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ORIGINAL PAPER

Fine mapping a major QTL for flag leaf size and yield-related traits in rice

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Abstract Leaf size is a major determinant of plant architecture and yield potential in crops. A previous study showed that the genomic region of chromosome 1 contains a major quantitative trait locus (QTL) for flag leaf size in a set of backcross recombinant inbred lines derived from two elite parental lines (Zhenshan 97 and 93-11). In the present study, the QTL (*qFL1*) was shown to explain a large proportion of the variation in flag leaf size (leaf length, width and area) in derived populations (BC_2F_3 and BC_3F_2) in multiple environments. Using a large segregating population, we narrowed the location of *qFL1* to a 31 kb region containing four predicted genes. Expression of one of these genes, *OsFTL1*, differed between leaves in near-isogenic lines carrying alleles of Zhenshan 97 and 93-11. *qFL1* had a pleiotropic effect on flag leaf size and yield-related traits. Conditional QTL analysis of the derived population (BC_3F_2) supports the assertion that *qFL1* is the QTL for flag leaf length and exhibits pleiotropy. Pyramiding of *qFL1* with two known genes (*GS3* and *Wx*) from 93-11 into Zhenshan 97 enlarged flag leaves, improved grain size and amylose content, and increased yield per plant, but slightly delayed heading date. These results provide a foundation for the functional characterization of the gene underlying the pleiotropic effects of *qFL1* and for genetic improvement of the plant architecture and yield potential of rice.

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Introduction

Given the importance of rice as a staple food, improvement of yield potential is an ultimate goal of rice breeding programs. As a principle organ in plants, leaf is involved in many fundamental physiological functions such as photosynthesis and transpiration. Leaf size (length, width and area) is a major determinant of plant architecture, and strongly affects yield performance in crops (Tsukaya 2005). The flag leaf, which is the last leaf to emerge before the panicle (inflorescence), is generally regarded as the panicle's major source of photosynthetic products, and is associated with 1,000-grain weight (TGW) and panicle weight (PW) and other yield-related traits in cereals such as rice (Cui et al. 2003; Mei et al. 2003; Ma et al. 2006). About 90% of rice grain yield depends on the photosynthetic rate after flowering, and over 50% of the carbohydrates that accumulate in grains is produced by flag leaves (Gladun and Karpov 1993). Despite the importance of flag leaf size in plant architecture and yield potential, the genetic mechanisms determining its characteristics are not well understood because leaf morphology is a complex trait controlled by many quantitative trait loci and is greatly influenced by the environment.

In the past two decades, a number of QTLs for leaf size have been detected on 12 chromosomes in various mapping populations of rice (Li et al. 1998; Shen et al. 2003; Kobayashi et al. 2006; Yue et al. 2006; Yoon et al. 2006; Tong et al. 2007; Farooq et al. 2010). Most QTL studies have involved *indica*/*japonica* populations in which a large

variation in leaf size is observed. For example, Yan et al. (1999) detected a QTL for flag leaf size on chromosome 4 in a doubled haploid population generated from a cross between IR64 and Azucena. Kobayashi et al. (2003) found that a QTL for leaf size in the same region stably increased flag leaf size over five rice-growing seasons using a recombinant inbred line (RIL) population derived from a cross between Milyang 23 and Akihikari. In a parallel study using a set of RILs from a cross between Lemont and Teqing and two BC populations derived from these RILs, two QTLs were detected for flag leaf length (FL) and one for flag leaf width (FW) (Mei et al. 2003). Among 180 RILs derived from a cross between Zhenshan 97 and IRAT109, seven and four QTLs were identified for leaf length and width, respectively (Yue et al. 2006). Furthermore, several QTLs influencing source leaves (e.g. flag leaf, second top leaf) have been mapped to the genomic regions similar to those associated with sink traits such as the number of spikelets per panicle (SPP), TGW and plant yield. This is consistent with high correlations between source leaves and yield components, implying possible pleiotropy or tight linkage of genes that affect source leaves and yield traits (Li et al. 1998; Erik et al. 2002; Ishimaru 2003; Thomson et al. 2003; Yoon et al. 2006). Although a number of QTLs for leaf size have been detected in rice, few have been delimited to small genomic regions, and none has been cloned. Thus, validation and functional characterization of these QTLs or genes is still required, not only to facilitate transfer of desirable QTLs without undesirable linkages, but also for better understanding of the genetic mechanisms regulating source–sink related traits.

Several mutants for leaf size with plant architecture alterations such as dwarfism have been identified genetically, and the some of the corresponding genes have been recently cloned in rice (http://www.gramene.org/). For example, three mutant genes—narrow leaf 7 (*nal7*), narrow leaf 1 (*nal1*), and narrow and rolled leaf 1(*nrl1*)—have been isolated by mutant analysis and map-based cloning. *Nal7*, which is located on chromosome 3, encodes a flavin-containing mono-oxygenase and a spontaneous mutation in this gene significantly decreased leaf width with curling and altered indole-3-acetic acid content (Fujino et al. 2008). *Nal1* maps to chromosome 4 and encodes a plant-specific protein of unknown biochemical function, mutation of which significantly reduced polar auxin transport capacity (Qi et al. 2008). *Nrl1* on chromosome 12 encodes cellulose synthase-like protein D4 (Hu et al. 2010). Functional analysis of these genes has revealed that multiple genetic pathways control leaf growth in a largely independent manner. A similar complex pathway regulating leaf growth has also been reported in *Arabidopsis thaliana* (Gonzalez et al. 2010).

In a previous study, a major QTL on the short arm of chromosome 1 accounted for a large proportion of the genetic variation for flag leaf size and yield traits in a backcross recombinant inbred line (BRIL) population (Wang et al. 2011b). A similar QTL region has also been reported to affect multiple traits such as leaf morphology, heading date (HD) and yield components in various populations of rice (Kobayashi et al. 2003; Tong et al. 2007; Ando et al. 2008; Liu et al. 2009). However, it remains to be determined whether this QTL region corresponds to a single gene with pleiotropic effects or multiple genes for multiple traits. To resolve the target genomic region of chromosome 1, we constructed a series of segregating populations and near-isogenic lines (NILs) derived from the BRIL with the goal of cloning the gene (or genes) underlying the flag leaf QTL. The main objectives of this study were to: (1) validate the effect of the QTL for flag leaf size in different environments; (2) fine map the major QTL for flag leaf size using a large F_2 population derived from the BRIL; and (3) investigate the effects of the QTL in NILs and its possible role in the genetic improvement of rice.

