# QTL Analysis for Yield Related Traits Using Populations Derived from an *indica-japonica* Hybrid in Rice (*Oryza sativa* L.)

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**Abstract**: Introgression has been achieved from a wild species *Oryza minuta* (2*n* = 48, BBCC, Acc No.101141) into *O. sativa* subsp. *indica* IR71033-121-15. This introgression line was developed at International Rice Research Institute (IRRI) through embryo rescue as well as three backcrosses using IR31917-45-3-2 as a recurrent parent. These two IR lines resemble each other but differ in several important agronomic traits, which can be attributable to *O. minuta* introgressions. Out of 530 STS markers tested for introgression analysis, at least 14 introgressed chromosomal segments from *O. minuta* were detected throughout 12 chromosomes. Most of the introgressed segments were quite small in size, as they were detected by single markers and flanking markers were negative for introgressions. A population from the cross between japonica cultivar Junambyeo and introgressed indica line IR71033-121-15, consisting of 146 plants, was evaluated for ten agronomic traits. Genotyping of $F_2$ lines and phenotyping from both $F_2$ and $F_3$ were considered for QTL analysis. A total of 11 single-locus QTLs (S-QTLs) were identified for ten traits, i.e. days to heading (DTH), panicle number (PN), spikelet fertility (SF), panicle length (PL), spikelet per panicle (SPP), grain length (GL), grain width (GW), grain length to width ratio (GLW) and grain thickness (GT) in both populations. The *O. minuta* derived alleles of QTLs, *spp6, gl3, glw3, glw5* and *gt12*, were detected in both populations indicating robust QTLs for these traits. QTLs from *O. minuta* introgression could be new sources of natural genetic variation for genetic improvement of rice.

**Keywords**: rice; pleiotropic effect; alien introgression; Sequence Tagged Site (STS) markers; *Oryza minuta*; quantitative trait locus (QTL)

The wild species of *Oryzae* are an important reservoir of genetic variability for agronomic traits such as biotic and abiotic stresses and for improved yield potential (Brar & Khush 1997; Xiao *et al*. 1998; Moncada *et al*. 2001; Yoon *et al*. 2006). Because the wild germplasm has not been thoroughly exploited yet, there is still a great potential to develop new modern rice varieties by incorporating new useful genes from wild races (Brar 2004). Recent progress in plant genome analysis has enabled us to examine naturally occurring allelic variation underlying complex traits. Quantitative trait locus (QTL) analysis can provide information relevant to agronomic traits using molecular markers to identify specific regions of the genome affecting any measurable trait (Tanksley 1993). To date, a large number of QTLs associated with various traits including yield and its components,

quality traits, environmental stress tolerance, and disease and insect resistances from interspecific crosses, have been identified in rice (Xiao *et al.* 1998; Xiong *et al.* 1999; Moncada *et al.* 2001; Brondani *et al.* 2002; Cai & Morishima 2002; Multani *et al.* 2003; Septiningsih *et al.* 2003; Thomson *et al.* 2003; Aluko *et al.* 2004; Jin *et al.* 2005; Suh *et al.* 2005; Yoon *et al.* 2006; Mei *et al.* 2006; Tian *et al.* 2006; Steele *et al.* 2006). However, most of these QTLs were identified by using AA genome species as a donor parent in wide hybridization due to the easy gene transfer through conventional hybridization and selection. However, introgressions from distantly related genomes other than the AA genome are usually difficult because of low crossability and thus failure of raising hybrid plants. Some of them can be achieved through embryo rescue with subsequent backcrossing (Brar & Khush 1997). Several useful 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, 2003; Ishii *et al.* 1994; Liu *et al.* 2002). The possible mechanism of gene transfer might be achieved by restricted reciprocal recombination detectable only at the molecular level (Jena & Kochert 1991).

An allotetraploid of *O. minuta* (2*n* = 48, BBCC, Acc No. 101141) belongs to the *O. officinalis* complex and has useful genes for resistances to brown plant hopper, sheath blight and blast (Brar & Khush 1997). But few studies have been conducted so far to transfer insect and disease resistances from *O. minuta* to cultivated rice, *O. sativa* (Amante *et al.* 1998). However, attempts to transfer QTLs from *O. minuta* to cultivars have been limited due to incompatibility between unrelated genomes.

We used an advanced backcross line IR71033-121-15 from a cross between *O. sativa* subsp. *indica* IR31917-45-3-2 and a wild species, *O. minuta*, as a donor parent, which was developed and made available at IRRI. Although introgressed lines, IR71033-121-15, resembled IR31917-45-3-2, several yield related traits were different from its recurrent parent. Our interest was whether *O. minuta* derived chromosomal segments in introgressed lines would provide us with new QTLs for agronomic traits. In the present study we report the mapping from a population derived from a cross between Junambyeo (a Korean elite japonica cultivar) and introgressed *indica* IR71033-121-15.

