Molecular Plant • Volume 1 • Number 5 • Pages 830–838 • September 2008 RESEARCH ARTICLE

Fine Mapping of *Spr3*, a Locus for Spreading Panicle from African Cultivated Rice (*Oryza glaberrima* Steud.)

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ABSTRACT A CSSL (chromosome segment substitution line), SG-64, carrying a segment of chromosome 4 from African cultivated rice (CG-14) in the genetic background of var. Wuyujing-7 (*japonica*), showed a spreading panicle, which was different significantly from that of Wuyujing-7 with an erect compact panicle. The gene controlling a spreading panicle is referred to as *Spr3*, and is mapped on chromosome 4. To uncover the genetic basis of *Spr3*, a large F_2 population derived from cross between SG-64 and Wuyujing-7 was constructed for fine mapping of the *Spr3* locus. The high-resolution linkage analysis revealed that the *Spr3* locus was narrowed down to a 4.6-kb region. The delimited genomic DNA regions of Wuyujing-7 and CG-14 were sequenced and compared. Sequence mutations between Wuyujing-7 and CG-14 were evident and the candidate genes for the locus were predicted. Publicly available databases were searched for homologous cDNA sequences. However, any coding regions or other meaningful sequences for the *Spr3* locus were not found within this delimited region. This result suggested that *Spr3* is an unknown genetic factor in controlling the outspreading of the primary branches in rice inflorescence. In addition, NIL(*Spr3*) exhibited seed shattering. The formation of spreading panicle was accompanied by a few undesirable traits and the spreading panicle links with seed shattering suggest that the spreading panicle was likely lost during the domestication and selection for high seed productivity of cultivated rice.

Key words: rice; spreading panicle; fine mapping; molecular marker.

INTRODUCTION

Diverse inflorescence architectures are common in flowering plants. Spatial and temporal arrangement of lateral organs originates from inflorescence meristem (IM) and, to a large extent, determines inflorescence architecture. Inflorescence lateral organs are produced by IM, and specific IM activity plays a primary role in inflorescence architecture specification. In rice inflorescence development, meristem fate undergoes drastic changes (Ikeda et al., 2004) during direct conversion from the shoot apical meristem (SAM). Unlike SAM activity, which often forms large leaves and axillary meristems that are small or dormant, IM often produces reduced leaves and dominant axillary meristems (Bortiri et al., 2006). In the course of rice inflorescence development, IM first forms bract primordia and primary branch meristems from the base of bracts. The secondary branch meristems are subsequently initiated from the primary branch meristems. Finally, the terminal and lateral spikelet meristems are produced on the rachis-branches (primary and/or secondary branches). The floral meristems derive from the rachis-branches to form floral organs (Takeoka et al., 1993; Komatsu et al., 2001; Ikeda et al., 2007). Thus, rice inflorescence architecture depends primarily on the number of rachis-branches, specifying the number of primary branches and spikelets (Ikeda et al., 2007), and on the length of primary branches of the inflorescence.

In grasses, especially in rice and maize, the regulatory pathways of inflorescence development are not fully elucidated. However, in recent years, progress in understanding the molecular genetic mechanisms of inflorescence development has been made. Some genes have been characterized and mutations in those genes can lead to the alteration of inflorescence architecture. *LAX panicle* (*LAX*) in rice (Komatsu et al., 2001, 2003b) and *barren stalk1* (*BA1*) in maize (Gallavotti et al., 2004) are required for the initiation and maintenance

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doi: 10.1093/mp/ssn045
Received 19 May 2008; accepted 23 June 2008

of axillary meristems. Both *LAX* and *BA1* encode orthologous basic helix-loop-helix transcription factors. Mutations in *LAX* strongly reduce the number of primary branches and spikelets, and, in *ba1* mutants, unbranched tassels lacking spikelets are produced. In maize, *BARREN INFLORESCENCE2* (*BIF2*) encodes a co-ortholog of the *PINOID* serine/threonine kinase. *BIF2* plays a key role in both axillary meristem and lateral primordia initiation in the inflorescence. A defect in branch meristem initiation, resulting from *bif2* mutants, leads to the development of fewer branches (McSteen and Hake, 2001; McSteen et al., 2007). An increased number of long primary branches and/or spikelets are observed in *ramosa 1* (*ra1*), *ra2*, *ra3*, and *bd1* mutants in maize, and in *fzp* mutant in rice. In *ra1*, *ra2*, and *ra3* mutants, the tassels exhibit an increased number of long primary branches. A presumed transcription factor, *Ra1* encodes a zinc-finger domain protein (Vollbrecht et al., 2005), and acts downstream of *Ra2*, a presumed transcription factor with a LOB domain (Bortiri et al., 2006). The trehalose-6-phosphate (T6P) metabolic protein (Ra3) (Satoh-Nagasawa et al., 2006) regulates *Ra1* directly or indirectly (Kellogg, 2007). *FRIZZY PANICLE* (*FZP*) encodes an ERF transcription factor, and is involved in the establishment of the floral meristem from the spikelet meristem and prevents the formation of axillary meristems. The mutation in *FZP* results in the formation of axillary meristems in rudimentary glume axils and they either arrest or develop into branches of higher order, instead of the subsequent establishment of floral meristem identity (Komatsu et al., 2001, 2003a). *BRANCHED SILKLESS* (*BD1*) in maize, encoding an ERF transcription factor, is the ortholog of the rice *FZP* gene. In the *bd1* mutant, indeterminate branches are produced in the place of spikelets of the ear and the number of spikelets is increased in the tassel (Colombo et al., 1998). Phenotypic evaluations of the *panicle phytomer1* (*pap1*) mutant reveals an increased number of primary branches and reduced length of the rachis in rice inflorescence (Takahashi et al., 1998). *ABERRANT PANICLE OGRANIZATION 1*(*APO1*), a temporal regulator of meristem identity and an ortholog of *Arabidopsis UNUSUAL FLORAL OGRAN* (*UFO*), encodes a F-box protein, regulates meristem fate, and plays a role in preventing the inflorescence meristems converted precociously to spikelet meristems. The *apo1* mutant exhibits an abnormal panicle phyllotaxy (distichous phyllotaxy), in contrast to the typical wild-type 2/5-spiral phyllotaxy, and a decrease in the number of primary branches (Ikeda et al., 2005, 2007). In summary, the above studies in rice and maize mutants indicate that mutation in specific regulatory gene functioning in inflorescence development pathways leads to changes in the number of primary branches and/or spikelets, the length of primary branches or panicle, and consequently an alteration of the inflorescence architecture.