Materials and methods

Population development and field trials

BRILs were developed from the cross between Zhenshan 97 (ZS97) and 93-11 as described previously by Wang et al. (2011b). The segregating F_2 population and derived NILs for fine mapping were developed by several rounds of backcrossing with marker-aided selection (MAS) (Fig. S1). Briefly, one line (06WP126) that carried the homozygous target QTL segment RM283–RM8083 on chromosome 1 from ZS97 along with other five non-target regions on chromosomes 3, 5, 6 and 8 (Fig. 1a) was chosen from the BRILs to backcross with 93-11. The resultant BC_2F_1 was selfed to obtain a BC_2F_2 population comprising 190 plants for genotyping, and their progeny (BC_2F_3 or BC_2F_4) were generated by self-pollination of BC_2F_2 for phenotyping. The BC_2F_2 population were grown at the experimental site of Huazhong Agricultural University at Wuhan, China (30.4°N, 114.2°E) in 2007, and their progeny (BC_2F_3) were grown at Hainan, China (18.2°N, 108.9°E) in 2008, according to a previously described design and management protocol (Wang et al. 2011b).

One plant (06WZ008-1) among the BC_2F_2 containing the target QTL region was again backcrossed with 93-11 to produce BC_3F_1. The BC_3F_1 was selfed to generate 198 BC_3F_2 individuals. The BC_3F_2 were grown at the experimental site of Ezhou, China (30.2°N, 114.5°E) in 2008. A large segregating population was generated from a single heterozygous plant (08WF4-6-7) in the BC_3F_2 that

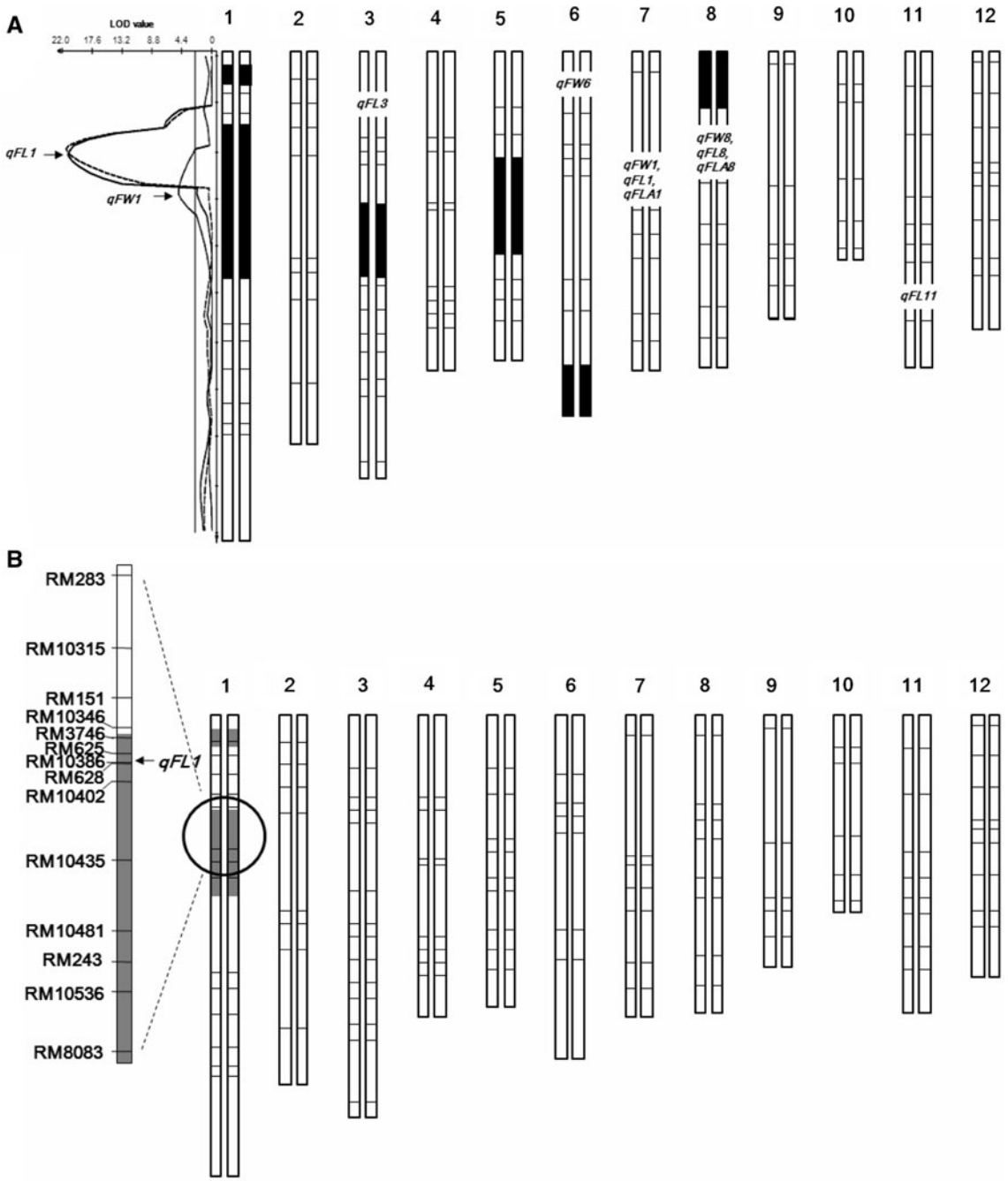


Fig. 1 Graphical genotypes of the target lines used to construct segregating populations for validation and fine mapping of leaf size quantitative trait loci (QTLs). **a** One line (06WP126) from the backcross recombination inbred lines containing six substitution segments, where only the *qFL1* region on chromosome 1 affecting leaf size has been previously reported (Wang et al. 2011b). The logarithm of odds (LOD) profile on the left indicates that this substitution region harbors *qFL1* for flag leaf length and *qFW1* for flag leaf width in the BC_2F_2. **b** An individual heterozygous at the *qFL1* region and another small fragment at the top of chromosome 1, with a background otherwise similar to 93-11, was selected to generate a large segregating population for fine mapping. The genetic map on the left shows the details markers in the *qFL1* region

harbored only the target region and another small substitution segment on the short arm of chromosome 1 with the genetic background of 93-11 (Fig. 1b; Fig. S1). Recombinant individuals and non-recombinants were selected through flanking markers (RM3746 and RM10402). Their progeny (BC_3F_3 or BC_3F_4) were analyzed at the experimental sites of Huazhong Agricultural University at Wuhan in 2009 and at Hainan in 2010, respectively.

NILs carrying homozygous alleles of ZS97 and 93-11 at the target QTL region RM3746–RM10402 (estimated

length of 0.54 Mb), designated NIL^{ZS97} and NIL^{93-11} respectively, were produced from the BC_3F_3. NIL^{ZS97} and NIL^{93-11} were cultivated at Hainan and Wuhan in 2009 following a randomized block design with four repeats. Each line was grown in a three-row plot with 10 plants in each row with a spacing of 16.7 × 26.6 cm.

