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ORIGINAL PAPER

Fine mapping *SPP1*, a QTL controlling the number of spikelets per panicle, to a BAC clone in rice (*Oryza sativa*)

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Abstract Near isogenic lines (NILs) can be used to efficiently handle a target quantitative trait locus (QTL) by blocking genetic background noise. One QTL, *SPP1*, which controls the number of spikelets per panicle (SPP), was located on chromosome 1, near *Gn1a*, a cloned gene for rice production in a recombinant inbred line population. NILs of the *SPP1* regions were quickly obtained by self-crossing recombinant inbred line 30 which is heterozygous around *SPP1*. Using a random NIL-F_2 population of 210 individuals, we mapped *SPP1* to a 2.2-cM interval between RM1195 and RM490, which explained 51.1% of SPP variation. The difference in SPP between the two homozygotes was 44. F_2-1456, one NIL-F_2 plant, was heterozygous in the *SPP1* region but was fixed in the region of *Gn1a* gene. This plant F_3 family showed a very wide variation in SPP, which suggested that it was *SPP1* but *Gn1a* affected the variation of SPP in this population. In a word, *SPP1* is a novel gene distinct from *Gn1a*. Four newly developed InDel markers were used for high-resolution mapping of *SPP1* with a large NIL-F_2 population. Finally, it was narrowed down to a bacterial artificial chromosome clone spanning 107 kb; 17 open reading frames have been identified in the region. Of them, LOC_Os01g12160, which encodes an IAA synthetase, is the most interesting candidate gene.

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Introduction

Grain yield is one of the most valuable traits in crop production. Pursuing high grain yield is one of the most important goals in rice (*Oryza sativa* L.) production. Grain yield is frequently replaced with grain yield per plant in quantitative trait locus (QTL) mapping owing to control of field experimental scale (Zhuang et al. 2002; Hittalmani et al. 2003). Grain yield per plant is determined by its three components, panicles per plant, 1,000-grain weight, and spikelets per panicle (SPP). Among the three components, SPP frequently makes the greatest contribution to grain yield and has received the most attention in genetic analysis. However, this trait is inherited in a quantitative manner and typically controlled by a number of major and minor QTL and is also affected by environment, which present a challenge in characterizing SPP. With QTL analysis based on molecular markers, SPP has been studied, and hundreds of QTL have been reported (Yu et al. 1997; Zhuang et al. 1997; Xing et al. 2002; Septiningsih et al. 2003). Many reports have provided evidence for a very complicated genetic basis of yield traits, which are affected simultaneously by QTL, epistasis, and environment in primary mapping populations (Li et al. 1997; Xing et al. 2002; Zhuang et al. 2002). Currently, QTL fine mapping and cloning are still difficult although many QTL for SPP have been mapped to chromosome regions.

In a primary mapping population with simultaneous segregation of multiple genetic factors, a given trait value is contributed not only by a given QTL but also by other QTL. For fine mapping of a QTL, genetic background noise should be avoided. Hence, QTL analysis in advanced populations is necessary for a good understanding of their characteristics. Near isogenic lines (NILs) has been proven to be an ideal population for QTL fine mapping and cloning.

Many QTL have been fine mapped and cloned using a large NIL population in which a major QTL can be visualized as a Mendelian factor (Ren et al. 2005; Song et al. 2007; Xing et al. 2008; Xue et al. 2008). There are three strategies for QTL-NILs construction. Consecutive backcrossing (CB-NILs) combined with marker aided selection (MAS) is the conventional method for developing NILs and has been widely used (Yamamoto et al. 1998, 2000; Li et al. 2004; Fan et al. 2006). However, CB-NILs construction is time consuming; usually taking 3 years to perform in rice after QTL information is obtained. The alternative method is to search directly for NILs according to trait performance (TP-NILs) in advanced recombinant inbred lines (RILs) (Zhang et al. 2006). Its genetic principle is that only a very small portion of the genomic regions of a RIL (e.g., F_6 or F_7) segregates, and if the segregating region contains a major QTL, trait variation would be exhibited within the RIL. Compared with CB-NILs development, TP-NILs development is faster. In most cases, however, TP-NILs is effective for only major QTL because variation of the trait controlled by major QTL is easily observed. The third strategy for NILs' development is the heterogeneous inbred family (HIF-NILs) method (Tuinstra et al. 1997), which is based on molecular marker screening for a small heterozygous genomic region. HIF-NILs' development is effective for the target QTL whether it is a major or minor QTL. The genetic principle of TP-NILs and HIF-NILs is similar. Indeed, both TP-NILs and HIF-NILs result in the selection of a RIL that is heterozygous in a small genomic region, but TP-NILs selection is based on a phenotypic screening, whereas HIF-NILs selection is based on a molecular marker screening.

