Theor Appl Genet (2006) 112: 570–580
DOI 10.1007/s00122-005-0165-2

ORIGINAL PAPER

Feng Tian · De Jun Li · Qiang Fu · Zuo Feng Zhu
Yong Cai Fu · Xiang Kun Wang · Chuan Qing Sun

Construction of introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*Oryza sativa* L.) background and characterization of introgressed segments associated with yield-related traits

Received: 6 June 2005 / Accepted: 13 November 2005 / Published online: 6 December 2005

Abstract Introgression lines (ILs) are useful tools for precise mapping of quantitative trait loci (QTLs) and the evaluation of gene action or interaction in theoretical studies. A set of 159 ILs carrying variant introgressed segments from Chinese common wild rice (*Oryza rufipogon* Griff.), collected from Dongxiang county, Jiangxi Province, in the background of Indica cultivar (*Oryza sativa* L.), Guichao 2, was developed using 126 polymorphic simple sequence repeats (SSR) loci. The 159 ILs represented 67.5% of the genome of *O. rufipogon*. All the ILs have the proportions of the recurrent parent ranging from 92.4 to 99.9%, with an average of 97.4%. The average proportion of the donor genome for the BC_4F_4 population was about 2.2%. The mean numbers of homozygous and heterozygous donor segments were 2 (ranging 0–8) and 1 (ranging 0–7), respectively, and the majority of these segments had sizes less than 10 cM. QTL analysis was conducted based on evaluation of yield-related traits of the 159 ILs at two sites, in Beijing and Hainan. For 6 out of 17 QTLs identified at two sites corresponding to three traits (panicles per plant, grains per panicle and filled grains per plant, respectively), the QTLs derived from *O. rufipogon* were usually associated with an improvement of the target trait, although the overall phenotypic characters of *O. rufipogon* were inferior to that of the recurrent parent. Of the 17 QTLs, 5 specific QTLs strongly associated with more than one trait were observed. Further analysis of the high-yielding and low-yielding ILs revealed that the high-yielding ILs contained relatively less introgressed segments than the low-yielding ILs, and that the yield increase or decrease was mainly due to the number of grain. On the other hand, low-yielding ILs contained more negative QTLs or disharmonious interactions between QTLs which masked trait-enchancing QTLs. These ILs will be useful in identifying the traits of yield, tolerance to low temperature and drought stress, and detecting favorable genes of common wild rice.

Communicated by T. Sasaki

F. Tian · D. J. Li · Q. Fu · Z. F. Zhu · Y. C. Fu
X. K. Wang · C. Q. Sun (✉)
Department of Plant Genetic and Breeding
and State Key Laboratory of Agrobiotechnology,
China Agricultural University, 100094 Beijing,
People's Republic of China
E-mail: suncq@cau.edu.cn
Tel.: +86-10-62731811
Fax: +86-10-62731811

F. Tian · D. J. Li · Q. Fu · Z. F. Zhu · Y. C. Fu
X. K. Wang · C. Q. Sun
Beijing Key Laboratory of Crop Genetic Improvement,
Key Laboratory of Crop Genetic Improvement and Genome
of Ministry of Agriculture, China Agricultural University,
100094 Beijing, People's Republic of China

Introduction

Oryza is an agronomically important genus containing species with highly diverse morphological characteristics (Clayton and Renvoize 1986). Included in this genus is cultivated rice (*Oryza sativa* L.), which constitutes the staple food for more than half of the world's population (Coffman and Herrera 1980), while other wild species of *Oryza* with $2n = 24$ or 48 chromosomes including AA, BB, CC, BBCC, CCDD, EE, FF, GG, and HHJJ genomes are potential reservoirs of useful genes (Aggarwal et al. 1997). Wild species of *Oryza* with AA genome, which is similar to that of *O. sativa*, constitute the primary gene pool; of these, common wild rice (*Oryza rufipogon* Griff.) is the wild ancestor of cultivated rice (Second 1982; Oka 1988; Wang et al. 1992). During the course of domestication from wild rice to cultivated rice, profound changes of agronomic traits and genetic diversity occurred (Sun et al. 2001). Using RFLP markers, Sun et al. (2001, 2002) compared the genetic diversity of common wild rice and cultivated rice, and the results indicated that the number of alleles of cultivated rice was only 60% that of wild rice, suggesting that

during the course of domestication from wild rice to cultivated rice many alleles were lost, leading to lower genetic diversity of the cultivated rice. Currently, rice breeding faces the problem of yield plateaus, caused by narrow genetic basis of parental materials (Rangel et al. 1996; Tanksley and McCouch 1997). Exploitation and utilization of the favorable alleles of wild rice that were lost or weakened in cultivated rice might be able to overcome the yield plateaus. Major genes for resistance to grassy stunt virus and those to bacterial blight have been transferred from *Oryza nivara* and *Oryza longistaminata* to cultivated rice, respectively, by conventional plant breeding methods (Khush et al. 1977, 1990). A new gene, *Xa*-23, for resistance to bacterial blight, was discovered from common wild rice of Guangxi (Zhang et al. 2000). Using advanced backcross quantitative trait loci (AB-QTL) analysis, Xiao et al. (1996, 1998) found two QTLs that could increase yield by 17 and 18% from low-yielding wild rice from Malaysia. Li et al. (2002) identified two high-yielding QTLs from Dongxiang common wild rice of China using the same method.

Introgression lines (ILs), or substitution lines, are the results of using marker-assisted selection (MAS) to introgress small chromosomal segments from the donor into the recurrent parent by consecutive backcrossing and selfing. Eshed and Zamir (1994) constructed the first complete set of substitution lines in tomato consisting of near isogenic lines (NILs) carrying single *Lycopersicon pennellii* chromosomal segment in an otherwise homogeneous background of *Lycopersicon esculentum*, representing the entire wild tomato genome. Paterson et al. (1990) proposed substitution mapping as a method for fine mapping of QTLs. In rice, introgression lines carrying *Oryza glaberrima* were constructed (Doi et al. 1997; Sobrizal et al. 1999). In other plant species such as *Brassica napus*, *Arabdopsis* and barley, ILs were also constructed (Howell et al. 1996; Koumproglou et al. 2002; Matus et al. 2003). Using substitution lines by substitution mapping, several authors demonstrated that ILs are powerful tools for the identification of new genes (Eshed and Zamir 1994, 1995, 1996; Chetelat et al. 1995; Chetelat and Meglic 2000; Kubo et al. 2002) to distinguish pleiotropy versus linkage as well as pseudo-overdominance versus true-dominance (Yamamoto et al. 1998; Monforte and Tanksley 2000), and to eliminate the linkage drag and set the basis for the map-based cloning of QTLs (Grandillo et al. 1996; Alpert and Tanksley 1996; Yamamoto et al. 2000; Yano et al. 2000; Takahashi et al. 2001).

