

Identification of *Indica* rice chromosome segments for the improvement of *Japonica* inbreds and hybrids

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Received: 14 January 2010 / Accepted: 22 December 2011 / Published online: 7 February 2012
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Abstract Exploitation of heterosis has brought significant advance in plant breeding and agricultural production, although its genetic basis is still poorly understood. In this study, a total of 66 chromosome segment substitution (CSS) lines, derived from a cross between *japonica* rice inbred line Asominori (as the recurrent parent) and *indica* rice inbred line IR24 (as the donor parent), were used to investigate the genetic basis of heterosis in *indica* × *japonica* inter-sub-specific rice hybrids. Each CSS line was crossed with the background parent Asominori, and the heterosis of F₁ hybrids was estimated by comparing the F₁ performance with its two parental lines. Field experiments were carried out across six different environments to evaluate yield and yield-related traits in the 66 CSS lines and their 66 corresponding F₁ hybrids. Quantitative trait loci (QTL) analyses were conducted using a likelihood ratio test based on the stepwise regression. Thirty-six QTL were identified with significant effects in CSSL, 21 with significant effects in hybrids and 13 with significant effects in both. On the basis

of average dominance degree, of all the 70 QTL affecting yield-related agronomic traits, 28.6% (20) showed an overdominance, 35.7% (25) a partial dominance and 30% (21) an additive effect, indicating that all effects contribute to trait variation in *japonica*–*indica* rice hybrids. Effects of these QTL were examined to identify *Indica* rice chromosome segments of interest for the improvement of *japonica* inbred lines and hybrids.

Introduction

Heterosis is defined as the superior performance of a hybrid (such as F₁ hybrids) over its parents (Shull 1908), and is usually measured as either mid-parent heterosis (MPH) or best-parent heterosis (BPH). Heterosis has been exploited extensively in breeding hybrid varieties in several crop species over the past decades, especially in maize (Duvick 2001) and rice (Yuan 2003; Ma and Yuan 2003). However, there remains a poor understanding of its genetic basis (Coors and Pandey 1999; Lippman and Zamir 2007).

Several hypotheses have been proposed to explain the genetic mechanism of heterosis, among which are dominance, overdominance, and epistasis (Crow 1999; Goodnight 1999). The dominance hypothesis attributes heterosis to favorable dominant alleles in one parent overwhelming the effect of unfavorable recessive alleles in the other (Davenport 1908; Bruce 1910; Jones 1917; Xiao et al. 1995; Cockerham and Zeng 1996). The overdominance hypothesis ascribes heterosis to the superior fitness of heterozygous genotypes over either homozygous genotypes (East 1908; Shull 1908; East 1936; Crow 1948; Stuber 1994; Semel et al. 2006); “pseudo-overdominance” refers to a particular situation where tightly linked genes with favorable dominant alleles in the parental lines create an

Communicated by A. Charcosset.

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apparent overdominance when combined in the hybrid (Jones 1917, Crow 1952). Finally, the interaction of alleles from the two parents at different loci is taken into account by the epistasis hypothesis (Williams 1959; Schnell and Cockerham 1992; Stuber et al. 1992; Yu et al. 1997; Li et al. 1997, 2001; Luo et al. 2001; Mei et al. 2005; Melchinger et al. 2007; Kusterer et al. 2007).

Molecular markers and genetic linkage maps have greatly facilitated the identification of individual loci conditioning heterosis and the estimation of underlying gene action. Quantitative trait loci (QTL) mapping has been proved to be an informative approach in dissecting complex traits and heterosis in crops (Stuber et al. 1992; Xiao et al. 1996; Yu et al. 1997; Li et al. 1997, 2001, 2008; Mei et al. 2003, 2005). In the aforementioned studies, non-permanent segregating populations such as F_2 , $F_{2,3}$, BC_1F_2 generations and permanent populations such as recombinant inbred lines (RILs) or double haploid lines (DH) were usually used. In an F_2 or BC_1F_2 population, each genotype is represented by only one individual. When these populations are used, it is not possible to make repeated observations at the level of the individual or to carry out multiple trials; also $F_{2,3}$ populations have only half heterozygosity of F_2 population (Mei et al. 2005).

Hua et al. (2002) proposed an “immortalized F_2 ” population that is generated from random intermating between the RILs. One “immortalized F_2 ” population could be tested repeatedly, but may exhibit skewed frequencies of marker locus compared with the true F_2 population. RIL populations can also be used to explain the genetic basis of heterosis on the basis of a mating design to the parents, i.e. NCIII design (Stuber et al. 1992; Schön et al. 2010). However, such RIL or DH based experiments do not make it possible to compare the heterosis effects of different chromosome segments in the same genetic background. Genome-wide chromosome segment substitution (CSS) lines or introgression lines (ILs) population is composed of a series of overlapping chromosome segment substitution lines that are built up with one parent as genetic background line and another as a chromosome segments donor. CSS lines make it possible to compare the effects of different chromosome segments in the same genetic background. They therefore could partly overcome the limitations of F_2 , $F_{2,3}$, BC_1F_2 , RIL or DH population mentioned above (Yu et al. 2005) and/or complement information from these designs.

In this study, we tested a set of 66 rice CSS lines and their F_1 hybrids with the background parent across six environments with the objectives: (1) to understand the genetic basis of heterosis, and (2) to identify genomic regions of interest for the improvement of *japonica* rice inbred lines or *japonica* rice hybrids.

Materials and methods

Genetic populations

A panel of 66 CSS lines was generated in a *japonica* cultivar Asominori genetic background, by selecting 19 recombinant inbred lines in which the genetic background was at least 60% of Asominori, and back-crossing these lines to Asominori, without selection, to the BC_3 generation. A whole-genome survey, based on 116 RFLP markers, was used to select the set of 66 CSS lines, designated by CSS line 1–66 (for details concerning the development of this CSS lines population, see Tsunematsu et al. 1996; Kubo et al. 1999a, Kubo et al. 2002). Combining with the McCouch et al. (2002) rice consensus map, the RFLP markers were replaced with 137 SSR markers evenly distributing across all 12 chromosomes as shown in Fig. 1. The donor segments of IR24 in the 66 CSS lines were rescreened by these SSR markers in 2008 and used in our QTL mapping study. Each CSS line inherited 6–8 introgressed segments from the donor parent. Each CSS line was crossed with cv. Asominori to form the F_1 hybrid.

Field experimental design

The two parents, 66 CSS lines and 66 F_1 hybrids were grown in six different environments, i.e., Nanjing in 2003, 2004, 2007 and 2008, and Nanchang in 2007 and 2008, abbreviated as E1–E6. Each entry plot contained two rows, each of ten individual plants. A randomized block design with two replications was applied in each environment; every single parental CSSL line and its corresponding F_1 hybrid which were planted side by side within each block. The recurrent parent Asominori and donor parent IR24 were planted with two replications together with the 66 CSSLs and their F_1 hybrids. The 20 individual plants of each genotype in one entry plot were planted with spacing of 16.5×16.5 cm. A wide-row spacing of 23.5 cm was set between the plots. The fertilizer management and control of diseases and insect pests were applied as recommended.

