Genetic analysis of heterotic loci detected in a cross between *indica* and *japonica* rice (*Oryza sativa* L.)

Xiao Yun Xin, Wen Xiang Wang, Jin Shui Yang and Xiao Jin Luo*

State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Fudan University, Shanghai 200433, China

The study on the genetic basis of heterosis has received significant attention in recent years. In this study, using a set of introgression lines (ILs) and corresponding testcross F_1 populations, we investigated heterotic loci (HL) associated with six yield-related traits in both *Oryza sativa* L. subsp. *indica* and *japonica*. A total of 41 HL were detected on the basis of mid-parent heterosis values with single-point analysis. The F_1 test-cross population showed superiority in most yield-related traits and was characterized by a high frequency of overdominant HL. Thirty-eight of the 41 HL were overdominant, and in the absence of epistasis, three HL were dominant, suggesting that heterotic effects at the single-locus level mainly appeared to be overdominant in rice. Twenty-four HL had a real positive effect, suggesting that they are viable candidates for the improvement of rice yield potential. Compared with the quantitative trait loci (QTLs) detected in the ILs, only six out of the 41 (14.6%) HL were detected in QTL analysis under the same statistical threshold, indicating that heterosis and trait performance may be conditioned by different sets of loci.

Key Words: introgression lines, QTLs, heterotic loci, overdominant.

Introduction

Use of heterosis has become a major strategy for increasing the productivity of plants and animals (Hua *et al*. 2003); however, the study of the genetic basis of heterosis has fallen far behind the exploitation of heterosis. Dominance (Bruce 1910, Jones 1917) and overdominance (Shull 1908) are two hypotheses proposed a century ago to explain the genetic basis of heterosis. Recent advances in genome research involving a number of molecular-marker techniques and the availability of high-density molecular linkage maps, together with developments in analytical methods (Lander and Botstein 1989, Zeng 1994), have facilitated the analysis of the genetic basis of quantitative traits.

Recently, many quantitative trait loci (QTL) mapping studies have provided insight into the genetic basis of heterosis (Frascaroli *et al*. 2007, Garcia *et al*. 2008, Hua *et al*. 2003, Li *et al*. 2001, Li *et al*. 2008, Luo *et al*. 2001, Luo *et al*. 2009a, Mei *et al*. 2003, 2005, Melchinger *et al*. 2007, 2008, Stuber *et al*. 1992, Xiao *et al*. 1995, Yu *et al*. 1997), and resulted in different explanations for this phenomenon, such as dominance, overdominance, epistasis and so on. We analyzed previous studies and found that differences in segregating populations and/or statistical methods caused the different conclusions. Furthermore, one known problem in establishing the genetic basis of heterosis has been the use of

whole-genome segregating populations, where interactions often mask the effects of individual loci (Semel *et al*. 2006).

A solution to this problem is the use of introgression lines (ILs), in which small chromosomal segments are introgressed from the donor into the recurrent parent by consecutive backcrossing and selfing (Eshed and Zamir 1994, 1995). Consequently, ILs provide more precise estimates of the genetic effects of introgression against a relatively uniform and elite lineage background (Tanksley and Nelson 1996) and are therefore well-suited for genetic analysis of heterosis. Analysis of overlapping chromosomal segments in ILs has proven to be a powerful strategy to more precisely map QTL and validate the QTL mapped in early generations or in genome-wide segregating populations (Paterson *et al*. 1990). In addition, several reports have indicated that ILs are a powerful tool for identifying new genes (Eshed and Zamir 1994, 1995, He *et al*. 2006, Luo *et al*. 2009b), distinguishing pleiotropy from linkage (Yamamoto *et al*. 1998), eliminating QTL linkage drag, and for map-based cloning (Alpert and Tanksley 1996).

Recently, we analyzed heterotic loci (HL) between wild and cultivated rice associated with six yield-related traits in a set of 265 ILs, and our results supported the overdominant model involving a single functional Mendelian locus in the absence of epistasis (the results have been submitted to *Genetics Research*). In this study, we further investigated the genetic basis of rice heterosis using a set of 70 ILs from an intersubspecific cross. The lines were generated from a cross between IR24, a commercial *indica* cultivar, as the recurrent parent, and Asominori, a typical *japonica* cultivar, as

the donor parent. On the basis of the set of 70 ILs and 175 testcross F_1s (derived from the cross between the ILs), QTL and HL associated with yield and yield-related traits between the ILs and testcross F_1s were analyzed. The genetic effects and main features of the HL are discussed.

Materials and Methods

Experimental population development

In this study, an IL population composed of 70 lines carrying variant introgressed segments of Asominori (*japonica*) was used. The IL population included the *indica* cultivar IR24 background and covered the entire *japonica* genome with overlapping introgressed segments of each line. The detailed characteristics of ILs were presented in Aida *et al.* (1997) and Kubo *et al.* (2002). Only the heterozygous introgressed segments were all eliminated through associating ILs selfing and corresponding markers analysis. Additionally, 175 F_1 testcross individuals, derived from crosses among the 70 ILs as described below, were evaluated.

Crosses were made between lines chosen by random permutations of the 70 ILs. In each round of permutation, the 70 ILs were randomly divided into two groups, and the lines in the two groups were paired up at random to provide the parents for 35 crosses. Each of the 70 ILs was used only once in each round of pairing and crossing. This procedure was repeated five times, resulting in a population consisting of 175 crosses. The design resembled that previously reported by Hua *et al.* (2003). Crosses to generate the F_1 generation were completed in the summer of 2004 in Beijing and the winter of 2004 in Sanya (18°20′N, 109°50′E, Hainan Province, China).