To validate the effects of the pyramided QTLs on the improvement of ZS97, five BRILs were backcrossed with ZS97; each contained one, two or three 93-11 introgression regions with the known genes, *GS3* for grain shape (Wang et al. 2011a), *Wx* for grain amylose content (AC) (Zhou et al. 2003) and *Ghd8*, which regulates grain yield, plant height (PH) and HD (Yan et al. 2011). Their relevant progeny were consecutively backcrossed to ZS97 three times. In each round of backcrossing, tightly linked or genic markers for *qFL1*, *GS3*, *Wx* and *Ghd8* (Table S1) were used to ensure the heterozygosity or presence of 93-11 alleles at the given loci. Five improved lines (ZS1, ZS2, ZS3, ZS4, and ZS5) carrying the homozygous 93-11 alleles at the target loci within the ZS97 background were developed. These five improved lines, together with 93-11 and ZS97, were assessed in Wuhan in 2010, following the same design as described above for the NILs. The phenotypic effect of the QTL(s) was determined by the difference between the line containing that introduced gene(s) and the control ZS97.

Trait measurement

At the maturity stage, FL, FW, the second top leaf length (sLL) and width (sLW) were measured on the main stem of each plant; a derived trait, flag leaf area (FLA) was calculated as previously described in Wang et al. (2011b). At the harvest stage, SPP, panicle length (PL), second branch number (SBN), PW, and TGW were determined following the Standard Evaluation System for Rice (http://www.knowledgebank.irri.org/ses/). Days from sowing to the time when the first panicle emerged from the flag leaf sheath (HD), PH, grain length (GL), grain width (GW), yield per plant (YD) and AC were also measured for the NILs and the improved lines.

PCR and sequencing markers

Simple sequence repeats (SSR) markers were selected covering the target region based on the published linkage map of rice (http://www.gramene.org). The extraction of microquantities of DNA, polymerase chain reaction (PCR) conditions, PCR product electrophoresis and silver staining procedure for SSR markers are described in Wang et al. (2011a, b). Based on the reference Nipponbare and 93-11 genomic sequences, primers were designed for sequencing 93-11 and ZS97 to detect single nucleotide polymorphisms (SNP) and insertions and deletions (Indel) in the target region (Table S2). The PCR amplified fragments were sequenced directly using BigDye Terminator Cycle Sequencing v3.1 (Applied Biosystems, Foster, CA, USA) after digestion and purification according to the manufacturer's specifications. The SNPs and Indels were used as additional markers to detect recombination points for fine mapping.

RNA isolation and quantitative real-time PCR

The RNA of leaves at three growth stages (three-leaf seedling at 20 days after sowing, 40 days after transplantation into rice paddy and seven days before heading) was extracted using a TRIzol Reagent Kit (Invitrogen, Carlsbad, CA, USA) and treated with DNase I. cDNA was synthesized from 2 μg RNA using SuperScript III Reverse Transcriptase. Quantitative analysis of gene expression was performed using SYBR Premix Ex Taq™ (TaKaRa, Dalian, China) on an Applied Biosystems 7500 Real-Time PCR System. The relative expression of each transcript was obtained by comparison with the expression of the rice gene *ubiquitin* (Xue et al. 2008). The primers used for real-time PCR are listed in Table S2.

Data analysis

Composite interval mapping analysis of QTL in the BRILs and the derived backcross populations was performed with WinQTLcart (Wang et al. 2007). To reduce the probability of type I errors less than 5%, the logarithm of odds (LOD) score was determined from 1,000 replicates (Churchill et al. 1994). The proportion of observed phenotypic variation attributable to a particular QTL was estimated from the coefficient of determination (R^2).

Conditional QTL analysis of the relation between flag leaf and yield components was performed using Genad.exe and Gencond1.exe software (http://www.cab.zju.edu.cn/ics/faculty/zhujun.htm; Zhu 1995). Conditional phenotypic values (T1|T2) were obtained by a mixed model approach in the BC_3F_2 population, where T1|T2 represents trait 1 conditional on trait 2 (e.g. TGW|FL means 1,000-grain weight conditional on flag leaf length). The conditional values were then analyzed using WinQTLcart. To compare the means of each line with the controls or ZS97, Dunnett's test was performed using Statistica (StatSoft, 1997).

Results

qFL1 for flag leaf size stably expressed

A major QTL was confirmed for flag leaf size in the RM283–RM8083 region of chromosome 1, showing stably

significant effects on three flag leaf traits (FL, FW and FLA) in the derived backcross populations (BC_2F_3 and BC_3F_2) over three growing seasons (Table 1). This major QTL explained the greatest proportion (39.2%) of the variance in FL in the BC_3F_2 and is hereafter designated *qFL1*.

To further investigate *qFL1*, 14 polymorphic SSR markers distributed within the target region RM283–RM8083 (Fig. 1b) were used to genotype 198 BC_3F_2 individuals. Using the phenotypes of their families (BC_3F_3), a QTL was detected in the interval RM3746–RM10481 with an LOD peak around RM10386 that influenced three leaf traits (FL, FW and FLA), SPP and PW (Fig. 2), suggesting it might be a pleiotropic QTL for flag leaf size and yield components. In addition, the *qFL1* region contained another QTL (*qFW1.2*) with small effect on FW in the BC_2F_2 and BC_3F_2 populations (Figs. 1a, 2). As seen in Fig. 3a, FW in group 3 was similar to that in group 5 and both were significantly different from the controls, suggesting that there are two tightly linked QTLs for leaf width in this region. Two tightly linked QTLs (*qsLL1* and *qsLW1*) for sLL and sLW were also detected independently in the BC_2F_4. Notably, *qFL1* and *qsLL1* occupied the same location (Fig. 2; Fig. S2).

