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# *Du1*, encoding a novel Prp1 protein, regulates starch biosynthesis through affecting the splicing of $Wx^b$ pre-mRNAs in rice (*Oryza sativa* L.)

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**Abstract** Starch is the major component of cereal grains. In rice, starch properties determine the eating and cooking quality. The dull endosperm of rice grains is a classical morphological and agronomical trait that has long been exploited for breeding and genetics study. To understand the molecular mechanism that regulates the starch biosynthesis in rice grains, we characterized a classic rice mutant *dull endosperm1* (*du1*) and isolated *Du1* through a map-based cloning approach. *Du1*, encoding a member of pre-mRNA processing (Prp1) family, is expressed mainly in panicles. *Du1* specifically affects the splicing efficiency of $Wx^b$ and regulates starch biosynthesis by mediating the expression of starch biosynthesis genes. Analysis of *du1wx* shows that *Du1* acts upstream of $Wx^b$. These results strongly suggest that Du1 may function as a regulator of the starch biosynthesis by affecting the splicing of $Wx^b$ and the expression of other genes involved in the rice starch biosynthetic pathways.

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**Keywords** *Du1* · Prp1 · Splicing efficiency · Starch biosynthesis · Rice (*Oryza sativa* L.)

**Abbreviations**

| | |
|---|---|
| AC | Amylose content |
| AGPase | ADP-glucose pyrophosphorylase |
| ASV | Alkali spreading values |
| BAC | Bacterial artificial chromosome |
| CAPS | Cleaved amplified polymorphic sequences |
| DAP | Days after pollinating |
| DBE | Starch debraching enzyme |
| *du1* | *Dull endosperm1* |
| Du1L | Du1-like |
| GBSS | Granule-bound starch synthase |
| GC | Gel consistency |
| Prp | Pre-mRNA processing |
| QTL | Quantitative trait locus |
| SBE | Starch branching enzyme |
| SR | Serine–arginine-rich |
| SSS | Soluble starch synthase |
| STS | Sequence tagged site |
| TPR | Tetratric peptide repeats |
| UTR | Untranslated region |

## Introduction

Starch comprises ~90% of the dry weight of rice grain, the staple food for more than half of the world's population. Starch properties determine various aspects of rice quality, especially eating and cooking quality (Bao et al. 2004). Varieties different in amylose contents (ACs) have long been utilized to improve grain quality in breeding. High AC, in general, lowers rice eating quality, especially the

stickiness of cooked rice, because high AC rice becomes dry and fluffy after cooking. Genetic and molecular marker-based QTL analyses have revealed that AC is mainly controlled by a major locus *Wx* and multi minor loci (Aluko et al. 2004; Bao et al. 2002; Fan et al. 2005; Hirano and Sano 2000; Lanceras et al. 2000; Okuno 1983; Septiningsih et al. 2003; Tan et al. 1999; Yano et al. 1988), including the 11 *dull endosperm* loci, *du1* to *du11* (Kinoshita 1987; Koh 1997; Satoh and Omura 1981, 1986). The *du1* mutant was first reported in 1981 (Satoh and Omura 1981), which has a lower endosperm AC and intermediate degree of translucence when its grains are dry. Biochemical analysis suggested that the relative low AC in the *du1* mutant is caused by a decreased level of Wx protein (Sano et al. 1985).

At least two functional alleles of the *Wx* gene, $Wx^a$ and $Wx^b$, have been identified in Asian cultivated rice (*Oryza sativa* L.). The abundance of mature *Wx* transcripts and Wx protein in the $Wx^a$ grains are about 10-fold higher than in $Wx^b$ seeds. $Wx^a$ is widely distributed in *O. sativa* spp. *indica*, a subspecies with higher AC, whereas $Wx^b$ mainly in *japonica*, a subspecies with intermediate AC. It has been shown that the low level of the mature transcripts of $Wx^b$ is largely caused by a single nucleotide substitution of G-to-T at the splicing donor site of the first intron in $Wx^b$, which results in the inefficient splicing of the $Wx^b$ pre-mRNA and the activation of two cryptic splice sites in exon 1 (Cai et al. 1998; Frances et al. 1998; Isshiki et al. 1998).

Although *du1* has been extensively exploited in rice breeding (Qian et al. 1996), the understanding of starch biosynthesis in *du* mutants is still very limited. We report here the map-based cloning of *Du1* and the elucidation of the molecular mechanism that *Du1* regulates starch biosynthesis through altering the splicing efficiency of $Wx^b$ pre-mRNA in rice.

## Materials and methods

### Plant materials

The *du1* mutant line *kl704* (spp. *japonica*) was crossed with a *japonica* cultivar *Xiushui11* (Qian et al. 1991) and an isogenic line carrying the *du1* mutation was generated by 12 rounds of backcrosses with *Xiushui11*. For convenience, this isogenic line was renamed as the *du1* mutant in this work and *Xiushui11* was regarded as the wild type. To map and clone the *Du1* gene, *du1* was crossed with an *indica* variety, *Minghui63*, to construct an $F_2$ mapping population.