In addition, the outgrowth (exsertion) direction of primary branches during inflorescence development is another important factor influencing inflorescence structure. Four loci, a recessive gene *spr1* (Kinoshita and Takamure, 1986), and dominant genes *Spr2* (Mitra and Ganguli, 1932), *Spr3(t)* (Eiguchi and Sano, 1990), and *Spr4(t)* (Sanchez and Khush, 1997), for the spreading panicle, which is characterized by nearly perpendicular outgrowth of the primary branches of the panicle relative to the rachis, and giving the panicle a spreading appearance (Sanchez and Khush, 1997), were reported in the past few decades. These four loci confer specific inflorescence architecture in the mutants, which is significantly different from those of cultivated rice. Additional reports during the last decade, including map-based cloning and functional dissecting, have not been published. In this study, a chromosome segment substitution line (CSSL), denoted as SG-64, was found, showing a spreading panicle by screening a set of CSSLs. We mapped the locus on the long arm of the rice chromosome 4 and confirmed the locus using a small-scale F_2 population. The locus may correspond to *Spr3(t)* and we designated it as *Spr3*. The *Spr3* was then targeted for fine mapping and functional dissection in this study.

RESULTS

Preliminary Mapping of *Spr3*

A CSSL, SG-64, exhibited a spreading panicle at the caryopsis ripening stage and carried a CG-14 homozygous substitution chromosomal segment on the long arm of chromosome 4. We speculated that this substitution chromosomal segment was associated with the formation of the spreading panicle. The 158-plant F_2 population was constructed to confirm our speculation. The 158 F_2 individuals were genotyped using RM5503 and L4160 markers, and phenotyped at the caryopsis ripening stage. One hundred and twenty of the 158 individuals exhibited the spreading panicle and 38 exhibited the compact panicle. A 3:1 ($\chi^2 = 0.076 < \chi^2_{0.05} = 3.84$) segregation ratio was determined, indicating the compact panicle was controlled by a single recessive gene. These results confirmed that this CG-14 homozygous substitution chromosomal segment between the markers RM5503 and L4160 was responsible for the spreading panicle (Figure 1B). This region was then targeted to pursue mapping *Spr3*. Seven markers in the target region were used to construct a linkage map for *Spr3* on the basis of the 200-plant F_2 population (Figure 1B and Table 1). The *Spr3* was mapped on the region between the markers L4213 and L4218, which was physically located between 33.50 and 33.53 Mb on assembled rice chromosome 4 genome sequences according to Build 4.0 pseudomolecules of the Nipponbare genome released by IRGSP in 2005 (IRGSP, 2005).

Fine Mapping of *Spr3*

One hundred and thirty-seven recombinants were identified by the assaying of the total of the 2754-plant F_2 population and the *Spr3* locus was narrowed down to the 14.5-kb interval defined by the markers L4359 and L4293 on a BAC clone, OSJNBa0071I13 (Figure 1C). Eight markers were developed in the target region flanked by L4359 and L4293 markers (Figure 1C and Table 1) and were used for further fine mapping of the *Spr3* locus. The subsequent recombinant screening work

