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Mapping quantitative trait loci for yield components and morphological traits in an advanced backcross population between *Oryza grandiglumis* and the *O. sativa japonica* cultivar Hwaseongbyeo

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Abstract Introgression has been achieved from wild species *Oryza grandiglumis* ($2n = 48$, CCDD, Acc. No. 101154) into *O. sativa* subsp. *japonica* cv. Hwaseongbyeo as a recurrent parent. An advanced introgression (backcross) line, HG101, produced from a single plant from BC_5F_3 families resembled Hwaseongbyeo, but it showed differences from Hwaseongbyeo in several traits, including days to heading and culm length. To detect the introgressions, 450 microsatellite markers of known chromosomal position were used for the parental survey. Of the 450 markers, 51 (11.3%) detected *O. grandiglumis* segments in HG101. To characterize the effects of alien genes introgressed into HG101, an $F_{2:3}$ population (150 families) from the cross Hwaseongbyeo/HG101 was developed and evaluated for 13 agronomic traits. Several lines outperformed Hwaseongbyeo in several traits, including days to heading. Genotypes were determined for 150 F_2 plants using simple sequence repeat markers. Qualitative trait locus (QTL) analysis was carried out to determine the relationship between marker genotype and the traits evaluated. A total of 39 QTL and 1 gene conferring resistance to blast isolate were identified using single-point analysis. Phenotypic variation associated with each QTL ranged from 4.2 to 30.5%. For 18 (46.2%) of the QTL identified in this study, the *O. grandiglumis*-derived alleles contributed a desirable agronomic effect despite the overall undesirable characteristics of the wild phenotype. Favorable wild alleles were detected for days to heading, spikelets per panicle, and grain shape traits. Grain shape QTL for grain weight, thickness, and width identified in the $F_{2:3}$ lines were further confirmed based on the F_4 progeny test. The confirmed locus, *tgw2* for grain weight is of particular interest because of its independence from undesirable height and maturity. Several QTL controlling amylose content and grain traits have not been detected in the previous QTL studies between *Oryza* cultivars, indicating potentially novel alleles from *O. grandiglumis*. The QTL detected in this study could be a rich source of natural genetic variation underlying the evolution and breeding of rice.

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Introduction

The genus *Oryza* has 2 cultivated and 22 diploid or allotetraploid wild species (Vaughan et al. 2003). The wild species of *Oryza* are an important reservoir of genetic variability for agronomic traits such as biotic and abiotic stresses and for improved yield potential (Brar and Khush 1997; Xiao et al. 1998; Moncada et al. 2001). The cultivated species *Oryza sativa* and *O. glaberrima* share a common AA genome with closely related wild species, and these wild species can easily be crossed with *O. sativa* by conventional crossing methods. Valuable genes from AA genome wild species could be transferred into the cultivated rice (Xiao et al. 1998; Moncada et al. 2001; Nguyen et al. 2003). However, it is difficult to transfer genes from wild species with other genomes into cultivated rice genotypes because of the crossability and recombination barriers. Nevertheless, several beneficial genes have been successfully transferred from distantly related species such as *O. officinalis* (CC), *O. australiensis* (EE), and *O. minuta* (BBCC) using advanced techniques of tissue culture and chromosome manipulation (Jena et al. 1992; Ishii et al. 1994; Brar and Khush 1997; Liu et al. 2002; Jena et al. 2003). The possible

mechanism of gene transfer was by restricted reciprocal recombination detectable only at the molecular level (Jena et al. 1992).

An allotetraploid CCDD genome species, *O. grandiglumis* is endemic to Central and South America. This species has large panicles and has potentially useful genes for higher biomass production and resistance to diseases and insect pests (Heinrichs et al. 1985; Yu et al. 1997; Vaughan et al. 2003). A few studies have been conducted to detect genetic variability between *O. grandiglumis* and *O. glumaepatula* (Akimoto and Morishima 2003), and among three CCDD genome species endemic to Central and South America at the molecular level (Jena and Kochert 1991; Wang et al. 1992). However, attempts to transfer genes from *O. grandiglumis* to cultivars have been limited mainly due to incompatibility between unrelated genomes (Yu et al. 1997).

Recent progress in plant genome analysis has made it possible to examine naturally occurring allelic variation underlying complex traits. Quantitative trait loci (QTL) analysis can provide information relevant to agricultural traits by using molecular markers to identify specific regions of the genome affecting any measurable trait (Tanksley 1993). Several QTL, including yield, yield components, and morphological traits from *O. sativa* cultivars, have been identified in the past decade (McCouch and Doerge 1995; Yano and Sasaki 1997). Also, map-based cloning of QTL in tomato and rice confirm the feasibility of using QTL to uncover the molecular nature of quantitative variation (Frary et al. 2000; Yano et al. 2000).

This study was carried out using an introgression line, "HG101", developed from the cross between *O. sativa* cv. Hwaseongbyeo and *O. grandiglumis*, (1) to identify the regions of the genome related to the agronomic traits of interest, (2) to identify the trait-improving alleles from *O. grandiglumis* and selectively transfer novel alleles into the background of Hwaseongbyeo, and (3) to determine the conservation of QTL across other rice cultivars.

To our knowledge, this study represents the first report of a genetic study conducted on an interspecific cross of *O. sativa*/*O. grandiglumis*.

Materials and methods

Plant materials and field trials

Interspecific backcross progenies were developed from the cross between the elite *japonica* cultivar Hwaseongbyeo used as a recurrent parent and the wild species *O. grandiglumis* (IRGC Acc. No. 101154) with the CCDD genome as a donor parent. Among them, one BC_5F_6 introgression line (HG101) was selected. HG101 was produced from a single plant from BC_5F_3 families, which was subsequently self-pollinated for three more generations. HG101 differed from Hwaseongbyeo in several traits, including days to heading, and these differences can be attributed to the *O. grandiglumis* chromosome segments introgressed into HG101. To evaluate the effects of these segments on the agronomic traits, 150 $F_{2:3}$ families were developed from the cross Hwaseongbyeo/HG101.