### Field trials and phenotypic evaluations

Two separate experiments were conducted at the Experiment Stations of China Agricultural University (CAU, Beijing, 39°54′N, 116°28′E) and the Anhui Academy of Agricultural Sciences (AAAS, Hefei, 31°51′N, 117°18′E) in 2005. In the CAU environments, the ILs, parents, and testcross F_1s were sown in the seedling nursery on May 7, 2005. The 25-d-old seedlings were transplanted into 6-row plots (72 plants), each consisting of two rows of F_1 hybrids and the corresponding two IL parents in fixed order: IL_i, F_1 and IL_j (j = I + n, namely j > I, n = 1, 2, ……, 69). Planting of experimental units followed a complete randomized block design with two replications. All materials were planted with 13.3 cm spacing between plants within each row and 26.4 cm spacing between rows. Field management followed normal rice production conditions. In the AAAS environment, all of the materials were sown on June 5, 2005 and transplanted into the field after 25 d. The field arrangement was the same as the CAU environment.

Morphological features characteristic of hybrid rice were used to identify the F_1 hybrids, including plant height, heading date, and tillers, among others. Parents were used as the baseline for comparison, and observations were made throughout the growing season. Several appropriate RFLP markers were chosen to definitively identify the F_1 hybrid plants. Five plants from the middle of each row were harvested at maturity in both the ILs and F_1 individually, and the following traits were scored: spikelet number per panicle (SP), filled grain number per panicle (GP), percent seed set (SSP), 1000-grain weight (GW), panicle number per plant (PP) and grain yield per plant (YP).

DNA extraction and molecular marker analysis

DNA was extracted from fresh leaves according to the CTAB method (Murray and Thompson 1980) with minor modifications. The RFLP markers analyzed in this study were from a previous publication (Saito *et al.* 1991). The molecular marker order was based on the rice linkage map described by Saito *et al.* (1991) and Gramene (http://www.gramene.org/db/markers/marker_view) (Fig. 1). A total of 87 polymorphic RFLP markers were used to genotype the 70
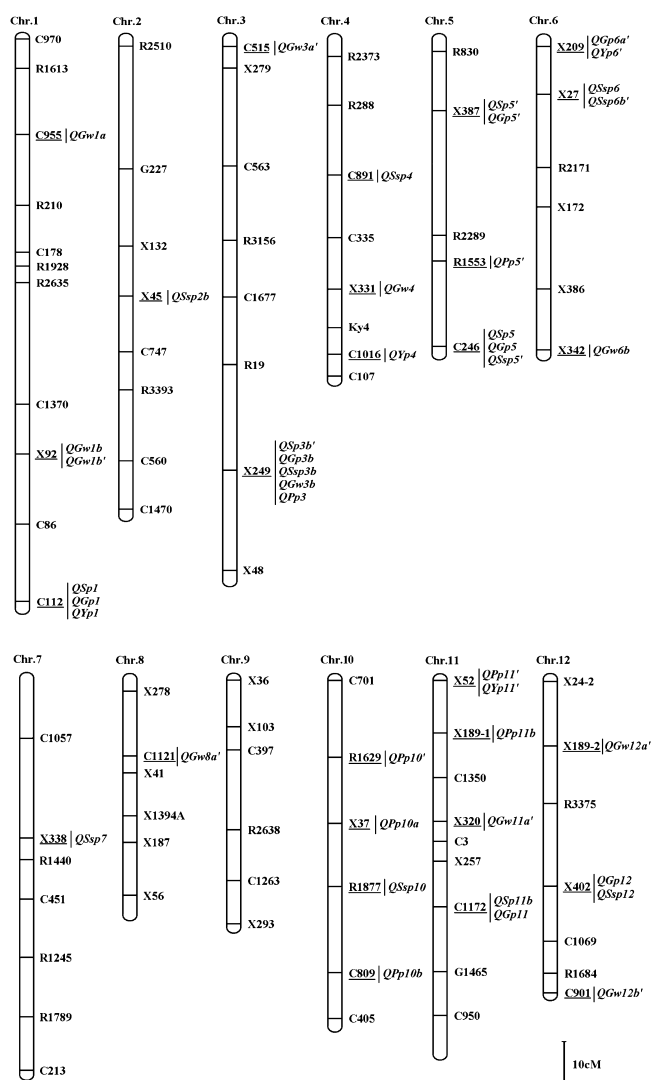
Fig. 1. Genomic locations of putative HL associated with yield-related traits detected using single-point analysis in the testcross F_1s. Underlined markers correspond to HL at right.

ILs and the recurrent parent IR24. The F_1 testcross genotypes were deduced on the basis of the genotype of its corresponding parental IL.

Analysis method of mapping HL

The direct trait measurement values from the six yield-related traits obtained from the ILs were used to identify the associated QTLs. The mid-parental heterosis values, $H_{MP} = F_1 - (IL_i + IL_j)/2$, (where F_1 is the testcross F_1 mean value for the measured trait, and IL_i and IL_j are the corresponding IL parent mean values for the same measured trait) were used to identify the loci affecting heterosis (HL is a locus that demonstrates a significant difference between the heterozygote and the mean of two corresponding homozygotes; i.e., HL is the QTL for heterosis) in the six yield-related traits. The testcross F_1 trait measurements were used to identify the loci affecting testcross F_1 performance.

On the basis of the IL structure, QTLs or HL can be mapped on introgressed chromosome segments. One representative marker of each specific introgressed segment was defined as a QTL or HL. The association between the phenotypic and RFLP data was investigated by single-point analysis using the software package Map Manager QTXb17 (Manly et al. 2001).

The genetic effects of HL from IL populations (advanced backcross population) were analyzed by single-locus association using QTXb17. The statistical a priori threshold for main effect loci was $P < 0.01$. If a group of similar markers was associated with the same trait of similar effect(s) in both magnitude and direction, the marker (or locus) exhibiting the lowest P-value was chosen as the locus associated with the trait(s).