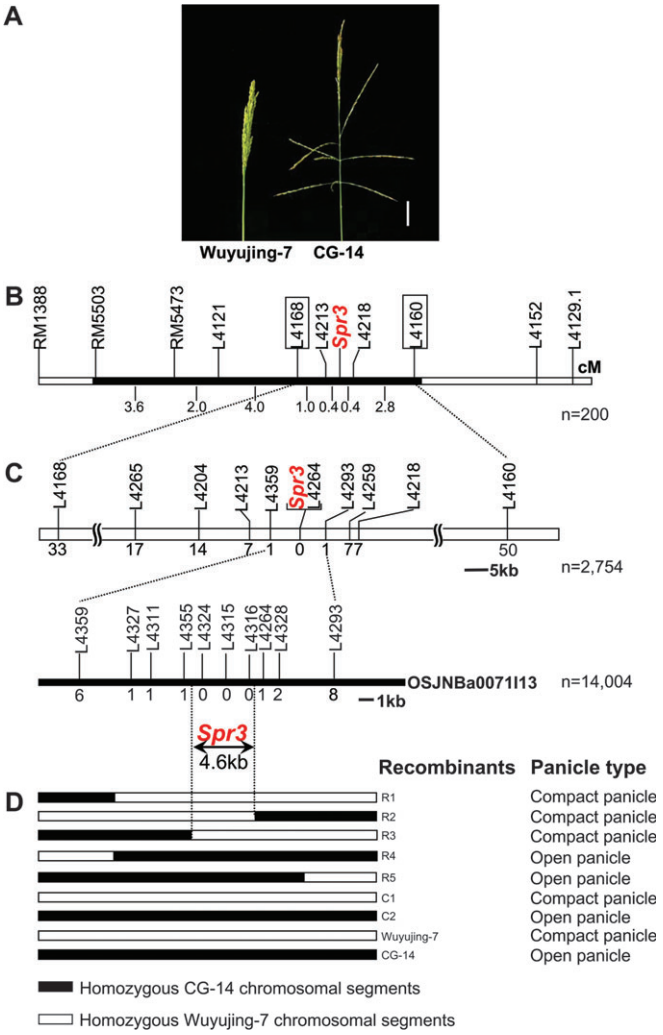

Figure 1. Fine Mapping of *Spr3*.

(A) Panicle phenotypes of parents Wuyujing-7 and CG-14. Scar bar, 5 cm.

(B) Location of *Spr3* on rice chromosome 4 in 200 BC_3F_2 plants in which a region (filled bar) is segregated. Numbers under the linkage map indicate genetic distance between adjacent markers.

(C) High-resolution linkage map of the *Spr3* region produced with 2754 and 14 004 BC_3F_2 plants (a total of 16 758), respectively. The number of recombinants between markers and *Spr3* locus is indicated under the linkage map. Filled bar shows a part of the BAC clone, OSJNBa0071I13. Two lines between L4355 and L4264 markers delimited the region that is responsible for the variation of inflorescence architecture between the two parents.

(D) Progeny testing of homozygous recombinants delimited the *Spr3* locus to the region between markers L4355 and L4264. To the right are the phenotypes of each kind of recombinant and the phenotypes of two parents and the controls.

identified 20 recombinants in the target region from the 14 004-plant F_2 population and the *Spr3* locus was more precisely positioned in the interval between L4355 and L4264 in detecting the one recombinant event that occurred between markers L4355 and L4324, and the other occurred between markers L4316 and L4264 (Figure 1C and 1D). The genotype and phenotype of each recombinant were confirmed by F_3 progeny analysis. These results showed that the *Spr3* was precisely mapped in the 4.6-kb interval defined by markers L4355 and L4264.

Candidate Gene Prediction and Sequence Analysis

The counterpart DNA sequences of the interval defined by the markers L4359 and L4264 of Nipponbare were predicted with FGENESH of Softberry (www.softberry.com/berry.phtml). This DNA sequences were also used to perform similarity blast searches at NCBI (www.ncbi.nlm.nih.gov/). However, these results showed that any coding regions were not found in the delimited area. To further understand the function of *Spr3*, DNA sequences of the 7.6-kb interval defined by markers L4359 and L4264 (Figure 1C and 1D) of Wuyujing-7 and CG-14 were sequenced and compared to Nipponbare (*japonica* cultivar). The results revealed little sequence divergence between Wuyujing-7 and Nipponbare (data not shown). However, a great number of sequence divergences, including nucleotide substitutions, insertions, and deletions, were found between Wuyujing-7 and CG-14. The sequence divergence for the 4.6-kb region defined by markers L4355 and L4264 (Figure 1C and 1D) in Wuyujing-7 and CG-14 are listed in Table 2. The 7.6-kb DNA sequences of Wuyujing-7 and CG-14 were subsequently blasted with a known cDNA clone database (http://cdna01.dna.affrc.go.jp/cDNA/). The results of blast did not generate any useful related cDNA information. A review of the rice annotation databases RAP-DB (http://rapdb.dna.affrc.go.jp/), Gramene (http://www.gramene.org/), and TIGR (http://www.tigr.org/) resulted in the failure to obtain any annotations for the *Spr3* locus. These results suggested that *Spr3* may be an unknown gene or may be an intergenic sequence acting as a *cis*-regulator in regulating the transcription of its target genes. Therefore, additional efforts are warranted to determine the genetic basis of the *Spr3* locus. In addition, the *Spr3* sequence data of CG-14 were deposited in the GenBank database (accession number, EU365774).

Spr3 Locus Is Associated with the Formation of a Spreading Panicle

NIL(*Spr3*), containing a small *Spr3* region of CG-14 in Wuyujing-7 genetic background, was bred to investigate the effect of *Spr3* on rice inflorescence architecture. It is interesting to note that the linkage relationship between the spreading panicle and seed shattering was found in the NIL(*Spr3*), and a seed shattering associated gene, *sh4* (Eiguchi and Sano, 1990; Li et al., 2006), might be contained on the homozygous chromosome segment from the CG-14 in NIL(*Spr3*). In young inflorescence development, the primary branch primordia of NIL(*Spr3*) were indistinguishable from those of Wuyujing-7, which produced 2/5-spiral phyllotaxy primary branch primordia (Figure 2A and 2B). However, the primary branches of the spreading panicle spread out gradually after heading in comparison with the control (Figure 2C, 2E, 2G and 2D, 2F, 2H). Finally, the distinct variation in inflorescence architecture

Table 1. New Molecular Markers Were Developed from Sequence Data of Nipponbare at the Interval RM5503-L4160 of Chromosome 4 for Fine Mapping of the *Spr3* Locus.