From 150 F_3 families, 1 family, CR1242, was selected as the starting material for detailed characterization and NIL development of the grain weight QTL *tgw2*. This family was selected because it segregated at the target loci and had a significantly bigger grain weight than the other lines, and a relatively few nontarget background introgressions. This family was screened for two parent type homozygotes to produce 22 F_4 lines (6 for Hwaseongbyeo and 16 *O. glandiglumis* alleles, respectively) for evaluation of traits including grain weight.

Phenotypic evaluation

One hundred and fifty F_3 families were grown in the field during the summer of 2002 and 2003 at Chungnam National University, Deajeon, Korea. Twenty-two F_4 lines were grown during the summer of 2004. Each family was represented by a single row of 30 30-day-old seedlings planted with 15 cm between plants and 30 cm between rows in a randomized complete block design with two replications. A total of 13 agronomic traits were evaluated in the 150 lines. Three traits (blast resistance, grain yield, and amylose content) were evaluated in 2002. The other ten traits (days to heading, culm length, panicle number, panicle length, spikelets per panicle, grain length, grain width, grain thickness, length/width ratio of grain, and 1,000-grain weight) were evaluated in 2002 and 2003, respectively. Twenty-two F_4 lines were evaluated for the 1,000-grain weight.

One qualitative trait, *resistance to blast isolate*, was included. Fungal inoculation and disease evaluation of the 150 $F_{2:3}$ lines (20 plants per line) to one blast isolate, KI-313, were carried out as described in Kwon et al. (2002). The other 12 traits were quantitatively inherited and evaluated based on the commonly used methods in rice genetics and breeding. *Days to heading* (DTH) was evaluated as the number of days from seeding until 50% of the panicles of the 30 plants flowered. For culm length, panicle length, and panicles per plant, ten plants per line in the middle were measured, and the average of the measurements was used as the phenotype of each line. *Culm length* (CL) was measured in centimeters from the soil surface to the neck of tallest panicle. *Panicle length* (PL) was measured in centimeters from panicle neck to the panicle tip. *Panicle number* (PN) was calculated as the number of panicles per plant. Two representative panicles per plant (a total of 20 panicles per line) were bagged after panicle emergence to avoid seed shattering and evaluated for spikelets per panicle and percent seed set. *Spikelets per panicle* (SPP) was calculated as the average number of spikelets per panicle. *Grain length* (GL), *grain width* (GW), *grain thickness* (GT), *length*/*width*

ratio of grain (LW), and *1,000-grain weight* (TGW) were measured on 50 grains per plant (10 plants per line). *Yield per plant* (YD) was measured and calculated as the average weight per plant of bulked grain harvested from the ten plants per line. The 1,000-grain weight and the yield per plant were corrected for the 12% grain moisture content. *Amylose content* (AC) was measured as described by Williams et al. (1958) and Juliano (1971) using a sample of 100 g 100-mesh-sieved rice flour. Means for the two replications were calculated for each trait and used in data analysis.

Twenty-two F_4 lines from the CR1242 were grown during the summer of 2004. Each line was represented by a single row of 30 30-day-old seedlings planted with 15 cm between plants and 30 cm between rows in a randomized complete block design with two replications. The 22 F_4 lines were evaluated for traits using the aforementioned.

DNA extraction and SSR genotyping

DNA was extracted from fresh leaves of 150 F_2 plants. DNA extraction was performed as described in Causse et al. (1994). A total of 450 simple sequence repeat (SSR) markers of known chromosomal position were used to survey the parents for polymorphism (Temnykh et al. 2000, 2001; McCouch et al. 2002). The 51 markers showing polymorphism between Hwaseongbyeo and HG101 were used for genotype analysis of 150 F_2 plants. SSR analysis was performed according to the method described in Panaud et al. (1996), with the following modification in the PCR profile: 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and lastly 5 min at 72°C. The linkage map with the order and distance between markers was constructed based on two previously developed SSR maps (Temnykh et al. 2001; McCouch et al. 2002). When the marker segregation data was analyzed, deviation from the expected Mendelian ratios of 1 Hwaseongbyeo homo:2 hetero:1 HG101 homo alleles was examined by Chi-square tests.

DNA was extracted from fresh leaves of 22 F_3 plants of the CR1242 population, and their SSR genotypes of the target loci were determined.

QTL analysis

The chromosomal location of QTL was determined by single-point analysis (SPA). The primarily analysis using SPA was performed using QGene (Nelson 1997). In SPA, QTL was declared if the phenotype was associated with a marker locus at $P < 0.005$ or with two adjacent marker loci at $P < 0.05$. Due to limited introgressions from *O. grandiglumis* into HG101 and limited number of polymorphic markers between the parents, Hwaseongbyeo and HG101, interval mapping analysis could not be performed. The proportion of observed phenotypic variation attributable to a particular QTL was estimated by the coefficient of determination (R^2). The total phenotypic variation explained was estimated by fitting a model including all putative QTL for the respective trait simultaneously. Because the QTL detected with the 2002 data were in good agreement with those with the 2003 phenotypic data, the 2002 data were used in analysis. The effect of digenic interaction on each trait was analyzed with two-way ANOVA, using the SAS program.

The QTL locations from this study were compared with those of the QTL reported in 27 previous QTL studies across rice cultivars and wild species (http://www.gramene.org). In total, 446 previously reported QTL were found for the 12 traits, and QTL in the similar location on the same chromosome as a QTL in the present study were selected for comparisons.

