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ORIGINAL PAPER

Detection of QTLs with additive effects and additive-by-environment interaction effects on panicle number in rice (*Oryza sativa* L.) with single-segment substitution lines

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Abstract A novel population consisting of 35 single-segment substitution lines (SSSLs) originating from crosses between the recipient parent, Hua-jing-xian 74 (HJX74), and 17 donor parents was evaluated in six cropping season environments to reveal the genetic basis of genetic main effect (G) and genotype-by-environment interaction effect (GE) for panicle number (PN) in rice. Subsets of lines were grown in up to six environments. An indirect analysis method was applied, in which the total genetic effect was first partitioned into G and GE by using the mixed linear-model approach, and then QTL (quantitative trait locus) analyses on these effects were conducted separately. At least 18 QTLs for PN in rice were detected and identified on 9 of 12 rice chromosomes. A single QTL effect ($a + ae$) ranging from −1.5 to 1.2 was divided into two components, additive effect (a) and additive × environment interaction effect (ae). A total number of 9 and 16 QTLs were identified with a ranging from −0.4 to 0.6 and ae ranging from −1.0 to 0.6, respectively, the former being stable but the latter unstable across environments. Three types of QTLs were suggested according to their effects expressed. Two QTLs (*Pn-1b* and *Pn-6d*) expressed stably across environments due to the association with only a, nine QTLs (*Pn-1a, Pn-3c, Pn-3d, Pn-4, Pn-6a, Pn-6b, Pn-8, Pn-9* and *Pn-12*) with only ae were unstable, and the remaining seven of QTLs were identified with both a and ae, which also were unstable across environments. This is the first report on the detection of QE (QTL-by-environment interaction effect) of QTLs with SSSLs. Our results illustrate the efficiency of characterizing QTLs and analyzing action of QTLs through SSSLs, and further demonstrate that QE is an important property of many QTLs. Information provided in this paper could be used in the application of marker-assisted selection to manipulate PN in rice.

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Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world. It has been estimated that more than 50% of the human population depends on rice as its main source of nutrition (Brar and Khush 2002). It is unique among cereals by having a storage protein, which is primarily made of glutelin, and has a more balanced amino acid profile than the prolamine-rich storage proteins found in most cereals (Juliano 1985). On the other hand, the rice genome is more than a resource for understanding the biology of a single species. It is a window into the structure and function of genes in other crop grasses as well. It has also become a useful plant for studying biology, as a model plant for monocots due to its small genome relative to those of other species of the Gramineae, synteny with other grasses such as wheat, barley, and maize, efficient transformation, dense molecular genetic maps, large sequence libraries and abundance of genetic resources (Motoyuki and Makoto 2002). For these reasons, rice has been the subject of numerous genetic and breeding studies over the past 100 years, and has provided much useful information for plant biology and plant breeding.

Panicle number (PN) in rice, an important agronomic character for grain production, is normally one of the main determinants of grain yield, even at adequate plant populations (Counce et al. 1992). The development of PN is affected by various environmental factors including plant nutrients, planting density, and climatic circumstances such as light, temperature and water supply. Scientists have paid increasing attention to PN in rice due to reduced tillering capacity being one of the main target traits for the super-rice ideotype (Khush 2000). Research using mutant materials confirmed that the PN in rice could be controlled by one single gene (Li et al. 2003a, b). Most studies by using traditional and molecular genetic analysis reported that rice PN was influenced by multiple quantitative trait loci (QTLs) (Ahmad et al. 1986; Li et al. 1997). QTLs for PN in rice have been identified on 10 of the 12 chromosomes of rice (Yan et al. 1998; Liao et al. 2001; Hittalmani et al. 2003; Jiang et al. 2004) using populations of recombinant inbred lines (RILs) or doubled haploid lines (DHLs). With such populations, it is difficult to differentiate quantitative trait locus (QTL) effects from background noise, particularly for QTLs with small and/or interacting effects (Eshed and Zamir 1995).