Marker	Marker type	Predicted size (bp)	Forward primer(5′–3′)	Reverse primer(5′–3′)	Restriction enzyme
L4121	Indel	364	AACTCGTTAGAGGTGCAG	GGAACCAAAGGTAGATGA	
L4168	Indel	478	CAAAGCCAAGGTAGATAGG	TTTGCCCACTAAACATCAG	
L4265	CAPS	1265	GCATGTCTACTGCTGTTGCC	CCGCTCATGACGTAGTGGAA	*Stu* I
L4204	CAPS	1582	CCCCACCTTGTGGTGCTAAT	AGTTTCCACGCCCAACTTTA	*Dra* I
L4213	CAPS	1609	AGGAGATGAGGCGAATACA	CACAACTTTCAGGGCTTTT	*Alu* I
L4359	Indel	342	GCATGGATATACTTGCTTACCGT	TGGGTATGGAATCCTACAGCGA	
L4327	Indel	324	CATCTCATGCGTCGTCGTCT	ACTAGCTAGATCCTCGGCAT	
L4311	CAPS	1397	ACGAGCACTACATTATTCATGA	CGGAGTACTTATCATTCTAGC	*Hinc* II
L4355	Indel	453	CAGGGCCTTTCTCTCAGCAT	GTATTTGATTTGTGTGTACAAGTAACT	
L4324	Indel	389	GGATTATGGGTTGCCTCCAA	GGCTGGTGAATAATGTGTGA	
L4315	CAPS	586	TGCAACGCATGCATGCGCTT	TAGCCTGCCACGACAATGTA	*Spe* I
L4316	CAPS	560	ACATTTCCGCCGAATTCATC	GTGCAATAGCATATCGATCT	*Asu* I
L4264	CAPS	1287	CTGGTGACATGAGCCACAGTTG	CCTTACTCTCGATCGAGGCC	*BseN* I
L4328	Indel	330	AGATTTAGGGTGCGCTCGTT	AGCTACCGGAATTCCTACGA	
L4293	CAPS	1365	GATATATCTCGATCGGTACCTC	AAGCCCTGAATTCAGACCAA	*Hin6* I
L4259	Indel	308	TTAACCAGCTTCTCCAACGA	ATCAGCAGCATCACCAACAC	
L4218	Indel	1690	CGAGGATTGACTACGACAT	TAAGTTCCACGGTTAGGTT	
L4160	Indel	216	TTGAGCATGAACGGTGTT	GTTAGATGGTGGTGAGACG	

was observed between NIL(*Spr3*), with a spreading panicle (Figure 2H), and Wuyujing-7 control, with a compact panicle at caryopsis ripening stage (Figure 2G). In spreading panicle architecture, the primary branches are nearly perpendicular to the inflorescence rachis, and therefore give the panicle a spreading appearance. In addition, the several basipetal primary branches were always formed in a cluster in spreading panicle (Figure 2H), similar to those observed in the donor parent, CG-14 (Figure 1A). These results indicated that the *Spr3* allele from CG-14 plays a primary role in the gradual spread of primary branches of inflorescence after heading. In other words, the spreading panicle of NIL(*Spr3*) was formed in late panicle development but the morphology and phyllotaxy of primary branch primordia were not altered in young panicle development (Figure 2A and 2B).

Spr3 Affects the Yield-Related Panicle Traits and Plant Height

Spr3 has a great impact on plant height and the yield-related panicle traits. The mean of each trait was compared between NIL(*Spr3*) and Wuyujing-7, respectively. We found that plant height, PL, PBNP, SBNP, GNP, FGNP, UGNP, and GD of NIL(*Spr3*) increased by 13.4, 19.0, 38.5, 65.6, 44.8, 34.7, 133.2, and 22.0% ($P < 0.01$), respectively (Figure 3A, 3B, and 3D–3J), whereas SSR and 100-GW of NIL(*Spr3*) decreased by 6.7 and 6.7% ($P < 0.01$), respectively (Figure 3K and 3L). Although PL, PBNP, SBNP, GNP, FGNP, UGNP, and GD of NIL(*Spr3*) increased significantly, the significant decrease in 100-GW and SSR and the significant increase in UGNP of NIL(*Spr3*) resulted in an insignificant difference in grain yield per plant (GYP) between NIL(*Spr3*) and Wuyujing-7 (Figure 3C).

