

Fine mapping and candidate gene analysis of *spd6*, responsible for small panicle and dwarfness in wild rice (*Oryza rufipogon* Griff.)

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Abstract Identification of genes in rice that affect production and quality is necessary for improving the critical global food source. CSSL58, a chromosome segment substitution line (CSSL) containing a chromosome segment of *Oryza rufipogon* in the genetic background of the *indica* cultivar Teqing showed significantly smaller panicles, fewer grains per panicle, smaller grains and dwarfness compared with the recurrent parent Teqing. Genetic analysis of the BC₄F₁ and BC₄F₂ generations, derived from a cross between CSSL58 and Teqing, showed that these traits are controlled by the recessive gene *spd6*, which mapped to the short arm of chromosome 6. Fine mapping and high-resolution linkage analysis using 24,120 BC₄F₃ plants and markers flanking *spd6* were carried out, and the gene was localized to a 22.4 kb region that contains four annotated genes according to the genome sequence of *japonica* Nipponbare. Phenotypic evaluation of the nearly isogenic line NIL(*spd6*) revealed that *spd6* from wild rice has pleiotropic effects on panicle number per plant, grain size, grain weight, grain number per panicle and plant height, suggesting that this gene might play an important role in the domestication of rice. The discovery of *spd6*

may ultimately be useful for the design and breeding of crops with high grain yield and quality.

Introduction

Food security for an ever-increasing global population largely depends on increased grain yield in crop plants (Gupta et al. 2006). Rice is the main cereal crop in many countries, and the yield depends on three main components: effective tillers per plant, grain weight (1,000 grain weight) and number of grains per panicle. Of these factors, grain number per panicle has been shown to have the greatest range of variation and is the major target for improvement in high yield rice breeding (Yamagishi et al. 2002). Grain size also has a large impact on grain yield and the rice's end-use quality, such as rice appearance and milling quality.

The origin of domesticated rice is still under debate. There are two domesticated rice species currently under cultivation, *Oryza sativa* and *O. glaberrima*. The former is distributed globally especially in Asia, while the latter is cultivated regionally in West Africa. There is some evidence that the *indica* and *japonica* subspecies of *O. sativa* have the same ancestor, *O. rufipogon* (Kovach et al. 2007). During the course of the domestication of wild rice, profound changes in agronomic traits and genetic diversity occurred. Panicle number per plant, grain weight (1,000 kernel weight) and grain number per panicle constitute important components of the rice domestication symbols. The grain size of the wild ancestor is consistently small, while domesticated grains vary in size. The panicle also changed from an open panicle with few

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secondary branches and grains to a densely packed panicle carrying more grains than wild ancestors (Sweeney and McCouch 2007).

The sequencing of the rice genome gave birth to a rapid surge in research on rice genetics and evolution (Vaughan et al. 2008). Grain yield and associated traits in rice have always been active areas of research in rice. Some genes involved in grain yield have been cloned: *MOC1* was shown to play an important role in the regulation of rice tillering (Li et al. 2003); *Gn1a* controls panicle size by influencing the number of grains (Ashikari et al. 2005); *GS3* (Fan et al. 2006), *GW2* (Song et al. 2007) and *qSW5* (Shomura et al. 2008; Weng et al. 2008) affect grain yield by regulating grain shape and weight; *Ghd7* has major effects on several traits in rice, including grains per panicle, plant height and heading date (Xue et al. 2008); and *GIF1* encodes a cell-wall invertase controlling rice grain filling and yield during early grain filling (Wang et al. 2008).

Plant height is another important component of grain yield that is related to lodging resistance and fertilizer endurance. To date, numerous rice dwarf mutants, some of which have been cloned and isolated, have been validated and characterized because of their agronomic importance. There are various reasons for the dwarf phenotype in plants. Recent molecular genetic approaches revealed that gibberellin (GA) and brassinosteroid (BR) are the two most important factors in determining plant height. In rice, for example, several GA-related semi-dwarf or dwarf mutants have been cloned: *sd1* (Sasaki et al. 2002), *d1* (Ueguchi-Tanaka et al. 2000), *d18* (Itoh et al. 2002), *d35* (Ogawa et al. 1996), *dgl1* (Komorisono et al. 2005), *gid1* (Ueguchi-Tanaka et al. 2005) and *gid2* (Sasaki et al. 2003). In parallel with studies on GA, the molecular mechanisms of brassinosteroid (BR) biosynthesis and signaling have been elucidated using BR-deficient- and BR-insensitive mutants of rice, such as: *brd1* (Mori et al. 2002), *brd2* (Hong et al. 2005), *d2* (Hong et al. 2003), *d11* (Tanabe et al. 2005), *d61* (Yamamuro et al. 2000) and *osdwarf4-1* (Sakamoto et al. 2006). Other rice dwarf mutants that are independent of GA and BRs have also been isolated and characterized: *lhd2* (Xiong et al. 2006), *d3* (Ishikawa et al. 2005), *d6* (Sato et al. 1999), *d10* (Arite et al. 2007), *d50* (Mase et al. 2005), *dbt1* (Sazuka et al. 2005) and *htd1* (Zou et al. 2006).