Recently, more and more rice SPP QTL are being fine mapped and cloned using NILs. In NIL-F_2, two QTL, *Gn1a* and *Gn1b*, were detected in the QTL *Gn1* region, and *Gn1a* was fine mapped to a 6.3-kb region. Gene cloning analysis shows that *Gn1a* is a gene for cytokinin oxidase/dehydrogenase (OsCKX2) which controls the grain number phenotype variation (Ashikari et al. 2005). *gpa7*, a QTL controlling SPP on chromosome 7, was narrowed down to a 35-kb region that contains five predicted genes (Tian et al. 2006). *qSPP7*, a major QTL for SPP, was fine mapped to a 0.2-cM region (Xing et al. 2008), renamed *Ghd7*, and finally cloned (Xue et al. 2008).

With the complete genome sequence of rice, chromosome-walking will be greatly reduced or even avoided in the process of map-based gene isolation, and the Rice Genome Automated Annotation System (http://RiceGAAS.dna.affrc.go.jp), combined with the high-resolution of gene fine mapping, is helpful in identifying the candidate gene. Now that thousands of QTL have been mapped during the last two decades, the focus of QTL research is being gradually transferred to QTL isolation from mapping in rice.

In our previous study, a population consisting of 190 RILs was developed for QTL mapping by single-seed descents from a cross between two *indica* cultivars, Zhenshan 97 (ZS97), a widely used maintainer for hybrid production, and Teqing (TQ), a high yielding variety in China. One major QTL, *SPP1*, was detected in the interval between RM490 and RM283 near *Gn1a* and *Gn1b* on chromosome 1. *SPP1* had an additive effect of 16.5 and 15.4 in two environments, respectively. Because of low resolution of QTL mapping in RILs, the relationship between *SPP1* and *Gn1a* and *Gn1b* is not clear. To characterize *SPP1*, we used in this study a straightforward HIF strategy to develop a NIL population and fine map it to a bacterial artificial chromosome (BAC) clone with a large NIL population.

Materials and methods

Experimental populations and phenotypic measurements

In the mapping population consisting of 190 RILs, the line RIL30 is homozygous at all investigated 176 marker loci except for the two markers RM490 and RM283, which flank the QTL *SPP1*. Naturally, selfing progenies of RIL30 (pseudo F_2) constitute a NIL-F_2 population of *SPP1*. In the summer of 2005, a total of 2,200 NIL-F_2 plants were planted into a bird-net-equipped field in the experimental farm at Huazhong Agricultural University, Wuhan, China. Ten seedlings (approximately 25 days old) per row were transplanted with a distance of 16.5 cm between plants within a row, and 26.4 cm between rows. A random F_2 population of 210 plants (subpopulation) from the 2,200 plants was used for QTL analysis. In May 2006, F_3 families of the 210 F_2 plants; the progeny of F_2-1456, which is a NIL-F_2 plant segregated at only *SPP1* but fixed at *Gn1a*; and the progeny of five recombinants (1208, 1367, 1599, 1758 and 1747) were sowed on a seedling bed. For QTL analysis on progeny test, 24 seedlings, approximately 25 days old, of each family were transplanted into the same experimental farm with the same planting density as that of NIL-F_2. Because of bad germination of 44 families, 166 families were used. 240 F_3 seedlings of F_2-1456 were transplanted into a 20-row plot. Sixty F_3 seedlings of each recombinant were transplanted into a five-row plot. Progenies from two homozygous F_2 with ZS97 alleles and TQ alleles at *SPP1* were grown as the controls (CK-Z and CK-T, respectively). At the ripening stage, all 2,200 NIL-F_2 plants, 20 plants in the middle of the two rows of each NIL-F_3, 180 plants except the plants seated in the boundaries of F_3-1456, and 50 plants except the plants seated in the boundaries of each recombinant were harvested individually to score the following traits: SPP as the total number of spikelets from the whole plant divided by its number

