with early heading or reduced HD differences, while the epistatic effects of the parental type (1L/2L and 1T/2T) tended to result in delayed heading or increased trait difference. Under the stress, the opposite was true.

QTLs for PH

Fourteen M-QTLs for PH were identified and mapped to 12 rice chromosomes, including 12 detected under the non-stress condition, 5 under stress, and 12 by height differences between the stress and non-stress conditions (Table 2, Fig. 1). These included three QTLs (*QPh1*, *QPh4a* and *QPh8*) of type I detected in both conditions. The Lemont alleles at these loci reduced height under the irrigated conditions but increased height under stress. Nine PH QTLs (*QPh2*, *QPh3*, *QPh5*, *QPh6a*, *QPh6b*, *QPh9*, *QPh10*, *QPh11* and *QPh12*) belonged to type II, which expressed under irrigation but not under stress. The Lemont alleles decreased height at all loci except *QPh6a*. Type III QTLs included *QPh4b* and *QPh7* that were induced by stress, and the Lemont alleles at both loci increased height. All PH QTLs except *QPh6a* and *QPh10* also contributed to PH differences of the ILs between the stress and non-stress conditions and the Lemont alleles at all these loci increased height differences.

Six pairs of E-QTLs affecting PH were identified, including two pairs detected under the irrigated condition and four pairs detected by height differences (Table 4). Under the non-stress condition, the two

Table 3 QTLs affecting grain yield (GY, in g m^{-2}) and its components, panicle number/m^2 (PN), 1,000-grain weight (GW, in g), detected by one-way ANOVA in the Teqing ILs evaluated under irrigated and water-stress conditions in 2001 and 2003

QTL	Ch.	Marker interval[a]	Parameters	2003			2001[b]							Population[c]
				Control	Stress	Difference	GY-c	GY-s	GY-d	PN-c	PN-s	GW-c	GW-s	
QGy1	1	OSR27 – RM212	*F* value	11.71		8.99	5.08					8.87		2
			Effect	−108.4		98.7	−43.5					4.5		
QGy5	5	RM509 – RM163	*F* value	18.92	5.67	11.77		4.36		5.78			5.34	3
			Effect	−144.51	−14.0	118.3		−38.7		−21.4			−2.8	
QGy6	6	OSR19 – RM204	*F* value	5.97	7.65					4.05				
			Effect	−72.0	−18.0					−17.0				
QGy7	7	RM234 – RM248	*F* value		8.63		5.11			8.18				
			Effect		60.4		41.6			32.8				
QGy8	8	RM223 – RM210	*F* value	7.78	12.59									1,2
			Effect	−89.8	27.5									
QGy9	9	RM242 – RM278	*F* value	24.52		20.86					5.79		5.85	2
			Effect	−147.1		140.1					−30.4		−3.5	
QGy10	10	RM271 – RM258	*F* value	9.51			4.5		4.18	3.99	3.94	9.93		1,2,3
			Effect	−111.4			−21.8		22.1	−10.0	−14.2	4.7		
QGy11a	11	RM20B – RM332	*F* value	12.05		11.87	6.52		4.56		5.35	6.49	3.97	
			Effect	−129.6		134.5	−49.8		43		−25.4	−2.9	−3.0	
QGy11b	11	RM209 – RM229	*F* value	11.12		12.03		3.96		5.85				2,3
			Effect	−99.2		111.1		−21.1		−21.0				
QGy12	12	OSR20 – RM277	*F* value	22.12		16.41					10.57			
			Effect	−121.3		110.6					−21.6			

[a] The underlined markers are those closer to the true QTL positions and the underlined numbers indicate that these marker-trait associations were detected at the subthresholds of $0.005 < P < 0.05$

[b] c, s and d represent the control, drought stress and difference between the stress and control