To overcome these limitations and achieve high-resolution mapping of QTLs, Eshed and Zamir (1995) proposed the application of introgression line (IL) populations. In rice, several permanent mapping populations, such as chromosome segment substitution lines (CSSLs) and backcross inbred lines (BILs) have been developed and have been used to detect many QTLs affecting heading date (Yano et al. 1997 2000, 2001; Yamamoto et al. 1998, 2000; Takahashi et al. 2001). Wan et al. (2003) used a mapping population of 66 *japonica* chromosome segment substitution lines in an *indica* genetic background to detect QTLs for leaf bronzing index, stem dry weight, plant height, root length and root dry weight under F_e^{2+} stress. Tian et al. (2006) constructed introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in a cultivated rice (*Oryza sativa* L.) background and characterized introgressed segments associated with yield-related traits. We have constructed a library of 1,123 single-segment substitution lines (SSSLs) in rice (Zhang et al. 2004; He et al. 2005a; Xi et al. 2006), and have used it to detect QTLs affecting many agronomic traits in rice by using the library (He et al. 2005b, c; Xi et al. 2006). As each of these studies was conducted in only one environment, it was not possible to estimate QTL-by-environment interaction effects (QE) (Zhu 1999; Wang et al. 1999).

Most plant traits are quantitative in nature, and are thought to be controlled by polygenes that have small effects and are easily affected by the environment. Thus genotype-by-environment interaction effect (GE) is a common phenomenon for quantitative traits (Falconer 1960). GE occurs when the deviations between two genotypes perform differently in different environments, and is thus described as differential genotypic sensitivities to environments (Falconer 1981). GE is also of great importance in plant evolution and breeding. In plant evolution, high level of GE allows plants better adaptation to their changing environments and the maintenance of genetic variation in populations (Jain and Marshall 1967). In plant breeding, GE has received considerable attention as it is closely related to the stability of varieties. Because of its importance, GE of quantitative traits has been the subject of extensive investigation (Baker 1988; Cooper and Hammer 1996). QTL analysis has made it possible to track the performance of individual QTL across environments, allowing GE to be dissected into its component of QE (Zhu 1999; Wang et al. 1999). Despite technical difficulties, QE has been revealed in many crops (Paterson et al. 1991; Zhuang et al. 1997; Jiang et al. 1999). Most of the previous studies inferred QTL-by-environment interaction by comparing QTLs detected in different environments, leading to results that may mix GE with G and that do not provide unbiased estimates of QE (Yan et al. 1999; Hittalmani et al. 2003; Li et al. 2003a, b).

Zhu (1998) proposed an indirect analysis methodology for QE, in which the total genetic effect is first partitioned into G and GE, and then QTLs are mapped for these effects separately. QTLs mapped for the G led to estimation of the genetic main effect of QTLs, independent of change in environmental conditions, while those mapped for GE led to the identification of QE that are significantly affected by variation in environmental conditions. Use of this approach in rice has provided valuable information about QE in a population of DHLs (Yan et al. 1999; Hittalmani et al. 2003; Li et al. 2003a, b). In the present study, each of 35 rice single-segment substitution lines selected from our library was evaluated in up to six environments. QTL analyses on PN were conducted first on total genetic effect (G + GE) estimated in data from individual environment, and then on G and GE separately. QTLs identified according to G + GE were expected to contain mixed effects of additive effect (*a*) and additive-by-environment interaction effect (*ae*), while QTLs obtained on G and GE were with *a* and *ae*, respectively. The aims of the study were to detect *a* and *ae* of QTLs, and to evaluate stability of the QTLs for PN in rice.

Materials and methods

Plant materials

The SSSLs in the library were developed by using of Hua-jing-xian 74 (HJX74), an elite *indica* variety from South

China, as recipient, and 24 varieties including 14 *indica* and 10 *japonica* varieties collected worldwide as donors (Zhang et al. 2004). Development of the SSSLs, through backcrossing and SSR marker selection, was described by He et al. (2005a) and Xi et al. (2006). For this study, 35 SSSLs were selected (Table 1), each containing only one chromosomal segment from a donor substituted in the HJX74 genetic background. The substituted segments distribute on 10 chromosomes and range in length from 2.6 to 96.2 cM with an average of 26.86 cM, and a total length of 940.35 cM (Fig. 1).