of tillers, the yield per plant as the total weight (g) of the grains from the entire plant, and the number of filled grains per panicle as the total number of grains from the entire plant divided by the number of tillers. The heading date of each plant was scored as the days from sowing to the first appearance of a panicle.

DNA extraction and molecular marker development

DNA was extracted from fresh leaves at the seedling stage using the CTAB method (Murray and Thompson 1980). Simple sequence repeat (SSR) markers in the *SPP1* region were identified from the Gramene database (http://www.gramene.org/). The SSR primers were designed according to the public database (Temnykh et al. 2000, 2001; McCouch et al. 2002). Four newly developed InDel markers are listed in Table 1 on the basis of the available public rice genome sequences (http://www.rgp.dna.affrc.go.jp; http://www.rise.genomics.org.cn/).

Data analysis

SSR marker assay was conducted as described by Wu and Tanksley (1993). The local molecular linkage map was constructed on the basis of the subpopulation of 210 plants using Mapmaker 3.0 (Lincoln et al. 1992) and the Kosambi function to calculate the genetic distance. Interval QTL mapping was performed with both F_2 and F_3 data with the use of Mapmaker/QTL 1.1 (Lander and Botstein 1989; Lincoln et al. 1993). In the progeny test, the arithmetic mean of each family was regarded as its phenotype value for QTL analysis.

Putative ORF prediction in 107 kb region

The putative open reading frame (ORF) in the 107-kb region was predicted by the online software FGENESH on the basis of the gene structure available on the Internet (http://linux1.softberry.com/berry.phtml?topic=fgenesh&group=programs&subgroup=gfind), and monocot plants were set as the model organism for prediction.

Results

Trait variation in the subpopulation

The two parents, Zhenshan 97 (ZS97) and Teqing (TQ), showed highly significant differences in SPP. ZS97 exhibited a small panicle of 91 spikelets, and TQ had a large panicle of more than 226 spikelets. The SPP showed a normal distribution whose variation range in F_2 was 129.4–243.5 (Fig. 1). However, a progeny test confirmed that 45 and 41 families expressed identical large and small SPP, respectively, whereas 80 families showed varied SPP. Frequencies of the three genotypes was fitted to the expected Mendelian ratio (1:2:1) for single locus segregation ($\chi^2 = 0.4$, $P = 0.81$). This analysis suggested that one QTL/gene controlled SPP in the F_2 population. In the F_2 subpopulation, no plant had an SPP less than the mean of ZS97 SPP minus twofold its standard deviation, and more than the mean of TQ SPP plus twofold its standard deviation. This finding indicates that no transgressive segregation was observed for SPP in either direction and that the QTL did not express over dominance. Meanwhile, the subpopulation headed within 1 week.

QTL analysis for *SPP1*

Five markers inside the region from RM283 to RM490 were used to genotype the F_2 population from RIL30 self-crossing, and a local linkage group covering 11.6 cM was constructed (Fig. 2). QTL analysis based on the F_2 population defined the *SPP1* to a 2.2-cM region between the markers RM1195 and RM490, which was closely linked to *Gn1a* flanked by RM1195 and RM3604 markers (Ashikari et al. 2005) (Fig. 2). The QTL explained 51.1% of phenotype variance with additive effect of 22 spikelets (Table 2).