Traits evaluated

At maturity, five healthy representative individuals were harvested from each block for further testing. The following traits were evaluated: grain weight per plant (GWP), number of grains per panicle (GPP), 1000-grain weight (TGW) (average of three samples from each of the five plants), and number of panicles per plant (PPP) (excluding panicles bearing less than five grains). Procedures followed Xiao et al. (1998).

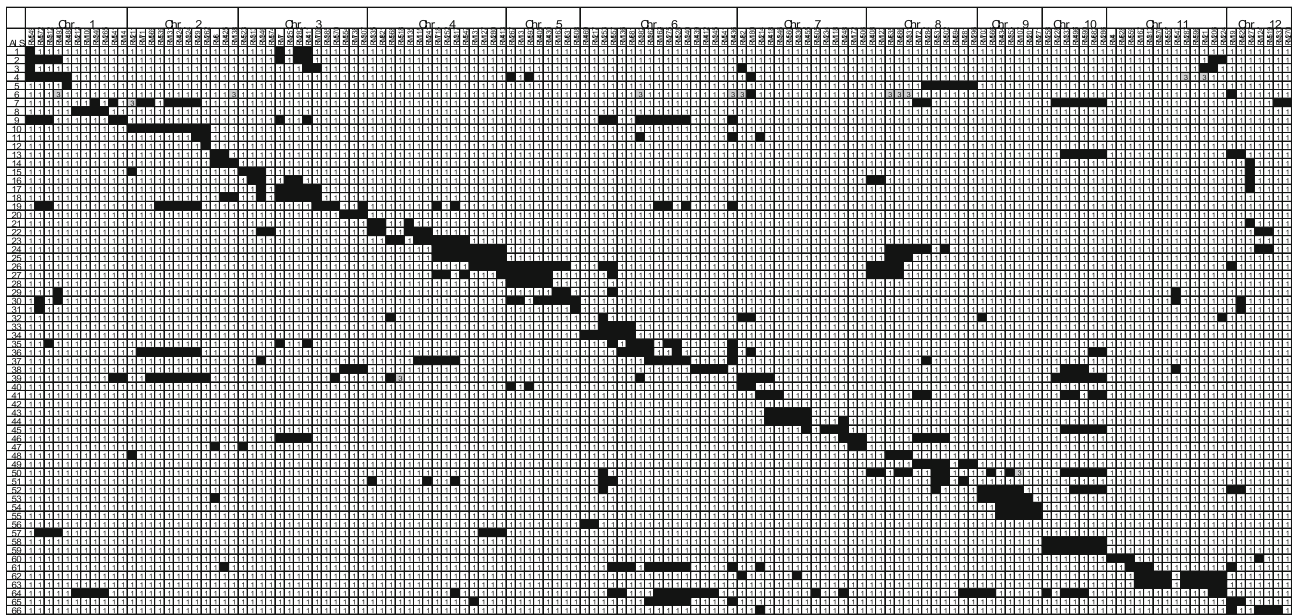


Fig. 1 Genotypes of the 66 CSS lines (*indica* cv. IR24 chromosome segment substitution lines with *Japonica* cv. Asominori genetic background) were rescreened by 137 SSR markers according to McCouch et al. (2002) consensus map between RFLP and SSR

markers. Genotype of Asominori (recipient parent) denotes as “1”, IR24 (donor parent) as “2” within heavy black squares, and heterozygous genotype as “3” within gray squares

Data analysis

Analysis of variance (ANOVA) was conducted for single environments and combined environments (locations \times years), where variance components and broad-sense heritability (H) were estimated. Assume n and r are the numbers of genotypes and replications, respectively, in one environment, and y_{jk} is the phenotypic value of the k th replication of j th genotype, the linear model used in ANOVA of each environment is,

$$y_{jk} = \mu + R_k + G_j + \varepsilon_{jk},$$

where μ is the overall mean, R_k is the replication effect, G_j is the genotypic effect, and ε_{jk} is the residual error. Let MS_G and MS_e represent the mean squares (MS) of genotype and random error, respectively, which can be estimated from ANOVA. Genotypic variance can be estimated as $\sigma_G^2 = \frac{MS_G - MS_e}{r}$, and error variance as $\sigma_e^2 = MS_e$. The plot level heritability can be estimated as, $H_P = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_e^2}$, and plot mean heritability can be calculated as, $H_{\bar{P}} = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_e^2/r}$. Both values have significant implications in plant breeding (Nyquist 1991; Holland et al. 2003).

Assume m , n , and r are the numbers of environments, genotypes and replications, respectively, and y_{ijk} is the phenotypic value of the k th replication of j th genotype at the i th environment, the linear model used in combined ANOVA is,

$$y_{ijk} = \mu + E_i + R_{k(i)} + G_j + GE_{ij} + \varepsilon_{ijk},$$

where μ is the overall mean, E_i is the environmental effect, $R_{k(i)}$ is the replication effect within environment, G_j is the genotypic effect, GE_{ij} is the GE interaction effect, and ε_{ijk} is the residual error. Let MS_G , MS_{GE} and MS_e represent the mean squares (MS) of genotype, GE interaction and random error, respectively, which can be estimated from the combined ANOVA. Under the random effect model, genotypic variance can be estimated as $\sigma_G^2 = \frac{MS_G - MS_e}{rm}$, variance of GE interaction as $\sigma_{GE}^2 = \frac{MS_{GE} - MS_e}{r}$, and error variance as $\sigma_e^2 = MS_e$. The broad-sense heritability at the plot level (or simply plot level heritability) can be estimated as, $H_P = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2 + \sigma_e^2}$. The broad-sense heritability of plot mean (or simply plot mean heritability) can also calculated as $H_{\bar{P}} = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{GE}^2/r + \sigma_e^2/rm}$.

Heterosis was evaluated by MPH, given by $F_1 - (\text{CSS line} + \text{cv. Asominori})/2$, where F_1 is the mean value of each individual hybrid, and CSS line the corresponding CSS line parent.

A likelihood ratio test based on the stepwise regression for QTL mapping with chromosome segment substitution lines.

The standard t test used in the idealized case that each CSS line contains a single chromosome segment from the donor parent (Belknap 2003) is not suitable for CSS line carrying more than one segment from the donor parent, which is the case of our material, as indicated in Fig. 1. Due to high intensity selection in the process of generating CSS lines, the gene and marker frequencies do not follow the same path as in a standard

unselected mapping population. Wang et al. (2006) proposed a likelihood ratio test based on the stepwise regression (abbreviated as RSTEP-LRT hereafter), where stepwise regression is used initially to select the most important chromosome segments for the trait of interest, and followed by the likelihood ratio test to calculate the LOD score of each segment, the largest p value for entering variables was set at 0.05, and the smallest p value for removing variables was set at 0.10 (Wang et al. 2006).