In this study, a set of 159 overlapping, homozygous ILs derived from a backcross between Guichao 2 (GC2), a high-yielding commercial Indica cultivar (*O. sativa*), as the recurrent parent and an accession of common wild rice collected from Dongxiang county, Jiangxi Province, China, as the donor, were constructed by MAS. Characterization of introgressed segments associated with yield-related traits from common wild rice to cultivated rice was conducted with 126 polymorphic loci from across the whole genome.

Materials and methods

Construction of ILs

Guichao 2, a high-yielding commercial Indica cultivar (*O. sativa*) and a common wild rice accession (*O. rufipogon*) collected from Dongxiang county, Jiangxi Province (DXCWR, the ratoon was collected from its original habitat), were used as recurrent parent and donor parent, respectively, in a backcrossing program. Dongxiang is the northernmost habitat of wild rice (28°14′N); the DXCWR is very tolerant to low temperature (–12.8°C). The F_1 plant, derived from a cross between GC2 and *O. rufipogon*, was backcrossed four times consecutively to GC2 until a BC_4 population was obtained. Then BC_4F_1 was genotyped with molecular markers and selfed four times consecutively to obtain BC_4F_4 population. In BC_4F_4 population, randomly selected plants were genotyped to construct ILs.

DNA extraction and simple sequence repeats (SSR) analysis

Fresh leaves were collected from the BC_4F_4 lines and ground in liquid nitrogen. DNA was extracted from the ground tissues by the Cetyltrimethyl ammonium bromide (CTAB) method (Rogers and Bendich 1988). SSR primers were synthesized according to the sequences published by Temnykh et al. (2000). A total volume of 25 μl reaction mixture was composed of 1 ng/μl template DNA, 10 mmol Tris–HCl (pH 9.0), 50 mmol KCl, 1.5 mmol $MgCl_2$, 0.1% Triton X-100, 2 μmol of each primer, 2.5 mM each of dNTP, and 1 U of Taq DNA polymerase (Promega). Amplification was performed on program for the initial denaturing step with 94°C for 5 min, followed by 35 cycles for 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, with a final extension at 72°C for 10 min. The PCR products were separated on 8% polyacrylamide denaturing gels and the bands were revealed using the sliver-staining protocol as described by Panaud et al. (1996).

Phenotypic evaluation

Two hundred and fourteen BC_4F_4 lines and the recurrent parent GC2 were planted at the Experiment Station of China Agricultural University, Beijing, China in the summer season of 2002, following a complete randomized block design, with two replications, four rows per plot, 11 plants per row, 20 cm between plants within each row and 30 cm between rows. The field management was similar to that under normal rice production conditions. At harvest time, ten plants in the middle of each plot were selected and evaluated for seven yield-related traits, including panicles per plant, grains per plant, grains per panicle, filled grains per panicle, seed

set rate, 1,000-grain weight and grain yield per plant. The 159 randomly selected individuals were selfed and planted as BC_4F_5 ILs at Sanya, Hainan Province, China in the autumn of 2002. The field plot design and evaluation of the agronomic traits at Sanya were similar to that described above at the site in Beijing. For subsequent analyses, the phenotypic values of seven yield-related traits evaluated were means of two replications.

Data analysis

The linkage map built by Temnykh et al. (2000) was used to estimate marker distances, the length of chromosomes and introgressed segments and the overall genome size for genome ratio calculations. The construction of graphical genotypes and the calculation of the percentage of the total genome in each BC_4F_4 plant were performed using the software GGT (van Berloo 1999). Basically, if two neighboring loci have alleles coming from the same parent, the interval between them was considered the length of the segment. If one locus has alleles from one parent and the locus beside it has alleles from the other parent, then half of the interval between them was considered to have the length of one parent and the other half from the other one (Young and Tanksley 1989).

The association between the phenotype and the marker genotype was investigated by single-point analysis using the software Map Manager QTXb17 (Manly et al. 2001). The statistical threshold for single-point analysis was $P < 0.01$. The results from this QTL analysis were also compared with those previously obtained QTLs using BC_4F_2 population from the same cross and the phenotypic data collected at the site in Beijing.

The average grain yield per plant of ILs at two sites showing significant difference ($P < 0.01$) from the recurrent parent GC2 was screened, of which the average grain yield per plant of ILs at two sites outperformed GC2 over 15% and was inferior to GC2 over 35%, were selected for further analysis. For all selected high-yielding and low-yielding ILs, mean percent phenotypic difference [$\Delta\% = 100$(IL−Guichao 2)/Guichao 2] of grain yield per plant and the number of grain was analyzed.

Results

Construction of ILs

Polymorphism between the two parents detected by SSR markers

A total of 346 SSR markers distributed throughout the 12 chromosomes of rice were used to detect polymorphism between GC2 and DXCWR. It was found that 121 SSR markers (35.0%) were polymorphic between these two parents, in which five SSR markers mapped to two locus showing polymorphism between parents. Thus, 126 polymorphic loci were used for IL analysis. The average distance between two flanking markers was 12.4 cM. The frequency of polymorphic SSR marker is rather low in some regions of the rice chromosomes; for example, of the 29 markers on chromosome 6, only seven were polymorphic.

Development of the ILs

To construct the ILs, the F_1 plant, derived from a cross between GC2 and *O. rufipogon*, was backcrossed four times consecutively to GC2 until a BC_4 population was obtained. First, the genotypes of 96 BC_4F_1 lines that originated from 94 BC_1 were surveyed by 87 SSR markers, and 86 plants were selected according to SSR genotypes and then selfed to generate BC_4F_2; then selfing was made two times to get BC_4F_4 population,

Table 1 Transition of *O. rufipogon* segments in introgression lines and cumulative proportion of donor genome represented by homozygous and heterozygous segments

Chromosome	Heterozygous segments		Homozygous segments		Percentage of homozygous segments	Maximum percentage genome coverage (Het + Homo)[a]
	Number of segments	Average segment length (cM)	Number of segments	Average segment length (cM)		
1	20	10.5	67	7.2	74.7	81.7
2	16	10.3	49	13.0	89.1	91.3
3	5	6.2	14	16.6	27.4	27.4
4	6	13.8	17	12.0	55.0	55.0
5	8	23.8	29	19.1	64.3	79.1
6	9	21.7	15	29.5	67.7	73.0
7	2	19.2	12	28.1	74.2	74.2
8	37	9.6	32	11.5	67.7	67.7
9	32	9.4	30	11.6	68.7	68.7
10	7	22.1	12	9.4	25.1	64.8
11	14	16.2	25	10.9	73.5	86.3
12	8	12.2	25	15.3	58.0	58.0
Average	0.96	12.3	2.06	13.3	61.6	67.5

[a]Based on the proportion of each chromosome's genetic length (in centi Morgan) represented by at least one introgression line

during which the individuals for selfing were randomly selected. In BC_4F_4 population, 214 plants were used to construct the ILs of DXCWR and evaluated for seven yield-related traits in Beijing. DNA samples from all 214 individuals were extracted and genotyped using 126 SSR loci showing polymorphism between the two parents. Of the 214 plants analyzed, 50 (23%) had no detectable *O. rufipogon*-specific SSR bands pattern. Finally, 159 unique ILs were obtained, and all of the alleles expect 18 from *O. rufipogon* were represented in at least one IL.