The genetic effects in the testcross F_1s were defined as follows: $d = H_{MP} = [F_1 - (IL_i + IL_j)/2]$; the trait mean values in the testcross F_1s were $\overline{F}_1 = (a + d)$, where a represents the additive effects from the performance values of testcross F_1. Subsequently, HL effects were inferred by comparison of the genetic effects on F_1 performance and mid-parent heterosis. HL with $d/a \leq 1$ were considered complete or partially dominant loci, and expected to generate an estimate of F_1 performance $(a + d)$ equal to or higher than twice the H_{MP} (d). HL with $d/a > 1$ that is, $2d$ $(2 \times H_{MP}) > a + d$ (F_1), or only detectable for H_{MP} were determined overdominant loci (Li et al. 2001, Mei et al. 2005, Melchinger et al. 1998).

Results

IL and F_1 testcross performance and H_{MP} for yield-related traits

The phenotypic data from the ILs and F_1 testcrosses showed a continuous distribution (Supplemental Fig. 1), which suggested that the yield-component traits were quantitative traits under multi-gene control. The performance statistics for ILs and testcross F_1s, and H_{MP} of F_1 testcrosses between two environments are provided in Table 1. Compared with ILs, testcross F_1s had a significantly ($P < 0.05$) greater mean value for each trait in the environment of the China Agricultural University (CAU) Experiment Station (Table 1), and clearly demonstrated hybrid vigor. In the environment of the AAAS Experiment Station, the F_1 mean value for each trait was also greater than that of ILs, while the differences were not significant ($P < 0.05$) for the GW and SSP traits. The highest heterosis was detected in grain yield per plant, while 1000-grain weight showed the lowest heterosis in both environments, consistent with the results from other studies (Hua et al. 2003, Li et al. 2001, Luo et al. 2001). The heterosis levels for all yield-related traits varied in the 175 testcross F_1s, from highly negative to highly positive.

Table 1. Summary of performance statistics for ILs, testcross F_1s and H_{MP} of F_1 testcrosses between two environments

| Locations | Trait[a] | Performance of ILs | | Performance of testcross F_1s | | H_{MP}[b] of testcross F_1s | | | | |
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	SE	%[c]	Range	%[d]
Beijing	SP	92.9 ± 17.8	52.7~128.7	104.3 ± 16.0*	66.2~168.8	10.9 ± 12.0	0.91	3.0	−7.7~100.2	−1.9~36.5
	GP	76.4 ± 14.4	44.7~108.8	92.1 ± 14.8*	56.7~153.2	14.3 ± 11.5	0.87	4.7	−6.4~85.4	−2.0~35.7
	SSP (%)	82.6 ± 7.4	54.7~94.4	88.3 ± 4.8*	58.2~96.0	4.4 ± 4.9	0.37	1.5	−12.8~20.3	−4.5~7.6
	GW (g)	22.9 ± 2.9	16.3~33.2	23.9 ± 1.7*	19.0~28.3	1.0 ± 1.4	0.10	1.1	−3.7~5.8	−3.6~7.4
	PP	10.0 ± 1.5	6.3~13.1	11.3 ± 2.0*	3.2~21.0	1.3 ± 2.0	0.15	3.4	−6.2~10.7	−16.4~25.7
	YP (g)	18.1 ± 3.3	10.7~24.9	25.1 ± 5.7*	8.9~48.0	6.8 ± 5.9	0.45	9.9	−10.3~30.0	−13.4~43.8
Hefei	SP	121.0 ± 15.7	86.5~160.4	130.2 ± 17.3*	82.9~188.1	9.5 ± 13.4	1.00	2.0	−15.8~63.4	−3.7~12.7
	GP	96.5 ± 16.4	45.0~131.9	104.3 ± 14.7*	70.4~146.4	8.1 ± 13.3	1.00	2.3	−35.6~41.3	−8.4~12.8
	SSP (%)	79.8 ± 9.5	50.3~95.9	80.4 ± 7.2	58.4~93.3	0.9 ± 8.5	0.64	0.3	−18.0~23.7	−7.2~9.4
	GW (g)	22.8 ± 2.1	18.3~28.5	22.5 ± 1.4	19.0~26.9	0.2 ± 1.0	0.54	0.1	−2.2~2.4	−2.1~2.7
	PP	11.0 ± 1.3	8.7~14.5	13.5 ± 2.5*	8.4~28.0	2.4 ± 2.6	0.07	5.6	−3.1~17.0	−6.7~38.6
	YP (g)	25.3 ± 4.5	12.5~37.0	33.3 ± 7.2*	18.1~64.6	7.8 ± 7.1	0.19	7.9	−6.8~38.2	−6.2~41.9

[a] Trait abbreviations: spikelet number per panicle (SP), filled grain number per panicle (GP), percent seed set (SSP), 1000-grain weight (GW), panicle number per plant (PP), grain yield per plant (YP).

[b] Mid-parental heterosis, $H_{MP} = F_1 - MP$, where MP was the mid-parental trait value $(IL_i + IL_j)/2$, F_1 was the performance value of testcross F_1.

[c] Rate of mid-parental heterosis = $(F_1 - MP)/MP*100\%$.

[d] Range of mid-parental heterosis rate.

* $P < 0.05$ significantly different between the mean performance of testcross F_1s and ILs.

Table 2. Phenotypic correlation (R) for six yield-related traits between IL and testcross F_1 performance values and H_{MP} values between two environments

Location	Trait[a]	Performance values of IL[b] and testcross F_1	H_{MP} values and performance values of testcross F_1	Performance values of IL[b] and H_{MP} values of testcross F_1
Beijing	SP	0.663	0.675	−0.104
	GP	0.636	0.695	−0.113
	SSP	0.470	0.502	−0.527
	GW	0.670	0.376	−0.436
	PP	0.238	0.866	−0.280
	YP	0.135	0.902	−0.306
	Mean	0.469	0.669	−0.294
Hefei	SP	0.637	0.730	−0.061
	GP	0.508	0.663	−0.307
	SSP	0.353	0.693	−0.430
	GW	0.721	0.374	−0.373
	PP	0.127	0.923	−0.264
	YP	0.259	0.909	−0.169
	Mean	0.434	0.715	−0.267

[a] See Table 1 for abbreviations.