## MATERIALS AND METHODS

### Plant materials

The mapping population used in this study was an $F_2$ population derived from a cross between IR71033-121-15 and Junambyeo, a Korean elite *japonica* cultivar. IR71033-121-15 was bred from backcrossing IR31917-45-3-2 three times to *O. minuta* aided with embryo rescue and generation advance. Though IR71033 mostly resembled the recurrent parent IR31917, it differed from IR31917 in several important agronomic traits including days to heading, spikelet fertility, panicle length, spikelet number per panicle and grain shape. An $F_1$ between IR71033 and Junambyeo showed about 60 percent spikelet fertility and early heading compared to the parents. Changes in grain shape were also found especially in grain length, width and thickness. This might originate from the introgression of *O. minuta* genomic segments.

### Phenotypic evaluation

146 $F_2$ plants with the parents IR71033 and Junambyeo were planted in the field one plant per hill at a distance of 30 × 15 cm using conventional transplanting methods during the summer of 2005 at the Experimental Farm of Seoul National University, Suwon, Korea. At the harvesting stage, they were evaluated for ten traits (days to heading, spikelet fertility, panicle length, spikelets per panicle, grain length, grain width, grain length to width ratio, grain thickness, tiller number and panicle number). Days to heading (DTH) were recorded as the number of days from sowing in the seedbed to heading of the first panicle of each $F_2$ plant. Panicle length (PL) was measured in centimetres from the panicle neck to the panicle tip. Spikelets per panicle (SPP) were counted as the number of spikelets of the biggest panicle in a plant. Spikelet fertility (SF) was calculated as a percentage: the number of fertile spikelets per panicle divided by the total number of spikelets per panicle. Grain length (GL), width (GW) and thickness (GT) were measured on ten fully ripened grains harvested from each $F_2$ plant using the Digimatic callipers (Mitutoyo Corp., Japan). Panicle number (PN) was counted at the panicle stage. Cultivation of $F_3$ families from $F_2$ lines followed the same cultural practices with three replications in 2006 for

Table 1. Chromosomal distribution of STS markers used in this study

| Chromosome | No. of markers | No. of polymorphic markers between Junambyeo & IR71033 | No of introgressed segments |
|---|---|---|---|
| 1 | 55 | 32 | 1 |
| 2 | 40 | 31 | 1 |
| 3 | 57 | 31 | 1 |
| 4 | 45 | 26 | 2 |
| 5 | 42 | 27 | 1 |
| 6 | 42 | 26 | 3 |
| 7 | 40 | 31 | 1 |
| 8 | 45 | 30 | 0 |
| 9 | 41 | 32 | 1 |
| 10 | 30 | 19 | 0 |
| 11 | 53 | 31 | 1 |
| 12 | 40 | 22 | 2 |
| Total | 530 | 338 | 14 |

phenotypic evaluation of the same traits by the same procedure followed in $F_2$.

### Detection of introgression and STS analysis

DNA was extracted from the leaf tissues of each plant of the mapping population according to the chloroform-based DNA extraction protocol (Causse *et al.* 1994). Sequence tagged site (STS) analysis of the $F_2$ population was performed according to the method described by Chen *et al.* (1997). A total of 530 STS markers of known chromosomal position designed in the Crop Molecular Breeding Lab, Seoul National University (unpublished) were surveyed for *O. minuta* introgression in the line IR71033 and for polymorphisms (Table 1). The process of detecting introgression is shown in Figure 1. For PCR amplification of STS markers, each 25 µl reaction mixture contained 50 ng DNA, 5 pmol of each primer, 2 µl PCR buffer [100mM Tris (pH 8.3), 500mM KCl, 15mM $MgCl_2$, 2 µg gelatine], 250µM of each dNTPs and 0.5 unit *Taq* polymerase. The MJ Research PCR system (Applied Biosystem) was used for DNA amplification. The thermocycler profile was as follows: 5 min at 94°C, 35 cycles of 1min at 94°C, 1 min at 48°C and 2 min at 72°C, and 5 min at 72°C for final extension. Amplified PCR products were resolved by electrophoresis in 3% agarose gel.

### Linkage and QTL analysis

Linkage analysis was performed with MAPMAKER/EXP 3.0 (Lander *et al.* 1987). The Kosambi mapping function was used to transform the recombination frequency to genetic distances (cM). Linkage groups were assigned to rice chromosomes according to the published rice map (Chen *et al.* 1997; Temnykh *et al.* 2000). Q-gene 3.06 (Nelson 1997) and SAS were used for statistical analysis. The association between phenotype and genotype

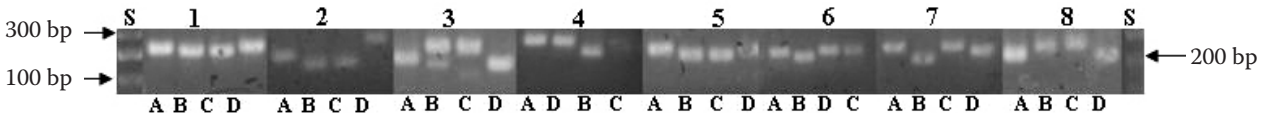


Figure 1. Introgression test of *O. minuta* segment at the loci tagged by the markers: (1) S601.7, (2) S6065.8, (3) S1027.3, (4) S5041.5, (5) S12094.6, (6) S6050.3, (7) S12011.5 and (8) S9083.2 in different chromosomes; tested lines A: IR71033-121-15, B: Junambyeo (accession #26428), C: IR31917-45-3-2 and D: *O. minuta* (accession #101141); introgression lines and *O. minuta* produced the same band size at these loci indicating positive introgression