QTL in CSS lines and their F_1 population were detected by software QTL IciMapping (Li et al. 2007; Wang 2009; freely available from <http://www.isbreeding.net>) according to the combined analysis of their marker genotypes shown in Fig. 1 and phenotypes data either in individual or combined environments. On the basis of the map shown as in Fig. 1 and two sets of phenotypic data collected in six environments, the effects of chromosome segments from donor parent in CSSL and heterozygous genotypes in F_1 hybrids were analyzed. The LOD threshold was set at 3.0 which represent the significant effect of QTL following the suggestion of Wang et al. (2006). QTL nomenclature followed the recommendations of McCouch (2008). The average dominance degree was given by ld/la , where d is the dominance effect, and a is the additive effect. Additive and dominance effects of the QTL were estimated following the classical quantitative genetics method. Assuming at one locus, the background parent has allele q , and donor parent has allele Q . From the CSSL population, we were able to calculate mean genotypic values of qq and QQ , represented by mp_qq and mp_QQ , respectively. From the $CSSL \times$ Background F_1 population, we were able to calculate mean genotypic values of qq and Qq , represented by mh_qq and mh_Qq , respectively. So we can have the genotypic values for the three QTL genotypes, i.e., mp_qq and mh_qq for qq , mh_Qq for Qq , and mp_QQ for QQ . To prevent effect of possible difference in means between lines per se and hybrids, we considered contrasts within each type of material: $mp_QQ - mp_qq = 2a$, $mh_Qq - mh_qq = d + a$; so that a and d can be estimated as, $a = (mp_QQ - mp_qq)/2$, $d = (mh_Qq - mh_qq) - (mp_QQ - mp_qq)/2$. The estimated additive and dominance effects were used to calculate ld/la and classify the QTL as either additive (A) ($ld/la < 0.2$), partial dominance (PD) ($0.2 \leq ld/la < 0.8$), complete dominance (CD) ($0.8 \leq ld/la < 1.2$), or overdominance (OD) ($ld/la \geq 1.2$), according to Stuber et al. (1987).

Results

Phenotypic performance of CSS lines and their F_1 hybrids

GWP, TGW and PPP showed positive heterosis (measured as MPH), while GPP showed negative MPH (Table 1).

This suggests that GPP may be a bottleneck for yield enhancement in inter-subspecific crosses between *indica* and *japonica* rice. A correlation analysis based on the mean values of the four yield-related traits across the six locations (Table 2) showed that GPP was positively and significantly correlated ($p < 0.01$) with GWP, indicating that the improvement of GPP trait could enhance yield level in rice. The correlations between PPP and GWP, GPP, TGW were significantly negative, indicating that PPP was a limitative factor for enhancing yield.

Variance components in CSS lines and their F_1 hybrids

Combined ANOVA of yield performance across years and locations showed that yield component traits were influenced by significant genotypic and environmental effects, as well as genotype by environment interactions (Table 3). ANOVA of yield-related traits among the CSS lines and F_1 hybrids showed that heritability varied between environments (Table 4). Heritability at plot mean level was higher than that at plot level because of replications in the experiment (Table 4). Heritability in the combined analysis was lower than in individual locations, illustrating a strong GxE interaction (Table 4). Trait PPP had uniformly low heritability, indicating that it is the more sensitive to the growing environment.

Detection of QTL and estimation of their additive and dominance effects

When the threshold of 3.0 was applied, a total of 22 QTL with significant additive effects in CSSL and/or combined additive-dominant effects in hybrids were detected for GWP in at least one environment (Table 5 in bold), located on chromosomes 1–8 and 12. Four QTL (*qGWP1.3*, *qGWP4.2*, *qGWP5.1* and *qGWP6.2*) had significant effects in CSSL and hybrids simultaneously, and the remaining 18 showed a significant effect either in CSSL or hybrids, demonstrating that a diverse genetic basis underlies this trait. Six QTL (*qGWP1.3*, *qGWP3.1*, *qGWP4.2*, *qGWP6.3*, *qGWP12.1* and *qGWP12.2*) showing positive effect in CSSL in five or six environments could increase grain weight per plant are appropriate targets for inbred line breeding of *japonica*. QTL *qGWP1.3* and *qGWP4.2* displayed significant positive effects in hybrids also and could also be useful for the improvement of *japonica* hybrids. The two QTL (*qGWP2.2*, *qGWP5.2*) with effects significant in hybrids only and positive in five or six environments are suitable in addition for hybrid breeding of *japonica* rice. Of all 22 QTL affecting trait of grain weight per plant, nine were classified as showing overdominance, nine as partial dominance, one as complete dominance, and three as additive.

Table 1 Phenotypic variation of four yield-related traits in CSS lines and F₁ hybrids

Trait	Environment	Asominori	CSS lines				F ₁ hybrids				
			Mean	SD	Min	Max	Mean	SD	Min	Max	MPH
GWP	E1	10.25	10.44	2.72	4.75	18.26	9.77	2.90	1.25	14.82	-0.57
	E2	19.31	17.69	3.07	10.08	26.37	20.08	4.71	2.52	28.37	1.58
	E3	23.81	23.99	4.64	11.55	38.42	28.02	5.92	8.47	42.02	4.12
	E4	25.25	25.41	3.52	16.30	33.70	27.03	4.37	10.20	32.10	1.71
	E5	5.72	9.22	3.86	1.93	17.45	9.06	3.71	0.17	17.86	1.59
	E6	15.91	16.08	3.98	2.49	22.94	16.16	5.70	0.20	26.36	0.17
	Average	16.71	17.14	3.63	7.85	26.19	18.35	4.55	3.80	26.92	1.43
GPP	E1	29.53	33.45	9.69	13.55	66.47	29.00	9.11	3.39	48.50	-2.49
	E2	55.53	57.65	10.90	24.19	80.02	56.04	14.59	7.09	78.45	-0.55
	E3	69.81	67.53	10.38	39.29	89.55	65.66	16.37	10.68	88.43	-3.01
	E4	74.50	75.64	14.23	39.99	109.78	71.89	18.87	6.40	92.40	-3.19
	E5	19.06	27.50	11.28	5.64	58.50	24.38	11.40	0.39	49.37	1.10
	E6	40.46	40.82	9.91	8.98	64.75	37.45	13.61	0.60	62.73	-3.19
	Average	48.15	50.43	11.07	21.94	78.18	47.40	13.99	4.76	69.98	-1.89
TGW	E1	26.48	25.86	2.36	19.98	31.68	25.93	1.51	22.94	29.46	-0.24
	E2	27.72	25.46	2.06	20.15	29.25	26.84	1.13	23.93	29.25	0.25
	E3	25.98	25.68	2.08	19.35	30.38	26.34	1.21	23.35	29.00	0.51
	E4	27.49	25.59	1.77	20.07	28.85	26.86	1.32	24.26	31.40	0.32
	E5	25.00	22.84	2.37	17.62	27.08	23.56	1.75	19.01	27.30	-0.36
	E6	27.12	25.25	1.85	19.13	29.63	26.39	1.36	21.43	28.58	0.21
	Average	26.63	25.11	2.08	19.38	29.48	25.99	1.38	22.49	29.17	0.12
PPP	E1	13.31	12.65	1.99	9.00	18.50	13.28	1.67	10.50	17.75	0.30
	E2	13.70	12.67	1.67	8.25	16.60	13.66	1.14	11.20	16.35	0.47
	E3	13.93	14.07	2.34	9.75	19.91	16.03	1.95	11.73	23.30	2.03
	E4	10.79	11.65	1.10	9.50	15.25	12.17	0.88	10.50	14.90	0.95
	E5	12.00	15.30	3.53	9.50	31.83	16.74	4.24	10.17	27.33	3.09
	E6	14.50	15.68	2.10	11.83	24.33	16.22	1.50	12.67	20.00	1.13
	Average	13.04	13.67	2.12	9.64	21.07	14.68	1.90	11.13	19.94	1.33