[c] 1, 2 and 3 indicated that the QTLs were previously detected in the populations of CT9993/IR62266 DH (Lanceras et al. 2004), IR64/Azucena DH (Lafitte and Courtois 2000) and Azucena/Bala RILs (Lafitte et al. 2004), respectively

Table 4 Epistatic QTL pairs affecting heading date (HD, in days), plant height (PH, in cm) and grain yield (GY, in g) in the Teqing ILs under irrigated and water-stress conditions

Trait	Condition	Ch	Marker 1[a]	Ch	Marker 2	LOD	R^2 (%)	Digenic genotypes and their epistatic effects,τ_{ij}^{b}			
								1L/2L	1L/2T	1T/2L	1T/2T
HD	Control	4	RM335	9	OSR12	3.62	2.38	2.3****(3)	−2.2****(22)	−1.8***(18)	0.8(182)
		8	**RM72**	10	RM271	5.73	4.20	2.9***(2)	−2.0*(12)	−2.6**(25)	1.5(208)
	Stress	1	RM212	10	RM271	3.7	2.96	−2.4****(6)	1.9***(8)	2.1***(18)	−0.8(211)
	Difference	1	RM129	4	***Ph***	4.08	5.67	2.0***(2)	−0.9(22)	−1.5**(11)	2.0***(206)
		2	RM221	3	RM85	3.07	5.21	2.5***(2)	−1.8*(9)	−1.7*(21)	1.8*(210)
PH	Control	3	**RM22**	3	RM282	7.00	10.55	3.1****(3)	−1.6(6)	−1.5(10)	3.3****(230)
		5	**RM592**	12	RM247	6.07	6.31	4.7****(4)	−4.2***(21)	−3.8***(14)	1.9(207)
	Difference	1	**RM246**	5	OSR35	5.48	6.39	−2.2***(5)	1.6**(23)	0.9(14)	−2.5***(193)
		1	RM212	12	RM309	3.81	5.12	−2.3**(6)	1.8*(18)	1.2(15)	−2.1**(203)
		3	**RM22**	3	RM282	4.12	5.69	−3.2**(3)	1.3(6)	1.7*(10)	−3.7***(230)
		4	**RM280**	9	RM205	3.62	5.77	−2.5***(10)	1.8*(16)	1.2(8)	−2.4***(193)
GY	Control	1	RM259	2	RM154	4.99	8.71	206.0**(4)	−135.4*(9)	−147.3*(9)	145.8*(197)
		1	**OSR27**	8	RM137	6.38	10.69	116.7***(3)	−64.5(18)	−68.0(15)	123.0***(205)
	Stress	3	RM156	5	RM289	2.66	2.38	26.2***(2)	−20.8**(12)	−25.6***(36)	9.7(199)
	Difference	5	RM574	7	RM11	3.23	5.00	−155.9**(2)*	117.0*(24)	128.7**(15)	−89.0(207)
		6	RM253	11	**RM229**	4.39	9.39	−111.2**(5)	57.2(20)	74.3(14)	−118.3**(209)

[a] Bold markers are the main-effect QTLs detected either in the control or in the drought stress (Table. 2, 3)

[b] L and T represent homozygous Lemont and Teqing alleles at the interacting markers, 1 and 2 represent markers 1 and 2. *, **, *** and **** indicate the significance levels of $P < 0.05$, 0.01, 0.001 and 0.0001 for the epistatic effects based on *t* tests (Li et al. 1997). Data in parentheses represent the number of plants in each category

significant epistatic effects of the recombinant type reduced height, while the two significant epistatic effects of the parental type (1L/2L and 1T/2T) all resulted in increased height. For the four pairs of E-QTLs detected by the trait differences, the parental type (1L/2L and 1T/2T) interaction reduced height difference while the recombinant type interaction increased height difference.