was investigated using single-point analysis. To determine empirical significance thresholds for declaring a QTL, 1000 permutations were done to calculate LOD thresholds for each trait at $P = 0.05$ and $P = 0.01$. Analysis of epistatic interaction between two loci (E-QTL) was performed with software QTLmapper 1.0 (Wang *et al*. 1999). Single point analysis (SPA) was conducted to determine the effect of each marker on each trait and the correlation between traits. The percent phenotype variance (%) associated with each significant QTL was calculated from the regression of each marker-phenotype combination. The total phenotypic variation explained was estimated by fitting a model including all putative QTLs for the respective trait simultaneously. Locations of these trait-improving QTLs were compared with those identified across rice cultivars and wild species using a genomics DB, http://www.gramene.org. QTLs were named according to McCouch *et al.* (1997).

## RESULTS

### Plant and grain characters

The phenotypic data for each trait in parents and two populations are shown in Table 2 and Figure 2. All traits varied widely with aberrant segregations and the amount of variation was quite similar in two populations. The skewness value was more or less than 1.0 for almost all traits, suggesting that the populations fitted a normal distribution. However, transgressive segregation was observed for the other traits and especially pronounced for the higher values of DTH, PN, SPP, GL and GT.

Significant correlations ($P < 0.01$) were observed between traits. Table 3 summarizes the correlation coefficients between the eight traits evaluated. Significant negative correlation was found between DTH, SPP, GL, GLW and GT and positive correlation was between DTH, PN and SF. Significant correlations were also detected between the traits associated with grain morphology, GL, GW, GLW, and GT. For most of the correlations, the directions (+ or –) and degree of correlation were similar like in previous studies (Xiao *et al.* 1998; Thomson *et al.* 2003; Yoon *et al.* 2006). In agreement with previous studies, SPP showed a negative correlation with DTH and SF while the correlation of this trait with PL was positive. GLW was positively correlated with GL but its correlation with GW was negative. GT showed a positive correlation with GL but a negative correlation with GW.

### Construction of a framework linkage map

A total of 530 STS markers were used to survey polymorphism between the parents. 338 (63.8%) markers showed polymorphism between the introgression parent, IR71033-121-15 and Junambyeo. Fourteen introgressed segments were identified throughout 12 chromosomes (Table 1). The introgressed segments were small in size as they are identified by a single marker. With the $F_2$ mapping

Table 2. Descriptive statistics of eight traits for parents and two populations between Junambyeo and IR71033

| Traits[a] | Parents (mean ± SD) | | $F_3$ population | | | $F_2$ population | | |
|---|---|---|---|---|---|---|---|---|
| | Junambyeo | IR71033 | mean ± SD | range | skew | mean ± SD | range | skew |
| DTH | 75.5 ± 0.7 | 83 ± 1.4 | 84.3 ± 18.9 | 67–146 | 1.30 | 98.0 ± 24.5 | 61.0–148.0 | 0.31 |
| PN | 13.3 ± 0.8 | 14.1 ± 2.1 | 11.8 ± 1.9 | 7.2–18.8 | 0.24 | 11.9 ± 2.0 | 7.5–18.0 | –0.31 |
| SF | 1.4 ± 0.1 | 4.6 ± 0.1 | 31.1 ± 18.9 | 1.3–95.5 | 1.60 | 46.2 ± 30.2 | 6.4–99.0 | 0.47 |
| SPP | 174.2 ± 32.8 | 204 ± 33.5 | 108.5 ± 40.4 | 239–151 | –0.26 | 81.5 ± 54.8 | 10–288 | 0.30 |
| GL | 6.8 ± 0.4 | 10.4 ± 0.6 | 9.0 ± 0.6 | 7.1–10.9 | –0.07 | 8.7 ± 0.6 | 6.6–10.1 | 0.02 |
| GW | 3.2 ± 0.4 | 2.6 ± 0.14 | 2.9 ± 0.2 | 2.3–3.5 | –0.01 | 2.9 ± 0.2 | 2.3–3.6 | 0.18 |
| GLW | 2.7 ± 0.8 | 4.8 ± 0.9 | 3.1 ± 0.3 | 2.1–4.2 | 0.53 | 4.2 ± 0.3 | 3.1–5.1 | –0.40 |
| GT | 2.2 ± 0.2 | 2.0 ± 0.2 | 2.0 ± 0.1 | 1.7–2.5 | 0.81 | 2.05 ± 0.1 | 1.7–2.5 | 0.85 |

[a]DTH – days to heading; PN – panicle number; SF – spikelet fertility; SPP – spikelet per panicle; GL – grain length; GW – grain width; GLW – grain length-width ratio; GT – grain thickness