Results

Morphology and DNA polymorphism of HG101

Among the BC_5F_5 population developed, HG101 was the most divergent in plant morphology and DNA polymorphism from Hwaseongbyeo (Yu et al. 1997). HG101 differed from Hwaseongbyeo in several traits, including culm length and days to heading (Table 1; Ahn et al. 2001, 2003). HG101 showed delayed heading and short culm length compared with Hwaseongbyeo. Also, the grain characteristics of HG101 were different from those of Hwaseongbyeo. This might be responsible for the high DNA polymorphism of 11.3% between

Table 1 Comparison of the agronomic characteristics of the parents and HG101

Acc.	Trait										
	DTH	CL	PL	SH	GL	GW	TGW	AC	Blast isolate reaction		
									KI-313	KI-409	KI-1113a
Oryza grandiglumis	180	174	45	1	7.61	1.99	9.9	–	R	R	R
Hwaseongbyeo	108	81	20	9	5.03	2.84	21.5	20.5	S	S	S
HG101	117	75	21	9	5.53	3.08	26.3	15.5	R	R	R

DTH days to heading, *CL* culm length, *PL* panicle length, *SH* shattering (evaluated using a 1 to 9 scale; 1 = easy, 9 = hard), *GL* grain length, *GW* grain width, *TGW* 1,000-grain weight, *AC* amylose content, *R* resistant, *S* susceptible

Hwaseongbyeo and HG101 (51×100/450 = 11.3%) which is much higher than the expected ratio of 1.56% after five continuous backcrosses. The most notable feature of HG101 is the absence of undesirable traits of *O. grandiglumis*, such as grain shattering and tall plant stature.

Correlations among traits

Significant correlations ($P < 0.05$, $P < 0.01$) were observed among many traits. Table 2 summarizes the correlation coefficients among the 12 traits evaluated with the $F_{2:3}$ family. Negative correlation was found between *dth* and *pn*, and *dth* and *spp*. Significant correlations were found among the traits determining plant morphology, *cl*, *pl*, and *spp*. Significant correlations were also detected among the traits associated with grain morphology, *gl*, *gt*, *gw*, and *lw*. For most of the correlations, the direction (+ and −) and degree of correlation was similar with previous studies (Xiao et al. 1998; Thomson et al. 2003). In agreement with previous studies, yield showed positive correlations with culm length, panicle length, and spikelets per panicle. Negative correlations existed between days to heading and panicle length, 1,000-grain weight and panicle length, and 1,000-grain weight and panicles per plant.

Trait distribution

The frequency distributions of phenotypes for each trait in the parents and F_3 families are shown in Fig. 1. A large amount of variation was observed between the two parents for most of the traits. The 150 F_3 families showed continuous distribution for all the measured traits except for grain width and grain length/width ratio. In most cases, transgressive lines that fell beyond the high or low mean of the two parents were observed. Four traits, *cl*, *pl*, *pn*, and *spp* showed the most transgressive segregation, with the average value of the lines falling outside the means of the two parents.

Genetic analysis of blast resistance

HG101 was resistant to three *Pyricularia grisea* isolates that were collected in Korea (Ryu et al. 1987). Several independent studies demonstrated that resistance to the isolate in several rice accessions is under dominant gene control and the resistance gene to an isolate KI-313 was mapped using molecular markers (Ahn et al. 2000; Kim et al. 1995). The F_1 plant of the cross between HG101 and Hwaseongbyeo was resistant to three isolates, indicating that the resistance is under dominant gene control. For the KI-313 isolate, segregation analysis based on disease evaluation of the 150 $F_{2:3}$ lines for the race showed a 1:2:1 (45:65:40) ratio ($\chi^2 = 3.0$, $0.10 < P < 0.50$) for resistant, segregating, and susceptible lines, thereby confirming that a single dominant gene governs the resistance to KI-313 in HG101. Linkage analysis showed that the gene, *Pi-18* (Ahn et al. 2000) conferring resistance to blast isolate KI-313 was linked to RM144 at the distal end of the long arm of chromosome 11 (Fig. 2). The linkage relationship of the genes conferring resistance to two isolates remains to be clarified.

Marker segregation

The F_2 plants were genotyped with 51 markers that detected *O. grandiglumis* alleles in HG101 (Fig. 2). Significant segregation distortion was observed in this study for 5 (10.2%) marker loci at a significance level of $P < 0.05$. Of these, three marker loci were skewed towards the Hwaseongbyeo alleles and two loci were skewed towards the *O. grandiglumis* alleles (Fig. 2).

QTL identification

Significant QTL were identified for 12 agronomic traits as summarized in Table 3. A total of 39 QTL and 1 gene controlling blast resistance were identified using

Table 2 Significant correlation coefficients (r) among 12 traits in the $F_{2:3}$ family

Trait	DTH	CL	PL	PN	AC	SPP	GT	TGW	GL	GW	LW
CL	−0.09										
PL	−0.21	0.29									
PN	−0.34	0.32	0.11								
AC	−0.10	0.04	0.10	–							
SPP	−0.40	0.26	0.26	0.04	0.06						
GT	0.07	−0.14	−0.26	−0.28	0.03	−0.14					
TGW	0.12	−0.10	−0.18	−0.37	0.06	−0.02	0.82				
GL	−0.03	−0.12	0.07	−0.26	−0.06	−0.02	0.50	0.55			
GW	0.02	−0.15	−0.29	−0.35	0.07	0.01	0.88	0.80	0.49		
LW	−0.05	0.08	0.37	0.20	−0.13	−0.03	−0.63	−0.50	0.17	−0.77	
YD	−0.03	0.22	0.20	0.03	0.02	0.33	−0.07	−0.02	−0.13	−0.08	−0.02

DTH days to heading, *CL* culm length, *PL* panicle length, *PN* panicle number, *AC* amylose content, *SPP* spikelets per panicle, *GT* grain thickness, *TGW* 1,000-grain weight, *YD* yield, *GL* grain length, *GW* grain width, *LW* grain length/width ratio
$r = 0.159$ at $P = 0.05$; $r = 0.208$ at $P = 0.01$