GWP grain weight per plant, GPP grains per panicle, TGW 1000-grain weight, PPP panicles per plant, SD standard deviation of mean value, MPH mid-parent heterosis, given by $F_1 - (\text{CSS line} + \text{cv. Asominori})/2$

Table 2 Phenotypic correlations between mean values of yield-related traits in CSS lines \times cv. Asominori F₁ hybrids across environments

Trait	GWP	GPP	TGW	PPP
GWP	1			
GPP	0.92**	1		
TGW	0.07	0.03	1	
PPP	-0.14	-0.34**	-0.25*	1

GWP grain weight per plant, GPP grains per panicle, TGW 1000-grain weight, PPP panicles per plant

*, ** Significance levels of 0.05 and 0.01, respectively

When the threshold of 3.0 was applied, a total of 17 QTL with significant additive effects in CSSL and/or combined additive-dominant effects in hybrids were detected for GPP in at least one environment (Table 6 in

bold), located on chromosomes 1, 2, 4–8, 10 and 12. QTL *qGPP5* and *qGPP7.3* had significant effects in CSSL and hybrids simultaneously. Six QTL (*qGPP1.2*, *qGPP4.2*, *qGPP7.3*, *qGPP8.2*, *qGPP10* and *qGPP12.1*) showed significant positive effects in CSSL in five or six environments and appear suitable for inbred line breeding. Among these, only *qGPP7.3* displayed a significant positive effect in hybrids also and might also be useful for the improvement of *japonica* hybrids. Only one QTL (*qGPP2*) showed significant effects in hybrids only, with positive effects in five or six environments, and appeared suitable for hybrid breeding of *japonica* rice. Of all QTL affecting GPP, five of the 17 QTL were classified as showing overdominance, six as partial dominance, one as complete dominance, and five as additive.

When the threshold of 3.0 was applied, a total of 20 QTL with significant additive effects in CSSL and/or

Table 3 Analysis of variance of yield-related traits in CSS lines population and F₁ hybrids

Source	Degree of freedom	Mean square (MS)				F value			
		GWP	TGW	PPP	GPP	GWP	GPP	TGW	PPP
CSS lines									
Environment	5	755.6	32.8	51.8	4,626.7	132.4**	185.6**	43.1**	13.3**
Rep (Env)	6	30.2	5.5	35.4	49.7	5.3**	2.0	51.2**	9.1**
Genotype	61	88.8	38.9	29.1	1,087.9	15.6**	43.7**	7.3**	7.5**
GE interaction	305	14.7	2.7	6.4	79.7	2.6**	3.2**	3.6**	1.6**
Pooled error	366	5.7	0.8	3.9	24.9				
CSS F ₁ hybrids									
Environment	5	961.4	26.6	61.3	5,511.0	147.7**	180.2**	29.7**	12.0**
Rep (Env)	6	15.9	2.4	9.7	21.2	2.4*	0.7	2.7*	1.9
Genotype	61	169.3	12.7	15.5	1,538.4	26.0**	51.8**	14.2**	3.0**
GE interaction	305	18.5	2.2	8.2	107.6	2.9**	3.5**	2.5**	1.6**
Pooled error	366	6.5	0.9	5.1	30.6				

In this table, $m = 6$, $n = 62$ (due to some missing data), and $r = 2$

m Number of environments, n number of tested lines, r number of replications, *GWP* grain weight per plant, *GPP* grains per panicle, *TGW* 1000-grain weight, *PPP* panicles per plant

*, ** Significance levels of 0.05 and 0.01, respectively

Table 4 Broad-sense heritability of yield-related traits in CSS lines and F₁ hybrids in individual and combined environments

Trait	Population	Level	Environment						Combined
			E1	E2	E3	E4	E5	E6	
GWP	CSS lines	H_P	88.91	95.14	87.43	75.47	74.20	89.72	37.69
		$H_{\bar{P}}$	94.13	97.51	93.29	86.02	85.19	94.58	69.39
	F ₁ hybrids	H_P	88.19	98.02	82.03	79.59	88.84	89.95	50.09
		$H_{\bar{P}}$	93.73	99.00	90.13	88.63	94.09	94.71	77.99
GPP	CSS lines	H_P	94.83	95.90	86.12	88.85	81.43	95.96	61.63
		$H_{\bar{P}}$	97.35	97.91	92.54	94.10	89.76	97.94	84.19
	F ₁ hybrids	H_P	89.05	96.75	83.61	89.82	90.95	96.41	63.31
		$H_{\bar{P}}$	94.21	98.35	91.07	94.63	95.26	98.17	84.54
TGW	CSS lines	H_P	94.63	96.36	86.86	94.38	85.24	96.81	63.58
		$H_{\bar{P}}$	97.24	98.14	92.97	97.11	92.03	98.38	84.63
	F ₁ hybrids	H_P	88.87	91.85	76.63	79.39	69.70	93.04	36.18
		$H_{\bar{P}}$	94.11	95.75	86.77	88.51	82.15	96.40	68.56
PPP	CSS lines	H_P	79.47	94.27	64.05	75.15	75.15	83.93	27.00
		$H_{\bar{P}}$	88.56	97.05	78.08	85.81	85.81	91.26	66.97
	F ₁ hybrids	H_P	78.34	87.24	64.17	61.05	52.69	73.56	8.41
		$H_{\bar{P}}$	87.85	93.19	78.18	75.81	69.02	84.76	33.90

GWP grain weight per plant, *GPP* grains per panicle, *TGW* 1000-grain weight, *PPP* panicles per plant

H_P broad-sense heritability at the plot level, $H_{\bar{P}}$ broad-sense heritability at the plot mean level

combined additive-dominant effects in hybrids were detected for TGW in at least one environment (Table 7 in bold), located on chromosomes 1–8, 10 and 12. Six QTL showed significant effects in CSSL and hybrids simultaneously, ten had significant effects in CSSL only and three had significant effects in hybrids only. Four QTL

(*qTGW3.2*, *qTGW7.1*, *qTGW7.2* and *qTGW7.3*) showed significant positive effects in CSSL, with positive effects in five or six environments, and might be suitable for improvement of TGW trait in inbred line breeding. None of these had stable positive effects in hybrids. QTL *qTGW3.3* displayed a significant effect in hybrids only, with positive

Table 5 Additive and dominant effects of QTL affecting GWP in CSS lines and F₁ hybrids populations across six environments