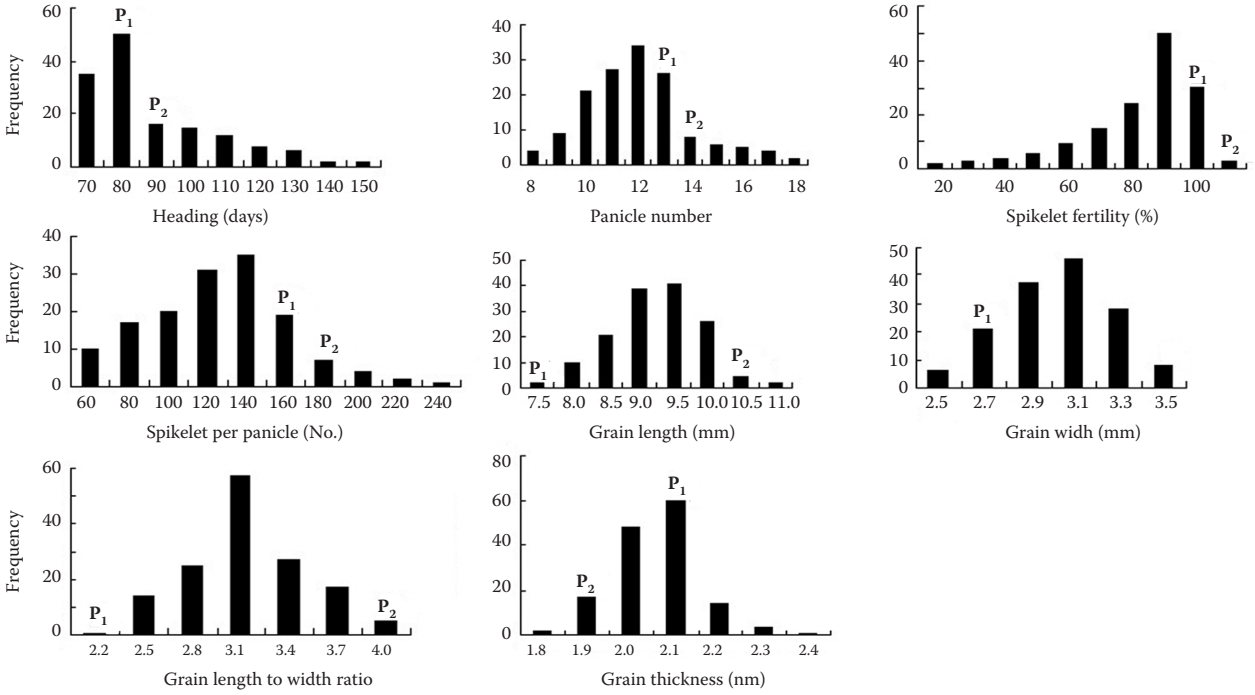


Figure 2. Frequency distribution of agronomic traits in $F_{2:3}$ QTL populations; $P_1$: Junambyeo, $P_2$: IR71033; phenotypic values of both parents are designated by arrows

population containing 146 individuals, a framework linkage map with 147 STS markers was constructed to identify the QTLs governing DTH, PN, SF and grain-related traits (Figure 3). The map covered 1825.9 cM over all 12 chromosomes with an average interval of 12.0 cM between the adjacent markers. The order of the markers in each chromosome was consistent with the order of the Nipponbare/Kasalath map (Harushima *et al*. 1998). Significant segregation distortion was observed for 41 marker loci (28%) out of 147 markers used at a significance level of $P < 0.05$. Some of the distorted markers were randomly distributed along the chromosomes, but mostly occurred in clusters of at least three adjacent loci, which were located on chromosomes 3, 4, 6, 11, 12 (Figure 4).

## Single-locus QTL analysis

Several significant QTLs were identified for eight agronomic traits and are summarized in Table 4. A total of 17 QTLs, 1 to 5 QTLs for each trait, were identified using single-point analysis (SPA) in $F_3$ population and 16 QTLs with 1 to 4 QTLs for each trait were identified in $F_2$ populations. 11 QTL loci shared a similar region in both populations indicating robust QTL for all traits analyzed. The phenotypic variation explained (PVE) by each QTL ranged from 9.7% to 28.1% in $F_3$ population and from 9.9% to 36.9% in $F_2$ population (Table 4). Most of the QTLs detected in this study were located in the same or adjacent regions (Figure 3). Of them, eight QTLs were located on the introgressed segments from *O. minuta* on chromosomes 3, 5, 6, and 12.

***Days to heading (DTH)***: Two QTLs were identified on chromosome 6 and 8, accounting for 34.9% to 36.9% of the phenotypic variation in both $F_2$ and $F_3$, respectively. Among them *qtl6* shared a similar region in both populations. For these QTL loci, the wild alleles contributed earliness at this locus.

***Panicle number (PN)***: Two QTLs were identified on chromosome 4 and 6 in both populations for panicle number, which explained 15.5% and 19.1% of PVE, respectively. An increasing effect on chromosome 4 was contributed by IR71033, while there was an decreasing effect on chromosome 6.

***Spikelet fertility (SF)***: One QTL on chromosome 6 in both populations, in which *O. minuta* alleles were associated with decreasing fertility. The phenotypic variation explained by this QTL was 19.2% in $F_3$ and 34.5% in $F_2$.