[b] Performance values of ILs are indicated average performance values of IL_i and IL_j.

Relationships among the mean trait values of ILs, H_{MP}, and F_1 performance

Table 2 shows the correlation coefficients among testcross F_1 mean values, mid-parent heterosis values, and parental IL mean values for yield-related traits. Highly positive correlations between H_{MP} and F_1 performance were found for most traits in both environments; the average R (correlation coefficient) was 0.669 in CAU environments and 0.715 in AAAS environments. A general negative correlation trend was evident between IL and H_{MP} trait values; the average R was −0.294 in CAU environments and −0.267 in AAAS environments. A positive correlation between IL trait values and the F_1s was observed, while the correlation coefficients appeared to show a larger difference among traits, with an average R of 0.469 (range of 0.135 for YP to 0.670 for GW) in CAU environments and 0.434 (range of 0.127 for PP to 0.721 for GW) in AAAS environments.

QTLs for 6 yield-related traits in ILs performance, F_1 testcross performance, and heterosis (H_{MP})

The IL phenotypic data for the six yield-related traits were used to identify the associated QTLs. Twenty-five QTLs were detected (Table 3), but only two of the 25 QTLs were common between the two environments. Trait phenotypic values from F_1 testcrosses and estimated H_{MP} values were used to infer the QTLs contributing to F_1 testcross performance and heterosis. Forty-eight QTLs influencing F_1 testcross performance and 41 HL associated with H_{MP} values were detected for the yield-related traits (Table 3 and Fig. 1). Nine of 48 F_1 performance QTLs and two of the 41 HL were common to the two environments.

QTLs for spikelets per panicle: Six QTLs were identified in ILs in the two environments, with two QTLs were identified in both environments, and three QTLs contributed to an increasing effect. Five HL and 11 loci affecting F_1 performance were detected in testcross F_1s, and four of these loci (near markers C112, C246, C1172 and X249) were simultaneously detected in both cases. Of these loci associated with F_1 performance, only three (near markers X249, R1877 and C1350) were identified in both environments and seven loci appeared to have positive effects on SP. Interestingly, all five HL exhibited a positive effect, and only one locus (near marker C112 on chromosome 1) was also detected by QTL analysis of ILs. Furthermore, a comparison of the genetic effects of loci detected in both H_{MP} and F_1 testcross performance indicated a d/a > 1 in *qSp11b* and *qSp3b'*, suggesting overdominant loci and dominant effects in *qSp1* and *qSp5*. The *qSp5'* was only associated with mid-parent heterosis and showed overdominant expression.

QTLs for filled grains per panicle: Four QTLs were detected in ILs in the two environments and two enhanced the phenotype. Seven HL and 10 loci affecting F_1 performance were detected in testcross F_1s and four were detected in both cases. Of these loci associated with mid-parent heterosis and/or F_1 performance, nine loci had significant positive effects and only four loci appeared to have negative effects. Furthermore, one locus influencing both mid-parent heterosis and F_1 performance, near marker C112 on chromosome 1, was also detected in QTL analysis of ILs, and contributed an increasing effect in ILs and positive heterosis in testcross F_1s on filled grains per panicle. Notably, all the HL appeared overdominant.

QTLs for percent seed set: Three QTLs were detected in ILs in both environments, and only one derived from *japonica* showed a positive effect on seed set percent. Eight HL and five loci affecting F_1 performance were detected in testcross F_1s, and only one was detected in both cases and appeared to be overdominant. The remaining seven HL were only mapped in mid-parent heterosis and exhibited overdominance. Only one HL was common to the two environments, and only one overlap between HL and IL QTLs was detected.

QTLs for 1000-grain weight: Five QTLs were detected in ILs in both environments, and only one exhibited a positive effect. Ten HL and 12 QTL affecting F_1 performance were detected in testcross F_1s and two were detected in both cases. Furthermore, nine out of 10 HL appeared to be overdominant and the remaining locus (*qGw3b*) appeared dominant. Interestingly, the HL, *qGw1b*, was also detected in QTL analysis of ILs and showed a decreasing effect when homozygous, while showing a significant positive heterosis effect when heterozygous.

QTLs for panicles per plant: In total, four QTLs were detected in ILs in the two environments, and two contributed a positive effect. Seven HL and seven QTLs affecting F_1 performance were mapped in the testcross population, and three were detected in both cases. Furthermore, all 7 HL appeared to be overdominant, and six showed a significant positive

Table 3. QTLs affecting six yield-related traits identified in ILs, testcross F_1s and H_{MP}