Fine mapping of qFL1

Two thousand six hundred individuals of the segregating population derived from a single plant heterozygous only at the *qFL1* region (Fig. 1b) were screened for recombinants

Table 1 The QTL (*qFL1*) detected stably affecting flag leaf length (FL), flag leaf width (FW) and flag leaf area (FLA) in the 93-11/ZS97 BRIL and derived populations across 5-year-location trials

Exp[a]	Pop[b]		Flag leaf length				Flag leaf width				Flag leaf area			
	Type	Size	LOD	*A*	*D*	R^2	LOD	*A*	*D*	R^2	LOD	*A*	*D*	R^2
2006 H	BRIL	244	18.2	−1.7	NA	30.1	4.1	−0.1	NA	8.5	24.5	−5.6	NA	37.7
2006 W	BRIL	244	21.6	−3.2	NA	26.6	5.0	−0.1	NA	7.3	21.3	−9.2	NA	26.7
2007 W	BC_2F_2	190	9.0	−2.9	−0.2	18.5	5.5	−0.1	−0.02	9.2	10.0	−8.5	−0.5	18.0
2008 H	BC_2F_3	90	9.2	−1.6	−0.5	32.5	3.9	−0.04	−0.01	24.0	8.5	−3.0	−0.7	31.9
2008 E	BC_3F_2	198	26.5	−3.6	−0.8	39.2	7.8	−0.1	−0.01	10.7	19.8	−8.5	−2.6	31.1

A additive effect, the negative value indicates the increased effect from 93-11 allele, *D* dominance effect, R^2 the total phenotypic variation (%) explained by the QTL, NA not available

[a] The year and location of experiments. H, W and E represent Hainan, Wuhan and Ezhou, respectively

[b] The populations used for quantitative trait loci (QTL) analysis, of which the backcross recombination inbred lines (BRIL) has been described in Wang et al. (2011b)

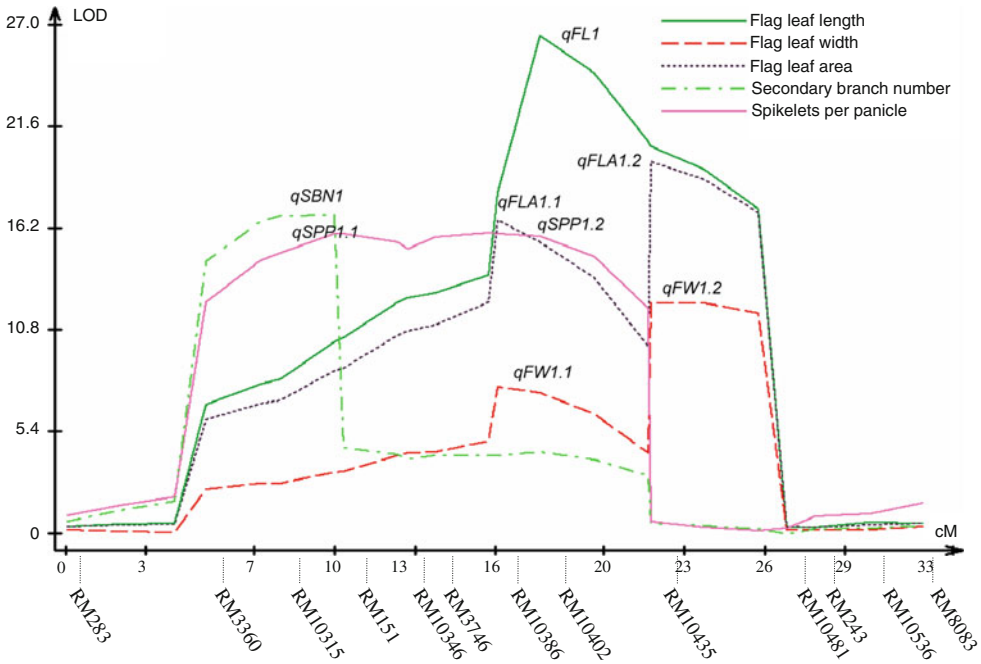
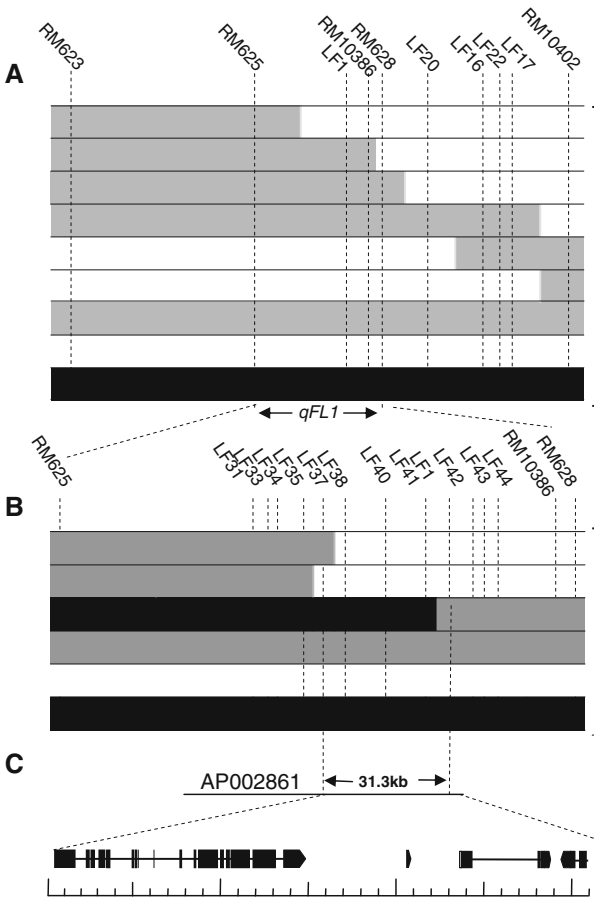


Fig. 2 Logarithm of odds profile of the quantitative trait loci (QTL) region on chromosome 1 in the BC_3F_2 population, showing a QTL (*qFL1*) for flag leaf length (FL), flag leaf length (FW) and flag leaf area (FLA), and a tightly linked QTL for secondary branch number (SBN) and a QTL closely linked for FW



Group	No.	FL (cm)	FW (cm)	FLA(cm²)
1	1	23.62[b]	1.94[b]	45.82[b]
2	1	26.99[c]	1.97[c]	53.17[c]
3	1	25.80[c]	2.00[c]	51.60[c]
4	2	26.30[c]	2.04[a]	53.65[c]
5	3	22.98[b]	1.98[c]	45.50[b]
6	8	22.69[b]	1.95[b]	44.25[b]
7	2	25.48[c]	1.96[b]	49.94[c]
CK1	13	23.07[b]	1.92[b]	44.29[b]
CK2	16	28.23[a]	2.06[a]	58.15[a]

Group	No.	FL (cm)	FW (cm)	FLA(cm²)
1	2	23.38[b]	2.10[b]	48.65[b]
2	1	25.10[b]	2.17[b]	54.62[b]
3	1	27.14[a]	2.38[a]	64.40[a]
4	2	26.45[a]	2.15[b]	57.02[a]
CK1	4	23.78[b]	2.18[b]	51.84[b]
CK2	4	27.51[a]	2.28[a]	62.72[a]

Fig. 3 Fine mapping of *qFL1* using a segregating population derived from the backcross recombination inbred lines of 93-11 and ZS97. **a** *qFL1* was narrowed down to RM625–RM628 by progeny analyses of seven recombinants. **b** Four recombinants further delimited *qFL1* to a 31.3 kb interval defined by markers LF37 and LF1. **c** Four candidate genes were predicted in this interval. The *superscript letters* (*a*, *b* and *c*) on the left panel indicate significant differences in the traits of the recombinants compared with those of the controls CK1, CK2 and both CK1 and CK2 at a level of 0.01. "Group" indicates genotype category. "No." represents the number of families investigated for each group

between RM623 (or RM3746) and RM10402. Eighteen informative recombinants were identified with eight markers (five sequencing markers and three SSRs) within this region (Fig. 3a) and grouped into seven genotypes according to the positions of recombinant breakpoints and allelic composition. Multiple comparisons of the flag leaf size of the recombinant genotypes, using the two non-recombinants as controls (CK1 and CK2), initially placed *qFL1* in a 110 kb region between RM625 and RM628 (Fig. 3a).