Here, we report our research on the *spd6* gene, which affects several agronomic traits. We found that the *spd6* allele from the wild rice (*O. rufipogon*) was associated with significantly smaller panicles containing fewer spikelets, small grains, low spikelet fertility and shorter plants compared with the recurrent parent Teqing. We carried out fine mapping and localized *spd6* to a 22.4 kb target region on chromosome 6. The potential importance of *spd6* in rice domestication and evolution is also discussed.

Materials and methods

Plant materials

CSSL58 is one of a set of 133 chromosome segment substitution lines (CSSLs) that were developed from backcross progenies (BC₃F₂) derived from a cross between a high yield *indica* variety, Teqing (*O. sativa* L.) as the recurrent parent and wild rice (*O. rufipogon* Griff.) collected from Hainan Province, China, as the donor parent. When phenotypically evaluated at two sites, Sanya and Shanghai, China, the CSSL58 plants containing a chromosome segment from wild rice showed significantly smaller panicles with fewer and shorter grains when compared with the recurrent parent Teqing. The wild rice segment was located at approximately 27.9 cM intervals, delimited by the RM8120 and RM3370 markers. A small BC₄F₂ population consisting of 250 plants was obtained by selfing the BC₄F₁ plants generated from CSSL58 'female' and Teqing 'male' parents and grown in a paddy field in Sanya (18°N, 109°E), Hainan Province, China, in the winter of 2004. Heterozygous plants were selected to produce a larger BC₄F₃ population for fine mapping of *spd6*. In total, about 26,617 BC₄F₃ plants were grown as above in Shanghai and Sanya alternately from the winter of 2005 to the winter of 2007. Each plant was genotyped using molecular markers. The BC₄F₄ recombinant lines derived from the BC₄F₃ population were used for progeny testing. We also developed a nearly isogenic line, NIL(*spd6*) from the BC₄F₂ population. In addition, another BC₃F₃ population from a cross between CSSL58 and *japonica* cultivar Zhonghua 11, with Zhonghua 11 as the recurrent parent, was constructed and grown as described above.

DNA extraction and molecular marker analysis

Microquantities of DNA were extracted from fresh leaves of each individual using a previously reported method (Lin et al. 2002) with minor modifications. In brief, a small piece of rice leaf was ground in a 1.5 ml tube containing 400 µl of 100 mM Tris/HCl, 1 M KCl and 10 mM EDTA and incubated for 20 min at 75°C. Three hundred microliters of each mixture was transferred to a 96-well PCR plates and centrifuged. Crude DNA in the supernatant was precipitated with an equal volume of isopropanol in another 96-well PCR plate, rinsed with 70% ethanol, centrifuged again and the pellet was redissolved in 60 µl H₂O. The SSR markers between RM8120 and RM3370 in the target region were used for preliminary mapping (McCouch et al. 2002). To obtain a high-density linkage map for fine mapping in the target region, new insertion/deletion (indel) and CAPS markers were developed according to the publicly available rice genome sequence

Table 1 Sequence of newly developed primers according to the sequence dates of *japonica* Nipponbare

Marker	Marker type	Product size in Nipponbare (bp)	Forward primer (5′–3′)	Forward primer (5′–3′)	Restriction enzyme
CX05	CAPS	1,196	TGTTCCATCCAAACCATC	GTGTCTCGCCTCACTTCA	<i>TaqI</i>
CX08	CAPS	1,231	ACAGAATTAAGGGCAAAG	TGAAGAAAGACGCAGGTA	<i>Acil</i>
CX09	CAPS	1,114	GCAATTTTCATGGGTGTTA	CTTTATTCCCTTGTGGTG	<i>ApaLI</i>
JX6024	CAPS	905	ATAGCGGAAATACCAGAA	AAGCCTTGTCTAACGAAA	<i>DraI</i>
JX6036	CAPS	679	GACCACGGCTAATGTATTCT	GCACAGTTCGTTCCTCC	<i>MspI</i>
Q5	CAPS	1,192	TTCAGGTGGCTCAACAAG	AATCACAAGTCCCGCAGT	<i>EcoRI</i>
Q14	CAPS	1,321	CAAGGTTGTTTCGCTCGTA	CGCCGTTTAGATTGAGAC	<i>HindIII</i>
SH03	CAPS	984	TAACGACAATAACTCCCC	CACAAACACAGCAAAAGA	<i>EcoRI</i>

(<http://rgp.dna.affrc.go.jp>). All newly developed markers used in fine mapping contained polymorphisms between CSSL58 and Teqing. The primer information for the eight markers is listed in Table 1.

Linkage map and gene mapping

All the available markers were used for the construction of a linkage map and for map-based cloning of *spd6*. Linkage analysis based on the 250-plant BC₄F₂ population was conducted using Mapmaker/Exp3.0 (Lander et al. 1987) to determine the order of the markers and the genetic distance between every two adjacent markers in the target region. In each recombinant family, the genotypes for all markers in the target region were determined and homozygous recombinants were identified. The recombinant plants were further confirmed by progeny testing.