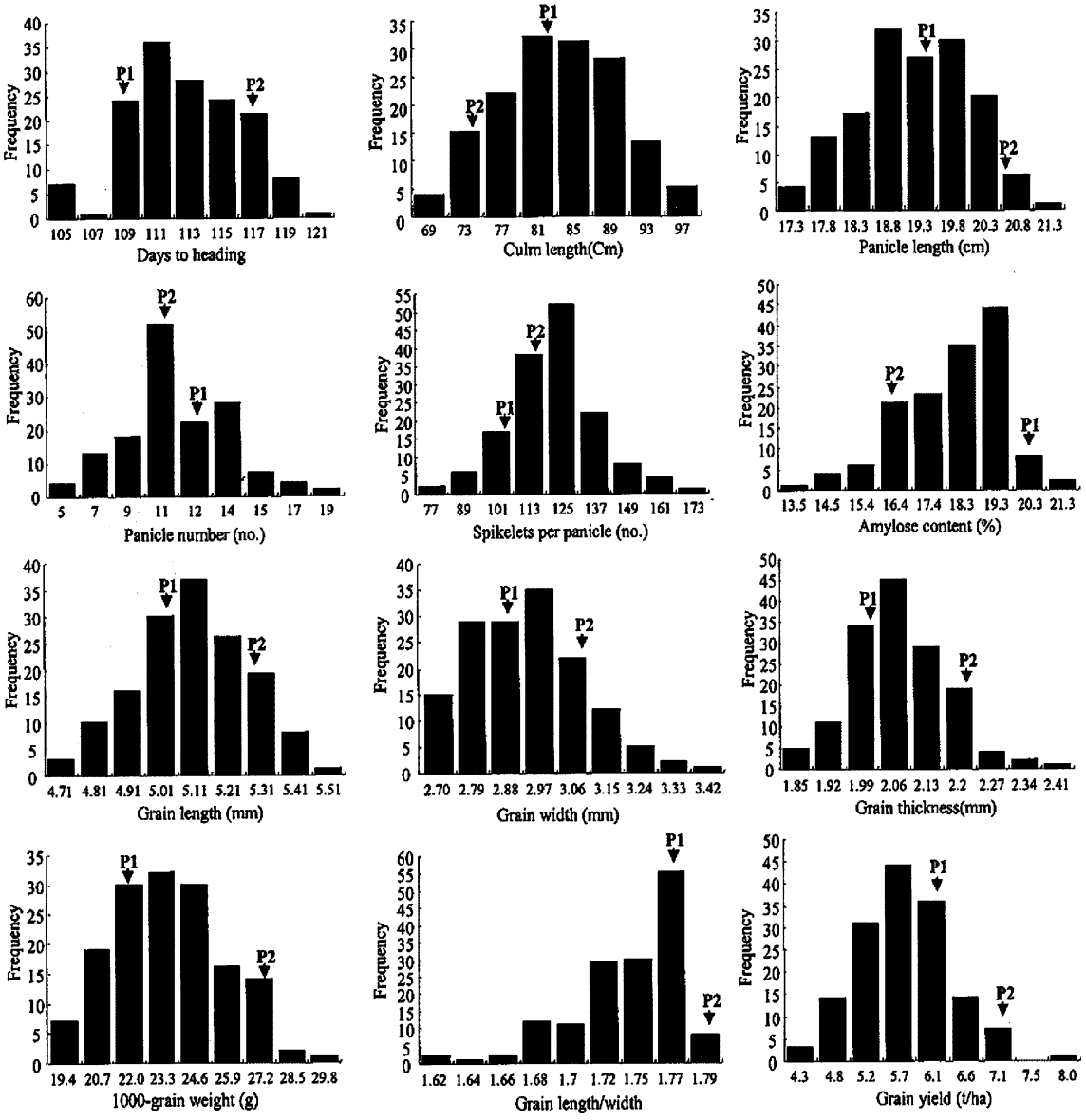

Fig. 1 Frequency distribution of 12 agronomic traits in the 150 F_3 lines (P_1 Hwayeongbyeo, P_2 HG101). The vertical axis of each figure represents the number of $F_{2:3}$ lines

SPA, and 1 to 6 QTL were detected for each trait. The phenotypic variation explained by each QTL (R^2) ranged from 4.2 to 30.5%. The effect of digenic interaction on the 12 traits analyzed with two-way ANOVA, using the SAS program, was not significant at the level of $P < 0.01$. Most of the QTL detected in this study were located in the same or adjacent regions (Fig. 2).

Days to heading (dth) Four QTL were identified for days to heading in the $F_{2:3}$ lines. The phenotypic variation explained by each QTL ranged from 4.9 to 19.5%. Collectively, these four QTL explained 30.8% of the phenotypic variation. The Hwaseongbyeo alleles contributed earliness at three loci, *dth3.1*, *dth3.2*, and *dth4*, and the *O. grandiglumis* allele contributed earliness at *dth6*.

Culm length (cl) Six QTL, *cl1.1*, *cl1.2*, *cl4.1*, *cl4.2*, *cl11*, and *cl12* were mapped to four chromosomes. At four loci, *cl1.1*, *cl1.2*, *cl4.1*, and *cl4.2*, the wild alleles decreased culm length, and at the other two loci, the wild alleles increased culm length. Collectively, these six QTL explained 50.6% of the phenotypic variation. The *cl1.2* is likely to correspond to *sd-1*, a semi-dwarf gene which has been previously located (Cho et al. 1994).

Panicle length (pl) One QTL on chromosome 6 was significantly associated with panicle length. The wild allele increased panicle length at *pl6*.

Panicle number (pn) One QTL on chromosome 11 was detected for panicle number. The *O. grandiglumis* allele increased panicle number at *pn11*.