Table 1 InDel markers developed for fine mapping of the *SPP1* locus

Marker	InDel size (bp)[a]	Size (bp)[b]	Forward primer (5′–3′)	Reverse primer (5′–3′)	Physical position
YN23	23	258	CCGATATCACCTGCTTGGAG	AGACTAACTGGACGGCGATG	AP001633[c], 121880[d]
YN27	6	192	TACCACTGAACCCACGTGTC	GCTGCCTTTGTTCTCACGTT	AP002094, 3900
YN34	12	348	TGTGCCATATCTGTGCCTGT	TCGGTTAGGTCGGTTAATTCC	AP002094, 111761
YN37	10	198	GACCTCCCTCCTATCGTAATGA	CAAGGAGGGAAGGACAACAA	AP002094, 57860

[a] InDel size difference between Nipponbare and 93-11 sequence

[b] The size of amplicon in Nipponbare

[c] The accession number of BAC clone where marker located

[d] The marker position in bp in corresponding BAC clone

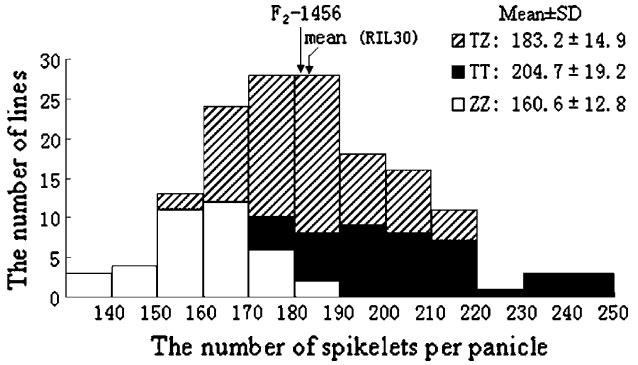

Fig. 1 Frequency distribution of number of spikelets per panicle in NIL-F_2 population. The three genotypes of homozygous Teqing (TT) and Zhenshan 97 (ZZ) and heterozygote (TZ) at *SPP1* were identified by progeny test

Meanwhile,QTL explaining 24.5 and 13.6% of phenotypic variance of grains per panicle (i.e., +21.4 grains per panicle) and total grain yield (i.e., +5.7 g per plant), respectively, were detected in the same interval, which suggest that the same gene is involved both in SPP and grains per panicle. However, no QTL affecting tillers per plant and 1,000-grain weight, two components of yield, was detected in this region. In the F_3 progeny test, the QTL could explain 63.4% of SPP variation, which explains more of the trait variation than in the F_2 population (Table 2). Similarly, the LOD value of the QTL for SPP detected in the F_3 progeny is higher than that in the F_2 population, but the additive effect is less than that of the F_2 population. *SPP1* acted as additive in both populations (Table 2). The position of the LOD peak for *SPP1* was 0.2 cM from RM490. No QTL was detected for heading date.

SPP1 distinct from *Gn1a* and *Gn1b*

SPP1 was genetically located in the region near *Gn1a* and *Gn1b* in the subpopulation. To clearly confirm that *SPP1* in this study was not the same as *Gn1a* or *Gn1b*, we investigated SPP variation using the progeny of F_2-1456, which segregates in the interval between RM8111 and RM490 but fixed in the other intervals containing *Gn1a* and *Gn1b*. A very wide variation of 124.3–233.5 in SPP was very similar