The ILs obtained carried 67.5% of the genome of *O. rufipogon* (Table 1) on the marker base. However, since the relationship between physical and genetic distances varied greatly within the genome, the proportion of *O. rufipogon* DNA transferred into cultivated rice cannot be accurately calculated. There were 17 ILs with a single heterozygous segment and 27 with a single homozygous one. Some ILs were NIL of the recurrent parent. For example, IL43 carried only one segment of donor parent on chromosome 1.

Number, length and position of introgressed segments

The 159 ILs carried 327 homozygous introgressed segments and 164 heterozygous ones, each of these ILs had 0–8 homozygous introgressed segments or 0–7 heterozygous introgressed ones, with an average of three segments per IL. There existed different introgressed frequencies among the 12 chromosomes; among 491 introgressed segments, 87 introgressed segments existed on chromosome 1, while only 14 on chromosome 7 (Fig. 1). Furthermore, the transmission and fixation of *O. rufipogon* introgressed segments varied for individual chromosomes. For example, up to 91.3% of chromosome 2 carried with *O. rufipogon* introgressed segments, while for chromosome 3, the coverage was only 27.4% (Table 1, Fig. 1).

A total of 491 introgressed segments were detected among the 159 ILs, the sizes ranged from 0.2 cM (on chromosome 2 of IL49) to 74.3 cM (on chromosome 2 of IL83), with an average of 13.4 cM. Most introgressed segments were detected by a single SSR marker. The majority of introgressed segments (49.7%) were less than 10 cM; another 43.0% were between 10 and 30 cM; and the proportions of large segments (more than 30 cM) were only 7.3% (Fig. 2).

The distribution of the introgressed segments along the chromosomes was not random. The majority of *O. rufipogon* introgressed segments were often terminal position, defined herein as including distance less than 15 cM from the end of the chromosome. Of 491 *O. rufipogon* segments detected, 334 (68.0%) were terminal, and 157 (32.0%) were interstitial. These results agreed with those indicated by Chetelat and Meglic (2000) and Jena et al. (1992) who also drew the same conclusion in tomato and rice, respectively.

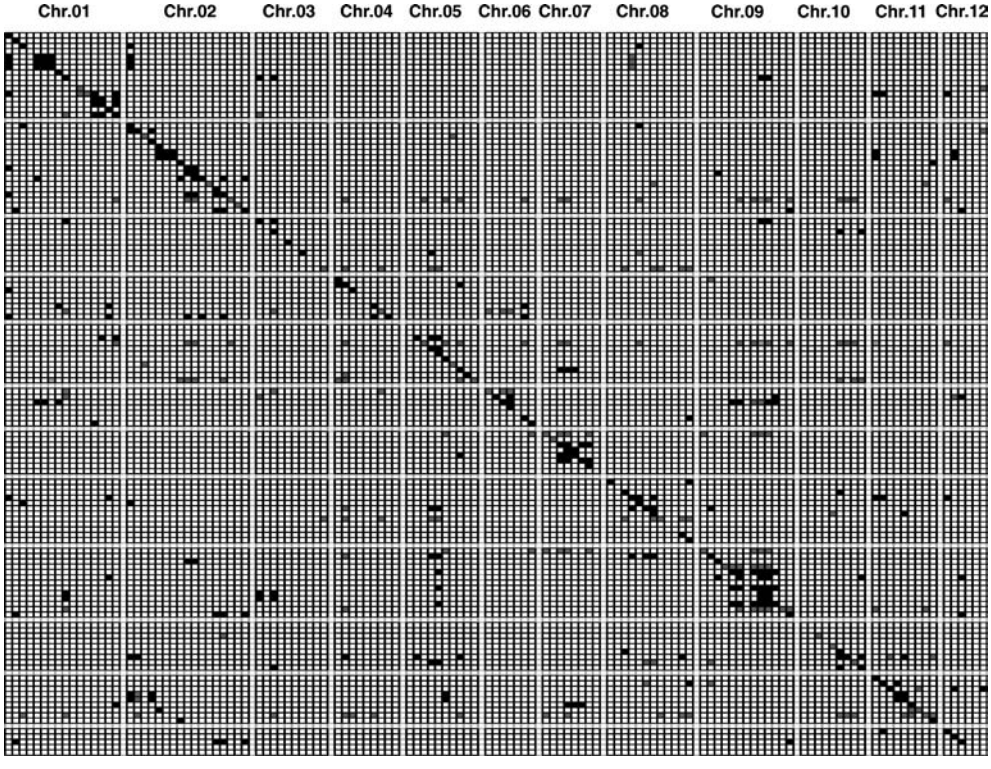

Fig. 1 Graphical representation of the introgression lines developed in this study. Each row represented a candidate introgression line and each column represented a SSR locus. The *black regions* indicated the regions homozygous for *O. rufipogon*; the *white regions* indicated the regions homozygous for Guicao 2 alleles; the *shaded regions* indicated heterozygous regions

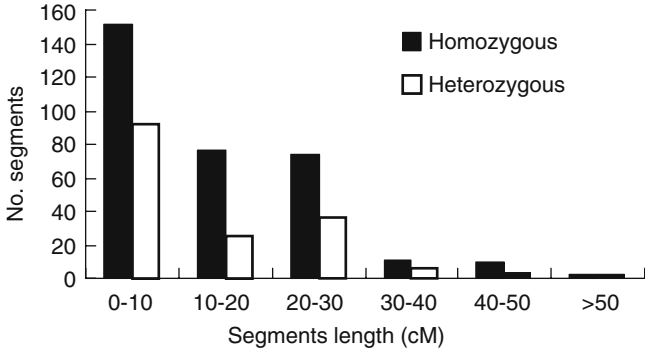

Fig. 2 Frequency of introgressed chromosome segments (homozygous, heterozygous) according to genetic length

Quantitative trait loci analysis of yield-related traits using the ILs

Trait segregation and field performances

As showed in Table 2, the values of the seven yield-related traits in BC_4F_4 and BC_4F_5 showed large range of variation at two sites. Phenotypic transgressive variation was observed for all traits. The variation range of the number of grain was the largest, while 1,000-grain weight the smallest. Taking all traits into consideration, the range of variation at Beijing site was larger than that at Hainan site.

Quantitative trait loci analysis

Quantitative trait loci analysis for seven yield-related traits was conducted separately at both sites using single-point analysis. A total of 37 QTLs were detected at the site in Beijing and 39 QTLs at the site in Hainan (data not shown). Seventeen significant QTLs were identified at both sites as summarized in Table 3 and Fig. 3.

Panicles per plant Two QTLs controlling panicles per plant, located near the marker RM220 on chromosome 1 and RM233a on chromosome 2, respectively, were detected at both sites. The alleles of both loci from common wild rice conferred a positive effect that increased panicles per plant. The phenotypic variance explained by these two QTLs varied from 6 to 14%.