DISCUSSION

The grass inflorescence in cereal crops provides the primary food source for humans and livestock, and seed productivity is closely allied with inflorescence architecture. Therefore, understanding the molecular genetic mechanisms underlying inflorescence architecture is of practical importance in genetically modifying the architecture of inflorescences to breed desirable crops in breeding programs. A CSSL, SG-64, showed an altered panicle appearance, designated as a spreading panicle. Four loci (*spr1*, *Spr2*, *Spr3(t)*, and *Spr4(t)*) for spreading panicle were previously reported (Kinoshita and Takamure, 1986; Mitra and Ganguli, 1932; Eiguchi and Sano, 1990; Sanchez and Khush, 1997). Among these genes, *Spr3(t)* was mapped on chromosome 4 (Eiguchi and Sano, 1990), but no further data have been reported for these loci. In a small-scale mapping population, we found that the chromosomal segment from donor parent, CG-14, was substituted in the vicinity of markers RM5503 and L4160 on the long arm of chromosome 4 and it was confirmed that this substitution segment was responsible for spreading panicle phenotype. In addition, based on chromosomal positions, the results suggested that the locus and *Spr3(t)* may be the same locus and we designated it as *Spr3.* The high-resolution linkage analysis using the large secondary F_2

Table 2. The Sequence Divergence of the Delimited Region between Wuyujing-7 and CG-14.

Wuyujing-7	CG-14	Wuyujing-7	CG-14	Wuyujing-7	CG-14
5[a] C	5T	1199G	1199A	3132G	3132C
19G	19A	1209G	1209T	3161G	3161A
53G	53A	1211C	1211T	3195A	3195G
74G	74A	1399A	1399T	3264G	3264A
75G	75 1-bp-deletion	1422T	1422C	3318G	3318T
111T	111C	1445T	1445C	3409T	3409C
242T	242C	1841T	1841C	3424A	3424C
256C	256T	2019C	2019T	3488G	3488 1-bp-deletion
265T	265A	2045A	2045G	3579T	3579C
281T	281C	2185A	2185T	3925T	3925A
291C	291T	2226T	2226G	3955G	3955A
315T	315C	2297T	2297C	4055C	4055A
316	316 16-bp-deletion	2317G	2317A	4122G	4122A
456T	456C	2455G	2455A	4161G	4161C
522	522 27-bp-deletion	2456	2456 3-bp-insertion	4168C	4168T
644G	644A	2511T	2511C	4202A	4202G
659	659 2-bp-insertion	2541T	2541G	4214T	4214A
662C	662G	2563C	2563T	4263C	4263A
740G	740A	2673A	2673G	4338A	4338G
750	750 1-bp-insertion	2697T	2697C	4355A	4355C
763	763 14-bp-deletion	2778C	2778T	4454T	4454A
797T	797 1-bp-deletion	2856C	2856T	4468A	4468G
830T	830C	2912A	2912T	4480	4480 2-bp-insertion
926A	926G	3102T	3102C	4601T	4601A
1194T	1194G	3113A	3113G		

a The number before the bases in the table indicates the mutation positions, which start from the site of the L4355 marker, in the delimited 4.6-kb region defined by L4355 and L4264 markers in Wuyujing-7.

population and newly developed markers revealed that *Spr3* was successfully narrowed down to a 4.6-kb region.

The 7.6-kb DNA sequences containing the 4.6-kb region defined by markers L4355 and L4264 in Wuyujing-7 and CG-14 were sequenced and compared, respectively. Polymorphisms, including nucleotide substitutions, insertions, and deletions, were found in the delimited region (Table 2). Softberry and other prediction software predicted the two 7.6-kb DNA sequences of Wuyujing-7 and CG-14. The related cDNA and sequence annotation data were searched based on cDNA clone and publicly available sequence annotation databases, respectively. However, these databases did not provide any useful information. Therefore, we were unable to establish whether any DNA sequence mutations in Wuyujing-7 were responsible for the variation in rice inflorescence architecture or the role that the locus played in the observed variability. We proposed three possible functions of the *Spr3* locus. First, the *Spr3* locus may be a new gene, and the gene structure is different from those of other recently described genes. Therefore, no data were available from published databases. Then, the *Spr3* locus may be a microRNA-coding region, regulating target genes that function in rice inflorescence development. Eleven *OsSPL* genes, predominantly expressed in the young panicle, were the putative targets of OsmiR156 (Xie et al., 2006). *Spr3* may serve as a gene encoding microRNA, controlling rice inflorescence development. Finally, the *Spr3* locus may be an intergenic enhancer or repressor, acting as a *cis*-acting element. In this case, *Spr3* would regulate transcription of its target gene functioning in rice inflorescence development. In maize, an intergenic sequence ~58–69 kb 5′ to the *teosinte branched1* (*tb1*) cDNA acted as an upstream enhancer in *tb1* transcription regulation, conferring pleiotropic effects on plant and inflorescence architecture (Clark et al., 2006). Konishi et al. (2006) identified a SNP ~12 kb 5′ to the *qSH1*, an ortholog of the *Arabidopsis REPLUMLESS* (*RPL*) gene. The SNP acted as a 5′-regulatory region of *qSH1* and resulted in the loss of seed shattering and associated with seed shattering among subspecies rice of *japonica*. Therefore, *Spr3* locus can be considered as a *cis*-regulator. However, additional experimental evidence is required to confirm these three hypotheses. In further works, we will identify more recombinants between the markers L4355 and L4264 to further narrow the *Spr3* locus