Phenotypic evaluation

Genetic and phenotypic identification of each plant derived from the BC₄F₄ families and the BC₄F₃ population were performed. Quantitative analysis of agronomic traits including plant height (PH), culm length (CL), tillers per plant (TPP), 1,000 grain weight (GW) and grain yield per plant (YD) for the two parents, NIL(*spd6*) and Teqing, were performed using 16 plants each. In addition, traits of the main panicles of those plants, including panicle length (PL), grain number per panicle (GNP), and spikelet fertility (SF) were also evaluated. To evaluate pollen viability, pre-flowering spikelets were gathered and dipped into 1% (v/v) of I₂ in 3% (v/v) KI, and then the anthers were removed from the spikelet and placed on glass slides. The anthers were crushed into powder and used to observe fertility using a light microscope. Pollen grains that were round in shape and stained black were considered to be living pollen. To evaluate stigma viability, anthers from spikelets on the top half of Teqing and NIL(*spd6*) panicles were removed a day prior to anthesis and covered with paper bags to prevent open

pollination. The stigmas were pollinated the next day with the excess pollen grains from Teqing. Twenty days after flowering, the spikelets in both emasculated and control plants were collected and analyzed.

Results

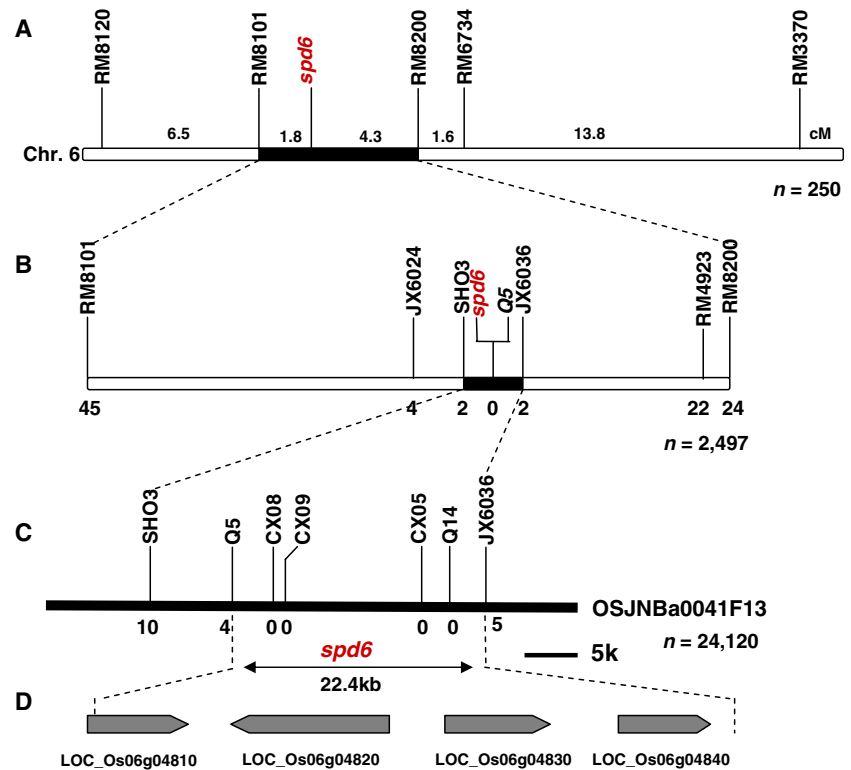
Detection and preliminary mapping of *spd6*

CSSL58 exhibited a small panicle, small grains and dwarfness at the heading stage, and it carried a chromosomal segment of about 27.9 cM derived from the genome of wild rice on the short arm of chromosome 6 in the genetic background of Teqing. Other 2 independent CSSLs containing the short arm of chromosome 6 also showed the phenotype of CSSL58; hence, we speculated that this target wild rice chromosomal segment co-segregated with the small panicle and dwarf phenotype. The small BC₄F₂ population, consisting of 250 individual plants derived from the cross between CSSL58 and Teqing, were used to confirm our speculation. The 250 plants were genotyped using SSR markers and phenotyped at the heading stage. The BC₄F₁ plants derived from the cross between CSSL58 and Teqing had the same phenotype as Teqing; 50 of the 250 BC₄F₂ plants showed the CSSL58 phenotype (3:1 ratio; $\chi^2 = 3.07 < \chi^2_{0.05} = 3.84$), indicating that the small panicle/dwarf plant was controlled by a single recessive gene referred to as *spd6*. These data confirmed that the chromosomal segment containing the recessive gene *spd6* from wild rice was responsible for the small panicle/dwarf phenotype. Further mapping of *spd6* was conducted using a total of five SSR markers (Fig. 1a). The *spd6* gene was validated and mapped primarily to a 6.1 cM region between the markers RM8101 and RM8200 (Fig. 1a).

Fine mapping of *spd6*

To further refine the position of *spd6*, we used a larger BC₄F₃ population containing 2,497 plants derived from the

Fig. 1 Genetic and physical maps of the *spd6* gene and candidate gene analysis.
a Linkage map of chromosome 6 constructed using 250 BC₄F₂ individuals. The *spd6* gene was mapped to the region between markers RM8101 and RM8200. Numbers show genetic distance between adjacent markers.
b Fine mapping of the *spd6* gene. The *spd6* gene was restricted to the region between markers SH03 and JX6036. The number of recombinants between the markers and *spd6* is indicated under the linkage map.
c High-resolution genetic map of *spd6*. The *spd6* gene was narrowed down to a 22.4 kb region in the BAC clone OSJNBa0041F13 between makers Q5 and JX6036 using a total of 24,120 plants from segregated populations.
d Candidate region of the *spd6* locus and the annotated gene in *japonica* Nipponbare from TIGR (<http://www.tigr.org/>)



heterozygous plants from the BC₄F₂ population. The location of the *spd6* gene was narrowed down to the 31.8 kb region defined by the markers SH03 and JX6036, according to the Build 5 pseudomolecules of the Nipponbare genome released by the International Rice Genome Sequencing Project (IRGSP) in 2008 (<http://rgp.dna.affrc.go.jp/E/IRGSP/Build5/build5.html>). Eight markers were newly developed and used in further fine mapping of the *spd6* gene (Table 1). We obtained 19 recombinants in the target region by screening 24,120 plants in the BC₄F₃ population plants using markers SH03 and JX6036. Finally, the *spd6* gene was precisely mapped to the 22.4 kb interval defined by markers Q5 and JX6036.