Grains per plant Two QTLs, located near markers RM84 on chromosome 1 and RM44 on chromosome 8, respectively, were associated with grains per plant. Both showed a decrease of grains per plant. The phenotypic variance explained by these two QTLs ranged from 4 to 8%.

Grains per panicle Two QTLs detected at both sites, located near markers RM249 on chromosome 5 and RM342a on chromosome 8, respectively, were associated with grains per panicle. Both contributed an increase of grains per panicle. The phenotypic variance explained by these two QTLs ranged from 6 to 28%.

Filled grains per panicle Three QTLs associated with filled grains per panicle were detected at both sites. The directions of their effects were similar at both sites. The phenotypic variance explained by individual QTL at both sites varied from 4 to 25%. Two QTLs derived from *O. rufipogon*, located near the marker RM342a on chromosome 8 and RM342b on chromosome 9, respectively, showed an increasing effect on filled grains per panicle, while another QTL contributed a decreasing effect.

Seed set rate Two QTLs detected at both sites, located near markers RM11 on chromosome 7 and OSR29 on chromosome 9, respectively, were associated with seed set rate. Both QTLs from common wild rice showed consistent negative effects that decreased seed set rate at both sites.

1,000-grain weight Four QTLs detected at both sites, located on chromosome 4, 5, 8, and 9, respectively, were associated with 1,000-grain weight. All QTLs derived from *O. rufipogon* caused a decrease of 1,000-grain weight. The phenotypic variance explained by individual QTL varied from 3 to 11%.

Grain yield per plant Two QTLs associated with grain yield per plant, located near markers RM11 on chro-

Table 2 Statistics of yield-related traits of Guichao 2 and the 159 ILs population in both locations

Trait	Guichao 2	ILs (Beijing)			ILs (Hainan)		
		Mean	CV (%)	Range	Mean	CV (%)	Range
Pa/pl	9.6	8.9 ± 2.1	23.1	5.40–18.30	8.9 ± 1.4	15.7	5.5–13.1
Gr/pl	1066.2	924.9 ± 215.4	23.3	393.6–1661.1	951.9 ± 196.9	20.7	373.3–1600.4
Gr/pa	112.6	109.5 ± 34.6	31.6	34.2–217.9	108.2 ± 23.8	22.0	56.6–189.2
Fgr/pa	92.6	88.4 ± 30.8	34.8	26.1–173.7	92.1 ± 20.6	22.4	43.8–165.5
Ssr (%)	80.0	79.5 ± 10.3	12.9	30.9–97.9	85.3 ± 5.0	5.9	66.7–93.5
Gr wt (g)	23.0	23.6 ± 2.1	9.0	13.05–27.1	22.5 ± 1.7	7.6	15.9–27.3
Gyi/pl	20.0	17.4 ± 5.1	29.3	3.3–29.1	18.3 ± 4.3	23.5	4.9–31.8

Pa/pl panicles per plant, *Gr/pl* grains per plant, *Gr/pa* grains per panicle, *Fgr/pa* filled grains per panicle, *Ssr* seed set rate, *Gr wt* 1,000-grain weight, *Gyi/pl* grain yield per plant, *CV* coefficient of variation

Table 3 Quantitative trait loci for yield-related traits detected in ILs at both sites

Trait	Locus	Marker	Chromosome	Beijing			Hainan		
				P	PV (%)	Add	*P*	PV (%)	Add
Panicle per plant	*qPN1*	RM220	1	0.000	14	1.9	0.002	6	2.1
	qPN2	RM233a	2	0.002	9	2.0	0.002	9	1.2
Grains per plant	*qGPL1*	RM84	1	0.004	8	−179.4	0.001	6	−91.8
	qGPL8	RM44	8	0.004	6	−134.7	0.010	4	−118.9
Grains per panicle	*qGPA5*	RM249	5	0.005	8	10.4	0.000	8	11.9
	qGPA8	RM342a	8	0.000	28	26.6	0.003	6	16.8
Filled grains per panicle	*qFG7*	RM11	7	0.007	5	−20.6	0.002	6	−16.2
	qFG8	RM342a	8	0.000	25	20.9	0.006	5	13.4
	qFG9	RM342b	9	0.010	4	17.4	0.008	5	13.0
Seed set rate	*qSS7*	RM11	7	0.010	4	−0.1	0.001	7	0.1
	qSS9	OSR29	9	0.002	6	−0.1	0.003	5	0.1
1,000-grain weight	*qGW4*	RM335	4	0.002	5	−0.7	0.004	5	−0.9
	qGW5	RM249	5	0.000	11	−1.2	0.001	8	−1.4
	qGW8	RM342a	8	0.001	8	−1.6	0.003	6	−1.2
	qGW9	RM342b	9	0.001	8	−1.7	0.001	7	−1.4
Yield per plant	*qYP7*	RM11	7	0.006	5	−3.5	0.000	8	−3.8
	qYP8	RM44	8	0.001	8	−3.6	0.010	4	−2.2

QTLs were detected with single-point analysis at $P < 0.01$
P The probability that the marker genotype had no effect on the trait, *PV* phenotypic variance explained by the QTLs, *Add* additive effect of allele from *O. rufipogon*