QTL	Chr.	Marker	Additive effect in CSSLs ^a						Dominant effect in F ₁ hybrids ^a						Mean value across six environments ^b			
			E1	E2	E3	E4	E5	E6	E1	E2	E3	E4	E5	E6	Add ^c	Dom ^d	d /a	
<i>qGWP1.1</i>	1	RM312	-0.27	-0.30	-0.12	-0.43	0.84	0.00	-0.02	0.15	-3.96	-1.77	-0.61	-0.07	0.48	-1.21	2.52	OD
<i>qGWP1.2</i>	1	RM493	0.41	-0.60	2.75	1.69	0.80	-0.28	0.18	0.58	-0.74	0.13	-0.45	0.26	0.77	0.25	0.32	PD
<i>qGWP1.3</i>	1	RM14	2.12	-1.39	0.27	0.04	1.38	0.10	1.22	0.06	-0.68	1.00	-0.95	0.74	0.27	0.36	PD	
<i>qGWP2.1</i>	2	RM211	0.08	0.46	0.92	0.38	-0.04	-0.78	-0.54	-0.37	-0.66	-1.12	-2.18	0.17	-2.30	13.50	OD	
<i>qGWP2.2</i>	2	RM5390	-1.50	-1.01	-0.49	-0.28	-1.09	0.36	3.03	3.34	4.57	3.22	-0.04	-1.06	4.29	4.04	OD	
<i>qGWP2.3</i>	2	RM262	-0.05	-2.63	-2.06	-0.49	-0.05	-0.57	-0.95	-1.02	-0.18	-0.73	2.81	-1.02	0.27	0.26	PD	
<i>qGWP3.1</i>	3	RM523	0.41	-1.08	0.46	0.10	2.50	0.16	0.63	0.86	1.28	0.95	0.82	0.21	0.43	2.02	OD	
<i>qGWP3.2</i>	3	RM7097	0.00	0.78	0.22	0.03	-0.88	-5.49	0.00	-1.42	0.06	-1.00	-0.61	0.14	-0.91	6.50	OD	
<i>qGWP4.1</i>	4	RM317	-1.34	0.57	-0.13	-0.01	-1.78	0.60	0.14	3.51	1.79	0.61	-0.15	-1.53	0.27	0.18	A	
<i>qGWP4.2</i>	4	RM5473	2.21	1.62	0.66	1.26	0.67	0.24	0.00	1.79	-0.48	-0.56	0.15	0.85	0.04	0.05	A	
<i>qGWP5.1</i>	5	RM267	0.30	-2.13	0.56	0.33	-0.30	-4.47	-2.58	0.02	-3.78	-3.35	-2.57	-0.77	-2.80	3.63	OD	
<i>qGWP5.2</i>	5	RM6313	-0.11	0.44	1.31	-0.38	0.82	1.51	0.87	3.08	2.49	2.06	3.94	0.51	2.95	5.78	OD	
<i>qGWP6.1</i>	6	RM217	-1.17	0.13	-0.01	-0.03	-1.46	-0.70	0.01	-0.19	-0.78	-0.12	-0.22	-1.09	-0.02	0.02	A	
<i>qGWP6.2</i>	6	RM253	-1.06	0.44	-0.05	-0.28	-0.13	-0.94	-0.27	-2.02	-0.90	-0.04	0.16	-0.04	0.12	2.93	OD	
<i>qGWP6.3</i>	6	RM345	0.50	1.26	-0.76	3.59	3.33	1.14	0.17	-5.32	1.23	-0.46	-0.24	1.32	-0.68	0.52	PD	
<i>qGWP7.1</i>	7	RM82	0.22	-0.14	0.59	0.10	-0.77	-1.87	-3.56	-7.47	-7.98	-6.35	-1.93	-0.47	-5.05	10.73	OD	
<i>qGWP7.2</i>	7	RM346	0.09	-1.16	0.33	-0.66	2.56	0.45	-0.20	-0.94	-0.77	-0.51	0.24	0.23	-0.20	0.86	CD	
<i>qGWP8.1</i>	8	RM331	-2.86	-2.83	-2.46	-3.86	-1.73	0.84	-0.35	-0.35	0.10	0.01	0.04	-2.63	-0.61	0.23	PD	
<i>qGWP8.2</i>	8	RM502	-3.08	-0.50	-1.84	-4.61	-1.67	-1.35	1.02	1.15	0.00	1.12	1.55	-2.84	0.89	0.31	PD	
<i>qGWP12.1</i>	12	RM286	0.68	0.73	7.20	1.64	0.76	0.10	-0.73	-1.73	0.27	0.16	2.12	2.13	0.52	0.24	PD	
<i>qGWP12.2</i>	12	RM19	0.59	0.81	1.24	0.70	2.63	0.04	0.08	-0.16	0.26	0.10	-0.34	0.68	0.22	0.32	PD	
<i>qGWP12.3</i>	12	RM270	0.14	-0.39	-4.94	-0.73	-1.90	-8.29	-1.64	-1.39	-2.69	-1.21	0.55	-0.86	-1.05	0.34	PD	

OD overdominance, PD partial dominance, CD complete dominance, A additive

^a QTL with LOD ≥ 3.0 were shown in bold. Tests reported in additive and dominant effects column correspond to QTL detection in CSSL and hybrids, respectively. Note that effect tested in hybrids includes both additive and dominance effects (see “Materials and methods”)

^b Additive and dominant value under header “Mean value across six environments” was using mean value across six environments of GWP trait as basic data to detect average additive and dominant effect of each QTL

^c Average value of additive effects across six environments

^d Average value of dominant effects across six environments

Table 6 Additive and dominant effects of QTL affecting GPP in CSS lines and F₁ hybrid populations across six environments

QTL	Chr.	Marker	Additive effect in CSSLs ^a						Dominant effect in F ₁ hybrids ^a						Mean value across six environments ^b			
			E1	E2	E3	E4	E5	E6	E1	E2	E3	E4	E5	E6	Add ^c	Dom ^d	ldl/dl	
<i>qGPP1.1</i>	1	RM5496	0.46	2.87	7.40	7.14	-0.41	-1.99	1.31	2.27	-2.14	-2.52	2.26	-0.59	0.72	-0.60	0.84	CD
<i>qGPP1.2</i>	1	RM14	7.37	1.29	2.34	4.00	14.25	1.23	2.32	0.91	0.84	4.07	-1.81	1.55	7.81	0.77	0.10	A
<i>qGPP2</i>	2	RM5390	-0.25	-0.27	1.06	1.70	-3.44	0.35	7.49	14.38	14.94	16.87	1.52	13.99	1.84	13.48	7.33	OD
<i>qGPP4.1</i>	4	RM317	-5.58	-9.85	-7.15	-8.65	-5.91	2.52	-0.64	7.52	3.27	2.41	0.13	0.02	-6.30	1.27	0.20	PD
<i>qGPP4.2</i>	4	RM5473	8.17	8.93	0.26	9.74	8.29	3.54	-1.37	0.66	1.72	-1.69	0.53	0.39	7.81	0.14	0.02	A
<i>qGPP5</i>	5	RM267	0.15	-1.38	-3.47	-1.62	-0.70	-10.51	-4.99	-9.58	-11.62	-13.87	-2.23	-7.52	-1.69	-8.49	5.02	OD
<i>qGPP6.1</i>	6	RM217	-3.84	-6.83	-5.85	-10.18	-8.77	-1.31	-0.07	-0.89	-1.48	-1.54	1.07	0.35	-6.34	-0.03	0.01	A
<i>qGPP6.2</i>	6	RM253	-2.26	-0.22	-1.58	1.22	0.94	1.02	-0.23	-0.90	-1.43	-0.72	0.78	-2.71	-0.29	0.38	1.32	OD
<i>qGPP7.1</i>	7	RM436	-1.00	0.77	1.16	0.01	-5.35	-1.19	0.75	-0.18	0.21	-0.35	-0.34	0.61	0.74	0.40	0.54	PD
<i>qGPP7.2</i>	7	RM82	-0.84	0.82	0.10	0.63	-1.37	-2.37	-9.01	-20.36	-21.34	-22.85	-3.76	-12.12	0.76	-16.07	21.14	OD
<i>qGPP7.3</i>	7	RM180	1.02	4.50	3.29	2.64	0.89	-1.23	2.41	0.67	0.75	4.00	-1.03	-1.36	3.40	0.95	0.28	PD
<i>qGPP8.1</i>	8	RM331	-9.80	-10.25	-10.83	-18.10	-9.71	0.03	-1.06	-0.30	-0.04	-0.66	-0.32	-2.75	-10.71	-0.99	0.09	A
<i>qGPP8.2</i>	8	RM284	1.15	4.41	2.35	8.95	1.04	-2.63	0.33	1.80	1.22	0.71	-0.20	1.34	1.55	0.73	0.47	PD
<i>qGPP8.3</i>	8	RM502	-9.83	-8.68	-3.74	-24.96	-11.64	-2.73	-0.18	-0.11	2.01	3.05	6.47	2.67	-11.86	2.75	0.23	PD
<i>qGPP10</i>	10	RM258	0.52	5.61	2.99	6.40	1.39	3.05	0.52	-1.25	1.14	-0.02	-0.02	1.26	3.29	0.33	0.10	A
<i>qGPP12.1</i>	12	RM19	4.19	-0.04	0.95	1.17	6.09	0.82	0.91	1.98	0.63	-0.12	-5.87	-0.26	2.03	0.84	0.41	PD
<i>qGPP12.2</i>	12	RM270	0.71	0.26	-4.96	1.63	-3.68	-17.34	-4.51	-3.77	-5.51	-5.98	6.01	-1.79	-2.12	-2.63	1.24	OD