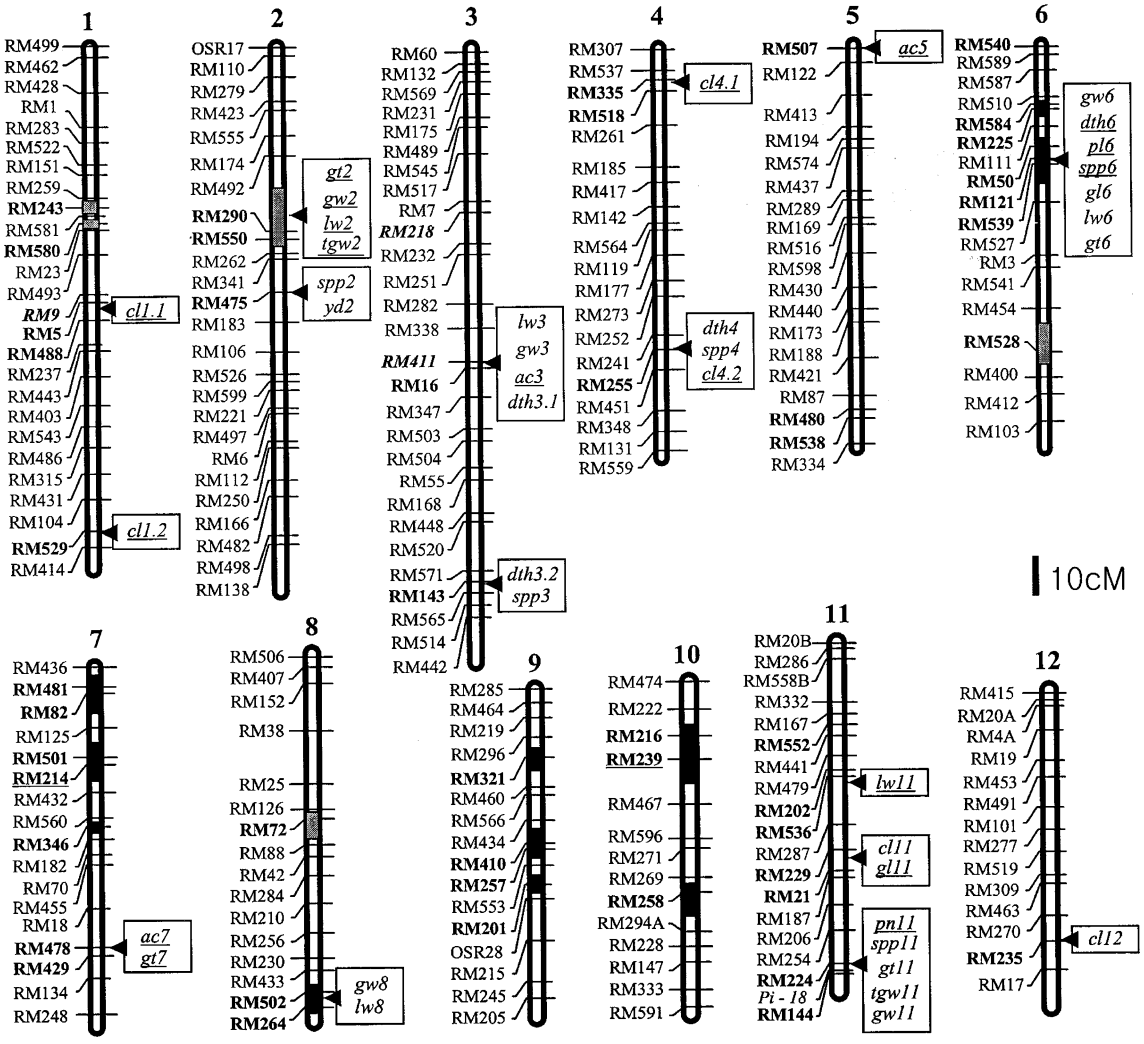

Fig. 2 A molecular map was developed using SSR markers polymorphic between Hwaseongbyeo and *Oryza grandiglumis*; SSR markers in *bold* mark the *O. grandiglumis*-specific genomic regions introgressed into HG101. Quantitative trait loci (*QTL*) detected by single-point analysis were represented at the right of the chromosomes. The QTL detected in the same interval are *boxed*. The markers showing segregation distortion are indicated in *italics* (in favor of Hwayeongbyeo), and *underlined* (in favor of *O. grandiglumis*) at the significance level of $P < 0.05$. QTL with the desirable allele from *O. grandiglumis* are *underlined*. Also, graphical genotype of CR1242 is denoted on the chromosomes. *Black*, *gray*, and *blank regions* mark the *O. grandiglumis*-specific, heterozygous for Hwaseongbyeo and *O. grandiglumis* alleles, and the Hwaseongbyeo genomic regions in CR1242, respectively

Spikelets per panicle (spp) Five QTL located on chromosomes 2, 3, 4, 6, and 11 were identified for spikelets per panicle. The wild allele increased spikelets per panicle at *spp6*. At the other four loci, the Hwaseongbyeo alleles increased spikelets per panicle.

Amylose content (ac) Three QTL located on chromosomes 3, 5, and 7 were identified for amylose content. They were clustered in the same regions as QTL associated with the grain trait QTL except for *ac5*. These three QTL accounted for 11.5% of the phenotypic variation.

Grain length (gl) Two QTL located on chromosomes 6 and 11 were detected for grain length. The phenotypic variation explained by each QTL ranged from 10.2 to 15.3%. The wild allele increased grain length at *gl6* but decreased at *gl11*.

Grain width (gw) Five QTL located on chromosomes 2, 3, 6, 8, and 11 were detected for grain width. The phenotypic variation explained by each QTL ranged from 4.9 to 7.7% and the Hwayeongbyeo alleles were associated with greater grain width in all cases except for *gw2*.

Grain thickness (gt) Four QTL were detected for grain thickness. The phenotypic variation explained by the QTL ranged from 4.2 to 17.2%. The wild allele increased grain thickness at two loci, *gt2* and *gt7* and decreased at the other two loci.

1,000-grain weight (tgw) Two QTL were detected for grain weight. The phenotypic variation explained by the QTL ranged from 5.0 to 13.4%. The wild allele increased grain weight at the *tgw2* locus but decreased at the *tgw11* locus.

Table 3 Quantitative trait loci (QTL) for 12 traits based on single-point analysis in an F_3 population