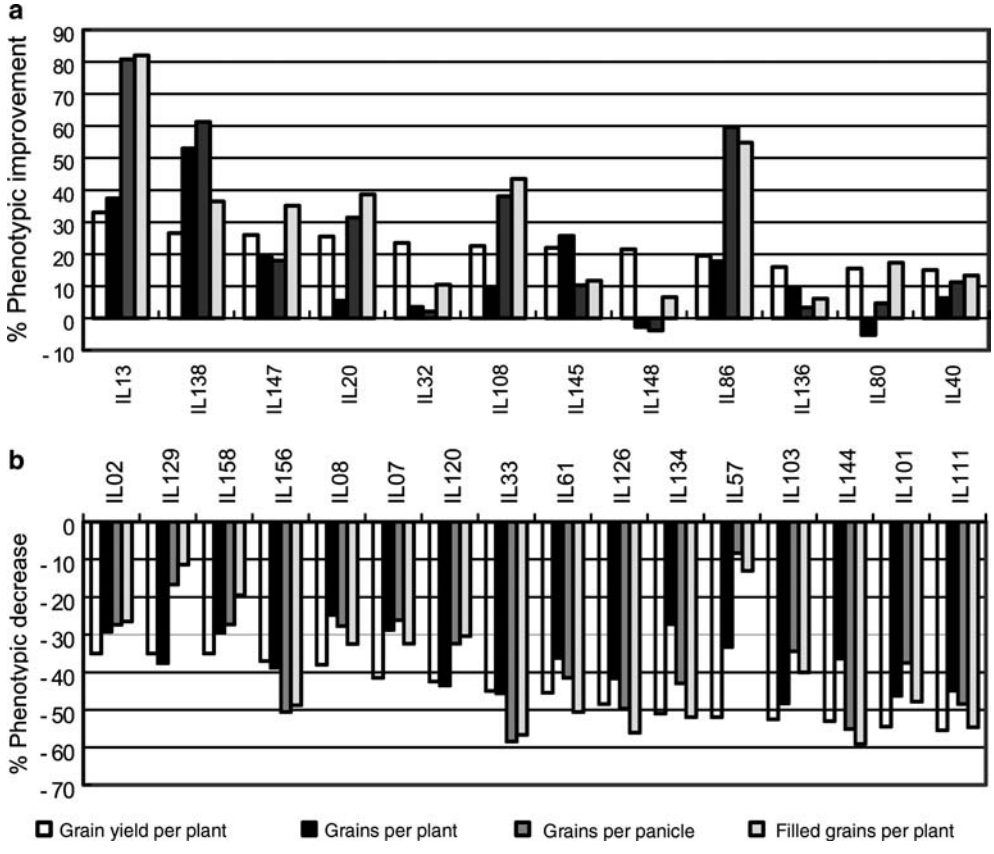

Fig. 3 Analysis of high-yielding ILs and low-yielding ILs. (**a**) Mean percent phenotypic improvement (Δ%) of high-yielding ILs over the recurrent parent Guichao 2 for grain yield per plant and the number of grain. (**b**) Mean percent phenotypic decrease (Δ%) of low-yielding ILs versus recurrent parent Guichao 2 for grain yield per plant and the number of grain. The percent phenotypic difference between the mean of an IL and the mean of the recurrent parent Guichao 2 was estimated as $\Delta\% = 100(\text{IL} - \text{Guichao 2})/\text{Guichao 2}$

mosome 7 and RM44 on chromosome 8, respectively, were detected at both sites, and the directions of their effects were similar at both sites. Both showed decreasing effects on grain yield per plant at both sites. The individual locus explained 4–8% of the phenotypic variance.

Analysis of high-yielding ILs

Twelve high-yielding ILs, of which average grain yield per plant at two sites outperformed the recurrent parent GC2 over 15%, were selected. Mean percent phenotypic improvement [$\Delta\% = 100(\text{IL} - \text{Guichao2})/\text{Guichao2}$] of the high-yielding ILs over GC2 for grain yield per plant and the number of grain was illustrated in Fig. 4a. Compared with GC2, these high-yielding ILs showed an apparent improvement of grain yield per plant by 15–33%. For filled grains per panicle, all of them outperformed the recurrent parent GC2 by 6.1–82.0%, with an average of 29.7%. No obvious tendency was observed on the performances of 1,000-grain weight, panicles per plant and seed set rate. As a result, it could be concluded that the yield increase of high-yielding ILs over the recurrent parent was mainly due to the increase of the number of grain.

The number of introgressed segments contained in the high-yielding ILs and QTLs included in the introgressions were summarized in Table 4a. On genomic constitution, most of the high-yielding ILs contained relatively less introgressed segments, with an average of 2. It was also found that most of them contained QTLs from *O. rufipogon* associated with the increase of the number of grain. It was noteworthy that the QTL from *O. rufipogon*, located near the marker RM249 on chromosome 5, could increase grains per panicle. An IL, IL13, only carrying a single introgression including marker RM249, could be considered as a QTL–NIL.

Analysis of low-yielding ILs

Sixteen ILs, of which average grain yield per plant at two sites was inferior to the recurrent parent GC2 over 35%, were selected. Mean percent phenotypic decrease ($\Delta\%$) of the low-yielding ILs versus GC2 for grain yield plant and the number of grain was illustrated in Fig. 4b. Compared with GC2, these ILs showed an apparent decrease of grain yield per plant by 35–54.5%. Analysis of yield components revealed that grains per plant, grains per panicle and filled grains per panicle of the low-yielding ILs were obviously less than those of GC2, with decreases of 24.8–48.3, 8.3–58.4, and 11.4–59.2%, respectively. However, no obvious tendency was observed on panicles per plant, 1,000-grain weight and seed set rate. Thus, it could be concluded that yield decrease of low-yielding ILs was due to the decrease of the number of grain.

The number of introgressed segments contained in the low-yielding ILs and QTLs included in the introgressions were summarized in Table 4b. On genomic

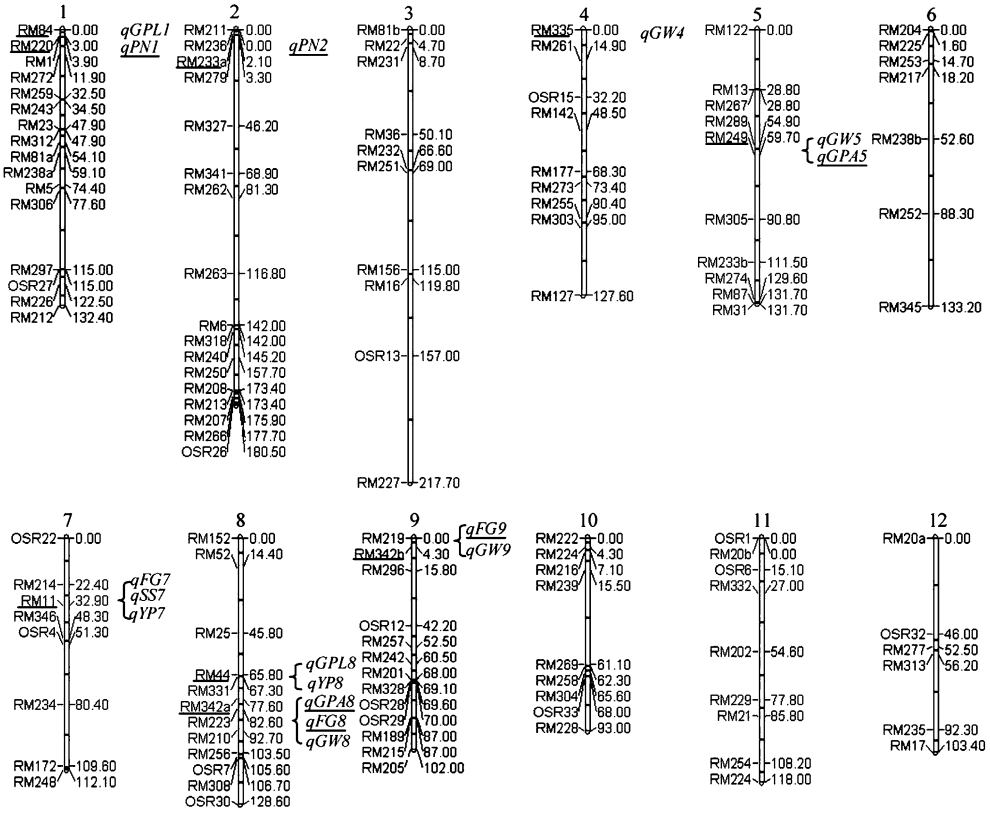

Fig. 4 Map locations of the putative QTLs associated with yield-related traits detected using single-point analysis at both sites, in Beijing and Hainan. See Table 3 for abbreviations of symbol of QTLs. SSR marker order is based on the rice linkage map described by Temnykh et al. (2000). *Underlined* QTLs indicate that the *O. rufipogon* derived alleles confer a positive effect on yield-related traits