***Spikelet per panicle (SPP)***: One QTL on chromosome 6 in both populations, which explained 19.7% and 28.1% of phenotypic variation in $F_3$ and $F_2$,

Table 3. Correlation coefficients between traits in the populations derived from a cross of IR71033 and Junambyeo

| Traits[a] | DTH | PN | SPP | SF | GL | GW | GLW |
|---|---|---|---|---|---|---|---|
| DTH | | | | | | | |
| PN | 0.31** | | | | | | |
| SPP | –0.65** | –0.38** | | | | | |
| SF | 0.74** | 0.37** | –0.84** | | | | |
| GL | –0.27* | –0.21* | 0.18* | –0.22* | | | |
| GW | 0.075 | –0.04 | –0.08 | 0.1 | –0.24* | | |
| GLW | –0.26* | –0.15 | –0.25* | –0.29* | 0.78** | –0.47** | |
| GT | –0.21* | –0.08 | 0.16 | –0.20* | 0.77** | –0.79** | 0.78** |

*and **indicate significant difference at 0.05 and 0.01 level, respectively

[a]DTH – days to heading; PN – panicle number; SF – spikelet fertility; SPP – spikelet per panicle; GL – grain length; GW – grain width; GLW – grain length-width ratio; GT – grain thickness

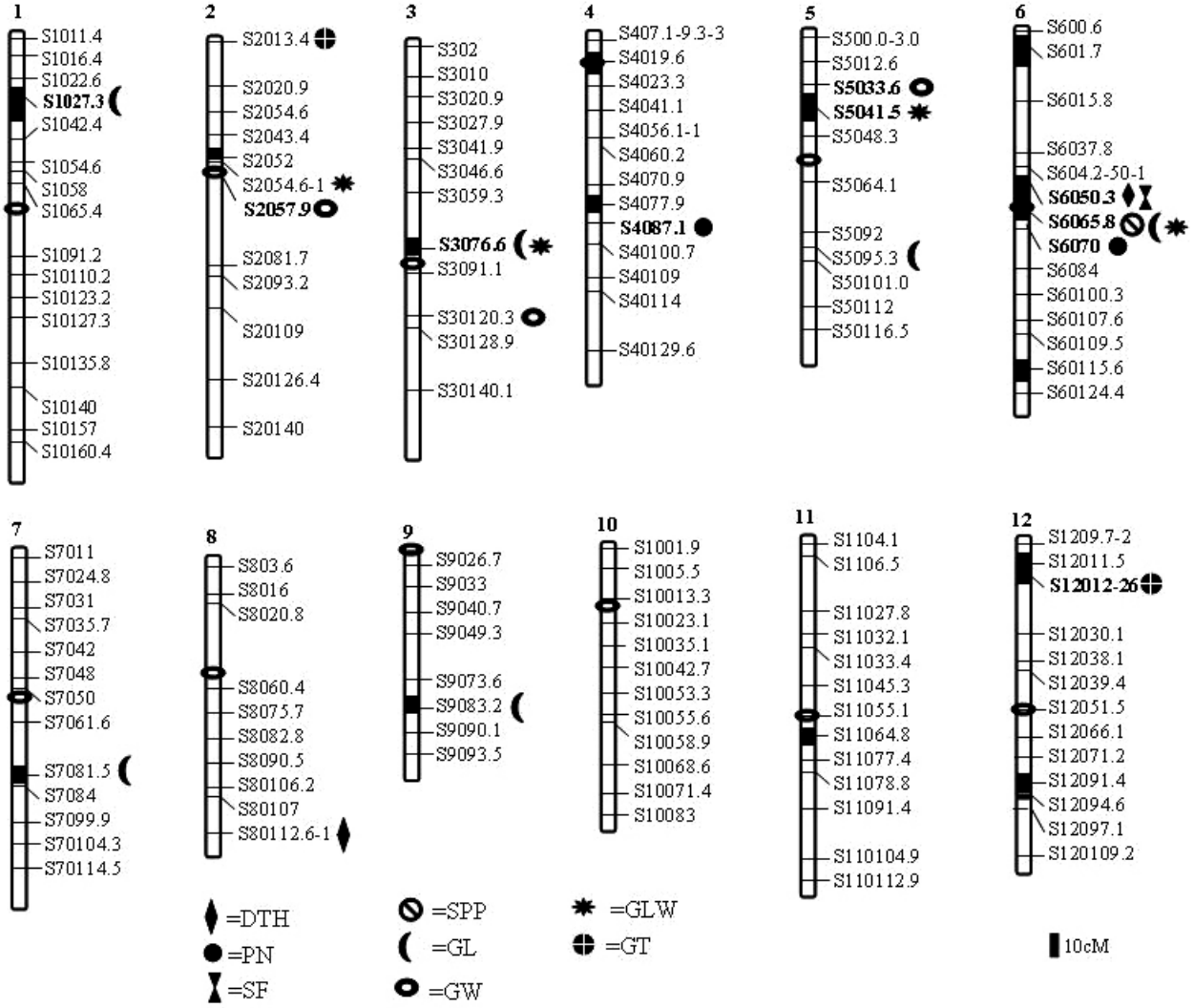


Figure 3. An STS linkage map of rice showing the locations of QTLs detected in this study; black solid bars and the circle indicating introgressed and centromeric region, respectively, and bold markers of QTL regions in both populations