QTLs for GY

A total of ten M-QTLs affecting GY were identified and mapped to nine rice chromosomes, including nine detected under the non-stress condition, four detected under stress, and six by GY differences between stress and non-stress conditions (Table 3, Fig. 1). Again, all these QTLs except *QGy8* were also identified in 2001. Of the QTLs identified in 2003, three QTLs (*QGy5*, *QGy6* and *QGy8*) belonged to type I, being detected under both stress and non-stress conditions. The Lemont allele at *QGy5* and *QGy6* decreased GY under both conditions, whereas at *QGy8* it reduced yield under irrigation but increased yield under stress. Six QTLs (*QGy1*, *QGy9*, *QGy10*, *QGy11a*, *QGy11b* and *QGy12*) belonged to type II that were detected under the normal irrigated condition but not with stress. The Lemont alleles at all these loci decreased GY. *QGy7* was the only type III QTL, being induced only by stress; the Lemont allele at this locus increased GY. Six M-QTLs (*QGy1*, *QGy5*, *QGy9*, *QGy11a*, *QGy11b*, and *QGy12*) also contributed significantly to GY differences of the ILs between stress and non-stress, and the Lemont alleles at all six loci increased yield differences. In 2001, 6 GY QTLs (*QGy1*, *QGy5*, *QGy7*, *QGy10*, *QGy11a* and *QGy11b*) and 13 QTLs for its component traits (eight for PN and five for GW) were identified in the same regions as GY QTLs detected 2003 (Table 3).

In addition, five pairs of E-QTLs affecting yield were identified, including two pairs detected under irrigation, one pair under stress, and two pairs detected by yield differences (Table 4). Under the non-stress and stress conditions, the epistatic effects of the recombinant type were all associated with reduced yield, while the epistatic effects of the parental type increased yield. Interestingly, the recombinant type interactions were associated with increased yield difference and the parental type interactions reduced yield differences.

Discussion

The recurrent parent, Teqing, is a high yielding paddy rice cultivar with a moderate level of DT (Lafitte et al. 2005). Thus, the stress level applied was severe, with both parents suffering a ~90% yield reduction. We used GY as the primary criteria for DT because that is of greatest interest to farmers growing rice in rainfed areas. Our results show that almost 10% of the ILs developed by random introgression from a non-DT donor, Lemont, outyielded their recurrent parent, Teqing. The presence of genetic diversity for DT hidden in the susceptible donor, Lemont, was also observed for a much larger sample of rice germplasm (Lafitte et al. 2005).

Differential QTL expression and their association with DT of rice

Many QTLs affecting HD and PH in rice interact with environments to a varied degree, and some respond differently to drought (Li et al. 2003). In this study, most QTLs showed pronounced differential expression either qualitatively or quantitatively in response to drought, evidenced by two observations. First, of the 36 identified M-QTLs (Table. 2, 3), 17 were observed only in the control and 7 others were detectable under drought, suggesting they were induced by stress. Second, of the 12 QTLs detected under both conditions, 4 (*QPh1*, *QPh4a*, *QPh8*, and *QGy8*) had effects in opposite directions, 3 (*QHd6*, *QHd10b*, and *QGy5*) had effects that differed significantly in magnitude, and only 5 (*QHd3*, *QHd4a*, *QHd7*, *QHd12*, and *QGy6*) behaved similarly under stress and non-stress conditions. Furthermore, we found that 6 (*QHd1*, *QHd3*, *QHd5*, *QHd6*, *QHd8*, and *QHd12*) of the 12 HD QTLs, 6 (*QPh2*, *QPh4a*, *QPh4b*, *QPh6b*, *QPh8*, and *QPh10*) of the 14 PH QTLs, and 6 (*QGy1*, *QGy5, QGy8, QGy9, QGy10* and *QGy11b*) of the 10 GY QTLs detected in this study located approximately the same regions of QTLs affecting DT identified previously (Lanceras et al. 2004; Lafitte and Courtois 2000; Lafitte et al. 2004; Babu et al. 2002).