Field trials

Phenotypic experiments were conducted at the experimental farm of South China Agricultural University, Guangzhou (at ~113° east longitude and ~23° north

Table 1 Thirty-five single-segment substitution lines (SSSLs) and their codes, donors and experimental environments

SSSL	Code	Donor	Experimental environment					
			E1 (2003F)	E2 (2004S)	E3 (2004F)	E4 (2005F)	E5 (2006S)	E6 (2006F)
W07-14-10-04	S1	Suoyunuo	+	+	+	+	+	+
W02-17-08-14	S2	Amol 3	+	+	+	+	+	+
W15-05-07-15	S3	American jasmine	+	+	+	+	+	+
W11-15-08-10-05	S4	Basmati 370	+	+	+	+	+	+
W08-15-06-04-04	S5	IR64	+	+	+	+	+	
W02-17-06-15	S6	Amol 3	+	+	+	+		
W11-15-09-03	S7	Basmati 370	+	+	+	+		
W18-06-02-02	S8	IRAT261	+	+	+	+		
W07-14-08-04	S9	Suoyunuo		+	+	+	+	+
W09-38-54-07-06-01	S10	Basmati 385		+	+	+	+	+
W14-18-06-06-02	S11	Lianjian 33	+	+	+	+	+	+
W17-10-06-01-08-07	S12	Ganxiangnuo		+	+	+	+	+
W20-20-05-19-07	S13	Chenglongshuijingmi		+	+	+	+	+
W23-07-06-01-01-08	S14	Lemont		+	+	+	+	+
W14-18-06-10-01	S15	Lianjian 33	+	+	+	+		
W17-10-07-05-12	S16	Ganxiangnuo		+	+	+	+	
W17-46-40-10-07-04	S17	Ganxiangnuo			+	+	+	+
W20-20-05-05-11	S18	Chenglongshuijingmi			+	+	+	+
W27-14-01-09-18	S19	IAPAR9		+	+	+		
W20-12-02-01-04	S20	Chenglongshuijingmi				+	+	+
D21(W15-05-07-15-03-S)	S21	American jasmine				+	+	+
W08-09-05-03	S22	IR64				+	+	
W08-16-03-59	S23	IR64	+	+				
W20-20-05-06	S24	Chenglongshuijingmi	+	+				
W27-14-06-20	S25	IAPAR9	+	+				
W23-07-06-10-06	S26	Lemont		+	+			
W04-45-50-04-05-01	S27	BG367			+	+		
W13-11-29-06-04-08-10	S28	Jiangxi-Si-Miao				+	+	
W08-18-09-09-06-02	S29	IR64				+	+	+
W19-18-09-06	S37	Kyeema	+	+				
W15-05-09-02-01	S38	American jasmine	+	+				
W15-05-09-06-04-02	S39	American jasmine		+	+			
W07-07-02-07-03	S40	Suoyunuo		+	+			
W06-26-21-04-03-02	S41	Katy		+	+			
W10-31-35-06-06-06	S42	Nangyangzhan					+	+

E1–E6 represent the six experimental environments. The numbers and letters in parentheses indicate the growing year and season (*S* for spring from March to July, or *F* for fall from July to November). '+' indicates the environments in which each line was evaluated