Traits[a]	Locations	QTLs	Markers	IL			F_1			H_{MP}[b]			d/a[d]
				PV[c]	P[c]	a[c]	PV[c]	P[c]	a + d[k]	PV[c]	P[c]	d[k]	
SP	Beijing	qSp1	C112	8	0.0095	10.11	8	0.0001	15.96	4	0.0066	8.02	1.01
		qSp2	R3393				4	0.0065	8.07				
		qSp3a	C515	15	0.0015	−19.29							
		qSp3b	X249				5	0.0021	8.99				
		qSp4	R2373				4	0.0072	11.44				
		qSp5	C246				10	0.0000	34.98	5	0.0021	17.45	1.00
		qSp9	CH63				4	0.0051	−8.85				
		qSp10	R1877				8	0.0001	−12.53				
		qSp11a	C1350				4	0.0070	−11.49				
		qSp11b	C1172				6	0.0007	24.22	3	0.0091	13.55	1.12
	Hefei	qSp1a'	X92	12	0.0043	11.13							
		qSp1b'	C112	9	0.0096	9.80							
		qSp3a'	C515	11	0.0057	−15.02							
		qSp3b'	X249				4	0.0096	8.89	10	0.0001	11.22	2.52
		qSp5'	X387							10	0.0001	10.57	
		qSp6'	X342	11	0.0075	9.45	7	0.0009	12.93				
		qSp8'	X278				8	0.0008	−28.20				
		qSp10'	R1877	13	0.0031	−13.16	10	0.0001	−16.40				
		qSp11'	C1350	7	0.0090	−9.50	15	0.0000	−25.36				
GP	Beijing	qGp1	C112	8	0.0098	8.04	8	0.0001	13.96	7	0.0003	10.12	1.45
		qGp3a	C515	15	0.0014	−15.81							
		qGp3b	X249				8	0.0002	10.12	6	0.0011	6.86	1.36
		qGp4a	R2373				4	0.0079	10.47				
		qGp4b	C891	14	0.0022	9.86							
		qGp5	C246				11	0.0000	32.60	6	0.0010	18.82	1.15
		qGp10	X37				9	0.0001	−11.15				
		qGp11	C1172				5	0.0015	20.92	3	0.0094	12.88	1.23
		qGp12	X402							4	0.0056	−6.66	
	Hefei	qGp1'	X92				5	0.0056	11.23				
		qGp3'	X249				4	0.0099	6.47				
		qGp5'	X387	8	0.0096	−8.43				5	0.0091	7.27	
		qGp6a'	X209							5	0.0072	−7.85	
		qGp6b'	X342				5	0.0051	8.49				
		qGp8'	C1121				8	0.0005	18.15				
		qGp10'	X37				7	0.0009	−9.99				
		qGp11'	C3				6	0.0022	−14.43				
SSP	Beijing	qSsp1	X92	18	0.0005	−6.32	2	0.0097	−2.36				
		qSsp2a	G227				12	0.0000	−5.05				
		qSsp2b	X45	10	0.0096	−4.28				5	0.0021	3.11	
		qSsp3a	R3156				18	0.0000	−12.11				
		qSsp3b	X249							5	0.0025	2.73	
		qSsp4	C891							4	0.0069	−2.44	
		qSsp6	X27							2	0.0090	−1.31	
		qSsp7	X338							4	0.0056	3.75	
		qSsp10	R1877							4	0.0096	−2.53	
		qSsp12	X402							4	0.0051	−2.88	
	Hefei	qSsp5'	C246				7	0.0018	−15.70	7	0.0016	−16.40	2.09
		qSsp6a'	X209	12	0.0044	6.02							
		qSsp6b'	X27							5	0.0045	−3.24	
		qSsp11'	C1172				5	0.0051	−9.03				
GW	Beijing	qGw1a	C955							8	0.0001	1.81	
		qGw1b	X92	21	0.0001	−2.68				18	0.0000	1.93	
		qGw2	C560				4	0.0068	1.11				
		qGw3a	R19				10	0.0000	−1.47				
		qGw3b	X249				10	0.0000	−1.30	3	0.0094	−0.59	0.91

Table 3. (continued)

Traits[a]	Locations	QTLs	Markers	IL			F₁			H_MP[b]			d/a[d]
				PV[c]	P[c]	a[c]	PV[c]	P[c]	a + d[k]	PV[c]	P[c]	d[k]	
		qGw4	X331				4	0.0050	−1.09	8	0.0001	−1.22	2.24
		qGw5	X387				4	0.0064	−0.90				
		qGw6a	X209				3	0.0090	0.80				
		qGw6b	X342							15	0.0000	1.44	
		qGw8a	C1121	12	0.0058	−2.29	5	0.0035	−1.34				
		qGw8b	X56				9	0.0000	−1.71				
		qGw10	R1877				4	0.0072	0.89				
		qGw12	C901				4	0.0091	−0.93				
	Hefei	qGw1a'	R210				5	0.0076	0.89				
		qGw1b'	X92							6	0.0021	0.91	
		qGw3a'	C515							6	0.0034	−1.01	
		qGw3b'	R19				6	0.0027	−0.94				
		qGw5'	X387				6	0.0029	−0.89				
		qGw6'	X209	10	0.0088	1.25	8	0.0008	1.05				
		qGw8a'	C1121							7	0.0015	1.21	
		qGw8b'	X56	11	0.0067	−1.64	3	0.0092	−1.05				
		qGw11a'	X320							11	0.0000	1.24	
		qGw11b'	C1172	9	0.0097	−1.86	9	0.0002	−2.35				
		qGw12a'	X189-2							5	0.0064	0.99	
		qGw12b'	C901							6	0.0031	−0.75	
PP	Beijing	qPp1	C178				5	0.0035	−1.17				
		qPp2a	X45	17	0.0007	−1.13							
		qPp2b	C560				5	0.0030	−1.46				
		qPp3	X249				3	0.0098	−0.92	5	0.0017	−1.16	2.52
		qPp7	X338				5	0.0039	1.59				
		qPp10a	X37				5	0.0020	1.16	5	0.0021	1.17	2.02
		qPp10b	C809							4	0.0050	0.83	
		qPp10c	C405				5	0.0034	0.88				
		qPp11a	X320	11	0.0079	1.14							
		qPp11b	X189-1							4	0.0094	1.29	
	Hefei	qPp5'	R1553				8	0.0004	2.83	7	0.0012	2.67	1.89
		qPp7'	X338	11	0.0055	1.06							
		qPp10'	R1629							4	0.0099	1.42	
		qPp11'	X52							10	0.0001	6.98	
		qPp12'	X402	15	0.0014	−0.96							
YP	Beijing	qYp1	C112				3	0.0092	3.04	6	0.0006	4.91	3.23
		qYp4	C1016							5	0.0037	−6.09	
		qYp7	RH45	12	0.0050	3.23							
		qYp12	R3375				7	0.0003	3.15				
	Hefei	qYp1'	R210	11	0.0070	3.02							
		qYp6'	X209	24	0.0000	4.04				4	0.0091	−3.77	
		qYp11'	X52				6	0.0026	15.25	7	0.0009	16.41	2.15

[a] See Table 1 for abbreviations.