Candidate genes in the 22.4 kb target region

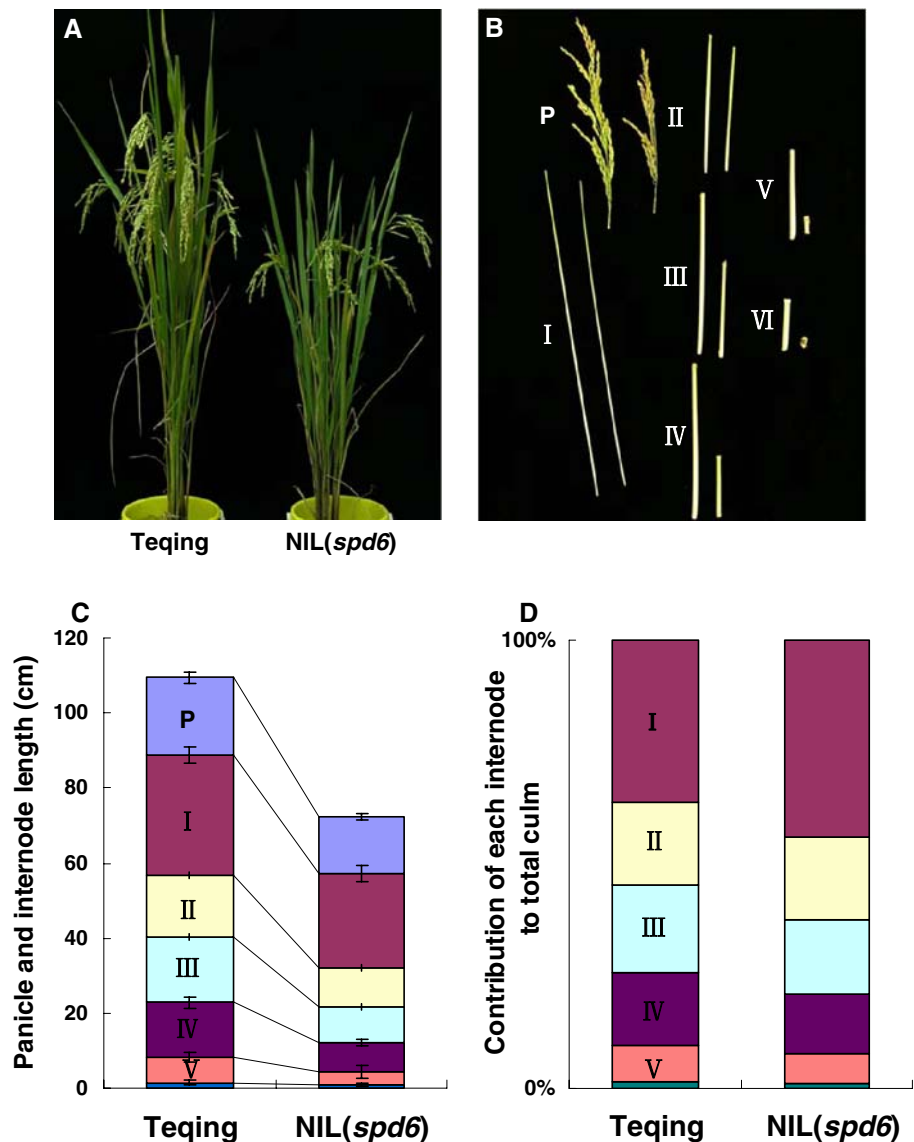
According to available sequence annotation databases (<http://www.tigr.org/>; <http://www.rgp.dna.affrc.go.jp/>), there are four annotated genes (LOC_Os06g04810, LOC_Os06g04820, LOC_Os06g04830 and LOC_Os06g04840) in the 22.4 kb target region. LOC_Os06g04810, LOC_Os06g04830 and LOC_Os06g04840 were leucine-rich repeat proteins and classified as putative *HcrVf3* proteins. LOC_Os06g04820 was expressed and had a corresponding full-length cDNA (AK063371) in GenBank, which was a gene classified as a putative leucine carboxyl methyltransferase-1 (LCMF-1) with 13 exons and a transcript length of 1,315 bp.

Characterization of *spd6*

The *spd6* gene exhibited pleiotropism by influencing almost every visibly morphological phenotype. The NIL(*spd6*) line, containing a small wild rice homozygous chromosomal segment of about 6.0 cM in the Teqing genetic background, was used to characterize the magnitude and behavior of the *O. rufipogon*-derived allele in the domesticated *indica* rice Teqing.