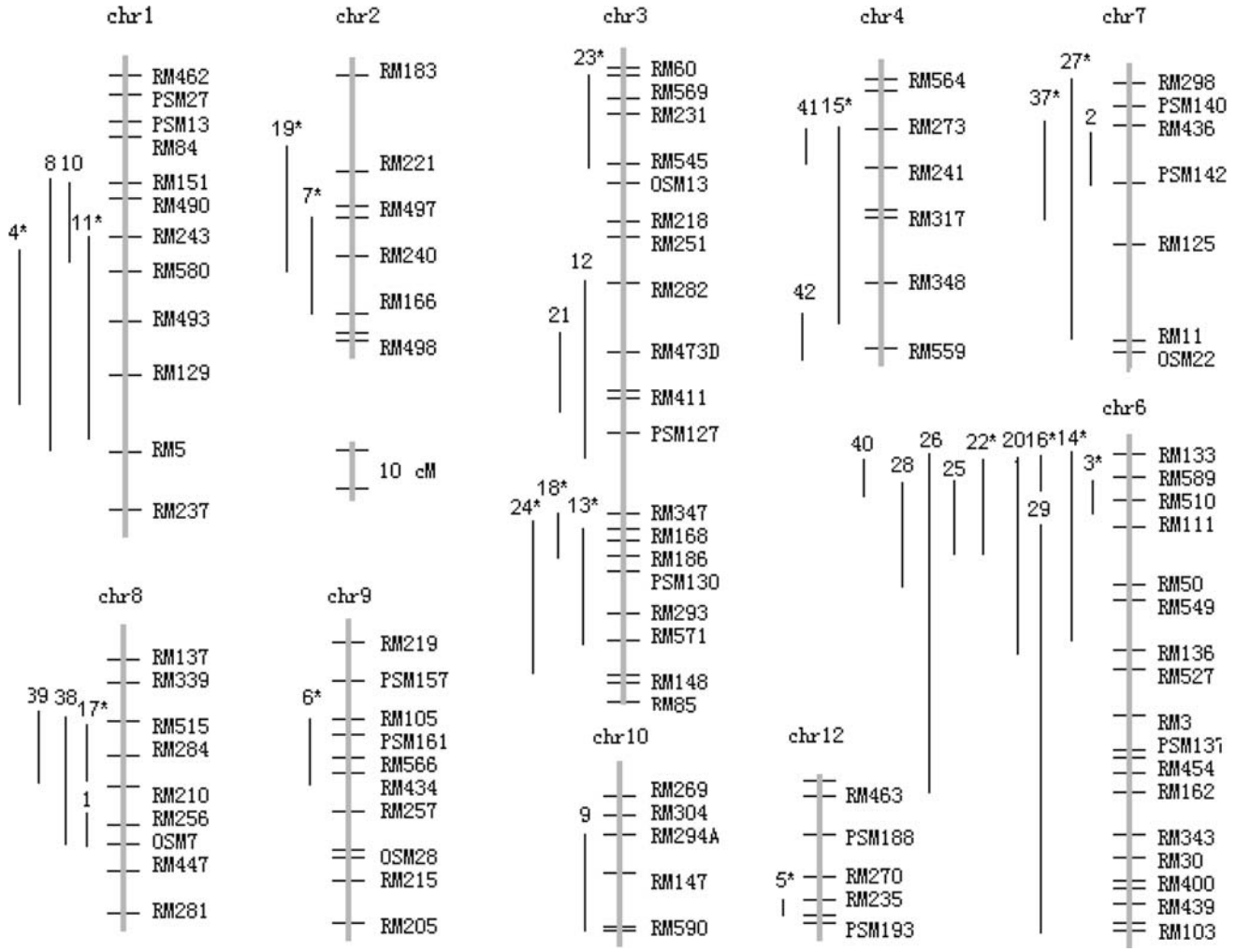

Fig. 1 The distribution and the lengths of substituted chromosome segments in 35 single-segment substitution lines (SSSLs). The substituted segments are represented by *vertical lines*. The number at the top of each vertical line is the number of the SSSL carrying that segment. The number with '*' indicates the SSSL carrying a QTL on its substituted segment. *Codes* on the right of each chromosome designate molecular marker loci

latitude), China, in spring (from March to July) 2004 and 2006 and autumn (from July to November) 2003, 2004, 2005 and 2006. HJX74 was grown in all six environments, and each of the SSSLs was grown in at least two of the environments (Table 1). In each experiment, the germinated seeds were sown in a seedling bed and seedlings were transplanted to a paddy field 20 days later, with two plants per hill spaced at 16.7 cm × 20.0 cm. Each plot consisted of thirteen 6.2 m long rows with 32 hills, and all plots were arranged in a randomized complete block design with three replications. The management of the field experiments was in accordance with local standard practices. At maturity, the number of panicles (PN) was counted for each of 20 hills from the middle of each plot, and the average PN value of the 20 hills was used as raw data in the analysis.

Mixed linear models for estimating G effects and GE interaction effects

For a genetic experiment conducted only in one environment, the phenotypic performance of the *j*th genetic entry in the *k*th block can be expressed by

$$y_{jk} = \mu + G_j + B_k + \varepsilon_{jk} \quad (1)$$

where, μ = population mean, fixed; G_j = genetic main effect of *j*th genotype, $G_j \sim N(0, \sigma_G^2)$; B_k = block effect of *k*th block, $B_k \sim N(0, \sigma_B^2)$; and ε_{hjk} = residual effect, $\varepsilon_{hjk} \sim N(0, \sigma_\varepsilon^2)$.