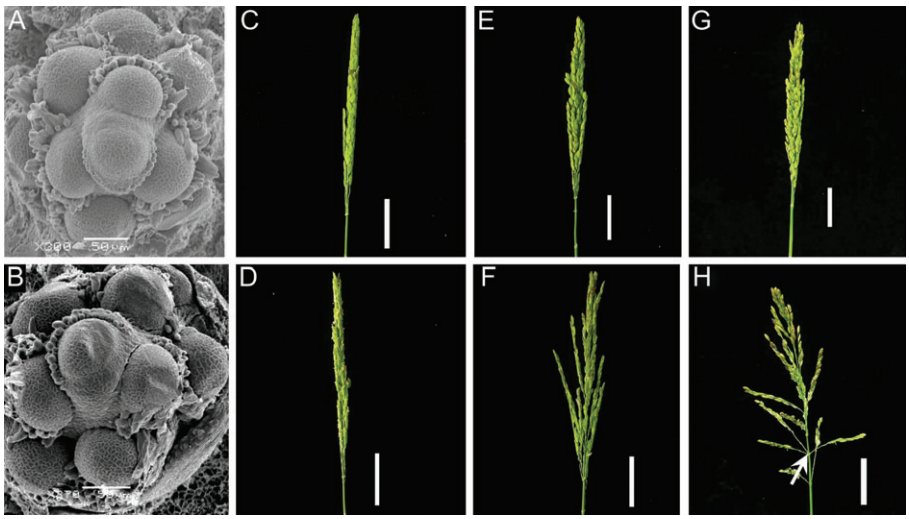

Figure 2. Formation of Specification Inflorescence Architecture in Wuyujing-7 and NIL(*Spr3*).
Upper panels (A,C,E,G) indicate the formation of inflorescence architecture in Wuyujing-7. Lower panels (B,D,F,H) indicate the formation of inflorescence architecture in NIL(*Spr3*).
(A, B) SEM image of young inflorescence showing the stage of initiating primary-branch primordia.
(C, D) Inflorescence at heading to anthesis stage.
(E, F) Inflorescence at filling stage, 7 d after anthesis.
(G, H) Inflorescence at ripening stage, 19 d after anthesis. Scale bars, 50 μm in panels (A) and (B), and 5 cm in panels (C–H). The arrow in (H) indicates forming several primary branches in a cluster.

into a smaller region using a larger F_2 population. Meanwhile, a 4.6-kb DNA fragment will be divided into several fragments and will be transformed into Wuyujing-7 to confirm which fragment complements the spreading panicle in the Wuyujing-7 genetic background, and ultimately determine which sequence divergence is responsible for the variation of the inflorescence architecture.

Rice (*Oryza sativa* L.) agronomic traits underwent profound changes and genetic diversity occurred during the course of domestication from wild rice (*Oryza rufipogon* Griff.) to cultivated rice (Sun et al., 2001). These traits, including seed numbers, fertility, plant architecture, seed shape, flowering time, seed color, and seed shattering, have been artificially selected during the course of domestication (Konishi et al., 2006). Spreading panicle was found to be rare in cultivated rice, and common in *Oryza glaberrima* Steud., *Oryza. rufipogon* Griff., and in the progenies of cross between *Oryza glaberrima* Steud. × *japonica* (plant material of this study), *Oryza. rufipogon* Griff. × *indica*, and *indica* × *japonica*, respectively, in the previous construction of CSSLs in our laboratory (data not shown). The formation of spreading panicle in *Oryza glaberrima* Steud. is always accompanied by some undesirable traits, such as the production of far fewer secondary branches on the panicles (Jones et al., 1997), low yield and/or seed shattering (WARDA, 1993). Previous reports suggested that *Spr3(t)*, which may be the same locus as *Spr3*, and *sh4*, a gene associated with seed shattering, are linked on the long arm of chromosome 4 (Eiguchi and Sano, 1990; Li et al., 2006). The linkage relationship between spreading panicle and seed shattering led to the indirect artificial elimination of the *Spr3* locus in the course of domestication of non-shattering species or it was directly lost in the course of domestication of high seed productivity cultivars.

NIL(*Spr3*) in Wuyujing-7 genetic background forms a spreading panicle with more desirable yield-related panicle phenotypes, such as, the increasing in PBNP, SBNP, GNP, FGNP, UGNP, and GD (Figure 3F), unlike those that were observed in *Oryza glaberrima* Steud. (Jones et al., 1997). However, these increases did not lead to an increase in grain yield per plant in NIL(*Spr3*) due to the formation of a spreading panicle accompanied by a decrease in 100-grain weight and seed setting rate and an increase in unfilled grain number per panicle. This suggested that a pyramiding of the *Spr3* locus with QTLs for high seed productivity, such as *GW2* for grain weight (Song et al., 2007) and *Gn1a* for number of grains per panicle (Ashikari et al., 2005), has substantial potential to increase the production of rice in breeding programs. Moreover, further isolation and analysis of the *Spr3* locus can provide enhanced understanding of this new unknown genetic factor and the molecular mechanisms in the formation of the spreading panicle, and lay the solid foundation for genetically modifying the architecture of inflorescences to breed a desirable variety of crops for high seed yield and high seed quality in breeding programs. Finally, the evolutionary relationships among diverse inflorescence architectures formed by the *Spr3* locus natural mutation may provide valuable insights into the origins of inflorescence types across angiosperms.