To position the QTL more precisely, eleven additional SNP or Indel markers were developed within this region (Table S2) and used to genotype six recombinants containing recombinant points in the region of RM625 and RM628. These analyses further delimited *qFL1* to a 31.1 kb region defined by markers LF37 and LF1 (Fig. 3b), which is on a single bacterial artificial chromosome clone AP002861 (http://www.gramene.org/Oryza_sativa/) (Fig. 3c). This narrow region contains four predicted genes (LOC_Os01g11920, LOC_Os01g11930, LOC_Os01g11940 and LOC_Os01g11946) based on the genome annotated database (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse). They encode retrotransposon protein, hypothetical protein, *FLOWERING LOCUS T* (*FT*)-Like homolog and ATP-binding cassette (ABC) protein, respectively. The database contains full-length cDNA or expressed sequence tags for the latter two genes in rice, but not for the former two. Furthermore, expression data for LOC_Os01g11920 (a putative retrotransposon) and LOC_Os01g11930 (hypothetical gene) indicated that these genes are hardly detected in young leaves at tiller stage and flag leaves (http://crep.ncpgr.cn/). Comparison of the sequences of LOC_Os01g11930, *FT*-Like homolog (*OsFTL1*) and ABC transporter between 93-11 and ZS97 revealed that there is no difference in LOC_Os01g11930, however, there is one difference between the *OsFTL* alleles and two between the ABC transporter alleles. One nucleotide difference in the first exon in *OsFTL1* causes an amino acid difference,

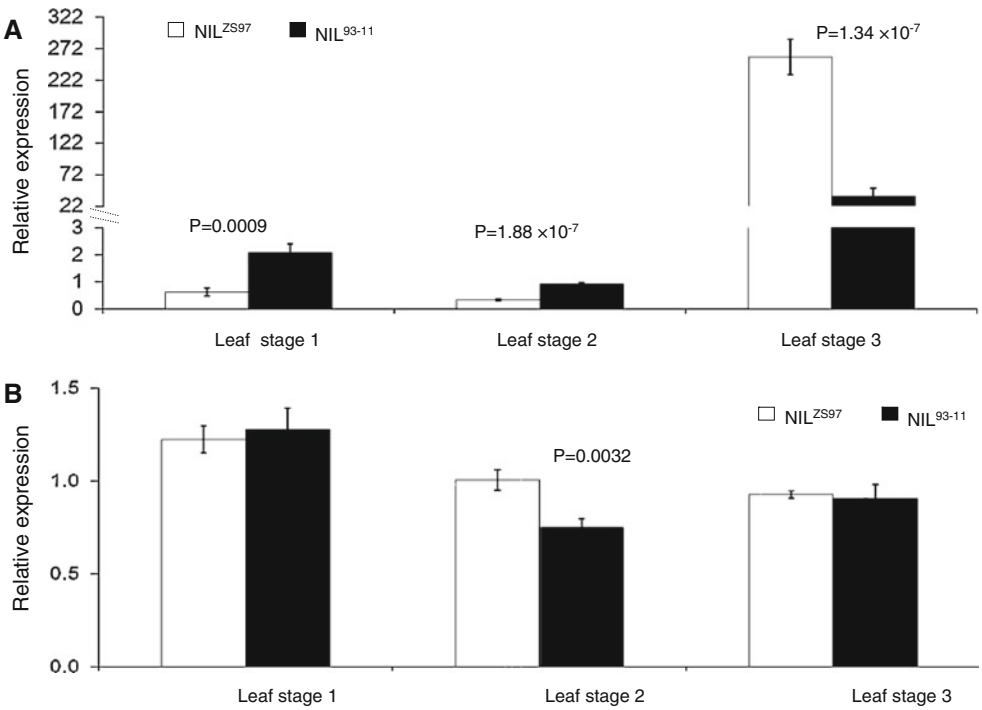


Fig. 4 Relative expression of the two candidate genes **a** LOC_Os01g11940 and **b** LOC_Os01g11946 at three growth stages. The differences between NIL^{ZS97} and NIL^{93-11} are shown at the young leaf stage of rice seedlings (leaf stage 1), about 40 days after transplantation into rice paddy (leaf stage 2), and the mature flag leaf stage about seven days before heading (leaf stage 3). *P* values are given for *t* tests comparing the means of three biological replicates

aspartic acid in 93-11 and alanine in ZS97. One substitution in the first exon of ABC transporter results in a difference of a leucine (93-11) and a serine (ZS97), and one in the ninth exon causes a difference of an alanine (93-11) and a threonine (ZS97). These results suggest that *OsFTL1* and the ABC transporter are the most likely candidates underlying *qFL1*.

Expression patterns of candidate genes

Real-time PCR was performed to determine the patterns of expression of the two most likely candidate genes (*OsFTL1* and ABC transporter) in rice leaves at three growth stages. *OsFTL1* was expressed at a high level in flag leaves one week before heading, and at a low level in young leaves at the seedling stage and at the vegetative growth stage 40 days after transplantation (Fig. 4a), indicating that *OsFTL1* is expressed in leaves and may be associated with initiation of flowering. Relative to *OsFTL1*, expression of the ABC transporter was lower at all three growth stages (Fig. 4b). Expression of *OsFTL1* differed significantly between NIL^{ZS97} and NIL^{93-11} at all three growth stages, whereas expression of the ABC transporter differed only at the vegetative growth stage (Fig. 4b).

Conditional QTL analysis of qFL1

To determine whether *qFL1* is responsible for FL, FW, FLA and yield-related traits (i.e. TGW, PW, SPP, SBN), conditional QTL analysis was performed on the 198 BC_3F_2 individuals. The LOD scores for the four yield-related traits decreased significantly and that for FLA was reduced almost to zero when they were conditional on FL. By contrast, the LOD scores for FL, FLA and the yield-related traits showed little change when they were conditional on FW (Fig. 5). These results suggest that *qFL1* is primarily responsible for FL with effects on FLA and yield-related traits.