The abbreviations for phenotypic traits are the same as in Table 1

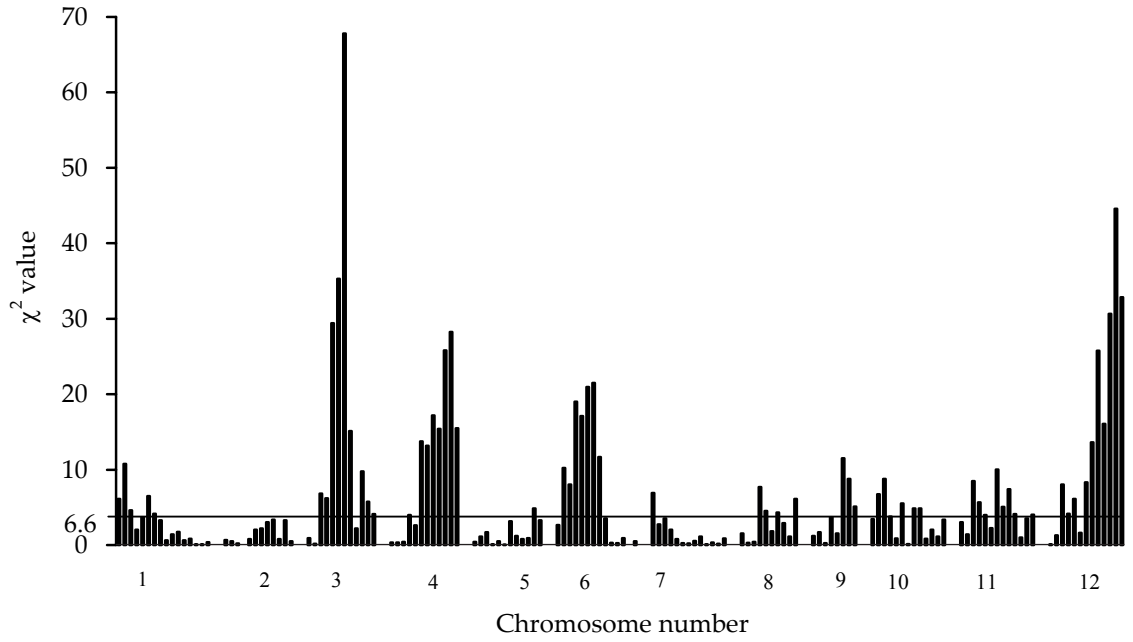


Figure 4. Segregation distortion detected on 12 rice chromosomes; chi-square values of each marker are shown on y-axis; the value of $\chi^2 > 6.6$ corresponds to the significance threshold at $P = 0.01$ level

respectively. The introgressed *O. minuta* allele increased spikelets per panicle at *spp6*.

***Grain length (GL)***: Five QTLs in $F_3$ and two QTLs in $F_2$ were detected, which explained 42.0% and 11.5% of the phenotypic variation, respectively. The introgressed *O. minuta* allele increased grain length at all loci. Among them *gl3* was identified in both populations.

***Grain width (GW)***: Two QTLs for GW in $F_3$ and 3 QTLs in $F_2$ population, among them *gw2* and *gw5* were detected in both populations. The *O. minuta* allele increased grain width in *gw2* locus but the increasing effect of the other three loci in both populations was contributed by the IR71033 allele. Total PVE for GW was 24.5% in $F_3$ and 31.8% in $F_2$.

***Grain length to width ratio (GLW)***: Three QTLs for GLW in both populations were detected on the same position. Among them *glw3* and *glw5* were positively contributed by the *O. minuta* allele. Another three loci contributed positively by the wild allele but they did not share in both populations. Total PVE for this trait was 34.3% in $F_3$ and 42.9% in $F_2$ population.

***Grain thickness (GT)***: One QTL for GT was detected on chromosome 12 in both populations. The wild allele increased grain thickness in both populations and PVE by these two QTLs was 27.1% in $F_3$ and 10.7% in $F_2$. The other locus *gt2* was detected in $F_2$ only and the PVE value was 9.9%.

### Epistatic interaction between randomly paired two loci (E-QTLs)

A total of 24 statistically significant ($P < 0.001$) E-QTLs including one intra-chromosomal in $F_3$ and 28 E-QTLs including 4 intra-chromosomal E-QTLs in $F_2$ were identified between random markers (Table 5) for all traits. The PVE of the trait E-QTL ranged from 7.2% to 42.8%. Phenotypic variation between the two generations was quite similar. Among 147 markers distributed throughout the genome that appeared to be involved in 76 (50.6%) significant ($P < 0.05$) interactions, the majority of them, 63 (42%) did not appear to have any main effects on the relevant traits, but they influenced the trait(s) predominantly through interactions (data not shown).