[b] H_{MP} is the mid-parental heterosis of testcross F_1 calculated from $H_{MP} = F_1 − MP$, where $MP = (IL_i + IL_j)/2$.

[c] PV, phenotypic variance explained by the locus; P, probability that the marker genotype had no effect on the trait; *a*, additive effects from the performance values of ILs; $a + d$, additive and dominance effects from the performance values of testcross F_1; *d*, dominance effect from H_{MP} values.

[d] d/a, ratio of dominant and additive effects. HL with $d/a \leq 1$ were referred to as complete or partial dominant loci. By contrast, HL with $d/a > 1$ were referred to as overdominant loci (Melchinger *et al.* 1998, Li *et al.* 2001, Mei *et al.* 2005).

heterotic effect on panicles per plant. Interestingly, no HL or F_1 performance QTLs were detected in the QTL analysis above.

QTLs for grain yield per plant: Three QTLs were detected in ILs in the two environments, all of which contributed increasing effects. Four HL and three F_1 performance QTLs were detected in testcross F_1s and two were detected in both cases. All of the HL appeared to be overdominant. No HL or F_1 performance QTLs were detected in the QTL analysis above.

Table 3 indicates the genetic overlap of H_{MP} and QTLs detected in the F_1 testcrosses. Analysis detected 48 QTLs influencing F_1 performance; 16 were associated with H_{MP} and 32 QTLs with additive and dominant effects were detected for F_1 performance. At the single locus level, 38 of the 41 HL (92.7%) were overdominant and three appeared dominant. In IL QTL analysis, six of the 41 HL (14.6%) were resolved at the same statistical threshold and showed less genetic overlap with the yield-related trait QTLs.

Discussion

The complex nature of heterosis makes it difficult to divide into individual components, particularly in F_2, recombinant inbred and backcrossed populations, largely because of epistatic interactions among the many segregating loci throughout the genome (Li *et al*. 2001, Luo *et al*. 2001, Semel *et al*. 2006); therefore, it is difficult to define specific heterotic phenotypes and the individual genomic loci that control them. However, the IL population allowed us to partition heterosis into defined genomic regions, largely eliminating genome-wide epistasis. To understand the action types of heterotic loci, QTLs and HL associated with yield and yield-related traits were investigated using a set of 70 *japonica* introgression lines against an *indica* background and 175 testcross F_1s derived from the crosses between the ILs. A total of 41 HL for six yield-related traits were identified. The heterotic effects were determined as the combined effects of both additive and dominant gene actions, estimated from the performance values of testcross F_1s and the dominance effects estimated from their mid-parent heterosis (H_{MP}) values. On the basis of this strategy, we characterized the gene action type at each HL, and the 41 HL revealed two different genetic effects, dominant or overdominant. These HL data indicated that overdominance was the major underlying factor of heterosis in the absence of epistasis. Notably, Semel *et al*. (2006) carried out quantitative genetic and phenotypic analyses on an IL population of tomato (*Solanum lycopersicum*) carrying a single chromosomal segment from the distantly related wild species *Solanum pennellii*. In the absence of epistasis, at a single locus level, overdominant loci had greater effects on tomato yield and fitness.

To analyze a set of 265 ILs and their testcross F_1 of *O. rufipogon* Griff. against the background of the *indica* high-yielding cultivar Guichao 2 (*O. sativa* L.), Luo *et al*. (2011) identified 42 HL associated with six yield-related traits in wild and cultivated rice. Furthermore, 38 were overdominant and, in the absence of epistasis, four HL were dominant (the results have been submitted to *Genetics Research*). To confirm the validity of overdominant HL, Luo (2006), Luo *et al*. (2011) evaluated several key HL, such as *hyp2* (near RM236 on chromosome 2) and *hsp11* (near RM224 on chromosome 11). The two loci coincided with the locations of *qGY2-1* and *qGY11-2*, respectively, two yield-improved QTLs mapped by Li *et al*. (2002). Luo (2006) and He *et al*. (2006) narrowed *qGY2-1* (correspond-

ing to *hyp2*) down to a 102.9-kb region by constructing NILs that differed in only a single QTL. Notably, Luo (2006) confirmed that *qGY2-1* was an overdominant heterotic locus. At another locus (*qGY11-2*), Luo *et al*. (2009b, 2011) identified a QTL and/or HL (*hsp11*) associated with the number of spikelets per panicle, and narrowed *hsp11* down to a 702-kb interval between markers RM224 and RM3577 using chromosome fragment substitution analysis. Genetic analysis indicated that *hsp11* appeared to be weak overdominant effects. In this study, the reproducibility of key lines and HL need to be further evaluated. Investigating F_2 progeny derived from the F_1 of target ILs to check whether there was epistasis between introgressed regions and confirming the genetic effect of heterotic loci will be our next research. Therefore, our present study favored, in the absence of epistasis, at a single locus level, overdominance as major genetic basis of HL in rice. To confirm this conclusion, further work, such as fine mapping and cloning, needs to be performed.

Many QTL mapping studies have shown that genetic effects in F_1 hybrid rice appear to fit one of three different models: additive, dominance and overdominance at a single locus level (Hua *et al*. 2003, Li *et al*. 2001, Li *et al*. 2008, Luo *et al*. 2001, Luo *et al*. 2009a, Mei *et al*. 2003, 2005). Our results are consistent with previous studies. We detected 48 QTLs influencing F_1 performance, and 16 were associated with H_{MP} and revealed dominant or overdominant effects, the remaining 32 appearing to have additive and dominant effects. To analyze the H_{MP} data, we identified 41 HL. Of them, 25 loci only influenced H_{MP} and appeared to be overdominant. The cause of heterosis of intersubspecific hybrids or intrasubspecific hybrids might show certain differences. In intersubspecific hybrids, many researches have shown that the overdominant effects were more advantageous than dominant effects (Li *et al*. 2001, Luo *et al*. 2001, Mei *et al*. 2003, 2005). Li *et al*. (2008) simultaneously analyzed the heterosis of intersubspecific (IJ) hybrids and intrasubspecific (II) hybrids using backcross populations of RIL, and found that the ratio of overdominant loci in II hybrids was higher than in IJ hybrids.