Trait	QTL	Marker	Chr.	*P*	R^2(%)	Mean			
						H/H[a]	H/G[a]	G/G[a]	Allele effect[b]
Days to heading	*dth3.1*	RM143	3	0.0050	5.3	111	113	113	1.0
	dth3.2	RM16–RM411	3	0.0153	4.9	111	112	114	1.5
	dth4	RM255	4	0.0047	6.7	111	112	114	1.5
	dth6	RM539	6	0.0000	19.5	115	113	110	−2.5
	Total				30.8				
Culm length	*cl1.1*	RM5–RM488	1	0.0009	9.1	86	82	81	−2.5
	cl1.2	RM529	1	0.0000	30.5	87	83	77	−5.0
	cl4.1	RM335–RM518	4	0.0107	6.0	84	83	81	−1.5
	cl4.2	RM255	4	0.0026	8.1	85	83	80	−2.5
	cl11	RM229–RM21	11	0.0100	4.7	80	83	84	2.0
	cl12	RM235	12	0.0040	4.6	81	83	85	2.0
	Total				48.7				
Panicle length	*pl6*	RM539–RM121	6	0.0021	8.2	19.2	19.7	20.0	0.4
Panicle no.	*pn11*	RM224 - RM144	11	0.0235	5.6	11	11	12	0.5
Spikelets /panicle	*spp2*	RM475	2	0.0037	4.4	125	122	115	−5.0
	spp3	RM143	3	0.0040	6.7	128	118	118	−5.0
	spp4	RM255	4	0.0032	4.8	127	120	117	−5.0
	spp6	RM539–RM121	6	0.0089	6.3	113	121	127	7.0
	spp11	RM224–RM144	11	0.0038	7.4	121	127	115	−3.0
	Total				15.5				
Amylose content	*ac3*	RM16–RM411	3	0.0496	5.4	18.6	18.1	17.5	−0.6
	ac5	RM507	5	0.0047	6.6	18.6	18.0	17.5	−0.6
	ac7	RM478–RM429	7	0.0031	7.7	18.7	18.1	17.6	−0.6
	Total				11.5				
Grain length	*gl6*	RM539–RM121	6	0.0224	10.2	5.01	5.12	5.15	0.07
	gl11	RM229–RM21	11	0.0000	15.3	5.18	5.11	5.00	−0.09
	Total				18.0				
Grain width	*gw2*	RM290–RM550	2	0.0072	6.7	2.92	2.92	3.02	0.05
	gw3	RM16–RM411	3	0.0257	4.9	2.96	2.95	2.87	−0.05
	gw6	RM50–RM121	6	0.0068	6.7	2.99	2.92	2.89	−0.05
	gw8	RM502–RM264	8	0.0105	6.1	2.99	2.92	2.90	−0.05
	gw11	RM224–RM144	11	0.0042	7.7	2.99	2.92	2.89	−0.05
	Total				21.3				
Grain thickness	*gt2*	RM290–RM550	2	0.0000	17.2	1.99	2.08	2.11	0.11
	gt6	RM50–RM121	6	0.0295	4.8	2.10	2.06	2.05	−0.03
	gt7	RM429 - RM478	7	0.0253	4.2	2.04	2.08	2.10	0.03
	gt11	RM224–RM144	11	0.0218	4.8	2.10	2.07	2.05	−0.03
	Total				21.0				
1000-grain weight	*tgw2*	RM290–RM550	2	0.0000	13.4	22.1	23.8	24.3	1.1
	tgw11	RM224–RM144	11	0.0243	5.0	24.0	23.7	22.9	−0.55
	Total				15.2				
Grain length/width Ratio	*lw2*	RM290–RM550	2	0.0000	13.4	1.79	1.73	1.71	−0.04
	lw3	RM411–RM16	3	0.0006	8.9	1.71	1.74	1.78	0.04
	lw6	RM50–RM539	6	0.0015	8.6	1.71	1.75	1.77	0.03
	lw8	RM502–RM264	8	0.0024	8.0	1.71	1.75	1.76	0.03
	lw11	RM202–RM536	11	0.0024	8.9	1.75	1.75	1.70	−0.03
	Total				32.1				
Yield	*yd2*	RM475	2	0.0001	11.5	590	583	529	−30.5

[a]H/H, H/G, G/G; Hwaseongbyeo homozygotes, Hwaseongbyeo/HG101 heterozygotes, and HG101 homozygotes, respectively
[b]The additive effect is the one associated with substitution of a 'Hwaseongbyeo' allele by the corresponding *O. grandiglumis* allele

Grain length/width ratio (lw) Five QTL located on chromosomes 2, 3, 6, 8, and 11 were identified for the length/width ratio of the grain, and the phenotypic variation explained by the QTL ranged from 8.0 to 13.4%. The wild allele increased the ratio at three loci, *lw3*, *lw6*, and *lw8* but decreased at the other loci.

Yield per plant (yd) One QTL on chromosome 2 was detected for grain yield, which explained 11.5% of the phenotypic variation. The wild allele decreased grain yield at *yd2*.

Confirmation of QTL for grain shape traits

CR1242, the F_3 source population for the characterization of the QTL including *tgw2*, had large seeds and contained *O. grandiglumis* introgression across the region marked by RM290 and RM550 (Fig. 2). CR1242 also contained 16 additional background introgressions located on 7 of the 12 chromosomes (Fig. 2). In 22 F_4 lines derived from CR1242, the difference in 1,000-grain weight between lines having an *O. grandiglumis* introgression in the target region and those having

Hwaseongbyeo DNA was as large as 3.7 g (Table 4). And the differences in grain thickness, grain width, grain length/width ratio, panicle length, and grain yield/plant between F_4 lines having an *O. grandiglumis* introgression in the target region and those having Hwaseongbyeo alleles were significant. However, the differences in traits including days to heading between F_4 lines having an *O. grandiglumis* introgression in the target region and those having Hwaseongbyeo alleles were not significant. These results confirmed primary QTL observations based on F_3 lines and demonstrated that transgressive segregants could be identified in subsequent generation. To determine the size of the introgression segment and fine map *tgw2*, ten additional SSR markers polymorphic between Hwaseongbyeo and *O. grandiglumis* were mapped in the 22 F_4 lines (Fig. 3). Six lines were found to be fixed for Hwaseongbyeo alleles in the introgressed region defined by markers RM3390-RM13104. The other 16 lines homozygous for *O. grandiglumis* alleles in the target region were homozygous for Hwaseongbyeo at RM7288 and RM13104 indicating that recombinations occurred between RM1358 and RM7288, and between RM550 and RM13104 during the backcross breeding program. The precise boundary of the centromere on chromosome 2 was resolved to be between 13,570,041 and 13,857,135 bp with a total amount of 201,035 bp by using CentO satellite DNA (IRGSP 2005). On the basis of available sequence information (http://www.gramene.org/), the introgressed segment does not appear to harbor the centromere because the SSR marker RM13104 is located above the centromere. As indicated in Table 4, there were significant differences in TGW between the lines having an *O. grandiglumis* introgression in the target region and those having Hwaseongbyeo DNA. This allowed us to conclude that *tgw2* was located in the interval RM7288–RM13104, a region of approximately 4.4 Mb in size. It should be noted that RM7288 and RM13104 represented the outside borders of the introgression.