## DISCUSSION

Out of 530 STS markers used in the parental polymorphism survey, 338 (63.8%) markers were polymorphic between the introgressed *indica* line IR71033 and *japonica* Junambyeo. The rate of polymorphism was similar to that in previous reports on interspecific and intersubspecific crosses (McCouch *et al.* 1988; Xiao *et al.* 1998; Doi *et al.* 1998; Suh *et al.* 2005). Fourteen alien segments were detected throughout 12 chromosomes. Alien segments were quite small in size because they were

Table 4. QTLs for some agronomic traits identified in this study

| Traits[a] | $F_3$ population | | | | | | $F_2$ population | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | QTL | Chr | marker | LOD | PVE | AE | QTL | Chr | marker | LOD | PVE | AE |
| DTH | ***dth6*** | 6 | S6050 | 10.5 | 28.1 | –15.2 | ***dth6*** | 6 | 6050.3 | 16.7 | 36.9 | –18.1 |
| | *dth8* | 8 | S80112 | 4.1 | 11.9 | –2.7 | | | | | | |
| | total | | | | 34.1 | | | | | | | |
| PN | ***pn4*** | 4 | S4087 | 3.2 | 9.7 | –1.2 | ***pn4*** | 4 | S4087 | 3.2 | 9.7 | –0.3 |
| | ***pn6*** | 6 | S6070 | 3.4 | 10.1 | 0.7 | ***pn6*** | 6 | S6070 | 3.3 | 10.1 | 0.8 |
| | total | | | | 15.5 | | | | | | 19.1 | |
| SF | ***sf6*** | 6 | S6050.3 | 6.8 | 19.2 | –13.6 | ***sf6*** | 6 | S6050.3 | 17.6 | 34.5 | –23.4 |
| SPP | ***spp6*** | 6 | S6065.8 | 6.8 | 19.7 | 28.5 | ***spp6*** | 6 | S6065.8 | 13.1 | 28.1 | 34.9 |
| GL | ***gl3**** | 3 | S3076 | 6.1 | 17.5 | 0.3 | *gl1** | 1 | S1027.3 | 4.1 | 10.6 | 0.3 |
| | *gl5** | 5 | S5095 | 4.3 | 12.7 | 0.3 | ***gl3**** | 3 | S3076.6 | 4.9 | 11.6 | 0.3 |
| | *gl6** | 6 | S6065 | 3.8 | 11.2 | 0.3 | | | | | | |
| | *gl7** | 7 | S7081 | 6.2 | 17.7 | 0.4 | | | | | | |
| | *gl9** | 9 | S9083 | 2.7 | 8.11 | 0.2 | | | | | | |
| | total | | | | 42 | | | | | | 11.5 | |
| GW | ***gw2**** | 2 | S2057 | 5.1 | 14.8 | –0.1 | ***gw2**** | 2 | S2057 | 4.4 | 12.1 | –0.1 |
| | ***gw5*** | 5 | S5033 | 4.5 | 13.3 | –0.1 | *gw3* | 3 | S30120.3 | 4.1 | 17.3 | –0.1 |
| | | | | | | | ***gw5*** | 5 | S5033.6 | 7.5 | 11.1 | –0.1 |
| | total | | | | 24.5 | | | | | | 31.8 | |
| GLW | *glw2** | 2 | S2054 | 3.8 | 11.2 | 0.2 | *glw1** | 1 | S1027.3 | 3.3 | 9.9 | 2.2 |
| | ***glw3**** | 3 | S3076.6 | 4.4 | 12.7 | 0.2 | ***glw3**** | 3 | S3076.6 | 7.5 | 20.9 | 1.1 |
| | ***glw5*** | 5 | S5041 | 5.2 | 15.1 | 0.2 | ***glw5**** | 5 | S5041 | 6.7 | 17.2 | 0.1 |
| | | | | | | | *glw6** | 6 | S6065.8 | 5.2 | 14.3 | –0.1 |
| | total | | | | 34.3 | | | | | | 42.9 | |
| GT | ***gt12**** | 12 | S12012 | 9.9 | 27.1 | 0.1 | *gt2* | 2 | S2013.4 | 4 | 9.9 | 1.0 |
| | | | | | | | ***gt12**** | 12 | S12012 | 4.4 | 10.7 | 0.2 |
| | total | | | | | | | | | | 19.1 | |

*Potentially novel allele from *O. minuta* introgression

[a]DTH – days to heading; PN – panicle number; SF – spikelet fertility; SPP – spikelet per panicle; GL – grain length; GW – grain width; GLW – grain length-width ratio; GT – grain thickness

PVE – Percentage of phenotypic variance explained by the QTL; AE – allelic effect; bold letters – QTLs are robust QTLs for the traits

detected by single markers and flanking markers were negative for introgression.

There were significant distortions in the segregation of markers on chromosome 1, 3, 4, 6 and 12. Lorieux *et al.* (2000) also reported a strong segregation distortion in mapping analyses based on an interspecific hybrid-progeny population.