Although QTLs induced only by drought may be associated with mechanism(s) of rice stress response, they may not necessarily contribute to DT. Then, an important question arises regarding which QTLs are expected to be able to contribute to DT of rice. We believe that those QTLs that can reduce trait difference between stress and non-stress conditions should have contributed to DT because of their obvious contribution to trait stability. Allelic differences at 22 (61.1%) of the M-QTLs identified in this study, including 4 HD QTLs (*QHd4b*, *QHd5*, *QHd8* and *QHd9*), 12 PH QTLs (all except *QPh6a* and *QPh10*) and 6 GY QTLs (*QGy1*, *QGy5*, *QGy9*, *QGy11a*, *QGy11b*, and *QGy12*), were of this group, evidenced by their associations with the trait differences of the ILs between the stress and non-stress conditions. It was striking to note that the Teqing alleles at all these PH and GY M-QTLs increased PH and yield under the irrigated condition and simultaneously contributed to the trait stability (reduced trait difference). For the four HD QTLs, the Lemont allele at three loci reduced HD difference whereas the Teqing allele at *QHd8* reduced HD difference.

The second group of DT QTLs comprised those that behaved similarly across water levels, such as *QHd3*, *QHd4a*, *QHd7*, *QHd12* for HD and *QGy6* for GY. Conversely, QTLs that behaved very differently across the stress conditions are expected to contribute to trait instability and drought susceptibility. These included *QHd6* and *QHd10b* for HD, *QPh1* for PH, and *QGy5* and *QGy8* for GY, respectively.

Different adaptation strategies of rice to drought and their implications in breeding for DT

In this study, the most striking phenotypic changes of rice plants caused by drought were reduced height, delayed flowering and high sterility, consistent with previous reports (Yoshida and de los Reyes 1976; Lafitte and Courtois 2000). Interestingly, we found that the parents of the ILs responded very differently to water stress, in which drought accelerated flowering of Lemont but delayed flowering of Teqing. Delayed and accelerated flowering represent two major contrasting responses to drought observed in a diverse set of rice germplasm, and reflect two different adaptive strategies (Lafitte et al. 2005). In some circumstances, acceleration of flowering might allow rice plants to complete the life cycle before the stress gets too severe, so that plants can escape the stress to a certain degree. This response to stress has been observed in other cereals (Araus et al. 2002). Flowering delay, when associated with true drought tolerance, would allow plants to survive longer under stress, with the critical flowering stage occurring after the resumption of rains. Our observation that the Lemont alleles at all HD QTLs except *QHd5* resulted in early heading under the stress condition is consistent with the putative adaptation of Lemont to stress by escaping (earlier heading under stress). In contrast, the adaptation of Teqing to drought was apparently achieved by drought tolerance, as evidenced by the consistent associations of the Teqing alleles at most PH/GY QTLs with increased yield potential and trait stability. The virtually complete separation of the two adaptive strategies in the non-DT parental lines suggests a possible negative regulation between the two underlying genetic systems, which remains to be elucidated.

In practice, stable yields can be achieved either by drought escape or by DT. Thus, while it remains a mystery why the two contrasting and genetically complex adaptation strategies exist separately in the non-DT parents, our results indicated that the genetic systems underlying the two adaptation strategies appeared to be largely non-overlapping. Our observation that the strong epistasis between four DT QTLs (*QPh1*, *QPh3*, *QPh4b*, and *QGy11b*) and some background loci and the consistent associations of DT (reduced trait difference in PH and GY) with the parental type interaction, suggest the expected presence of strong genetic background effects on the expression of these DT QTLs involved. Thus, caution should be taken when one wishes to transfer the ‘DT’ Teqing alleles at these loci into the japonica genetic background. Otherwise, a wide range of opportunities exist for breeding DT rice cultivars by combining the different DT strategies by pyramiding the identified QTLs that truly contribute to DT, but the challenge is how to put large numbers of DT QTLs together efficiently in the context of a breeding program.

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