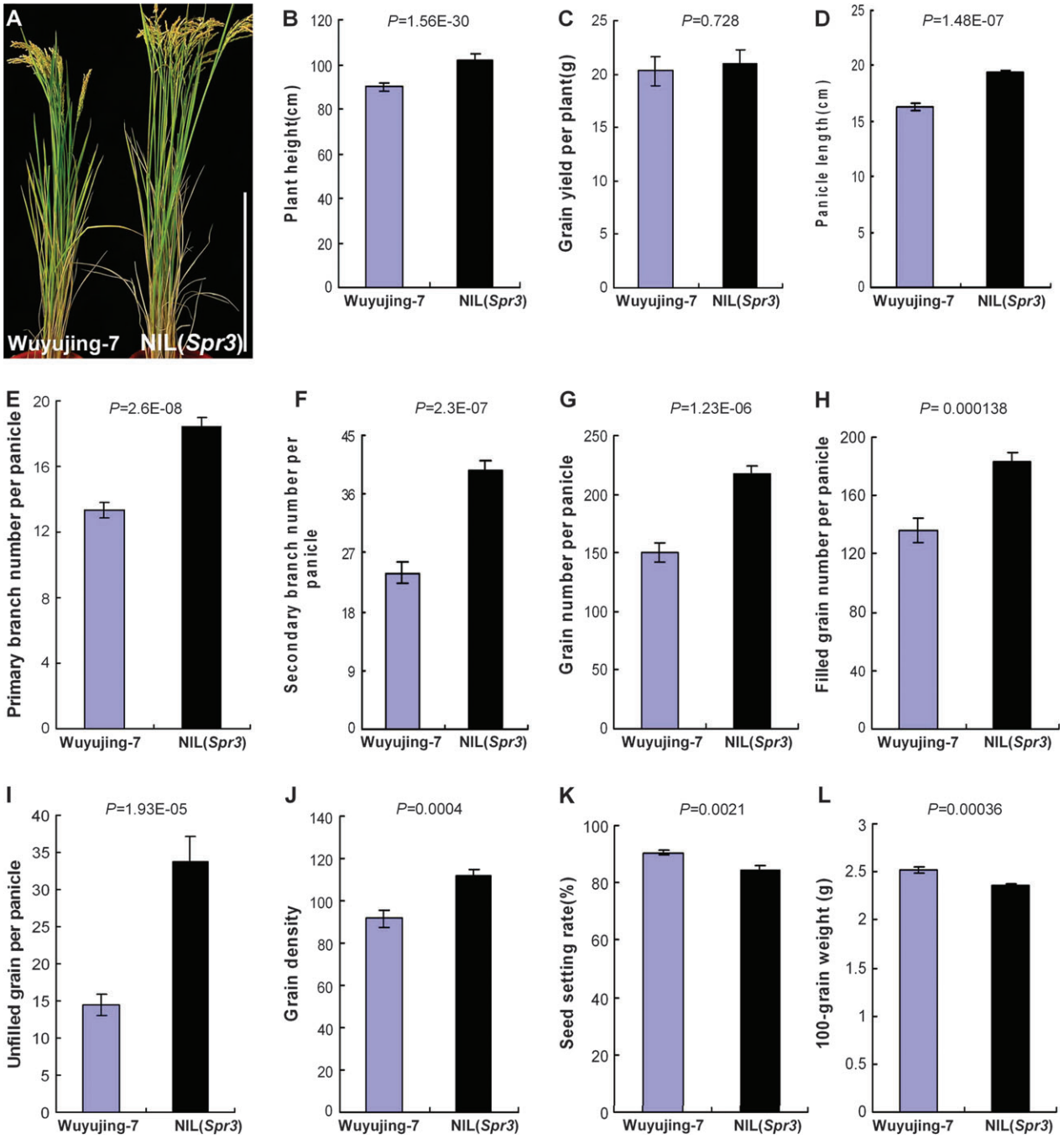

Figure 3. Phenotypic Characterization of Plant Height and Panicle Traits in Wuyujing-7 and NIL(*Spr3*).

(A, B) Comparison of plant type and plant height in Wuyujing-7 and NIL(*Spr3*) plants (n = 31). Scale bar, 40 cm.
(C) Comparison of grain yield per plant in Wuyujing-7 and NIL(*Spr3*).
(D) Comparison of panicle length in Wuyujing-7 and NIL(*Spr3*).
(E) Comparison of primary branch number per panicle in Wuyujing-7 and NIL(*Spr3*).
(F) Comparison of second branch per panicle in Wuyujing-7 and NIL(*Spr3*).
(G) Comparison of grain number per plant in Wuyujing-7 and NIL(*Spr3*).
(H) Comparison of filled grain number per panicle in Wuyujing-7 and NIL(*Spr3*).
(I) Comparison of unfilled grain per panicle in Wuyujing-7 and NIL(*Spr3*).
(J) Comparison of grain density in Wuyujing-7 and NIL(*Spr3*).
(K) Comparison of seed setting rate in Wuyujing-7 and NIL(*Spr3*).
(L) Comparison of 100-grain weight in Wuyujing-7 and NIL(*Spr3*). All traits except plant height were measured in 15 plants in Wuyujing-7 and NIL(*Spr3*), respectively. All data are given as mean ± S.E. A student's *t*-test was used to generate the *P*-values in (B–L).