OD overdominance, PD partial dominance, CD complete dominance, A additive

^a QTL with LOD ≥ 3.0 were shown in bold. Tests reported in additive and dominant effects column correspond to QTL detection in CSSL and hybrids, respectively. Note that effect tested in hybrids includes both additive and dominance effects (see “Materials and methods”)

^b Additive and dominant value under header “Mean value across six environments” was using mean value across six environments of GPP trait as basic data to detect average additive and dominant effect of each QTL

^c Average value of additive effects across six environments

^d Average value of dominant effects across six environments

Table 7 Additive and dominant effects of QTL affecting TGW in CSS lines and F₁ hybrid populations across six environments

QTL	Chr.	Marker	Additive effect in CSSLs ^a						Dominant effect in F ₁ hybrids ^a						Mean value across six environments ^b		
			E1	E2	E3	E4	E5	E6	E1	E2	E3	E4	E5	E6	Add ^c	Dom ^d	ld/ld ^e
<i>qTGW1</i>	1	RM14	-1.82	0.26	0.09	0.05	0.22	-1.41	0.12	0.03	-0.08	-0.09	-0.15	-1.03	0.01	0.01	A
<i>qTGW2.1</i>	2	RM211	0.22	0.22	0.15	-0.11	0.01	0.15	0.07	-0.02	0.78	0.02	0.27	-0.13	0.05	0.42	PD
<i>qTGW2.2</i>	2	RM5390	-0.32	-1.36	-0.62	-0.15	-1.49	-1.32	-0.19	-0.31	-1.74	0.22	-0.16	-0.56	-0.19	0.34	PD
<i>qTGW2.3</i>	2	RM6	-0.36	-1.01	-0.54	-0.50	-0.12	-0.54	0.09	0.10	0.01	0.14	0.15	-0.45	0.04	0.08	A
<i>qTGW2.4</i>	2	RM138	-1.00	-1.38	-0.48	-1.24	-0.35	-0.49	0.78	-0.59	0.18	0.03	0.33	-1.32	0.08	0.06	A
<i>qTGW3.1</i>	3	RM5748	-0.45	-1.17	-0.42	-1.13	-0.07	0.11	-0.03	-0.02	-0.15	-0.33	0.07	-0.57	-0.08	0.14	A
<i>qTGW3.2</i>	3	RM411	0.47	1.09	0.25	0.85	0.33	0.14	-0.21	0.03	-0.67	-0.23	0.04	0.13	-0.10	0.73	PD
<i>qTGW3.3</i>	3	RM7097	0.00	0.09	0.26	0.10	0.25	0.17	0.00	0.28	2.25	0.01	1.33	0.03	0.35	11.57	OD
<i>qTGW4.1</i>	4	RM252	-0.55	-0.62	-0.28	-0.04	-0.31	-0.23	0.16	-0.04	0.01	-0.04	0.05	-0.06	-0.04	0.62	PD
<i>qTGW4.2</i>	4	RM317	-1.15	-0.43	-0.45	-0.21	-1.77	-0.68	-0.06	-0.13	-0.04	0.14	-0.05	-0.24	-0.11	0.46	PD
<i>qTGW5</i>	5	RM267	-0.48	-0.03	0.46	0.25	-0.35	-0.19	-0.17	0.10	0.47	-0.03	-1.54	0.14	0.02	0.13	A
<i>qTGW6.1</i>	6	RM217	-1.02	-0.45	-0.45	0.09	-1.16	-0.14	0.17	-0.17	0.09	0.05	-0.18	0.03	0.06	2.13	OD
<i>qTGW6.2</i>	6	RM3827	-1.39	-1.30	-1.64	-0.85	-2.01	-0.58	-0.15	-0.07	0.03	0.08	-0.04	-1.06	0.01	0.01	A
<i>qTGW7.1</i>	7	RM436	1.11	0.42	0.26	0.33	-0.27	0.89	-0.24	-0.09	-0.08	-0.17	-0.14	0.44	-0.10	0.24	PD
<i>qTGW7.2</i>	7	RM82	0.11	1.16	0.65	0.74	0.33	0.35	0.18	0.22	1.31	-0.56	-0.22	0.55	0.06	0.12	A
<i>qTGW7.3</i>	7	RM248	0.37	2.38	0.71	0.01	0.33	0.18	0.91	-0.04	0.23	0.07	0.31	0.02	0.04	1.95	OD
<i>qTGW8.1</i>	8	RM506	-0.16	-0.85	-0.17	-0.69	-1.13	-0.76	-0.06	0.04	0.08	0.70	-0.07	-0.80	0.14	0.18	A
<i>qTGW8.2</i>	8	RM331	-1.92	-1.69	-1.31	-0.48	-2.30	-0.14	0.11	0.66	0.09	0.03	0.20	-1.25	0.21	0.17	A
<i>qTGW10</i>	10	RM258	-0.92	-0.92	-1.28	-0.72	0.00	0.00	-0.01	-0.04	-0.12	-0.20	0.01	-0.17	-0.08	0.49	PD
<i>qTGW12</i>	12	RM270	-0.42	-0.27	-0.99	-1.81	-0.70	-3.42	-0.05	0.11	0.01	-0.02	0.26	-1.73	0.06	0.03	A

OD overdominance, PD partial dominance, CD complete dominance, A additive

^a QTL with LOD ≥ 3.0 were shown in bold. Tests reported in additive and dominant effects column correspond to QTL detection in CSSL and hybrids, respectively. Note that effect tested in hybrids includes both additive and dominance effects (see “Materials and methods”)

^b Additive and dominant value under header “Mean value across six environments” was using mean value across six environments of TGW trait as basic data to detect average additive and dominant effect of each QTL

^c Average value of additive effects across six environments

^d Average value of dominant effects across six environments

effects in six environments, is suitable in addition for hybrid breeding of *japonica* rice. Of all QTL affecting TGW, 3 of the 20 QTL were classified as showing overdominance, seven as partial dominance, and ten as additive.