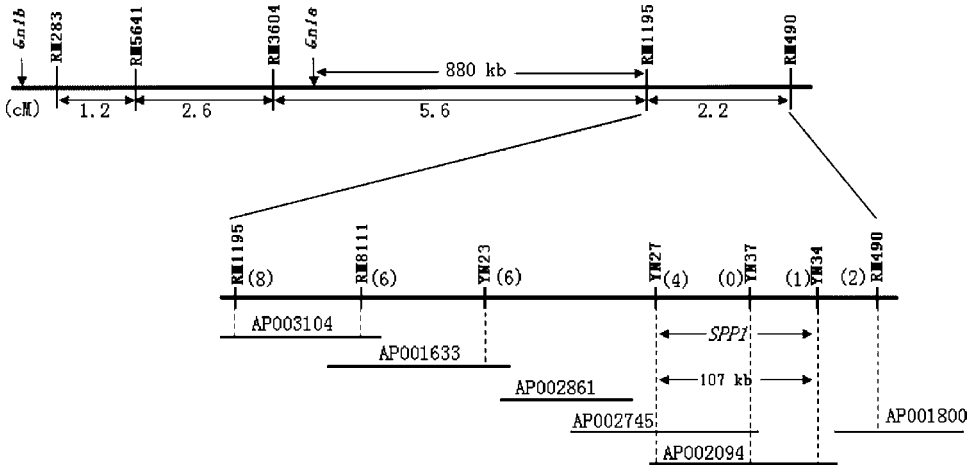

Fig. 2 The local genetic linkage map and high-resolution physical map for *SPP1* region *numbers in brackets* on the right/left side of markers indicate the number of recombinants between *SPP1* and the respective markers. The *short horizontal lines* represent BAC/PAC clones of cv. Nipponbare with their accession numbers (http://www.gramene.org/). The *dashed lines* indicate the relative position of the corresponding marker on BAC/PAC clones

Table 2 *SPP1* effects on SPP, grains per panicle, and yield per plant in the subpopulation and its progenies

Trait	Generation	QTL position[a]	LOD	A[b]	D	Var (%)[d]
Spikelets per panicle	F_2	RM1195 + 2.0 cM	23.95	22.05	0.11 Ns[c]	51.1
	F_3	RM1195 + 1.6 cM	35.52	10.81	0.55 Ns	63.4
Grains per panicle	F_2	RM1195 + 2.0 cM	10.0	21.4	9.6	24.5
	F_3	RM1195 + 1.6 cM	6.65	5.27	1.09	19.8
Yield per plant	F_2	RM1195 + 2.0 cM	5.5	5.7	0.7 Ns	13.6
	F_3	RM1195 + 1.6 cM	7.03	1.2	0.35 Ns	19.4
Spikelets per panicle	F_3-1456	RM1195 + 1.9 cM	12.40	21.53	2.16 Ns	37.0

[a] Position of QTL means the LOD peak position of QTL

[b] Additive effect; positive additive effect indicates that the Teqing allele increased the trait value

[c] *Ns* non significance at $P = 0.05$

[d] Percentage of total phenotypic variance explained by the QTL

to that in F_2 population. Averaged SPP of TT and ZZ genotypes were 204.8 and 161.4 with standard deviations of 21.3 and 21.0, respectively. One QTL was still identified in the population, which explains 37% of the phenotypic variance (Table 2). This finding suggested that neither *Gn1a* nor *Gn1b* controlled SPP variation in this population. Their physical positions are illustrated in Fig. 2. *Gn1a* was located about 880 kb from RM1195, which was about 1.2 Mb from *SPP1* (Fig. 2).

SPP1 fine mapping

For fine mapping of *SPP1*, 2,200 selfing progenies of RIL30 were grown. Only 250 plants (less than one-eighth) with the smallest panicle of less than 150 spikelets, which were assumed to be Zhenshan 97 homozygotes at *SPP1*, were used for recombinant screening with markers RM1195 and RM490. Eight and two recombinants were identified between RM1195 and *SPP1*, and RM490 and *SPP1*, respectively. To enhance the resolution of the *SPP1* local linkage map, we used one SSR (RM8111) and four InDel markers (Table 1). These five markers were further used to screen the ten recombinants: six recombinants were identified between YN23/RM8111 and *SPP1*, four between YN27 and *SPP1*, and one between YN34 and *SPP1* (Fig. 2). YN37 was found to co-segregate with *SPP1* (Fig. 2). In case the trait measurement yielded false Zhenshan 97 homozygous plants, progeny tests of the five recombinants (1208, 1367, 1599, 1758, and 1747) between YN27 and YN34 were conducted. Each recombinant progeny showed an identical small SPP, which was highly significantly smaller than the control Teqing homozygotes, but showed no difference with the control Zhenshan 97 homozygotes (Table 3). This result confirmed the Zhenshan 97 homozygous identity of the five recombinants at *SPP1*. Therefore, *SPP1* was narrowed down to the region of approximately 107 kb bounded by YN27 and YN34 (Fig. 2). One Nipponbare BAC (AP002094) exactly covered the region.