Effect of qFL1 in NILs

Comparison of NIL^{ZS97} and NIL^{93-11} revealed highly significant differences in the yield-related traits between the pairwise lines measured during the two environmental trials. Flag leaf size was greater in NIL^{93-11} than in NIL^{ZS97}, and secondary branch number, SPP and PW were also increased, TGW was decreased. Notably, HD was delayed slightly in Wuhan, but not changed in Hainan (Fig. 6). These findings indicate that the alleles of '93-11' at *qFL1* affect flag leaf size and panicle traits.

Improvement of ZS97 by pyramiding desirable alleles

Five improved lines (ZS1, ZS2, ZS3, ZS4 and ZS5) carrying homozygous alleles of 93-11 at one, two or three target loci (i.e. *qFL1* and *GS3*, *Wx* and *Ghd8*) were developed and evaluated. Although the genomic composition of these five lines was similar to that of ZS97, they differed significantly

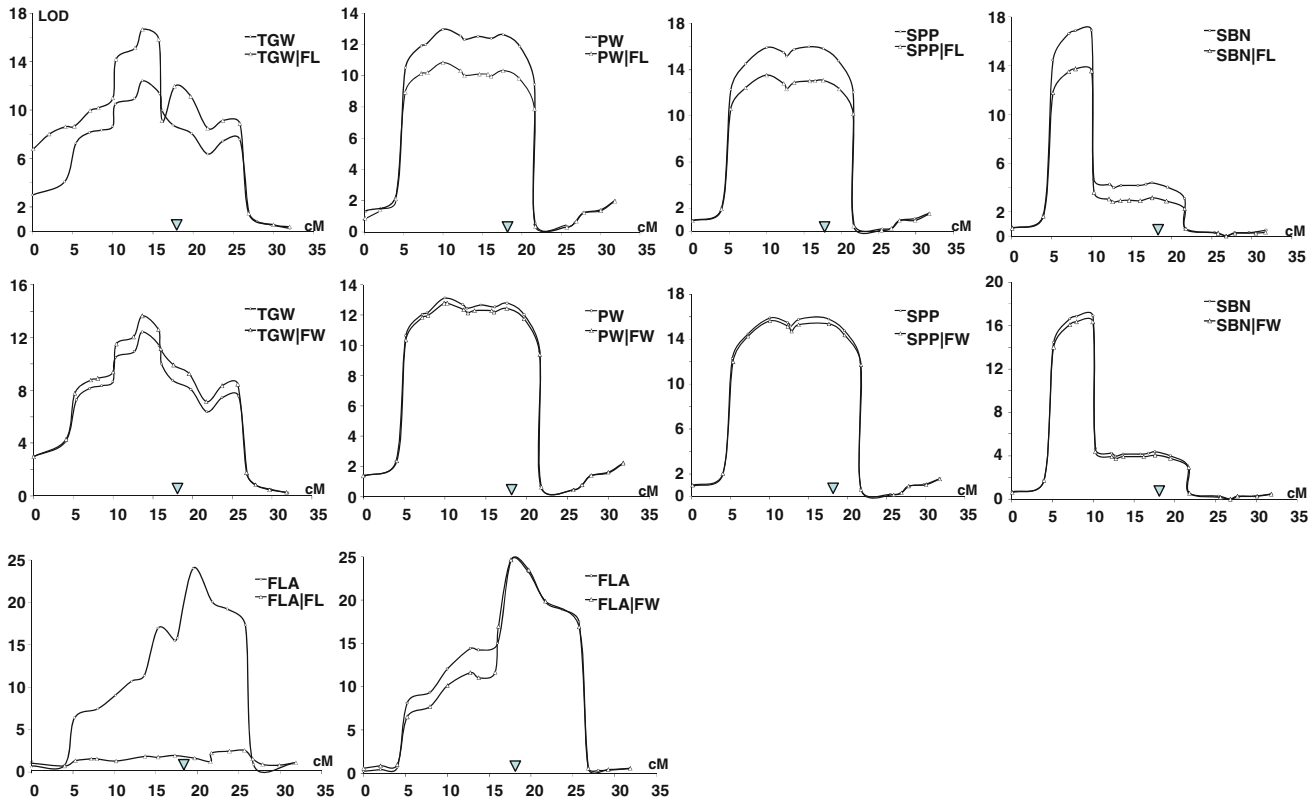


Fig. 5 Conditional quantitative trait locus analysis of the BC_3F_2 population suggests that *qFL1* has a pleiotropic effect on 1,000-grain weight (TGW), panicle weight (PW), spikelets per panicle (SPP), second branch number (SBN) and flag leaf area (FLA). The location of *qFL1* on the x-axis genetic map is indicated by the triangle

from ZS97 in several important traits. As seen in Table 2, ZS1 containing only *GS3* of 93-11 had greater grain length and TGW than ZS97, but there were no differences in FL, FW and SPP. ZS2 containing only *Ghd8* had significant increases in FL, FW, and yield-related traits. ZS3 carrying the 93-11 alleles at *qFL1* and *GS3* had larger flag leaves and grains (GL, GW and TGW) and greater SPP than ZS97, but there were little differences in PH and HD. ZS4 (*qFL1* + *GS3* + *Wx*) had larger flag leaves and grains, lower AC, and a higher YD with an increase of 52.5%. ZS5 (*qFL1* + *Wx* + *Ghd8*) had larger flag leaves and a much greater yield with an increase of 64.6%; however, HD was delayed by 17.4% compared to ZS97. The increased grain size and lower AC in the improved lines were caused by the introduced segments of 93-11 encompassing *GS3* and *Wx*. These results confirm that *qFL1* alleles from 93-11 increase leaf size and yield, whereas *Wx* and *GS3* alleles from 93-11 improve grain quality. Notably, the phenotypic effects of *qFL1* and *Ghd8* on FL in ZS5 statistically equated to the sum of each in ZS2 and ZS3. A similar pattern of the effects was shown on SPP (Table 2). These results suggest that both *qFL1* and *Ghd8* from 93-11 increased FL and SPP, and these two genes were additive in their effects on leaf size and yield components.