Li *et al*. (2001) and Luo *et al*. (2001) analyzed the correlation between RILs and backcrossed populations for yield-related traits, and considered that backcross F_1 performance was mainly determined by dominant gene action. A subsequent study (Mei *et al*. 2005) supported evidence that backcross F_1 performance was largely determined by non-additive gene action. Our study employed a different experimental design; random crossing between ILs to establish a testcross population. We investigated the correlation coefficients among testcross F_1 mean values, mid-parent heterosis values and parental IL mean values, and found a highly positive correlation between testcross F_1 performance and H_{MP} values and a lower positive correlation between IL and F_1 testcross performance values for PP and YP in both environments (Table 2). This indicated that dominant gene action rather than additive gene action was a substantial contributor

to F_1 testcross performance for traits PP and YP, and these results were consistent with the high H_{MP} values in the testcross F_1s. In contrast, a lower positive correlation between testcross F_1 performance and H_{MP} values and a highly positive correlation between IL performance values and F_1 testcross performance values were found with the GW trait, suggesting that additive gene action was a substantial contributor to F_1 testcross performance for GW. Moreover, the GW trait appeared to exhibit lower heterosis in testcross F_1 population in both environments. The negative correlation between IL performance values and H_{MP} values of the F_1 testcross population clearly indicated that additive and dominant gene action acted independently in the testcross population. Furthermore, QTL and HL analyses demonstrated that six of the 41 HL were detected in QTL analysis and exhibited less genetic overlap with QTLs, consistent with results reported by Hua *et al*. (2003); therefore, heterosis and trait performance may be conditioned by different sets of loci.

A number of trait-enhancing QTL alleles have been reported; however, few favorable HL have been identified. Utilizing heterosis from the cross between *indica* and *japonica* is a trend in hybrid rice breeding programs. Due to the complexity of heterosis, conditioned by various factors including genetic background and environments, the detection of favorable HL between *indica* and *japonica* is a new challenge for rice breeders. Our research indicates that introgression lines are a good tool to identify HL between *indica* and *japonica*. ILs give a more reliable implication for genetic improvement, mainly by benefitting from more precise estimation of genetic effects against a relatively uniform and probably elite background (Tanksley and Nelson 1996). For the population used in this study, derived from a cross between *indica* and *japonica*, hybrid weakness, sterility, and a large variation in heading date were observed. It might be possible to detect such recessive loci as heterotic loci. In fact, three loci (*qGp5'*, *qSsp2b* and *qGw1b*) appeared to have negative effects in IL analysis, but positive effects in H_{MP} analysis. Furthermore, the *qSsp2b* locus was located in a position similar to the hybrid breakdown locus *hbd2* reported by Matsubara *et al*. (2007). The three HL above are not viable candidates for the improvement of rice yield potential; however, analysis of the heterotic genetic basis of these recessive loci was viable. In this study, except for the three HL above, 24 HL showed significant positive heterotic effects on yield-related traits. In particular, grain yield per plant, the number of filled grains per panicle, and the number of panicles per plant showed positive heterosis. Further study including fine mapping of these HL and cloning for yield-related traits will facilitate the use of these characteristics in breeding programs.

Acknowledgments

Literature Cited

Aida, Y., H. Tsunematsu, K. Doi and A. Yoshimura (1997) Development of a series of introgression lines of *Japonica* in the background of *Indica* rice. Rice Genet. Newsl. 14: 41–43.

Alpert, K. and S.D. Tanksley (1996) High-resolution mapping and isolation of a yeast artificial chromosome contig containing *fw2.2*, a major fruit-weight quantitative trait locus in tomato. Proc. Natl. Acad. Sci. USA 93: 15503–15507.

Bruce, A.B. (1910) The Mendelian theory of heredity and the augmentation of vigor. Science 32: 627–628.

Eshed, Y. and D. Zamir (1994) Introgressions from *Lycopersicon pennellii* can improve the soluble-solids yield of tomato hybrids. Theor. Appl. Genet. 88: 891–897.

Eshed, Y. and D. Zamir (1995) Introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield associated QTL. Genetics 141: 1147–1162.

Frascaroli, E., M.A. Canè, P. Landi, G. Pea, L. Gianfranceschi, M. Villa, M. Morgante and M.E. Pè (2007) Classical genetic and QTL analysis of heterosis in a maize hybrid between two elite inbred lines. Genetics 176: 625–644.

Garcia, A.A.F., S.C. Wang, A.E. Melchinger and Z.B. Zeng (2008) Quantitative trait loci mapping and the genetic basis of heterosis in maize and rice. Genetics 180: 1707–1724.

He, G.M., X.J. Luo, F. Tian, K.G. Li, Z.F. Zhu, W. Su, X.Y. Qian, Y.C. Fu, X.K. Wang, C.Q. Sun *et al*. (2006) Haplotype variation in structure and expression of a gene cluster associated with a quantitative trait locus for improved yield in rice. Genome Res. 16: 618–626.

Hua, J.P., Y.Z. Xing, W.R. Wu, C.G. Xu, X.L. Sun, S.B. Yu and Q.F. Zhang (2003) Single-locus heterotic effects and dominance by dominance interaction can adequately explain the genetic basis of heterosis in an elite rice hybrid. Proc. Natl. Acad. Sci. USA 100: 2574–2579.

Jones, D.F. (1917) Dominance of linked factors as a means of accounting for heterosis. Proc. Natl. Acad. Sci. USA 3: 310–312.

Kubo, T., Y. Aida, K. Nakamura, H. Tsunematsu, K. Doi and A. Yoshimura (2002) Reciprocal chromosome segment substitution series derived from Japonica and Indica cross of rice (*Oryza sativa* L.). Breed. Sci. 52: 319–325.