Putative genes in 107-kb region

There are 17 predicted genes in the 107-kb region according to the rice genome automated annotation database (http://www.tigr.org/tdb/e2k1/osa1/irgsp.shtml) (Table 4). Of these, 11 genes have homology with rice full-length cDNAs or expressed sequence tags (ESTs). Among these 17 genes, 11 are of unknown function, one gene is a transposon protein gene, and the functional annotations of the remaining five genes are as follows: LOC_Os01g12020 is a putative nonspecific lipid-transfer protein (LTP) precursor gene; both LOC_Os01g12030 and LOC_Os01g12070 are putative endoglucanase 1 precursor genes; and LOC_Os01g12130 and LOC_Os01g12160 are putative MtN3 (*Medicago truncatula* nodulate gene 3) protein-like genes and indole-3-acetic acid-amido synthetase GH3.3 genes, respectively (Table 4). Accordingly, 17 genes were predicted with the use of the online software FGENESH in the 107-kb region (http://www.softberry.com). Of these, seven genes (Loc_Os01g12010, Loc_Os01g12030, Loc_Os01g12040, Loc_Os01g12060, Loc_Os01g12070, Loc_Os01g12140, and Loc_Os01g12150) are completely identical, and the other ten genes have a slight difference in either their gene framework or a different encoding sequence between the putative genes, as predicted by two different methods (Table 4).

Table 3 SPP performance in the progenies of recombinants

Recom binants	Spikelets per panicle		Marker				
	F_2 plant	F_2 progenies	RM8111	YN23	YN27	YN37	YN34
1208	135.60	149.1 ± 15.5[c]	TZ	TZ	TZ	ZZ	ZZ
1367	144.13	150.9 ± 12.9[c]	TZ	TZ	TZ	ZZ	ZZ
1599	148.25	150.9 ± 21.2[c]	TZ	TZ	TZ	ZZ	ZZ
1758	141.00	145.7 ± 16.0[c]	TZ	TZ	TZ	ZZ	ZZ
1747	131.67	151.8 ± 13.1[c]	ZZ	ZZ	ZZ	ZZ	TZ
CK-Z[a]	155.20	150.8 ± 14.5	ZZ	ZZ	ZZ	ZZ	ZZ
CK-T[b]	196.55	171.3 ± 22.8[d]	TT	TT	TT	TT	TT

ZZ, *TZ*, and *TT* indicate the Zhenshan 97 homozygotes, heterozygotes, and Teqing homozygotes at the locus of marker, respectively

[a] The control line with Zhenshan 97 allele of *SPP1*

[b] The control line with Teqing allele of *SPP1*

[c] No significant difference between the recombinant and CK-Z at $P = 0.05$

[d] Highly significant difference between the two controls CK-Z and CK-T, and between CK-T and recombinants at $P = 0.001$

Table 4 Comparison of putative genes in the 107-kb region from the rice genome automated annotation database with prediction by FGENESH

TIGR gene ID	Putative function	Homologous cDNA/EST	FGENESH gene	Comparison between TIGR gene and FGENESH gene	ID%[a]
LOC_Os01g11990	Expressed protein	AK108579	ORF1	Additional sequence prior to LOC_Os01g11990	81
LOC_Os01g12000	Expressed protein	AK073193	ORF2	Missing 54 bp at the proximal end of LOC_Os01g12000	52
LOC_Os01g12010	Hypothetical protein	No	ORF3	Same	100
LOC_Os01g12020	Nonspecific lipid-transfer protein precursor, putative	AK106863, AK109524	ORF4	Missing 12 bp at the proximal end of LOC_Os01g12020	99
LOC_Os01g12030	Endoglucanase 1 precursor, putative, expressed	AK109524	ORF5	Same	100
LOC_Os01g12040	Transposon protein, putative, mutator sub-class	AK067920	ORF6	Same	100
LOC_Os01g12060	Hypothetical protein	AK109722	ORF7	Same	100
LOC_Os01g12070	Endoglucanase 1 precursor, putative, expressed	AK103304	ORF8	Same	100
LOC_Os01g12080	Plant-specific domain TIGR01589 family protein	Have EST	ORF9	Additional 93 bp prior to LOC_Os01g12080	91
LOC_Os01g12090	Expressed protein	Have EST	ORF10	ORF10 have same start sequence but not stop sequence as LOC_Os01g12090	67
LOC_Os01g12100	Expressed protein	AK105221	ORF11	Sharing the same sequence, but with slightly different coding sequence	36
LOC_Os01g12110	Expressed protein	No	ORF12	Sharing the same sequence, but with slightly different coding sequence	97
LOC_Os01g12120	Hypothetical protein	No	ORF13	ORF13 has same start sequence but not stop sequence as LOC_Os01g12120	87
LOC_Os01g12130	MtN3 protein-like, putative, expressed	No	ORF14	Sharing the same sequence, but with slightly different coding sequence	70
LOC_Os01g12140	Conserved hypothetical protein	No	ORF15	Same	100
LOC_Os01g12150	Hypothetical protein	No	ORF16	Same	100
LOC_Os01g12160	Indole-3-acetic acid-amido synthetase GH3.3, putative	AK072125, AK120515	ORF17	Sharing the same sequence, but with slightly different coding sequence	86