For a genetic experiment conducted within multiple environments, the phenotypic performance of the *j*th genetic entry in the *k*th block within the *h*th environment can be expressed by

$$y_{hjk} = \mu + E_h + G_j + GE_{hj} + B_k + \varepsilon_{hjk} \quad (2)$$

where, μ = population mean, fixed; E_h = environment effect of hth environment, $E_h \sim N(0, \sigma_E^2)$; G_j = genetic main effect of jth genotype, $G_j \sim N(0, \sigma_G^2)$; GE_{hj} = genotype × environment interaction effect between jth genotype and hth environment, $GE_{hj} \sim N(0, \sigma_{GE}^2)$; B_k = block effect of kth block, $B_k \sim N(0, \sigma_B^2)$; and ε_{hjk} = residual effect, $\varepsilon_{hjk} \sim N(0, \sigma_\varepsilon^2)$. The minimum norm quadratic unbiased estimation (MINQUE) method with all prior values set at 1 (Zhu and Weir 1996) was used to estimate variance components for the trait. Values of G and GE were predicted by the Best Linear Unbiased Prediction (BLUP) method (Zhu and Weir 1996). All estimations were performed using the QGAStation software package (Chen and Zhu 2003).

QTL analyses

An indirect approach was conducted to analyze QTL effects (Zhu 1998). First, values of G and GE for HJX74 and all individual SSSLs on PN within each environment were estimated according to model (1) and model (2) mentioned above, respectively. Next, QTLs were mapped using these estimated values as input data separately. QTLs identified according to G in model (1) are expected to contain mixed effects of a and ae, and will be referred to here as with $a + ae$. QTLs obtained using G and GE from model (2) have a and ae, respectively. The estimates obtained for each SSSL were compared to those for HJX74 with one-tailed Duncan's multiple range tests (Chen and Zhu 2003) conducted at a significance level of 0.05. It was assumed that each SSSL affecting the trait carries only one QTL, and a significant QTL affecting PN was declared only if one type of the effect of SSSL is significantly different from the corresponding effect of HJX74 (Eshed and Zamir 1995). QTL effect values ($a + ae$, a and ae) were calculated as the differences of genetic effects between each SSSL and HJX74.

Results

Phenotypic variation for PN

The PN of the parent HJX74 ranged from 7.3 in environment E4 to 8.1 in environments E1 and E2, with standard deviations ranging from 0.08 to 0.56. The average PN of HJX74 was 8.0 in spring, 7.7 in fall, and 7.7 across all six environments. The average PN of the SSSLs was similar to that of HJX74 in all environments except for E1, where the average PN of the SSSLs was 7.7 compared to 8.1 for HJX74 (Table 2). Analysis of variance on phenotypic values of PN from all experimental environments indicated that variance components of the genotype (including HJX74 and SSSLs) and the GE were significant (data not shown), with relative contributions to the total phenotypic variation of 9.35 and 18.42%, respectively.

Table 2 Mean and standard deviations (SD) for the number of panicles per hill in the rice line HJX74 grown in six environments (E1 to E6) and means, standard deviations, maxima (Max) and minima (Min) for varying numbers (N) of single-segment substitution lines (SSSLs) evaluated in those environments

Environment	HJX74		SSSLs				
	Mean	SD	N	Mean	SD	Max	Min
E1	8.1	0.22	15	7.7	0.39	9.1	6.4
E2	8.1	0.09	26	8.1	0.42	8.8	7.3
E3	7.7	0.08	24	7.9	0.53	9.2	6.8
E4	7.3	0.56	25	7.1	0.46	8.2	6.3
E5	8.0	0.26	20	7.9	0.42	8.9	7.2
E6	7.4	0.29	16	7.5	0.22	8.0	7.1
All	7.7	0.43	126	7.7	0.59	9.2	6.3