Lander, E.S. and D. Botstein (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199.

Li, D.J., C.Q. Sun, Y.C. Fu, C. Li, Z.F. Zhu, L. Chen, H.W. Cai and X.K. Wang (2002) Identification and mapping of genes for improving yield from Chinese common wild rice (*O. rufipogon* Griff.) using advanced backcross QTL analysis. Chin. Sci. Bull. 18: 1533–1537.

Li, Z.K., L.J. Luo, H.W. Mei, Q.Y. Shu, R. Tablen, D.B. Zhong, C.S. Ying, J.W. Stansel, G.S. Khush and A.H. Paterson (2001) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. Genetics 158: 1737–1753.

Li, L.Z., K.Y. Lu, Z.M. Chen, T.M. Mu, Z.L. Hu and X.Q. Li (2008) Dominance, overdominance and epistasis condition the heterosis in two heterotic rice hybrids. Genetics 180: 1725–1742.

Luo, L.J., Z.K. Li, H.W. Mei, Q.Y. Shu, R. Tablen, D.B. Zhong, C.S. Ying, J.W. Stansel, G.S. Khush and A.H. Paterson (2001) Over-dominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. Genetics 158: 1705–1771.

Luo, X.J. (2006) Analysis of the heterotic loci between common wild rice (*O. rufipogon* Griff.) and cultivated rice (*O. sativa* L.) and fine-mapping the yield-enhancing QTL. Ph.D. degree thesis of China Agriculture University.

Luo, X.J., Y.C. Fu, P.J. Zhang, S. Wu, F. Tian, J.Y. Liu, Z.F. Zhu, J.S. Yang and C.Q. Sun (2009a) Additive and over-dominant effects resulting from epistatic loci are the primary genetic basis of heterosis in rice. J. Integr. Plant Biol. 51: 393–408.

Luo, X.J., F. Tian, Y.C. Fu, J.S. Yang and C.Q. Sun (2009b) Mapping QTL influencing panicle-related traits from Chinese common wild rice (*Oryza rufipogon* Griff.) using introgression lines. Plant Breed. 128: 559–567.

Luo, X.J., S. Wu, F. Tian, X.J. Za, X.X. Dong, Y.C. Fu, X.K. Wang, J.S. Yang and C.Q. Sun (2011) Identification heterotic loci associated with yield-related traits derived from Chinese common wild rice (*Oryza rufipogon* Griff.). Plant Sci. 181: 14–22.

Manly, K.F., J.R.H. Cudmore and J.M. Meer (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm. Genome 12: 930–932.

Matsubara, K., T. Ando, T. Mizubayashi, S. Ito and M. Yano (2007) Identification and linkage mapping of complementary recessive genes causing hybrid breakdown in an intraspecific rice cross. Theor. Appl. Genet. 115: 179–186.

Melchinger, A.E., H.F. Utz and C.C. Schön (1998) Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics 149: 383–403.

Melchinger, A.E., H.F. Utz, H.P. Piepho, Z.B. Zeng and C.C. Schon (2007) The role of epistasis in the manifestation of heterosis: a systems-oriented approach. Genetics 177: 1815–1825.

Melchinger, A.E., H.F. Utz and C.C. Schön (2008) Genetic expectations of quantitative trait loci main and interaction effects obtained with the triple testcross design and their relevance for the analysis of heterosis. Genetics 178: 2265–2274.

Mei, H.W., L.J. Luo, C.S. Ying, Y.P. Wang, X.Q. Yu, L.B. Guo, A.H. Paterson and Z.K. Li (2003) Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two testcross populations. Theor. Appl. Genet. 107: 89–101.

Mei, H.W., Z.K. Li, Q.Y. Shu, L.B. Guo, Y.P. Wang, X.Q. Yu, C.S. Ying and L.J. Luo (2005) Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two backcross populations. Theor. Appl. Genet. 110: 649–659.

Murray, M.G. and W.F. Thompson (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8: 4321–4325.

Paterson, A.H., J.W. DeVerna, B. Lanini and S.D. Tanksley (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. Genetics 124: 735–742.

Saito, A., M. Yano, N. Kishimoto, M. Nakagahra and A. Yoshimura (1991) Linkage map of restriction fragment length polymorphism in rice. Jpn. J. Breed. 41: 665–670.

Semel, Y., J. Nissenbaum, N. Menda, M. Zinder, U. Krieger, N. Issman, T. Pleban, Z. Lippman, A. Gur and D. Zamir (2006) Overdominant quantitative trait loci for yield and fitness in tomato. Proc. Natl. Acad. Sci. USA 103: 12981–12986.

Shull, G.H. (1908) The composition of a field of maize. J. Hered. 4: 296–301.

Stuber, C.W., S.E. Lincoln, D.W. Wolff, T. Helentjaris and E.S. Lander (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132: 832–838.

Tanksley, S.D. and J.C. Nelson (1996) Advanced backcross QTL analysis, a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor. Appl. Genet. 92: 191–203.

Xiao, J.H., J. Li, L.P. Yuan and S.D. Tanksley (1995) Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. Genetics 140: 745–754.

Yamamoto, T., Y. Kuboki, S.Y. Lin, T. Sasaki and M. Yano (1998) Fine mapping of quantitative trait loci *Hd-1*, *Hd-2* and *Hd-3*, controlling heading date of rice, as single Mendelian factor. Theor. Appl. Genet. 97: 37–44.

Yu, S.B., J.X. Li, C.G. Xu, Y.F. Tan, Y.J. Gao, X.H. Li, Q.F. Zhang and M.A. Saghai Maroof (1997) Importance of epistasis as the genetic basis of heterosis in elite rice hybrid. Proc. Natl. Acad. Sci. USA 94: 9226–9231.

Zeng, Z.B. (1994) Precision mapping of quantitative trait loci. Genetics 136: 1457–1468.