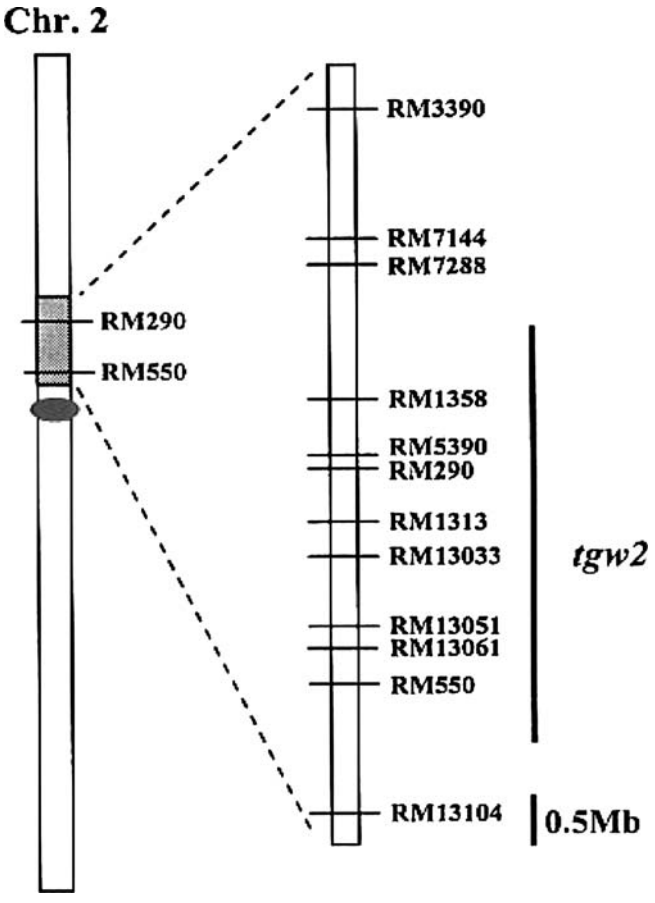

Fig. 3 Physical map of chromosome 2 showing introgression from *O. grandiglumis*. *Gray regions* indicate introgressed segments, and the *black circle* indicates approximate position of centromere with the *tgw2* QTL. Genotype analysis of the F_4 lines indicated that recombinations occurred between RM1358 and RM7288, and between RM550 and RM13104 during the backcross breeding program between Hwaseongbyeo and *O. grandiglumis*

Discussion

One of the objectives of this study was to identify novel beneficial alleles from *O. grandiglumis* for yield and yield components. We identified *O. grandiglumis* alleles related to yield components, including loci for days to heading. As was evidenced for culm length in this study, of particular interest is the demonstration that some of the novel genetic variation observed in the advanced backcross progenies of the interspecific cross involves positive transgressive variation. The fact that

Table 4 Comparison of means (±SD) for 11 traits of two genotypic classes at the target loci (RM1358 and RM550) in F_4 lines

Trait	Genotype			*F*	R^2
	H/H (6)	G/G (16)	Mean		
DTH	107 ± 0.7	108 ± 1.4	108 ± 1.2	0.73	3.5
CL	94 ± 2.1	91 ± 4.2	92 ± 4.1	4.15	17.2
PL	23.5 ± 0.5	22.3 ± 0.7	22.6 ± 0.8	13.8**	40.8
PN	12 ± 2.3	11 ± 0.6	11 ± 1.4	4.21	17.4
SPP	134 ± 17.8	143 ± 9.6	141 ± 13.1	2.48	11.0
TGW	22.9 ± 1.2	26.6 ± 1.0	25.6 ± 1.2	26.6***	57.1
GL	6.91 ± 0.08	6.91 ± 0.11	6.91 ± 0.10	0.003	0.0
GT	2.22 ± 0.05	2.40 ± 0.05	2.35 ± 0.09	45.7***	69.6
GW	3.34 ± 0.12	3.68 ± 0.06	3.59 ± 0.17	70.46***	77.9
LW	2.07 ± 0.06	1.88 ± 0.04	1.93 ± 0.10	36.31***	64.5
YD	21.4 ± 1.5	24.9 ± 2.8	24.0 ± 3.0	7.55*	27.4

Numbers in parentheses denote the number of lines examined *H*/*H* homozygous Hwaseongbyeo, *G*/*G* homozygous *O. grandiglumis*
Significant at $*P \le 0.05$, $**P \le 0.01$, $***P \le 0.001$

the trait-enhancing alleles come from the agronomically unfavorable wild parent provides evidence that phenotypic performance turns out to be a poor predictor of genetic potential. These results are comparable with those of the previous studies that reported trait-enhancing alleles from the wild species (Xiao et al. 1998; Moncada et al. 2001), although the wild accession was phenotypically inferior to *O. sativa*. For 18 (46.2%) of the 39 QTL identified in this study, the *O. grandiglumis*-derived alleles contributed a desirable effect in the elite cultivar background. Even though there was no yield-improving allele identified from *O. grandiglumis*, the finding of transgressive F_3 lines in yield might suggest that they were a consequence of introgressed genes that were not detected by QTL analysis. The number of QTL identified in this study is probably an underestimation because some of the chromosomal regions harboring *O. grandiglumis* introgressions might have been undetected with the microsatellite markers used. With more traits evaluated, it is expected that more trait-improving QTL from *O. grandiglumis* can be identified.