QTLs with $a + ae$ effects on PN

QTL mapping based on the data estimated in individual environments according to model (1) led to the identification of 18 QTLs with mixed effects of $a + ae$ in the SSSLs for PN in rice (Table 3, Fig. 1). Four QTLs were detected on each of chromosomes 3 and 6, two on each of chromosomes 1, 2 and 7 and one on each of chromosomes 4, 8, 9 and 12 (Fig. 1). Of 18 QTLs detected, 7 (QTLs *Pn-1a*, *Pn-1b*, *Pn-2a*, *Pn-2b*, *Pn-3a*, *Pn-3b* and *Pn-6a*) were detectable in three environments (out of 4, 5 or 6 environments in which the corresponding SSSLs were evaluated), 4 (QTLs *Pn-6c*, *Pn-6d*, *Pn-7a* and *Pn-7b*) in two environments (out of 2 or 4), and the remainder in only one environment (out of 2, 4 or 5) (Table 3). Some QTLs that were detected in multiple environments expressed different effects across environments, with differences observed in both the magnitudes and directions of effects (Table 3). QTL *Pn-6a* showed the most variation among environments, with effects ranging from −1.3 in E1 to 0.8 in E3. Three QTLs, *Pn-1b, Pn-6d and Pn-7a*, had quite consistent expression across environments (Table 3).

QTLs for PN with a effects

Quantitative trait locus mapping based on the G values estimated according to model (2) identified 9 QTLs with a

Table 3 Effects of QTLs on panicle number per hill in rice, as estimated by evaluating single-segment substitution lines (SSSLs) in various environments (E1 to E6)

QTL	SSSL code	$a + ae$					
		E1	E2	E3	E4	E5	E6
Pn-1a	S4	0.9^{***}		-0.7^{**}		-0.3^{*}	
Pn-1b	S11	-0.3^{*}			-0.3^{*}	-0.2^{*}	
Pn-2a	S7	-1.5^{****}		0.3^{*}	-0.3^{*}	–	–
Pn-2b	S19		0.4^{*}	0.7^{***}	0.6^{**}	–	–
Pn-3a	S13	–	0.4^{*}	0.8^{****}		0.5^{*}	
Pn-3b	S18	–	–	1.2^{****}		0.4^{*}	0.2^{*}
Pn-3c	S23		-0.4^{*}	–	–	–	–
Pn-3d	S24		-0.3^{*}	–	–	–	–
Pn-4	S15	-0.8^{***}				–	–
Pn-6a	S3	-1.3^{****}		0.8^{***}	0.5^{*}		
Pn-6b	S14	–				0.3^{*}	
Pn-6c	S16	–	0.3^{*}	0.7^{***}			–
Pn-6d	S22	–	–	–	-0.6^{**}	-0.4^{*}	–
Pn-7a	S27	–	–	0.3^{*}	0.4^{*}	–	–
Pn-7b	S37	-0.2^{*}	-0.4^{*}	–	–	–	–
Pn-8	S17	–	–			-0.3^{*}	
Pn-9	S6	-0.9^{***}				–	–
Pn-12	S5				-0.4^{*}		–

QTLs are designated by codes beginning with '*Pn-*'(for the trait panicle number) followed by the chromosome number and in some cases a letter to distinguish between two or more possible QTLs on the same chromosome. $a + ae$ is the confounded effect of the QTL estimated at a given environment

The sign indicates the direction of the effect of the donor allele. *, **, *** and **** show the significances at 0.05, 0.01, 0.005 and 0.001 of probability level, respectively. '–' indicates that a particular SSSL was not evaluated in a particular environment

that are stable across environments (Table 4). These QTLs were located on five rice chromosomes: one on chromosome 1 and two on each of chromosomes 2, 3, 6 and 7. At four QTLs (*Pn-1b, Pn-2a, Pn-6d* and *Pn-7b*) the alleles derived from the donor parents reduced PN (with effects ranging from -0.2 to -0.4). At the remaining five QTLs, the donor alleles increased PN (by between 0.3 and 0.6) (Table 4).