Discussion

Tightly linked QTL for leaf length and width

In the present study, one major QTL (*qFL1*) for FL was confirmed and delimited to a 31 kb region using advanced backcross populations (BC_2F_2 and BC_3F_2) derived from a BRIL of 93-11 and ZS97. Genetic dissection of the QTL region showed that *qFL1* and *qFW1* tightly linked for FL and FW, whereas *qFL1*/*qsLL1* and *qsLW1* are independent for sLL and sLW (Fig. S2). Two tightly linked QTLs in the same region were also detected, independent for FL and FW, in a set of 210 RILs from the cross between ZS97 and Minghui63 using a newly developed ultra-high density genetic map (Yu et al. 2011; Fig. S3). Several QTLs/genes for yield components adjacent to *qFL1* have been reported in other studies; for example, *Gn1a* and *Gn1b* (Ashikari et al. 2005) and *SPP1* for SPP (Liu et al. 2009). These findings suggest that at least two QTLs or genes in this narrow region are involved in the control of leaf size and/or spikelet number, and are responsible for the multiple effects of *qFL1* on leaf size and yield component traits (Table 2; Fig. 6).

The clustering of QTLs or genes for leaf size and grain yield in the *qFL1* region might be functionally related, and

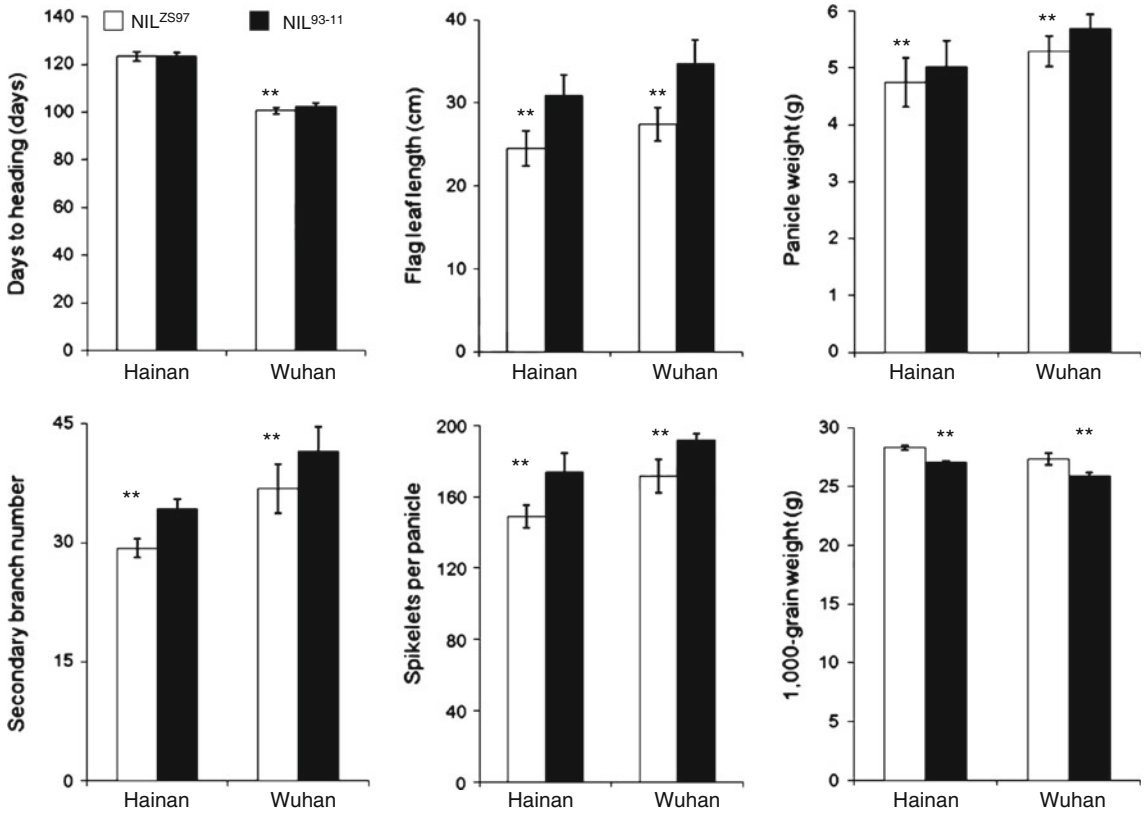


Fig. 6 Performance of near-isogenic lines NIL^{ZS97} and NIL$^{93-11}$ which contain the *qFL1* alleles from ZS97 and 93-11, respectively. *Asterisks* indicate significant differences in yield-related traits between paired NILs at a level of 0.01

confer an evolutionary advantage. Similar QTLs related to leaf size have been reported in the syntenic regions of other cereal species such as barley, sorghum and maize (http://www.gramene.org/; Tian et al. 2011). Notably, the barley *FT*-like gene *HvFT2* on chromosome 3H, which is highly homologous to *OsFTL1*, is associated with flowering time and grains per ear (Wang et al. 2010), and a QTL for FL and spike length has also been detected in the *HvFT2* region (Gyenis et al. 2007). In sorghum, overlapping QTLs for leaf width and 1,000-kernel weight have been identified in the region of chromosome 3S (Feltus et al. 2006), which is collinear with the genomic region of rice containing *OsFTL1*. Because leaf size is a key trait affecting plant architecture and productivity, further genetic and functional dissection of this hotspot QTL region will be of interest, not only to shed light on the molecular mechanisms regulating leaf development in plants, but also to facilitate genetic improvement of plant stature and yield in cereal crops.

Candidate genes for qFL1

The flag leaf QTL *qFL1* was narrowed down to a small region with the most likely candidate genes being *OsFTL1* and a gene encoding a putative ABC transporter. There are thirteen *FT*-like homologs in rice (*Oryza sativa*) (Izawa et al. 2002; Chardon and Damerval 2005). Among these, *OsFTL1* and other two *FT*-like genes, *OsFTL2* and *OsFTL3*, are known to be active and capable of promoting flowering in rice (Kojima et al. 2002; Monna et al. 2002; Komiya et al. 2009). Transgenic rice over-expressing *HvFT2*, which is highly homologous to *OsFTL1*, displays much earlier heading than control plants under both short-day and long-day conditions (Kikuchi et al. 2009). Recently, a QTL (*dth1.1*) for HD in the RM3746–RM10386 region containing *qFL1* was resolved into at least two separate QTLs (*dth1.1a* and *dth1.1b*) (Thomson et al. 2006). The alleles at *dth1.1* from a wild accession (*O. rufipogon*) affected flowering time and yield, but did not influence plant stature or panicle traits. In our present study, *OsFTL1* is expressed at a high level in leaves before heading (Fig. 4a), and differed between 93-11 and ZS97 by only one predicted amino acid. Furthermore, *qFL1* has a major effect on leaf size and panicle traits and a minor effect on HD. These results indicate that *OsFTL1* may relate to HD and could have a pleiotropic effect on leaf size and yield traits. However, further experiments such as transgenic testing must be performed to definitively prove that *OsFTL1* is responsible for the effect of *qFL1* on multiple traits in rice.