When the threshold of 3.0 was applied, a total of 11 QTL with significant additive effects in CSSL and/or combined additive-dominant effects in hybrids were detected for PPP in at least one environment (Table 8 in bold), located on chromosomes 1, 4 and 5–8. Only *qPPP4.2* showed a significant effect in CSSL and hybrids simultaneously. There were nine QTL showing significant effects in hybrids only, and one single QTL showing significant effects in CSSL only. QTL (*qPPP4.2* and *qPPP8*) with significant positive effects in CSSL in five or six environments might be used in improving PPP trait in *japonica* inbred lines. QTL *qPPP6* with effects significant in hybrids only and positive in five environments is suitable in addition for hybrid breeding of *japonica* rice. Of the 11 QTL affecting PPP, three were classified as showing overdominance, three as partial dominance, two as complete dominance, and three as additive.

Distribution of the phenotypic variation explained by QTL

The phenotypic variation explained (PVE) by QTL in CSS and hybrids was given in Fig. 2. Most QTL had a PVE <10% in individual environments. Thus, the inheritance of these yield-related traits is dominated by QTL each of minor effect confirming that these traits have a polygenic determinism. For GPP, the prevalence of negative dominant effect QTL such as *qGPP5* and *qGPP7.2* with PVE in hybrids ranging from 15% to over 20% may reflect the phenomenon of hybrid sterility, which is a common occurrence in *indica* × *japonica* hybrids.

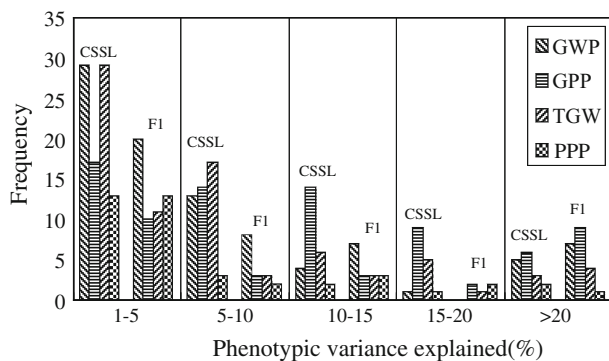


Fig. 2 The frequency of phenotypic variation explained by individual QTL showing significant effects in CSSL and F1 hybrids in individual environments. *GWP* grain weight per plant, *GPP* grains per panicle, *TGW* 1000-grain weight, *PPP* panicles per plant

Table 8 Additive and dominant effects of QTL affecting PPP in CSS lines and F₁ hybrid populations across six environments

QTL	Chr.	Marker	Additive effect in CSSLs ^a						Dominant effect in F ₁ hybrids ^a						Mean value across six environments ^b			
			E1	E2	E3	E4	E5	E6	E1	E2	E3	E4	E5	E6	Add ^c	Dom ^d	<i>l d a </i> ^e	CD
<i>qPPP1</i>	1	RM312	-0.16	0.28	0.53	0.05	0.72	0.16	0.03	-1.04	-0.78	-0.12	0.14	-0.09	0.18	-0.13	0.74	CD
<i>qPPP4.1</i>	4	RM252	0.31	-0.16	-0.56	0.00	0.67	0.42	0.05	-0.10	-0.23	0.01	-1.03	0.06	-0.21	3.55	OD	
<i>qPPP4.2</i>	4	RM317	1.76	1.22	-0.30	0.20	0.04	0.10	0.04	0.17	0.28	-0.10	-0.47	0.71	-0.03	0.04	A	
<i>qPPP4.3</i>	4	RM5473	-0.77	-0.72	-0.86	-0.29	-1.26	-0.48	0.12	0.64	-0.92	0.05	0.09	0.28	-0.73	-0.14	0.20	PD
<i>qPPP5.1</i>	5	RM267	0.24	-0.29	0.09	0.04	0.16	-0.01	-0.17	0.37	-0.35	0.85	0.50	0.01	0.05	5.00	OD	
<i>qPPP5.2</i>	5	RM430	-0.68	-0.07	-0.40	0.03	-0.79	-0.21	-0.04	0.19	-0.13	-0.76	-0.63	-0.31	-0.03	0.10	A	
<i>qPPP6</i>	6	RM217	-0.23	0.14	0.42	0.03	0.37	0.29	0.07	0.27	-0.19	0.08	0.16	0.06	0.03	0.43	PD	
<i>qPPP7.1</i>	7	RM436	0.14	-0.07	0.64	-0.06	0.11	-0.12	-0.35	-0.90	0.12	-0.07	0.77	0.13	-0.13	1.00	CD	
<i>qPPP7.2</i>	7	RM82	0.00	-0.22	-0.02	-0.27	-0.21	-0.24	-0.39	0.36	-0.22	0.76	0.30	-0.20	0.01	0.06	A	
<i>qPPP7.3</i>	7	RM346	-0.27	-0.64	-0.33	-0.27	-0.08	-0.18	-0.40	-0.47	-0.37	-0.25	-1.25	-0.38	-0.47	1.24	OD	
<i>qPPP8</i>	8	RM331	1.48	0.06	1.41	1.02	4.30	1.47	-0.52	-0.08	-0.13	-0.19	-0.46	1.57	-0.79	0.50	PD	

OD overdominance, PD partial dominance, CD complete dominance, A additive

^a QTL with LOD ≥3.0 were shown in bold. Tests reported in additive and dominant effects column correspond to QTL detection in CSSL and hybrids, respectively. Note that effect tested in hybrids includes both additive and dominance effects (see “Materials and methods”).

^b Additive and dominant value under header “Mean value across six environments” was using mean value across six environments of PPP trait as basic data to detect average additive and dominant effect of each QTL.

^c Average value of additive effects across six environments

^d Average value of dominant effects across six environments

Discussion

Usefulness of CSS lines in genetic investigations and breeding

CSS lines have obvious advantages over conventional genetic populations when used for heterosis QTL identification. Firstly, genetic interactions between alleles at different loci are limited to genes present on the introgressed segments, since the genetic background of each CSS line is mostly that of the recurrent (recipient) parent (Howell et al. 1996). Thus, target chromosome segments can be combined to study the interaction between genes or gene cluster at different chromosome segments by eliminating the influences of non-target chromosome segments. Secondly, when CSS lines are crossed with the recurrent parent, the genetic background of each F₁ hybrid remains largely homozygous—except at those locations where an introgression segment is present. This situation simplifies the dissection of the genetic basis of heterosis. Thus, the chromosome segments for heterosis can be tested through whole genome CSS lines and heterosis at different segments can be further studied. Thirdly, chromosome segments associated with hybrid sterility, hybrid weakness or unfavorable heterosis such as extremely long growth duration and immoderate tall plant height can be directly located, therefore facilitating selection against unfavorable alleles causing these traits. Finally, if commercially widely used parental lines are adopted as recipients or background parents, superior CSS lines can be directly used as parental lines for hybrid breeding.