Although the phenotype of *O. minuta* plants seemed generally inferior to IR71033 and Junambyeo from an agronomic viewpoint for the majority of the traits, the *O. minuta*-derived allele had a certain desirable effect on DTH, PN, SF, SPP, GL, GLW and GT. This kind of beneficial alleles from wild rice was reported in previous

Table 5. Epistatic interactions between random markers in both populations

| Traits[a] | Number of E-QTL in $F_3$ population | | Var (%) range | Number of E-QTL in $F_2$ population | | Var (%) range |
|---|---|---|---|---|---|---|
| | Inter-ch | Intra-ch | | Inter-ch | Intra-ch | |
| DTH | 0 | 1 | 44.3 | 1 | 0 | 42.8 |
| PN | 1 | 0 | 31.3 | 2 | 0 | 13.1–28.4 |
| SPP | 0 | 1 | 37.8 | 2 | 0 | 24.6–27.4 |
| SF | 4 | 0 | 6.1–14.3 | 3 | 0 | 7.2–33.6 |
| GL | 7 | 1 | 3.4–16.4 | 6 | 0 | 7.2–21.2 |
| GW | 2 | 1 | 6.1–14.2 | 4 | 0 | 8.7–16.5 |
| GLW | 7 | 0 | 5.0–15.6 | 3 | 1 | 11.7–18.2 |
| GT | 3 | 0 | 14.1–34.3 | 2 | 0 | 16.6–41.1 |
| Total | 24 | 4 | | 23 | 1 | |

[a]DTH – days to heading; PN – panicle number; SF – spikelet fertility; SPP – spikelet per panicle; GL – grain length; GW – grain width; GLW – grain length-width ratio; GT – grain thickness

QTL studies using wild rice (Xiao *et al.* 1998; Moncada *et al.* 2001; Brondani *et al.* 2002; Thomson *et al.* 2003; Jin *et al.* 2005). The QTL related with DTH on chromosome 6 was located in a similar region like in other studies (Yu *et al.* 2002; Ishimura *et al.* 2001). Among the two QTLs for panicle number detected in this study, *pn4* was identified in a similar region like a QTL for panicle number reported by Hittalamani *et al.* (2003) while the other QTL *pn6* shared a similar region reported by Liao *et al.* (2001), Kobayashi *et al.* (2003). QTL for spikelet fertility, *sf6* is in a similar location like *SPKFRT* (Zhuang *et al.* 2001). QTL for spikelets per panicle *spp6* shares the same position with previously reported QTL *qNSS6* from a cross between two temperate japonica rice cultivars, Akihikari and Koshihikari (Yamagishi *et al.* 2002). However, no report on QTLs that increase spikelets per panicle from wild introgression has been available for chromosome 6 yet. Two QTLs related to grain length were on chromosomes 1 and 3 and they did not share a similar position with any other reports on chromosome 3. The grain width QTL *gw5* was in a similar region like the QTLs of other studies (Xu *et al.* 2000; Ge *et al.* 2005; Wan *et al.* 2005) while another two QTLs on chromosomes 2 and 3 did not share a similar region with other previously reported QTLs. All 4 QTLs related to grain length to width ratio *glw1*, *glw3*, *glw5* and *glw6* on chromosome 1, 3, 5 and 6 did not share a similar positions with other QTLs reported. Among them *glw3* and *glw5* from wild introgression were detected in both populations. A QTL identified for grain thickness *gt12* did not share a similar region with other previously reported QTLs. The wild allele increased seed thickness at the *gt12* locus in both populations. The *O. minuta*-derived alleles of robust QTLs *spp6, gl3, glw3, glw5,* and *gt12,* having a positive effect, have not been reported in other previous QTL studies yet and they may be potentially useful for genetic improvement of rice. The number of QTLs related to *O. minuta* introgressions in this study might be underestimated due to the fact that some of the chromosomal regions harbouring some minor *O. minuta* introgressions might not be detected with the STS markers used.

Some QTLs detected on chromosomes 3 and 6 were clustered in chromosomal blocks indicating linkage or pleiotropic effects. Pleiotropism for spikelet fertility, 1000 seed weight, days to heading, seed length and yield per plant in chromosome 3 and 7 was also reported from *O. minuta* introgression QTL analysis (Jin *et al.* 2005). In this study, the QTL on chromosome 3 for *gl3* and *glw3*, which was derived from an introgression segment, seemed to be pleiotropic as the phenotype is related together. Two QTL blocks on chromosome 6, *dth6-sf6* and *spp6-gl6-glw6*, were derived from *O. minuta* chromosomal segments. Clusters of QTLs for seed fertility along with other four QTLs from *O. rufipogon* were also reported on chromo-

some 6 (Cai & Morishima 2002). It remains to be clarified whether the colocalization of QTLs was due to the linkage of independent genes or pleiotropic effects of the same genes. Therefore, additional genetic studies including high-resolution mapping and gene cloning are required to distinguish between linkage and pleiotropy.

A total of 28 significant E-QTLs in $F_3$ and 24 significant E-QTLs were identified for all characters studied. Of them, sixteen interactions, each involved a locus that had at least one significant QTL. The remaining 12 interactions although they did not include any of the significant QTLs had main effects on the relevant traits but they appeared to increase the overall contribution for the traits analyzed, suggesting that epistatic interactions are an important genetic basis for complex traits such as yield components. In this regard, when we adopt a marker-assisted selection (MAS) strategy in breeding programs, E-QTLs should be taken into consideration though the process for MAS might be more complicated.

The results obtained in this study indicate that *O. minuta* contains QTL alleles that are likely to improve agronomically important traits in elite japonica cultivars, the genetic variation of which is very low. It is proposed that NILs containing individual introgressions associated with positive QTLs from *O. minuta* be further 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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