METHODS

Plant Materials and Mapping Population

A set of 187 chromosome segment substitution lines (CSSLs) was constructed previously by introgressing chromosomal segments from CG-14 (*Oryza glaberrima* Steud.), an African cultivated rice with a spreading (open) panicle (Figure 1A), into *japonica* cultivar Wuyujing-7 (compact panicle) (Figure 1A) background based on marker-assisted selections (MAS) in BC_3F_2 populations (J.J. Luo, W. Hao and H.X. Lin, unpublished data). A CSSL, SG-64, exhibited a spreading panicle at caryopsis ripening stage and carried a CG-14 homozygous substitution chromosomal segment. The segment was located at approximately 14.2-cm intervals, delimited by markers RM5503 and L4160 on the long arm of chromosome 4. To elucidate the genetic basis of the variation, the heterozygous plants were harvested from a BC_3F_2 population grown at Sanya (18°N, 109°E), Hainan province, China, in the winter of 2003 and was used to construct an F_2 population. A small 158-plant F_2 population was grown in Shanghai (31°N, 121°E), China, in the summer of 2004. Each plant in the F_2 population was genotyped using markers RM5503 and L4160 and phenotyped at caryopsis ripening stage. The results of genotyping and phenotyping confirmed that the locus that was contained in the homozygous chromosome segment from the CG-14 in the interval defined by markers RM5503 and L4160 was responsible for the spreading panicle. Subsequently, a total of 16 758 plants of the F_2 population were planted for fine mapping in two locations, as above, each year, respectively, from the winter of 2004 to the summer of 2007. Two hundred plants were selected from the F_2 population and all plants were genotyped and phenotyped for the constructing linkage map and for the linkage analysis. Homozygous for CG-14 chromosomal segment and homozygous for Wuyujing-7 chromosomal segment, plants were selected from the large F_2 population as controls, such as C1 and C2 (Figure 1D). Meanwhile, we developed a nearly isogenic line, NIL(*Spr3*), containing a very small *Spr3* region (751 kb) of CG-14 defined by markers L4265 and L4160 (Figure 1C) in Wuyujing-7 genetic background from the BC_4F_2 generation for further trait evaluations.

DNA Extraction and Molecular Marker Analysis

Micro-quantities of DNA were extracted from fresh leaves according to the method of Lin et al. (2002). The molecular markers (RM5503 and L4160) in the target region were used for preliminary mapping (McCouch et al., 2002). To construct a high-density linkage map for fine mapping in the target region, new insertion/deletion (InDel) and CAPS markers were developed according to publicly available rice genome sequences (http://rgp.dna.affrc.go.jp). All newly developed markers were used to detect polymorphisms between Wuyujing-7 and CG-14 in the target region. Nineteen markers, including 10 InDels and eight CAPS showed polymorphisms and were used in further approaches in this study (Figure 1B and 1C, and Table 1).

Linkage Map and Gene Mapping

Newly developed informative markers and two polymorphic SSR markers (RM5503 and RM5473) (McCouch et al., 2002) were used to construct a linkage map and for map-based cloning of *Spr3*. The linkage analysis was done by Mapmaker/Exp 3.0 (Lander et al., 1987) on the basis of the 200-plant F_2 population to determine the marker order and the genetic distance between every two adjacent markers in the target region on the chromosome 4. The marker order was the same as that on the corresponding region of Nipponbare genome sequences (http://rgp.dna.affrc.go.jp) (Figure 1B). These informative molecular markers were used for genotyping each plant of the F_2 population, various recombinants in the target region were identified, and the linkage relationship between markers and *Spr3* locus was analyzed for gene mapping. The recombinant events in the F_2 population were further confirmed by F_3 progeny testing. By assaying the recombinant events, the *Spr3* locus was narrowed down to a very small region on the single BAC clone.

Phenotypic Evaluation

The panicle architecture of the 158 plants in the F_2 population (BC_3F_2) was individually evaluated in the summer of 2004 field investigations. In the large F_2 population (16 758 plants), the phenotype of each recombinant and controls were evaluated for fine mapping. Plant height, grain yield per plant (GYP), and yield-related panicle structure traits, including panicle length (PL), grain number per panicle (GNP), primary branch number per panicle (PBNP), secondary branch number per panicle (SBNP), filled grain number per panicle (FGNP), unfilled grain number per panicle (UGNP), 100-grain weight (100-GW), grain density (GD), and seed setting rate (SSR), of NIL(*Spr3*) and recurrent parent, Wuyujing-7, were surveyed in the summer of 2007. Plant heights of 31 NIL(*Spr3*) and 31 Wuyujing-7 plants were measured at caryopsis ripening stage, respectively, GYP and yield-related panicle traits were measured in 15 plants of each line, and their means were compared and analyzed employing a *t*-test.

SEM Analysis

The variation of the primary branch primordia between NIL(*Spr3*) and Wuyujing-7 in young panicle development was assayed by using scanning electron microscopy (SEM). The inflorescence meristems were dissected and fixed overnight at 4°C in FAA (formalin:glacial acetic:70%ethanol; 1:1:18), and dehydrated in a graded ethanol series. The samples were critical-point-dried, mounted, sputter-coated with platinum, and observed and photographed using a scanning electron microscope (JSM–6360LV; JEOL Ltd, Tokyo).

FUNDING

This work was supported by grants from the Ministry of Science and Technology of China (2006AA10Z147, 2006AA10A102, 2006AA10Z1F7), the Chinese Academy of Sciences and Science

(KSCX1-YW-03) and the Technology Committee of Shanghai Municipality (06XD14023).
No conflict of interest declared.

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