Heterosis of yield-related traits

Heterosis was commonly observed for the yield and yield-related traits. GPP is a relatively simple trait, which is highly correlated with grain yield, so it provides a good illustration of the genetic complexity of yield. The correlation between GPP and yield was the highest of the three yield-related traits that were considered (Table 2). The negative correlations between PPP and GWP, GPP, TGW create an apparent contradiction PPP and these three other yield-related factors. Understanding the complex relationship between yield and yield-related traits would clearly help plant breeders to breed for high yield. Meanwhile, the heritability of GPP is high (Table 4). TGW showed little heterosis, and was in general less variable than other yield components. Variation for yield-related traits provides opportunities for plant breeders, although G × E interaction at different level implies unstable performance of these traits, which is a problem for breeding programs serving a wide range of growing environments.

Maximizing the benefits of heterosis in *indica* × *japonica* hybrids is a key aim of hybrid rice breeding. Here, we were able to show that some of the introgressed *indica* segments carried stably expressed heterosis QTL, two steadily increasing GWP of hybrids over more than five locations (*qGWP2.2*, *qGWP5.2*), one increasing GPP of hybrids (*qGPP2*), one increasing TGW of hybrids (*qTGW3.3*), one increasing PPP of hybrids (*qPPP6*), and these represent useful leads in the search for hybrid yield improvement. On the other side, the Asominori alleles at QTL with negative additive effects might be useful in the improvement of *indica* inbred lines. Those at QTL with negative dominant effects might be useful in the improvement of *indica* hybrids. More and further research is needed to confirm these deductions because the genetic background is different.

Pleiotropic effects of individual loci or tight linkage between loci in the same region might be responsible for the significant negative correlation between the two main yield component traits GPP and PPP (Table 2). The negative correlation between GPP and PPP may reflect a source/sink limitation, in that competition for assimilate forces the plant to compromise between the mean number of grains which can be filled per panicle and the number of panicles which can be developed per plant. Such correlations are difficult for plant breeders to overcome. Seven of the QTL detected affected GPP and PPP simultaneously, and, as expected, had opposite effects (Tables 6, 8). Detailed information concerning the loci controlling the various yield components and their genetic relationships may help to improve yield potential by allowing the optimization of marker-assisted selection.

The genetic basis of heterosis through QTL mapping

Up to now, several studies have been conducted to try to understand the genetic basis of heterosis in rice (Xiao et al. 1995; Li et al. 2001; Luo et al. 2001b; Hua et al. 2002, 2003; Abdelkhalik et al. 2005). However, the causes underlying this important phenomenon have remained unclear and none of these studies quantified the gene action of QTL. According to the statistical method developed by Li et al. (2008), we employed it in this study to bring complementary elements on the causes of heterosis in rice. Our analyses allowed the identification of several QTL for each of the traits investigated. Most individual QTL explained modest variation (<10%), and only a small number of QTL in CSS lines and their F₁ derivatives contributed over 20% variation individually (Fig. 2), confirming that heterosis is a polygenic phenomenon (Kusterer et al. 2007).

About half of the 70 QTL showing additive effects in CSSL had effects stable and large enough to be considered as targets for inbred line improvement from the CSS lines.

In addition, ~30% of the QTL showing effects in hybrids were stably expressed, so that they could be useful in the context of *indica* × *japonica* hybrid breeding. The 13 QTL which showed significant effects in CSSL and hybrids also represented an interesting genetic resource in the context of inbred or hybrid improvement via a design-breeding approach, as described by Wang et al. (2007). The QTL on chromosomes 5 (*qGWP5.1*, *qGPP5*) and 7 (*qGWP7.2*, *qGPP7.2*) negatively affected GWP and GPP, perhaps because of tightly linkage with hybrid sterility genes such as *S-7*, *S-24(t)* or *S-31* (Wan et al. 1996; Kubo et al. 1999b; Zhao et al. 2007). The use of small chromosome segments in these regions might facilitate the identification of favorable linked QTL by avoiding the sterility in *indica* × *japonica* hybrids.

Dominance clearly plays an important role in determining heterosis in CSS F₁ hybrids. In our study, the proportion of QTL showing a gene action of overdominance was less than that with partial-to-complete dominance. This result was also found in the study conducted by Xiao et al. (1995). Across all the traits, overdominance occurred at 28.6% of the loci, 41.4% exhibited partial to complete dominance, and 30% additive effects. Thus, the various traits showed different genetic bases for heterosis, with GWP and GPP being dominated by overdominance and partial dominance, TGW by partial dominance, and PPP partly by complete dominance and partly by overdominance. These results are largely consistent with the relevant literature aforementioned. However, although a relatively lower portion of QTL demonstrated overdominance, QTL exhibiting high levels of overdominance effects are not necessarily indicative of true overdominance (Li et al. 2008), but rather can be the result of dominant alleles linked in repulsion (pseudo-overdominance).

Considering the characteristics of CSS lines population (with very low proportion of *Indica* genome introgression, see Fig. 1), we did not analyze epistasis between QTL. Analysis of epistasis was addressed globally considering that if heritability of a trait is equal to total PVE by the effects of detected QTL there is no epistasis in F₁ hybrids population. From combined analysis, broad-sense heritability of GWP, GPP, TGW, PPP at plot mean level was 77.99, 84.54, 68.56 and 33.90%, respectively, (Table 4). PVE of detected QTL for GWP, GPP, TGW, PPP was 88.97, 80.89, 68.52, and 27.58%, respectively. This suggests that epistasis globally plays a limited role but may affect to some extent GWP and PPP traits.

Chromosome segments substitution technology in the utilization of heterosis from inter-subspecific cross between *indica* and *japonica* rice.

The extent of heterosis expressed in inter-subspecific crosses follows the general trend: *indica/japonica* > *indicaljavanica* > *japonicaljavanica* > *indicalindica* > *japonical*

japonica (Yuan 2003). The *indicaljaponica* hybrids are characterized by pronounced heterosis, which can reach 30% in yield terms over what is achievable in intervarietal *indica* hybrids. Much effort has therefore been expended on exploiting this heterosis, but progress has been hindered by low seed set. The deployment of wide compatibility genes and the use of intermediate germplasm (instead of typical *indica* or *japonica* lines) are seen as the optimal way towards maximizing the use of intersubspecific hybrid heterosis. The cytoplasmic male sterility three-line system and photoperiod-thermo sensitive genic male sterility two-line system are the two major technologies currently used for hybrid rice breeding (Yuan 2003). Both systems are focused on *indica* × *indica* crosses, so further progress is difficult because of the narrow genetic bottleneck which this restriction has imposed.

Although it is possible that genomics of *indica* and *japonica* rice could be introgressed into each other through traditional backcross methods, heterosis is difficult to predict because of its complicated genetic basis (Yu et al. 2005). To simplify the situation, our conclusion is that the search for heterosis should be confined to a small number of chromosome segments, considering materials such as CSS lines. One feasible strategy therefore would be to use the parents of currently successful hybrids to create CSS lines, and use these lines as parents of inter-subspecific hybrids.

Acknowledgments We thank the editor and two anonymous reviewers for their constructive comments which significantly improved the manuscript. We also thank Professor A. Yoshimura, Kyushu University, Japan, for kindly providing us with the CSS lines population. This research is supported by the grants from the 863 Program of China (2009AA101101), the 973 Program of China (2011CB100102), the earmarked fund for Modern Agro-industry Technology Research System, Jiangsu Science and Technology Development Program (BE2009301-3), and PAPD project.

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