Figure 2a shows the gross morphology of NIL(*spd6*) (right) compared with the isogenic control Teqing (left) grown under natural long-day conditions in a paddy field in Shanghai. After heading, the NIL(*spd6*) plants reached ~66% of the height of the Teqing plant. Rice dwarf mutants can be categorized into six groups based on the elongation patterns of the upper four to five internodes (Takeda 1977). In order to determine the type of dwarf with which *spd6* might be associated, we measured the length of the panicles and internodes of Teqing and NIL(*spd6*). Elongation was suppressed in all internodes and panicles in NIL(*spd6*), resulting in this dwarf phenotype (Fig. 2a–c). From top to bottom, the upper five internodes in NIL(*spd6*) were reduced by 21, 37, 45.4, 46.5 and 47% compared with Teqing (Fig. 2b–d). In NIL(*spd6*), the length of each internode was reduced, and the internode elongation pattern was similar to that of the wild-type Teqing (Fig. 2b, c). By comparing to the internode elongation patterns of six groups of dwarf mutants described

Fig. 2 Morphology and characterization of plant type. **a** NIL(*spd6*) (right) and the recurrent parent, Teqing (left), at the milky dough stage of rice. **b** The appearance of the panicles and internodes of Teqing (left) and NIL(*spd6*) (right). **c** Comparison of the length of the panicles and internodes of NIL(*spd6*) (right) and Teqing (left). Data are averages of the lengths of the panicles and internodes of 16 main culms. In NIL(*spd6*), the lengths of almost all of the internodes were reduced, P panicle. The number of each internode is labeled. **d** Schematic representation of internode elongation patterns of Teqing and NIL(*spd6*)



previously (Takeda 1977), we associated *spd6* with the *dn*-type of dwarf mutants.

Several traits were phenotypically evaluated between Teqing and NIL(*spd6*) at two sites, Shanghai (31°N, 121°E) and Sanya (18°N, 109°E). The results showed that NIL(*spd6*) maintained a stable phenotype in all trials at both sites (Fig. 3). Morphologies of both Teqing and NIL(*spd6*) were affected by the photoperiod, with some differences between the plants in Sanya and in Shanghai, especially in plant height, tillers per plant, spikelet fertility and grain yield per plant (Fig. 3b, c, f, h). Although the length of NIL(*spd6*) panicles reached ~90% of that of Teqing, and NIL(*spd6*) plants also reached ~83.6% of the height of the Teqing plant in Sanya, the NIL(*spd6*) plants could be easily distinguished from the Teqing plants by their low spikelet fertility (32.6%). Figure 3a shows the panicle morphologies of NIL(*spd6*) (right) and Teqing

(left). NIL(*spd6*) showed shortened panicles (~72% length of that of Teqing in Shanghai) and decreased number of grains per panicle (~53.2% of that of Teqing in Shanghai).

NIL(*spd6*) also had small grains. The differences in grain length, width and weight among NIL(*spd6*), Teqing and wild rice are shown in Fig. 4a–d. The grains of NIL(*spd6*) were significantly shorter than those of the parent plants (Fig. 4b). The width of NIL(*spd6*) grains averaged 3.06 mm, between the 3.31 mm width of Teqing and 2.27-mm width of wild rice grains (Fig. 4c). The weight of the NIL(*spd6*) grains was almost equal to the mid-parent value, reaching 73.3% of the weight of the cultivated recurrent parent Teqing and they were 38.5% heavier than their wild donor parent *O. rufipogon* (Fig. 4d).

Figure 5 shows the result of pollen viability evaluations. Pollen grains that were round in shape and stained black were considered to be viable. About 99% of the pollen