QTLs for PN with *ae* interaction effects

There were 16 QTLs with significant *ae* for PN (Table 4): four on chromosome 3 and one to three on each of eight other chromosomes. QTL *Pn-1a* had no *a*, but showed significant interactions, with positive *ae* values in E1 and E6 and negative *ae* values in E3 and E5. Other environment-sensitive QTLs with significant *ae* values in fewer environments: QTLs *Pn-2a* and *Pn-6a* in three environments, QTL *Pn-3b* in two environments, and the remaining 12 QTLs in only one environment. All estimated *ae* values ranged from -1.0 of QTL *Pn-6a* to 0.6 of QTL *Pn-1a* in E1. QTL *Pn-6a* showed the largest variation among environments, with *ae* values of -1.0, 0.5 and 0.4 in E1, E3 and E4, respectively.

Discussion

QTL detection through SSSLs

In the present study, we used SSSLs as experimental materials and an indirect method to analyze data from six environments to map QTLs with additive and/or additive-by-environment interaction effects on PN in rice. For comparison, QTL mapping was also performed using data from each individual environment. Since each of 35 SSSLs used contained only one substituted segment from a donor in HJX74 genetic background, all the genetic variation between one of SSSLs and HJX74 can be associated with the substituted segment. In the development of the SSSLs, 574 SSR markers, distributed throughout the genome with an average interval of 2.7 cM were surveyed in the BC_2F_1 generation (Zhang et al. 2004), and polymorphic markers were re-examined in the BC_4F_1 and BC_4F_2 generations to ensure the uniformity of genetic background of the SSSLs (Xi et al. 2006). This should minimize the background genetic effects, providing more reliable QTL detection and estimation of QTL effects. QTLs affecting PN were detected in 10 distinct regions of the rice genome. This is more than in most previous QTL mapping studies in rice. For example, Yan et al. (1998) detected three QTLs for tiller number at maturity in a DHL population. Liao et al. (2001) detected six QTLs in a DHL population and nine QTLs in a RIL population in both field and pot experiments. And Hittalmani et al. (2003) detected a total of twenty significant QTLs for PN in a DHL population evaluated in nine locations across four countries in Asia, but the number of QTLs at any location varied from zero to three. QTLs in three regions on chromosomes 2, 4 and 12 detected here are likely in common with QTLs detected in previous studies, but other QTLs detected here have not previously been detected (Yan et al. 1998; Liao et al. 2001; Hittalmani et al. 2003). Given that the substituted chromosome segments used here did not cover the whole genome and that some segments were quite long, there remains scope for further work of this type, aimed at detecting QTLs elsewhere in the genome and/or determining whether any of the segments contain more than one QTL.

Table 4 QTL effect components on panicle number in rice, as estimated by evaluating single-segment substitution lines (SSSLs) in various experimental environments (E1 to E6)

QTL	SSSL code	*a*	*ae*					
			E1	E2	E3	E4	E5	E6
Pn-1a	S4		0.6^{****}		-0.5^{***}		-0.3^{*}	0.3^{*}
Pn-1b	S11	-0.2^{*}						
Pn-2a	S7	-0.3^{*}	-0.9^{****}	0.296^{*}	0.4^{**}		–	–
Pn-2b	S19	0.6^{****}			0.2^{*}		–	–
Pn-3a	S13	0.4^{****}	–		0.4^{*}			
Pn-3b	S18	0.5^{****}	–	–	0.6^{****}	-0.3^{*}		
Pn-3c	S23			-0.341^{*}	–	–	–	–
Pn-3d	S24			-0.305^{*}	–	–	–	–
Pn-4	S15		-0.5^{***}				–	–
Pn-6a	S3		-1.0^{****}		0.5^{***}	0.4^{*}		
Pn-6b	S14		–				0.3^{*}	
Pn-6c	S16	0.3^{*}	–		0.387^{*}			–
Pn-6d	S22	-0.4^{***}	–	–	–			–
Pn-7a	S27	0.3^{*}	–	–		0.3^{*}	–	–
Pn-7c	S37	-0.2^{*}		-0.321^{*}	–	–	–	–
Pn-8	S17		–	–			-0.3^{*}	
Pn-9	S6		-0.5^{***}				–	–
Pn-12	S5					-0.3^{*}		–

QTLs are designated by codes beginning with '*Pn-*' (for the trait panicle number) followed by the chromosome number and in some cases a letter to distinguish between two or more possible QTLs on the same chromosome. The sign indicates the direction of the effect of the donor allele. '*','**','*** and '****', show the significances at 0.05, 0.01, 0.005 and 0.001 of probability level, respectively. '–' indicates that a particular SSSL was not evaluated in a particular environment