[a] ID amino acid sequence identity between TIGR and FGENESH predictions

Discussion

A novel QTL controlling SPP

In light of the low resolution of QTL mapping in a primary population, comparison of QTL locations detected in different populations resulted in an equivocal conclusion as to whether one QTL or a QTL cluster controlling the same trait was located in the same chromosome region. Moreover, even though two linked genes were known to segregate in one primary population, it was still not easy to precisely map them because of impossible genotype inference at an individual QTL according to the target trait performance. However, with the help of advanced populations such as QTL-NILs, linked QTL could be identified separately because they can independently segregate in one NIL population. Currently, several QTL controlling the same trait have been confirmed to be tightly linked in the rice genome, and some of them have been isolated. For example, *Hd3*, a rice heading date QTL was identified in the F_2 population. Actually, two major QTL, *hd3a* and *hd3b*, which promote flowering in short-day conditions and inhibit heading in long-day conditions, respectively, were identified in the *Hd3* region in a NILs population (Monna et al. 2002a). *Xa4* and *Xa26*, two rice bacterial blight resistance genes, are tightly linked to each other in the long arm of chromosome 11 (Sun et al. 2004; Yang et al. 2003). *QTLph1* and *sd1*, which commonly control plant height, are tightly linked on chromosome 1 with a 1.7-Mb physical distance (Monna et al. 2002b; Ishimaru et al. 2004). In this study, *SPP1* was mapped to the interval of about 107 kb between RM8111 and RM490, which is closely linked to *Gn1a* and *Gn1b* controlling SPP (Ashikari et al. 2005). To genetically confirm that the SPP variation was not caused by *Gn1a* and *Gn1b*, we needed the NILs population that segregates in the region of *SPP1* but fixed in the region

covering *Gn1a* and *Gn1b*. F_2-1456 was the expected genotype in which only the *SPP1* region segregated. Its F_3 family still showed a very wide variation in SPP similar to that of populations from RIL30. Hence, it is suggested that SPP variation in the population is not caused by *Gn1a* and *Gn1b* but by *SPP1*. The left flanking marker of F_2-1456, YN27, has a 1.2-Mb physical distance to *Gn1a* on the left side. The distance between *SPP1* and *Gn1a* is more than 1.2 Mb. Moreover, *Gn1b*, located in the left side of *Gn1a*, is farther from YN27 than *Gn1a* (Fig. 2). Obviously, *SPP1* is distinct from *Gn1a* and *Gn1b*.