This study aimed to determine the degree of conservation of QTL across rice cultivars and environments. Eighteen of the 39 (46.2%) QTL exhibited effects in the opposite direction from that expected from the phenotypes of the parents. Location of these trait-improving QTL was compared with those identified across rice cultivars and wild species (http://www.gramene.org). For days to heading, one QTL on chromosome 6, in which the *O. grandiglumis* allele reduced the days to heading, shared the same genomic region as the heading date QTL in other studies (Xiao et al. 1998; Cai and Morishima 2002). This locus appears to coincide with the mutant photoperiod sensitivity locus, *se1*, also referred to as the QTL, *Hd1*, which was cloned by Yano et al. (2000). Among the six QTL for culm length detected in the present study, *cl1.2* was identified in a similar region as a QTL for culm length (Cai and Morishima 2002; Thomson et al. 2003). The finding that *cl1.2* explained 30.5% of the phenotypic variation in the present study suggests that this may correspond to *sd-1*, the semi-dwarf gene (Cho et al. 1994; Xiong et al. 1999; Thomson et al. 2003) that was cloned by Sasaki et al. (2002). The culm length QTL *cl1.1* shared a similar location as the QTL for culm length detected in other studies (Hittalmani et al. 2003; Septiningsih et al. 2003a). The other culm length QTL, except for *cl12*, have been reported in other previous studies (Moncada et al. 2001; Mei et al. 2003; Septiningsih et al. 2003b). The *O. grandiglumis* allele increased panicle length at QTL *pl6* in this study and it was localized in a similar location as a panicle length QTL in the study by Septiningsih et al. (2003a). The *O. grandiglimis* allele for spiklelets per panicle at *spp6* occurred in the same interval as *pl6* detected in this study, and no other study detected QTL for spikelets per panicle in this location. Several other QTL controlling amylose content and grain traits have not been detected in previous QTL studies between *Oryza* cultivars, indicating potentially novel alleles from *O. grandiglumis*. Genome-wide comparisons will be possible as more QTL are detected and accumulated in this study. For grain yield, several studies detected a QTL in the same region on chromosome 2. In two cases, this study and that by Brondani et al. (2002), the wild alleles decreased grain yield, while it was associated with increased grain yield in Thomson et al. (2003). These results suggest the possibility of an allelic series where wild accessions may harbor different alleles at these loci. In some cases, it appears that a wild allele is superior to one of the cultivated alleles, but possibly inferior to the different ones. It will be interesting to characterize the genes underlying these QTL to be able to determine how they interact with other genes/alleles in various genetic backgrounds. Such knowledge will be indispensable in predicting the effect of introgressing alleles from wild or exotic parents into diverse genetic backgrounds.

Similar genomic locations of QTL affecting different traits may be attributable to either pleiotropy of single genes or tight linkage of several genes that individually influence specific traits. In a previous study by Xiao et al. (1996), pleiotropy was suggested for three chromosomal regions that were simultaneously associated with 1,000-grain weight and grains per plant or 1,000-grain weight and grains per panicle. These yield components showed highly negative correlations, and three significant QTL associated with 1,000-grain weight were mapped to the same positions as three QTL affecting grains per plant and grains per panicle. In this study, one genomic region was associated with more than one trait, indicating linkage and/or pleiotropic effects. The *O. grandiglumis* allele at the locus RM143 on chromosome 3 increased the number of days to heading, and also decreased spikelets per panicle. The *O. grandiglumis* allele at the locus RM255 on chromosome 4 increased the number of days to heading, and also decreased culm length and spikelets per panicle. Interestingly, seven traits including three plant morphology and four grain traits were located at the loci near RM50 on chromosome 6. Also, the *O. grandiglumis* allele at the locus RM475 on chromosome 2 decreased grain yield and spikelets per panicle. To better understand the characteristics of this locus, development of further generations of near-isogenic lines (NILs) containing the fine-mapped QTL is underway.

Significant segregation distortion, deviation of the genotypic frequencies from their expected Mendelian ratio, was observed in this study for 5 (10.2%) marker loci. Of these, three marker loci were skewed towards the Hwayeongbyeo allele and two loci were skewed towards the *O. grandiglumis* alleles. Segregation distortion is a common feature of interspecific or intersubspecific crosses in rice (Harushima et al. 1996, 2002; Xu et al. 1997). Most of the skewed loci found in this study were located on the same or nearby regions as those reported by Xu et al. (1997). The segregation distortion in these genomic regions may be due to the presence of gene(s) governing sterility via gamete or zygote abortion. The

region on chromosome 3 has also been found to be similarly skewed in other studies. In the F_2 progenies from a cross between Nipponbare and Kasalath, the significant deviation towards the Kasalath genotype was found on chromosome 3, which is presumably due to the gametophyte gene (*ga-2*) (Harushima et al. 1996).

Because this study employed an advanced backcross line, HG101, in which relatively small chromosomal segments of *O. grandiglumis* are substituted in the Hwaseongbyeo genome, the primary QTL data should be reliable. Confirmation of the grain trait QTL, *tgw2, gw2, gt2*, and *lw2*, based on the progeny test provided the evidence for this assertion. As documented in this study, R^2 values increased with advanced generations, from 13.4% in the F_3 to 57.1% in the F_4 generation. As the number of spurious donor (i.e., *O. grandiglumis*) introgressions in the genetic background decreased, the proportion of the phenotypic variation that could be explained by the markers was greatly enhanced. This situation was similar to that discussed by Li et al. (2004) for *gw3.1* in rice, or by Paterson et al. (1990) for soluble solids concentration, fruit mass, and yield in tomato. The confirmed locus, *tgw2*, for grain weight is of particular interest because of its independence from undesirable height and maturity. The finding of the colocalization of the QTL for grain weight, grain width, and grain yield seems to imply that the increase in grain weight might be responsible for the increased grain yield potential in the nearly isogenic background of Hwaseongbyeo. Ishimaru (2003) also identified a grain weight QTL, *tgw6*, on chromosome 6 which is responsible for increased yield potential without any effects on plant type or grain quality in the Nipponbare genetic background. To better understand the genes contributing to these beneficial effects, we are pursuing a fine mapping strategy aimed at cloning the genes underlying the QTL for grain weight.

The results obtained in this study indicate that *O. grandiglumis* contains QTL alleles that are likely to improve agronomically important traits in elite cultivars. It is proposed that NILs containing individual introgressions associated with positive QTL from *O. grandiglumis* be developed from this population and evaluated in a wide range of environments, so that QTL versus environment interactions can be assessed.

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