Table 2 Performance (Mean ± SD) of the improved lines carrying 93-11 alleles at *qFL1*, *GS3*, *Wx*, and/or *Ghd8* in the genetic background of ZS97

Traits	ZS97	ZS1 (*GS3*)		ZS2 (*Ghd8*)		ZS3 (*qFL1* + *GS3*)		ZS4 (*qFL1* + *GS3* +*Wx*)		ZS5 (*qFL1* + *Wx* + *Ghd8*)	
	Values[a]	Values	Diff	Values	Diff	Values	Diff	Values	Diff	Values	Diff
FL (cm)	21.9 ± 1.5	23.0 ± 1.4	1.1	29.6 ± 4.5	7.7**	26.3 ± 2.0	4.4**	31.0 ± 2.0	9.1**	31.7 ± 3.4	9.8**
FW (cm)	1.4 ± 0.2	1.5 ± 0.03	0.1	1.8 ± 0.1	0.4**	1.6 ± 0.1	0.2**	1.6 ± 0.0	0.2**	1.7 ± 0.1	0.3**
GL (mm)	8.3 ± 0.1	9.2 ± 0.2	0.9**	8.3 ± 0.1	0.0	9.2 ± 0.2	0.9**	9.6 ± 0.2	1.3**	8.4 ± 0.2	0.1
GW (mm)	3.2 ± 0.1	2.9 ± 0.2	−0.3**	3.3 ± 0.2	0.1	2.8 ± 0.1	−0.4**	2.7 ± 0.1	−0.5**	3.3 ± 0.2	0.1
AC (%)	28.1 ± 0.2	26.0 ± 0.3	−2.1	27.0 ± 0.4	−1.1	25.7 ± 0.3	−2.4	14.9 ± 0.5	−13.2**	16.0 ± 0.3	−12.1**
SPP	105.6 ± 4.3	97.8 ± 9.2	−7.8	120.5 ± 7.5	14.9*	125.0 ± 5.8	19.4*	130.9 ± 5.7	25.3**	146.1 ± 7.6	40.5**
YD (g)	18.1 ± 1.8	19.4 ± 3.6	1.3	27.6 ± 3.8	9.5**	19.7 ± 2.8	1.6	27.6 ± 3.5	9.5**	29.8 ± 4.5	11.7**
TGW (g)	25.2 ± 0.4	27.2 ± 0.6	2.0**	27.2 ± 0.9	2.0**	26.2 ± 0.5	1.0**	27.1 ± 1.1	1.9**	27.3 ± 0.4	2.1**
PH (cm)	76.6 ± 2.8	74.6 ± 4.0	−2.0	88.1 ± 2.1	11.5**	74.5 ± 1.2	−2.1	84.5 ± 1.7	7.9**	88.4 ± 2.1	11.8**
HD (day)	69.0 ± 1.0	70.0 ± 1.0	0.9	75.1 ± 1.5	6.0**	70.0 ± 1.0	1.0	76.0 ± 1.0	7.0**	81.0 ± 1.0	12.0**

FL flag leaf length, *FW* flag leaf width, *GL* grain length, *GW* grain width, *AC* amylose content, *SPP* spikelets per panicle, *YD* yield per plant, *TGW* 1,000-grain weight, *PH* plant height, *HD* days to heading from sowing

Diff = Line − ZS97. *, ** indicate significant difference against ZS97 by *t* test at $P < 0.05$ and $P < 0.01$, respectively

[a] Mean ± SD, represents means and standard deviation of four replicated measurements for each line

At this time, we also cannot rule out the possibility that the ABC transporter has a role in leaf growth and yield formation because of differences in the predicted amino acids of the gene detected between 93-11 and ZS97. A few of plant ABC proteins have been reported to be involved in a wide range of biological processes including polar auxin transport, lipid catabolism, xenobiotic detoxification, disease resistance and stomatal function (Theodoulou 2000; Rea 2007; Shang et al. 2009). Recently, a tomato gene *sw4.1* encoding an ABC transporter has been reported to be potentially involved in auxin transport and the regulation of seed size (Orsi and Tanksley 2009). In *Arabidopsis*, an ABC transporter (*ABCG26*) is required for male fertility and pollen exine formation (Quilichini et al. 2010). The ABC transporter in the *qFL1* region belongs to the fatty acid elongase subfamily of half-size transporters with an integral membrane domain and an ABC domain. Although this type of transporter has been proposed to be involved in the export of fatty acid or toxic cyclic peptide-polyketides, its function is poorly understood (Garcia et al. 2004). Therefore, transgenic experiments should be carried out to explore the possible role of ABC transporter in leaf growth and yield formation in rice.

Implications for breeding

In the present study, *qFL1* was fine mapped using an advanced backcross population, and confirmed to influence leaf size and to have multiple effects on leaf size and yield traits in NILs. *qFL1* alleles from 93-11 increase leaf size and yield, and *Wx* and *GS3* alleles from 93-11 improve grain quality. These findings may be important for improving plant architecture and yield in rice. To assess whether the favorable alleles are expressed in other elite genetic backgrounds such as the parental line ZS97, the 93-11 alleles at *qFL1* and three other target genes (*GS3*, *Wx*, and *Ghd8*) were individually or jointly transferred into ZS97 using a backcross scheme with MAS. ZS97 is an elite hybrid parental line able to adapt to a wide range of environments, with a high combining ability and a shorter time to maturity. It was widely used as a parent for three-line hybrid rice breeding in China. However, its productivity and grain quality have failed to keep pace with current demands (Zhou et al. 2003). Our MAS pyramiding genes could be used to develop a new version of the line with better grain quality and a higher yield potential, as already performed for yield traits by MAS in other studies (Xie et al. 2006; Ando et al. 2008). The improved line ZS4 has larger flag leaves, more spikelets and better grain quality than the parent ZS97, but slightly delayed flowering time (Table 2), confirming that *qFL1*, *GS3* and *Wx* alleles from 93-11 increase flag leaf size and spikelets numbers and improve the grain quality of ZS97. The delayed

heading of ZS4 compared with that of ZS3 may result from tight linkage of *Hd3a* with *Wx* or its interaction with *qFL1*. In the pyramided line ZS5, the additive effects of *qFL1* and *Ghd8* increased yield and leaf size considerably, but delayed HD much more than in ZS97 and ZS4 (Table 2). It is preferable that the time to heading of a given cultivar remains the same or is increased only slightly when yield is improved. Therefore, lines with desirable 93-11 alleles at *qFL1*, *GS3*, and *Wx* in the genetic background of ZS97 will serve as excellent parental lines in hybrid rice breeding.

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