Table 4 The number of introgressed segments contained in the high-yielding ILs and low-yielding ILs and QTLs included in the introgressed segments

Number of IL	Gyi/pl(g)	Number of introgressed segments	QTLs included	
			Positive QTLs	Negative QTLs
A				
IL13	26.6	1	*qGPA5*	*qGW5*
IL138	25.3	4	*qGPA8*, *qFG8*, *qFG9*,	*qGW8*, *qGW9*
IL147	25.2	4	*qGPA5*, *qGPA8*, *qFG8*, *qFG9*	*qGW5*, *qGW8*, *qGW9*
IL20	25.1	2	*qPN2*	
IL32	24.7	2		
IL108	24.5	1		
IL145	24.4	2	*qGPA5*	*qGW5*
IL148	24.3	1		*qGPL1*
IL86	23.9	1		
IL136	23.2	1		
IL80	23.1	4	*qGPA8*, *qFG8*, *qFG9*	*qGW8*, *qGW9*
IL40	23.0	1		
B				
IL129	13.0	6	*qGPA8*, *qFG8*, *qFG9*	*qGPL1*, *qGW8*, *qGW9*
IL158	13.0	4		
IL156	12.6	2		*qSS9*
IL08	12.4	4		*qFG7*, *qSS7*, *qYP7*
IL07	11.7	5		*qFG7*, *qSS7*, *qYP7*
IL120	11.5	1		*qGPL8*, *qYP8*
IL33	11.0	7	*qPN1*	*qGPL1*, *qGPL8*, *qYP8*
IL61	10.9	6	*qPN1*	*qSS9*
IL126	10.3	8		*qGPL1*
IL134	9.8	2		*qFG7*, *qSS7*, *qYP7*
IL57	9.6	4		*qSS9*
IL103	9.5	2		
IL144	9.4	4	*qGPA5*	*qGPL8*, *qYP8*, *qGW5*
IL101	9.1	2		*qFG7*, *qSS7*, *qYP7*
IL111	8.9	6		*qSS9*

See Table 3 for abbreviations of symbol of QTLs*IL* Introgression line, *Gyi*/*pl* grain yield per plant

constitution, all low-yielding ILs contained relatively more introgressed segments than high-yielding ILs, with an average of 4.25, and most of them had more than one negative QTL. It was also found that a few low-yielding ILs contained both negative and positive QTLs simultaneously. For example, an IL, IL144, contained three negative QTLs, *qGPL8*, *qYP8* and *qGW5*, respectively, and a positive QTL *qGPA5*, but the ultimate yield traits were inferior to the recurrent parent.

Discussion

Genome coverage of the ILs

In the present study, SSR markers were used to detect chromosomal segments introgressed from *O. rufipogon* into 159 intogression lines. The ILs obtained carried only 67.5% of the genome of *O. rufipogon*, and a few chromosomal regions were not covered by the intogression lines. The similar results were also reported by Kubo et al. (2002) in chromosome substitution series derived from Japonica and Indica cross of rice. This might have occurred as no MAS was practiced during the course of backcrossing although a lot of labor was saved. Moreover, the hybrid sterility and gametophyte genes, or heading date genes in backcrossing could be considered as a factor. For example, the genome coverage of *O. rufipogon* on chromosome 3 was only 27.4%, and interestingly, the lower coverage on chromosome 3 was also reported by Kubo et al. (2002); in addition, Cai and Morishima (2002) detected a QTL for pollen fertility on chromosome 3 in the recombination inbred lines derived from a cross between Indica and *O. rufipogon*. Thus, the pollen fertility genes might be one of the factors affecting the coverage. Heading date did influence the development of ILs, for example, several lines never flowered, so a few candidate introgression may be lost; this may be another reason of low coverage on the chromosome 3, in fact, some QTLs for day to heading were located on chromosome 3 (http://www.gramene.org; Yano et al. 2000). In order to make ILs to represent the whole *O. rufipogon* genome, it is necessary to screen the BC_{2-4} generations by MAS to find the "lost" segments. In addition, 50 BC_4F_4 plants had no detectable *O. rufipogon*-specific SSR bands pattern based on current polymorphic SSR markers. Most of them were morphologically similar to GC2. However, those plants might still contain introgressions which could not be detected using the 126 SSR loci. Tanksley and Nelson (1996) suggested that QTL–NILs constructed subsequently based on QTL analysis could assess the existence and effect of QTL. Bernacchi et al. (1998a, b) identified QTLs for traits of agronomic importance from *Lycopersicon hirsutum*, and evaluated 23 NILs carrying single introgression for desirable wild QTL-alleles derived from *L. hirsutum* and *L. pimpinellifolium*, indicating that 22 out of the 25 (88%) quantitative factors showed the

phenotypic improvement. In the present study, of 159 intogression lines, 27 lines with a single homozygous segment and 17 with a single heterozygous one were obtained. The length of introgressed segments ranged from 1.6 to 26.25 cM, so they could be considered as NILs of the recurrent parent. In fact, several QTL–NILs have been selected based on results of QTL analysis. Field phenotypic evaluation revealed that most of the QTL–NILs showed apparent phenotypic difference from the recurrent parent GC2 for the target traits, suggesting that individual QTL detected in advanced backcross population will continue to exert their effects when isolated in NILs. For example, the QTL from *O. rufipogon* linked to marker RM249 on chromosome 5 showed a consistent increasing effect on the number of grain across generations; thus, we selected and evaluated phenotypically a QTL–NIL carrying only single RM249 marker introgression, and the results obtained at the Beijing site indicated that this QTL–NIL outperformed GC2 by 40.0% for grains per plant, 68.0% for grains per panicle, 78.6% for filled grains per panicle and 37.5% for grain yield per plant.

Yield-related QTLs from wild rice

Two molecular map-based studies have been conducted for simultaneous identification and transfer of wild QTL into a cultivated genetic background. One is the AB-QTL analysis (Tanksley and Nelson 1996), and a refinement of the AB-QTL method is the construction of exotic libraries (Zamir 2001) or ILs (Eshed and Zamir 1994).

Using AB-QTL method, several authors reported that 33–56% trait-enhancing QTL alleles were derived from the phenotypically inferior wild parent (Xiao et al. 1998; Moncada et al. 2001; Septiningsih et al. 2003; Thomson et al. 2003). Brondani et al. (2002) also found 15.7 and 9.1% of the QTLs detected at two locations, respectively, had positive alleles contributed by *O.glumaapatula*, a wild rice native to South America with AA genome same as cultivated rice.