Detection of QTLs with QE interaction effects

QTL-by-environment interaction is clearly important in affecting quantitative traits, and significant QE interactions have been reported (e.g. Paterson et al. 1991; Zhuang et al. 1997). In most previous mapping studies, the existence of QE interaction was inferred by comparing QTLs and their effects in multiple environments (e.g., Paterson et al. 1991; Bubeck et al. 1993). In this study, we applied a direct method of analysis conducted separately for each environment, and an indirect method (Zhu 1998) in which QTLs with QE were mapped using predicted total genotype × environment interaction effects. The two methods identified the same SSSLs as carrying QTLs affecting PN (Tables 3, 4). The direct method provided estimates of the effects of QTLs within a single environment, but effects can be mixed by both *a* and *ae* of QTLs. The indirect method allowed for separation of *a* and *ae*. A total of 16 QTLs were detected with significant *ae* values ranging from −1.0 to 0.6 on PN in rice (Table 4). Interactions of QTLs with environments included cases in which: (1) a QTL expresses in one environment but not in another; (2) a QTL expresses very differently and has opposite effects in different environments; and (3) a QTL expresses strongly in one environment but weakly in another. The total effect of each QTL at a specific environment (Table 3), as estimated by the direct method, tended to be approximately the sum of the *a* value and the *ae* value of the QTL at that environment (Table 4), as estimated by the indirect method.

Patterns of QE interaction for PN in rice

In any specific environment, the total effect of a QTL includes the main effect of the QTL and QE interaction effects for that environment. The QTL main effect is expressed in the same way across different environments and free from environmental influence, while the QE interaction effect is specific to a particular set of environmental conditions (Zhu 1998; Yan et al. 1999). Of the 18 QTLs detected in this study, two QTLs, *Pn-1b* and *Pn-6d* had significant *a* values but no significant *ae* values (Table 4). Because the expression of such QTLs is free from environmental interactions, their use in selection should improve trait performances across all environments similar to those two QTLs included in this study. As both of these QTLs had negative *a* values, the SSSLs in which they were detected (S11 for *Pn-1b* and S22 for *Pn-6d*) could be useful in breeding to reduce PN in rice (Table 4). A second type of QTLs is associated with *ae* only. This category includes nine of the QTLs (*Pn-1a, Pn-3c, Pn-3d, Pn-4, Pn-6a, Pn-6b, Pn-8, Pn-9* and *Pn-12*) detected in this study (Table 4). Since the expression of such QTLs is specific to a particular set of environmental conditions, they will be suitable for selection only for those

environmental conditions. The QTL *Pn-1a* provides an example of the complexity of QTL interactions, with positive *ae* values in E1 and E6, negative *ae* values in E3 and E5, and no significant *ae* values in E2 or E4 (Table 4). The third type of QTLs identified was associated with both *a* and *ae*. Seven QTLs (*Pn-2a, Pn-2b, Pn-3a, Pn-3b, Pn-6c, Pn-7a* and *Pn-7b*) identified in this study fell in this category. The expression of such QTLs is affected by environmental conditions. If the *a* value of such a QTL is in the desired direction (i.e., negative, for PN), and if there are no large *ae* values in the opposite direction, then selection for the donor allele could still contribute to improvement in performance across environments, albeit with variable responses in particular environments. For example, SSSL S37 might be a useful source of an allele at QTL *Pn-7c*. However, at QTLs with large *ae* values in opposing directions, selection for an allele with favorable effects in certain environments could lead to undesired results in other environments. For example, selection for the *Pn-2a* allele from SSSL S7 could reduce PN in environments similar to E1, but might increase PN in environments similar to E3. Agricultural researchers have long recognized the implications of genotype-by-environment interactions in breeding programs. Understanding QE would help breeders in deciding which QTL to use in their breeding programs while tailoring crop cultivars for specific or more diverse environments. This is the first report on detection of QE values of QTLs with SSSLs. It illustrates the value of analyzing action of QTLs through SSSLs with an indirect method that allows estimation of additive effects and QTL by environment interactions.

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