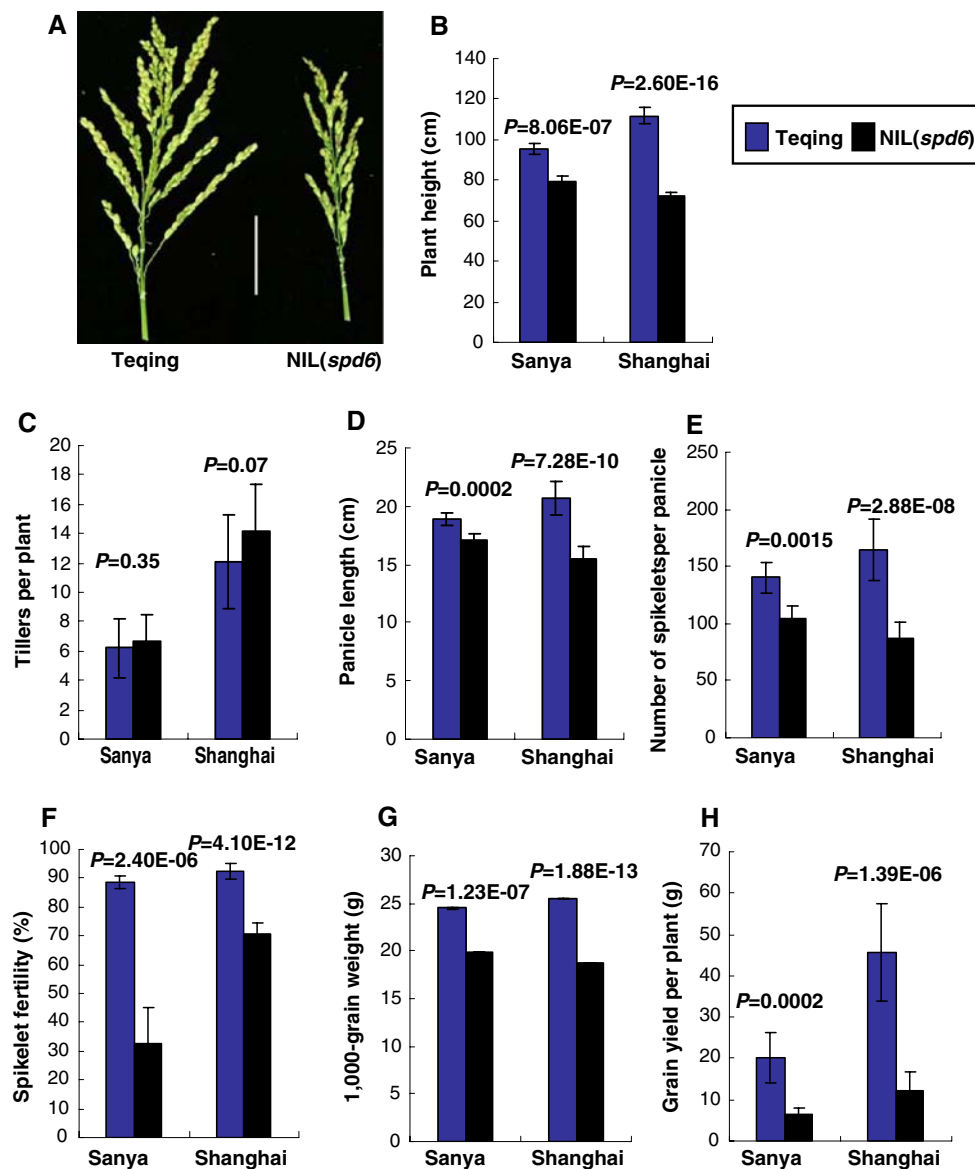


Fig. 3 Phenotypic evaluations of plant height and panicle traits for NIL(*spd6*) and the recurrent parent, Teqing at two sites, Sanya and Shanghai, China. **a** Panicle phenotypes of NIL(*spd6*) (right) and Teqing (left) at the milky stage. Scale bar 5 cm. **b** Comparison of plant height of NIL(*spd6*) and Teqing. **c** Comparison of tillers per plant of NIL(*spd6*) and Teqing. **d** Comparison of panicle length of NIL(*spd6*) and Teqing. **e** Comparison of spikelet number per panicle of NIL(*spd6*) and Teqing. **f** Comparison of spikelet fertility of

NIL(*spd6*) and Teqing. **g** Comparison of 1,000 grain weight of NIL(*spd6*) and Teqing. **h** Comparison of grain yield per plant of NIL(*spd6*) and Teqing. The data were obtained from the trials conducted at Sanya in 2005, and at Shanghai in 2006. Eight plants in Sanya and 16 plants in Shanghai were individually reaped and evaluated. Data are the averages of those plants or of the corresponding main panicles. All data are given as mean $\pm$ SE. *P* values were generated using a *t* test

grains of Teqing were viable, and they were of uniform size and shape (Fig. 5a). Meanwhile, 9.1% of the pollen grains of NIL(*spd6*) pollens were non-viable, and about 27% of them were smaller than normal controls (Fig. 5b). Therefore, these smaller pollen grains may be considered viable, but their pollen tubes may not be able to elongate long enough to fertilize. Low pollen tube growth and fertilization efficiency in NIL(*spd6*) may account for the low

spikelet fertility (Fig. 3f). The stigma viability was also tested, and there was no difference between Teqing and NIL(*spd6*) (data not shown).

Taken together, the above results show that the *spd6* gene from wild rice has pleiotropic effects on many important agronomic traits and suggest that *spd6* played an important role in development of agronomic traits during the domestication of rice.

Fig. 4 Morphology and characterization of grain type. **a** Grain phenotypes of Teqing (left), NIL(*spd6*) (middle) and wild rice (right). Scale bar 3 mm. **b** Comparison of grain length. **c** Comparison of grain width. **d** Comparison of 1,000 grain weight. The data were obtained from the trials conducted at Shanghai in 2006 [for Teqing and NIL(*spd6*)], and at Sanya in 2005 (for wild rice). Twenty grains were selected and measured. 1,000 grain weight was measured using 100 grains and three replicates. Columns with different letters were significantly different ($P < 0.01$, least significant difference test)