Oryza rufipogon ILs and yield-related QTLs analysis using ILs have not been reported yet. In this study, Dongxiang wild rice, originated from the northernmost habitat of common wild rice (28°14′N) and has very low seed yield and tolerant to very low temperature (–12.8°C),was used as the donor of ILs, and commercial cultivar ''GC2'', a yield record holder (15 ton/ha) for several years in Yunnan Province, China was used as the recurrent parent. These ILs provided a very good material for dissect yield-related QTLs in *O. rufipogon*. The results of analysis on seven yield-related traits using the 159 ILs demonstrated that there were trait-enhancing QTLs from DXCWR, and most of them were associated with the number of grain, for example, QTLs derived from *O. rufipogon* linked to RM249 marker on chromosome 5, RM342a on chromosome 8 and RM342b on chromosome 9 showed significant increase of the number of grain. Analysis of the high-yielding ILs revealed also that the yield increase of them was mainly due to the increase of the number of grain.

Comparative analysis of the genotypes of the high-yielding and low-yielding ILs demonstrated that high-yielding ILs averagely contained two introgressed segments, while low-yielding ILs contained an average of 4.25 ones. Most of the high-yielding ILs carried only positive QTLs from wild rice, while most of low-yielding ILs generally carried more than one negative QTL derived from wild rice. Although a few low-yielding ILs contained positive QTLs, their phenotypical traits were still apparently inferior to GC2, suggesting existence of disharmonious epistatic interaction with other negative loci. Further analysis of the low-yielding ILs revealed that the yield decrease of them was mainly due to the decrease of the number of grain, suggesting that the decrease of the number of grain was the main cause of low yield of wild rice. All these information demonstrated that characters of the low-yielding ILs revealed the genetic basis of wild rice, and that during the course of domestication from wild rice to cultivated rice, profound changes of agronomic traits and genetic diversity occurred. Under human selection, additive favorable alleles were gradually accumulated, unfavorable alleles were gradually removed, and linkage drag was gradually broken. In other words, cultivated rice retained most of the favorable alleles of wild rice, while removing most of the unfavorable alleles or allele combinations.

Three wild rice QTLs studies using the same *O. rufipogon* accession (IRGC105491) from Malaysia as the donor parent and different parent as the recurrent parent have been reported (Xiao et al. 1998; Moncada et al. 2001; Thomson et al. 2003). In this study, we used another accession of *O. rufipogon*, collected from Dongxiang county, Jiangxi Province, China as the donor. Compared with the results of the QTL analysis reported previously, four out of six QTLs (*qPN1* near RM220 on chromosome 1, *qPN2* near RM233a on chromosome 2, *qGPA8* and *qGFG8* near RM342a on chromosome 8, respectively) showing a trait-enhancing effect were in the same or similar regions as previously identified QTLs. For example, the allele from *O. rufipogon* increasing grain number at *qGPA8* and *qGFG8* near RM342a on chromosome 8 in this study was probably same with *gpl8.2* in Xiao et al. (1998) and *gpp8.1* in Thomson et al. (2003). Other two positive *O. rufipogon* alleles (RM249 on chromosome 5 and RM342b on chromosome 9) may be newly detected QTLs.

Efficiency of QTL detection: BC_2F_2 versus BC_4F_4

Quantitative trait loci for yield and its components from *O. rufipogon* have been analyzed by Xiao et al. (1998), Moncada et al. (2001), Thomson et al. (2003) and Septiningsih et al. (2003) using BC_2F_2 population using the same accession of *O. rufipogon* (IRGC105491) as the donor. The results of Septiningsih et al. (2003) demonstrated that the proportion of the genome corresponding

to *O. rufipogon* introgressions per individual ranged from 0 to 97%, with an overall average of 26.1%. The average segment length was 36.3 cM. In fact, there was higher genetic variance and epistatic interaction in BC_2F_2 that could reduce the power of detecting the effects of the QTLs. Many positive QTLs were probably masked by more predominant undesirable QTLs in BC_2F_2. For example, only 5 out of 47 QTLs were detected in both field environments in Septiningsih's study. The 159 ILs constructed in this study have very high proportions of the recurrent parent ranging from 92.4 to 99.9%, with an average of 97.4%, each of the individual line contained an average of three introgressed segments, and most of introgressed segments were less than 10 cM. Thus, the locations and effects of QTLs controlling yield-related traits could be precisely accessed using this set of ILs. In our results, a total of 17 out of 76 QTLs responsible for all traits evaluated were identified at two sites and the directions of their effects were the same at both sites. Compared with the results of QTL analysis by Li et al. (2002), relative less consistency between BC_4F_2 and BC_4F_4 or BC_4F_5 was found, and even opposite effect occurred. Overall, this information indicated that QTL analysis in early backcross generation was only a moderate predictor of the QTL analysis using advanced generation ILs.

Pleitropic effect versus linkage

Classic quantitative genetics assumed that trait correlation was due to the linkage of genes or the pleiotropic effects of a single locus. Many QTL analyses attempted to explain this issue. Xiao et al. (1996) proposed the explanation of highly negative correlation between 1,000-grain weight and grains per panicle based on pleiotropic effects. Monforte and Tanksley (2000) demonstrated that a introgression contained multiple QTLs affecting various agronomic and fruit traits and the effects could not be attributed to pleiotropic effects of a single locus by substitution mapping. In this study, of QTLs detected in BC_4F_4 and BC_4F_5, five specific marker regions were found strongly associated with more than one trait. Although conclusions might not be made about pleiotropy or gene linkage within these QTL regions, they represent interesting "hot spot" for major loci controlling such traits (Brondani et al. 2002). For better characterizing these loci, it is necessary to reduce the extent of the introgression and develop NILs containing fine mapped QTLs.

In conclusion, we constructed 159 ILs of *O. rufipogon* in a Indica background, and identified 17 yield-related QTLs from the *O. rufipogon* donor. This is the first report on construction of *O. rufipogon* ILs, and these ILs will be useful in identifying the traits of yield, tolerance to low temperature and drought stress, and detecting favorable genes of common wild rice. The ILs are available via Material Transfer Agreement for research purposes or via license for commercial purposes.

Acknowledgements We thank two anonymous reviewers for their valuable comments on the manuscript. This work was funded by the National Natural Science Foundation of China (No. 30270803), the "973" Project of the Ministry of Sciences and Technology of China (No. 2001CB108800), a grant from China National High-Tech Research and Development ("863") Program (No. 2003AA207040), and a grand from Protection and Utilization of Agricultural Wild Plants Program of the Ministry of Agriculture of China.