Possible candidate gene

In this study, *SPP1* was fine mapped to a BAC clone. However, there are still five recombinants to fine map with new markers in the 107-kb region. The SPP could be narrowed down to a mini-region, and then its candidate gene would be determined. By contrast, it is possible to infer the candidate gene with the help of bioinformatic analysis at this time. Among the five putative known function genes, LOC_Os01g1213 was predicted to encode MtN3 protein-like which is a transmembrane protein. The function of MtN3 family proteins is still unknown; they possibly take part in the development of root nodules and gene expressive activation of RAGs (recombination activation genes) (Gamas et al. 1996). However, *xa13*, a bacterial blight resistance gene, encodes an MtN3 family protein, which suggests a possible relationship to disease resistance (Chu et al. 2006). LOC_Os01g12020 is a putative nonspecific LTP encoding gene. LTP can transfer lipid between different membranes and possibly participate in engendering membrane (Dansen et al. 1999). Recent studies have shown that LTP contributes to stress resistance such as cold, salt, drought, and disease resistance, and its expression can be induced by biologic and abiotic stress (Cammue et al. 1995; Gaudet et al. 2003; Wu et al. 2004). Both LOC_Os01g12030 and LOC_Os01g12070 are putative endoglucanase 1 precursor genes, which are involved in cell wall disassembly during organ-aging stages including fruit ripening and organ abscission (Brummell et al. 1999; Lashbrook et al. 1994). Also, endoglucanase genes are associated with disease resistance (Flors et al. 2007). Therefore, these genes seem to have nothing to do with plant inflorescence development and cannot be a candidate for *SPP1*.

It is well known that IAA is the most universal auxin in plants. It can promote cell division and growth. In rice, the endogenous level of IAA in inflorescence tissue was 10–100 times higher than that in shoot tissue, which suggests that IAA may play an important role in regulating reproductive growth and the development of inflorescent meristem (Kobayashi et al. 1989). Guan et al. (2006) used the in vitro regeneration system of *Arabidopsis* to regenerate inflorescences when cytokinin and IAA were present. Hence, IAA is important for plant inflorescent development, which implies that IAA possibly regulates SSP in rice. In *Arabidopsis thaliana,* the *pin-formed (pin)* mutant had no flowers or had abnormal flowers on the inflorescence axis (Goto et al. 1991; Okada et al. 1991; Okada and Shimura 1994). The concentration of IAA and the activity of auxin polar transport in the inflorescence axis of *the pin* mutant were much lower than those of the wild-type (Enkheim ecotype) (Okada et al. 1991). Moreover, the application of inhibitors of IAA polar transport to the wild type plant of *Arabidopsis* induced the formation of an inflorescence axis similar to that of the *pin-formed* mutant (Okada et al. 1991; Okada and Shimura 1994). In addition, other evidence also showed that normal concentration of endogenous IAA and/or the activity of IAA polar transport is essential for the differentiation and formation of the floral meristem (Oka et al. 1999; Aloni et al. 2006). Hence, the LOC_Os01g12160, which codes IAA synthetase, is considered the most interesting candidate gene. Paying more attention to confirming the candidate gene function by transformation or by checking its mutation will be worthwhile.

Potential exploitation in rice yield improvement

Recently, several research groups have cloned the genes related to grain yield in rice. According to their results, the genes can be classified into two types: high grain yield accompanied by elongation of life cycle (type I) and high grain yield without a change in life cycle (type II). For example, *Ghd7* could produce more rice yield by delaying flowering time accompanied by an increase in plant height (Xue et al. 2008). However, yield in the *Gn1a* plant increased 40 grains per main panicle without a change in flowering time and plant height. Heading date is an important factor to determine seasonal and regional adaptation of rice. In terms of gene characterization, the rice growing region should be considered when exploiting type I genes for rice production. Otherwise, the plant cannot take full advantage of light and temperature for rice production, or rice cannot grow normally during the flowering period because of low temperature. Both cases would cause a large loss in yield. In a word, the exploitation of type I genes is dependent on rice growing regions and seasons. For type II genes, their exploitation has a large degree of freedom. Like *SPP1* reported in this study, it had no effect on seed setting and 1,000-grain weight (no QTL was detected in the NILs-F_2 population), but it had a major effect on SPP. Positive *SPP1* homozygotes could result in a about 10-g yield increase per plant compared with negative homozygotes. The positive allele of *SPP1*, theoretically, can be transferred to

rice varieties grown in any rice regions. Hence, type II gene is more favorable for rice breeding. In addition, *SPP1* showed dominant effects on SPP and grain yield, which indicated that the Teqing homozygote has more yield than the heterozygote. Hence, in theory, *SPP1* should be simultaneously transferred to both female and male parents to produce a better hybrid.

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