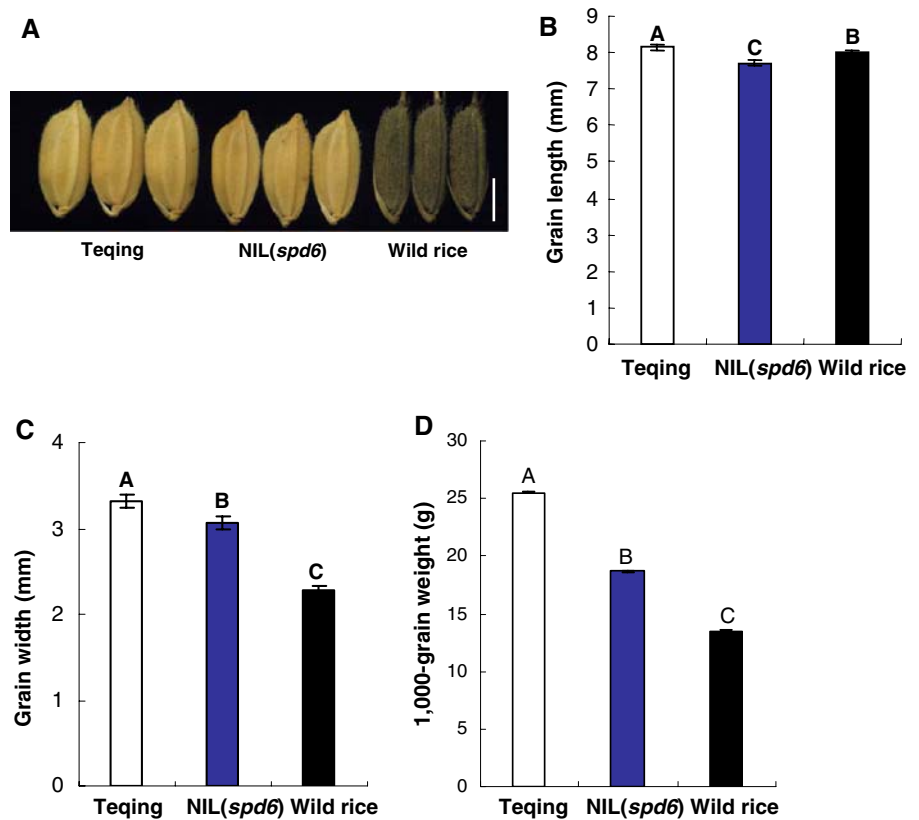
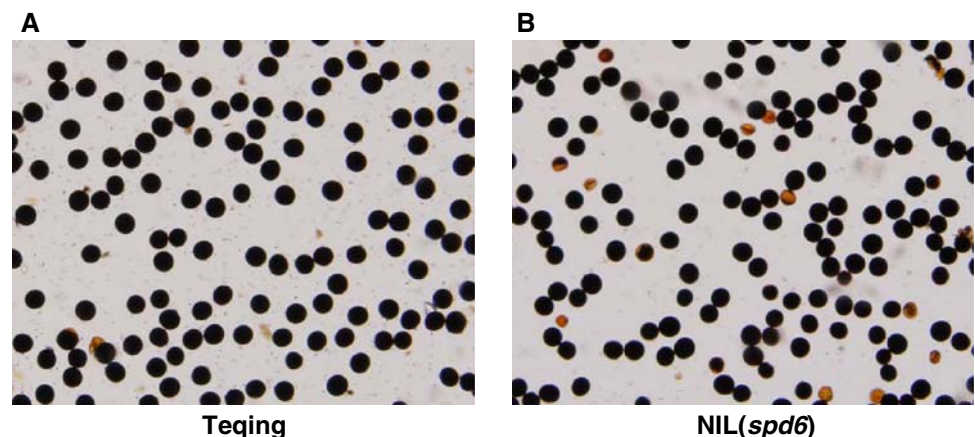


Fig. 5 Pollen grains stained with I_2 -KI solution from Teqing (a) and NIL(*spd6*) (b). Pollen grains staining black were considered viable, and those staining yellow or light red were considered sterile



Discussion

The most significant finding in our study was the delimitation of the *spd6* gene in wild rice to a DNA fragment of approximately 22.4 kb in length. This gene had effects on many characteristics, including panicle size, number of grains per panicle, grain weight and plant height. Identification of quantitative trait loci (QTL) has become a popular topic in genetics in recent years, and QTL mapping has become a very important tool for finding the genes that regulate complex quantitative traits. F_2 populations, recombinant inbred lines (RILs) and doubled haploid lines

(DHs) have been used widely for primary mapping. Advanced populations such as near isogenic lines (NILs) and chromosomal segment substitution lines (CSSLs) can be used for fine mapping QTLs to a locus as a Mendelian factor by blocking genetic background noise (Xing et al. 2008). We developed a set of chromosome segment substitution lines (CSSLs) from an *O. sativa*–*O. rufipogon* cross to clarify the genetic basis of complex traits, such as yield, plant architecture, response to environmental stresses and rice domestication. This set of CSSLs has been used for mapping and cloning of *PROG1*, a gene that regulates the architecture of wild rice and is involved in rice

domestication (Jin et al. 2008). This set of CSSLs also has been used to detect the QTLs for rice quality traits and a total of 15 QTLs was found (Hao et al. 2006). Therefore, this set of CSSLs is suitable for detecting the genes or QTLs for complex traits in wild rice by whole genome surveying.

Wild rice has adapted to unfavorable environments under natural biotic and abiotic stress tolerance selection, and it has been recognized as a natural gene bank that conserves many specific genes not presently found in cultivated rice. There is an urgent need to discover useful genes hidden in the wild rice genome and to apply these findings to improve agricultural traits in crop breeding. Here, we identified the *spd6* gene from wild rice (*O. rufipogon* Griff.). Although the NIL(*spd6*) showed a small panicle, small grain, low spikelet fertility and a dwarf phenotype, *spd6* may help to elucidate the rice domestication process. Two groups of wild rice (*O. rufipogon* Griff.) were found in China using SSR markers that were differentiated based on geography: one group consisted of populations of wild rice from Hainan Island, and the other group derived from provinces to the north (Zhou et al. 2003). The presence of *spd6* in all wild rice such as *O. rufipogon* Griff., and other species, is still under investigation, and this information may provide a unique chance to address several critical questions in rice evolution. The fact that plant height was separated in the populations from the crosses between CSSL58 and the *japonica* variety Zhonghua 11 (data not shown), and CSSL58 and the *indica* variety, Teqing, informed us that this gene had been functionally changed both in *indica* and *japonica*. Interestingly, this gene is recessive in wild rice, while the alleles at *spd6* in Teqing and Zhonghua 11 are dominant. It is unclear whether a recessive loss-of-function mutation at *SPD6* in the wild rice we collected from Hainan province, China, occurred naturally after the domestication of *indica* and *japonica* or whether a dominant gain-of-function mutant of *SPD6* was selected during rice domestication.