References

Aggarwal RK, Brar DS, Khush GS (1997) Two new genomes in the *Oryza* complex identified on the basis of molecular divergence analysis using total genomic DNA hybridization. Mol Gen Genet 254:1–12

Alpert K, Tanksley SD (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

van Berloo R (1999) Computer note. GGT: software for the display of graphical genotypes. J Hered 90:328–329

Bernacchi D, Beck-Bunn T, Eshed Y, Inai S, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley S (1998a) Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. Theor Appl Genet 97:381–397

Bernacchi D, Beck-Bunn T, Emmatty D, Eshed Y, Inai S, Lopez J, Petiard V, Sayama H, Uhlig J, Zamir D, Tanksley S (1998b) Advanced back-cross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium*.. Theor Appl Genet 97:170–180

Brondani C, Rangel PHN, Brondani RPV, Ferreira ME (2002) QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. Theor Appl Genet 104:1192–1203

Cai HW, Morishima H (2002) QTL clusters reflect character associations in wild and cultivated rice. Theor Appl Genet 104:1217–1228

Chetelat RT, Meglic V (2000) Molecular mapping of chromosome segments introgressed from *Solanum lycopersicoides* into cultivated tomato (*Lycopersicon esculentum*). Theor Appl Genet 100:232–241

Chetelat RT, De Verna JW, Bennett AB (1995) Introgression into tomato (*Lycopersicon esculentum*) of the *L. chmielewskii* sucrose accumulator gene (sucr) controlling fruit sugar composition. Theor Appl Genet 91:327–333

Clayton WD, Renvoize SA (1986) Genera *graminum*. Grasses of the world. Kew Bulletin Series VIII. Her Majesty's Stationary Office, London

Coffman WR, Herrera RM (1980) Rice. In: Fehr WR, Hadley HH (eds) Hybridization of crop plants. American Society of Agronomy-Crop Science of America, Madison, pp 101–131

Doi K, Iwata N, Yoshimura A (1997) The construction of chromosome introgression lines of African rice (*Oryza glaberrima* Steud.) in the background of japonica (*O. sativa* L.). Rice Genet Newsl 14:39–41

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

Eshed Y, Zamir D (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

Eshed Y, Zamir D (1996) Less-than-additive epistatic interactions of quantitative trait loci in tomato. Genetics 143:1807–1817

Grandillo S, Ku HM, Tanksley SD (1996) Characterization of *fs8.1*, a major QTL influencing fruit shape in tomato. Mol Breed 2:251–260

Howell PM, Marshall DF, Lydiate DJ (1996) Towards developing inter-varietal introgression lines in *Brassica napus* using marker-assisted selection. Genome 39:348–358

Khush GS, Ling KC, Aguiero VM (1977) Breeding for resistance to grassy stunt in rice. In: Proceedings of the 3rd international congress SABRAO. Canberra, Australia. Plant Breed, Paper I, pp 3–9

Khush GS, Bacalangco E, Ogawa T (1990) A new gene for resistance to bacterial blight from *O. longistaminata*. Rice Genet Newsl 7:121–122

Koumproglou R, Wilkes TM, Townson P, Wang XY, Beynon J, Pooni HS, Newbury HJ, Kearsey MJ (2002) STAIRS: a new genetic resource for functional genomic studies of Arabidopsis. Plant J 31:355–364

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

Li DJ, Sun CQ, Fu YC, Chen L, Zhu ZF, Li C, Cai HW, Wang XK (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

Manly KF, Cudmore RH Jr, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome 12:930–932

Matus I, Corey A, Filichkin T, Hayes PM, Vales MI, Kling J, Riera-Lizarazu O, Sato K, Powell W, Waugh R (2003) Development and characterization of recombinant chromosome introgression lines (RCSLs) using *Hordeum vulgare* subsp. *spontaneum* as a source of donor alleles in a *Hordeum vulgare* subsp. *vulgare* background. Genome 46:1010–1023

Moncada P, Martinez CP, Borrero J, Chatel M, Gauch H, Guimaraes E, Tohme J, McCouch SR (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment. Theor Appl Genet 102:41–52

Monforte AJ, Tanksley SD (2000) Fine mapping of a quantitative trait locus (QTL) from *Lycopersicon hirsutum* chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. Theor Appl Genet 100:471–479

Oka HI (1988) Origin of cultivated rice. Developments in crop science, vol 14. Elsevier, Amsterdam

Panaud O, Chen X, McCouch SR (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol Gen Genet 252:597–607

Paterson AH, Deverna JW, Lanini B, Tanksley SD (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes in an interspecies cross of tomato. Genetics 124:735–742

Rangel PH, Guimarães EP, Neves PCF (1996) Base genética das cultivares de arroz (*Oryza sativa* L.) irrigado do Brasil. Pesqui Agropec Bras 31:349–357

Rogers OS, Bendich AJ (1988) Extraction of DNA from plant tissue, plant molecular. Biol Manual A6:1–10

Second G (1982) Origin of the genetic diversity of cultivated rice (*Oryza* spp.), study of the polymorphism scored at 40 isozyme loci. Jpn J Genet 57:25–57

Septiningsih EM, Prasetiyono J, Lubis E, Tai TH, Tjubaryat T, Moeljopawiro S, McCouch SR (2003) Identification of quantitative trait loci for yield and yield components in an advanced backcross population derived from the *Oryza sativa* variety IR64 and the wild relative *O. rufipogon*. Theor Appl Genet 107:1419–1432

Sobrizal, Ikeda K, Sanchez PL, Doi K, Angles ER, Khush GS, Yoshimura A (1999) Development of *Oryza glumaepatula* introgression line in rice, *O. sativa* L. Rice Genet Newsl 16:107–108

Sun CQ, Wang XK, Yoshimura A, Iwata N (2001) Comparison of the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. Theor Appl Genet 102:157–162

Sun CQ, Wang XK, Yoshimura A, Doi K (2002) Genetic differentiation for muclear, mitochondrial and chloroplast genomes in common wild rice (*O. rufipogon* Griff.) and cultivated rice (*O. sativa* L.). Theor Appl Genet 104:1335–1345

Takahashi Y, Shomura A, Sasaki T, Yano M (2001) *Hd6*, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the α subnit of protein kinase CK2. Proc Natl Acad Sci USA 98:7922–7927

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

Tanksley SD, Nelson JC (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

Temnykh S, Park W, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100:697–712

Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, Lobos KB, Xu Y, Martinea CP, McCouch SR (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theor Appl Genet 107:479–493

Wang ZY, Second G, Tanksley SD (1992) Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. Theor Appl Genet 83:565–581

Xiao J, Grandillo S, Ahn SN, McCouch SR, Tanksley SD, Li J, Yuan L (1996) Genes from wild rice improve yield. Nature 384:223–224

Xiao J, Li J, Grandillo S, Ahn SN, Yuan L, Tanksley SD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150:899–909

Yamamoto T, Kuboki Y, Lin SY, Sasaki T, Yano M (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

Yamamoto T, Lin HY, Sasaki T, Yano M (2000) Identification of heading date quantitative trait loci *Hd6* and characterization of its epistatic interaction with *Hd2* in rice using advanced backcross progeny. Genetics 154:885–891

Yano M, Katayose Y, Ashikari M, Yamanouchi U, Monna L, Fuse T, Baba T, Yamamoto K, Umehara Y, Nagamura Y, Sasaki T (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. Plant Cell 12:2473–2483

Young ND, Tanksley SD (1989) Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor Appl Genet 77:95–101

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nat Rev Genet 2:983–989

Zhang Q, Zhao BY, Zhao KJ, Wang CL, Yang WC, Lin SC, Jue GS, Zhou YL, Li DY, Chen CB, Zhu LH (2000) Identification and mapping a new gene *Xa*-$23^{(t)}$ for resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) from *O.rufipogon*. Acta Agron Sin 26:536–542