Based on the available sequence annotation database (<http://www.tigr.org/>; <http://www.rgp.dna.affrc.go.jp/>), the candidate gene for *spd6* was identified. There are four predicted genes in the target region according to the genome sequence of Nipponbare (*O. sativa* ssp. *japonica*). Of these, the most likely candidate was LOC_Os06g04820, a putative leucine carboxyl methyltransferase-1 (LCMF-1). The carboxyl methyltransferase, which is known to methylate the carboxyl group of the C-terminal leucine residue of the catalytic protein phosphatase 2A (Leu³⁰⁹), was originally purified from porcine brain tissue (De Baere et al. 1999). Protein phosphatase 2A (PP2A) is a major cellular serine/threonine phosphatase that plays an important role in the regulation of cell growth and proliferation, primarily at the G₂/M transition, and in the development of

human cancers (Janssens and Goris 2001). LCMF-1 was also found to be necessary for normal progression and cell survival through mitosis and it is essential for embryonic development in mice (Lee and Pallas 2007). Corresponding research in plants has not been reported previously.

We have shown here that several plant traits are influenced by *spd6* and we speculate that LOC_Os06g04820 is a strong candidate for the underlying *spd6* locus. The *spd6* phenotype may be produced by the change in expression of target genes downstream of PP2A. The other three candidates LOC_Os06g04810, LOC_Os06g04830 and LOC_Os06g04840 were all leucine-rich repeats and putative *HcrVf3*-like proteins, according to information from GenBank. These genes were $\geq 45.9\%$ homologous in protein sequence. Because LRR domains are believed to be critical for the binding and recognition of molecules, these different candidate genes may recognize different plant pathogens. *HcrVf3* was cloned (Vinatzer et al. 1998) at the *Vf* locus from the *Vf*-containing *M. × domesticacv.* Florica. The *Vf* locus from *Malus floribunda* was an important source of resistance to apple scab disease, the most important disease affecting apples. This locus contained a cluster of four receptor-like genes, and *HcrVf3* was one of them. *HcrVf3* was either expressed at a very low level or was a non-functional pseudogene. It is not clear whether these candidate genes were responsible for plant disease resistance in rice, and there is no proof available that the *HcrVf* gene influences the development of the rice panicle and or plays a role in height. Therefore, LOC_Os06g04810, LOC_Os06g04830 and LOC_Os06g04840 likely do not represent the *spd6* gene. Of course, the gene underlying the *spd6* phenotype might have other candidates in light of the information available. Our analysis was conducted based on the genome sequence of the cultivated *japonica* rice Nipponbare, while *spd6* was derived from wild rice. Considering the DNA sequence diversity between cultivated and wild rice, some genes may have been lost in the cultivated rice during its domestication. We are conducting ongoing studies to completely sequence the region of the wild rice genome corresponding to the 22.4 kb target region in Nipponbare in order to determine the candidate. At the same time complement testing is being performed using these four candidate genes.

Thus far, several genes have been identified that likely play key roles in the development of rice panicle development. Apart from *Gn1a* (Ashikari et al. 2005) and *Ghd7* (Xue et al. 2008), *gpa7* was narrowed down to a 35 kb region and reported to play an important role in the regulation of grain number per panicle during the domestication of rice (Tian et al. 2006). *FZP* can prevent the formation of axillary meristem within the spikelet meristem and can permit the subsequent form of floral meristem. *FZP* encodes an ERF transcription factor and is the rice ortholog

of the maize *BD1* gene (Komatsu et al. 2003b). The *LAX* and *SPA* genes were identified as having overlapping functions as the main regulators of axillary meristem formation in rice (Komatsu et al. 2003a). *LAX* and *FZP* may operate in genetically independent pathways (Komatsu et al. 2001). The *LONELY GUY (LOG)* gene of rice encodes a novel cytokinin-activating enzyme that functions in the final step of bioactive cytokinin synthesis. The *log* gene resulted in a severe reduction of the panicle size and a decrease in the number of floral organs (Kurakawa et al. 2007). When compared with Teqing, NIL(*spd6*) not only significantly reduce panicle size, grain weight, number of grains per panicle and plant height, but also significantly reduce seed setting rate. The pleiotropism of *spd6* suggests that the alleles at *spd6* played an important role in rice panicle and spikelet morphogenesis during the domestication of rice and that *spd6* may be a model for the strong artificial selection used during the process of rice domestication. Studies of the genetic basis and function of *spd6* are underway.

A number of genes and QTLs have been identified that are involved in rice grain yield by influencing grain weight, grain number per panicle and plant height, among other traits. These results help us in understanding the molecular mechanisms underlying yield traits. In addition, our continuing work will also certainly be used to help improve grain yield and quality in crops through approaches such as marker-assisted selection (MAS), metabolic engineering and transgenic modification.

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