All the rice materials including *Xiushui11*, *Zhefu802* (*ZF802*), *Qiufengnuo* (*QFN*, a Chinese elite glutinous cultivar with a homozygous null mutation of *wx*), and *du1* plants were cultivated in the field either at the China National Rice Research Institute or at Hainan Island in the natural growing seasons. For sampling, panicles were harvested 18 days after pollination (DAP). Leaf blades, sheathes, culms, and roots were sampled at the day of pollination and grains at the mature stage. Each sample was a collection of four independent major tillers, each from a different plant. All samples except the mature grains were immediately frozen in liquid nitrogen and stored at –80°C until use.

### Genetic analysis and marker development

The *Du1* locus was primarily mapped to Chromosome 10 with two newly developed STS markers, P2 and P3. To fine map *Du1*, additional five STS and four CAPS makers were developed (Supplemental Table 1) based on the sequence differences between *indica* var. *93-11* and *japonica* var. *Nipponbare* (http://www.ncbi.nlm.nih.gov).

### Complementation test

An 8,285-bp genomic DNA fragment, containing an entire *Du1* coding region, a 3,051-bp upstream region, and a 2,114-bp downstream sequence, was inserted into the binary vector *pCAMBIA1300* to generate the transformation plasmid *p1300Du1* for complementation. The two plasmids, *p1300Du1* and its control *pCAMBIA1300*, were introduced into *Agrobacterium tumefaciens* EHA105 by electroporation and rice transformation was carried out as previously described (Li et al. 2003a). To identify the *Du1* transgene in the transgenic lines, a CAPS marker dudc was developed based on the nucleotide difference between the mutant and wild type plants (Supplemental Table 1).

### Sequence and phylogenetic analyses

The Prp1 family protein sequences were retrieved from SwissProt or GenBank and used for phylogenetic analyses. Multiple sequence alignments were conducted using ClustalX version 8.0 (Thompson et al. 1997). A neighbor-joining tree was built using MEGA version 2.1 adopting Poisson correction distance and the tree was presented using TreeView (Page 1996). Support for the tree obtained was assessed using the bootstrap method with 1,000 replicates. Similar topology was obtained by using the Protpas program in the Phylip package to estimate maximum parsimony and the Proml program in the Phylip package to estimate maximum likelihood (Felsenstein 2000). Alignments over the conserved regions of each family member produced trees of similar topology.

### RNA extraction and cDNA preparation

Total RNAs were isolation from seed endosperm and other tissues as described previously (Mou et al. 2000). Total RNA (1 μg) was first treated with 1 unit of RNase-free DNase (Invitrogen) at room temperature for 15 min, denatured at 70°C for 10 min in a 10 μl reaction mixture containing 1 μl 25 mM EDTA, and put on ice immediately. The first-strand cDNA was synthesized according to the manufacture's protocol of Reverse Transcription System (Promega).

### Quantitative real-time RT-PCR

Quantitative assay of transcript abundance was performed with 1 μl of each cDNA diluted with SYBR Green Master mix and assayed with an ABI 7900 sequence detection system according to the manufacture's protocol (Applied Biosystems). To distinguish the spliced transcripts from the intron-containing transcripts, gene-specific primers were designed and listed in Supplemental Table 2. The amplicon of *Actin* mRNA was used as an internal control. The relative quantification method ($\Delta\Delta C_T$) was used to evaluate the relative abundance of transcripts (Lan et al. 2004).

### Analysis of starch properties

Approximately 40 days after heading, rice grains were harvested, air-dried and stored at room temperature for 3 months before milling. Twelve grains of milled rice were selected for measuring the alkali spreading values (ASV), and 10 g grains were ground to flour and used to measure the AC and gel consistency (GC). AC (%) was measured as described previously with slight modification (Juliano 1971). Briefly, samples were boiled for 10 min in the volumetric flasks to completely disperse the grain powder and the optical density of the amylose-iodine blue was measured at 620 nm using a spectrophotometer. GC was measured using 100 mg of milled rice flour. The flour was first wetted with 0.2 ml of 95% ethanol containing 0.025% (w/v) thymol blue in 11 × 100 mm culture tubes, followed by adding 2 ml of 0.2 N KOH, and mixed vigorously. Tubes were covered with glass marbles, heated in a boiling water bath for 8 min, mixed again, and kept in ice water bath for 20 min. Finally, the tubes were laid horizontally against a ruled graphing paper and gel length was measured after 1 h. ASV was determined by incubating six milled grains in 10 ml of 1.7% KOH at 28°C for 23 h with two replicates. The degree of spreading was rated using the following 7-point semi-quantitative criteria: 1, grain not affected; 2, grain swollen; 3, grain swollen, collar incomplete and narrow; 4, grain swollen, collar complete and wide; 5, grain splitted, collar complete and wide; 6, grain dispersed, merging with collar; 7, grain completely dispersed and intermingled.

### Sequence analysis

Bioinformatic analyses were performed using the Lasergene software package (DNASTAR, Inc., Madison, WI). Blast searches were performed using the Tblastn program (http://www.ncbi.nlm. nih.gov/BLAST/) with the default parameters.

## Results

### Amylose content of *du1* grains

The phenotype of *du1* mutant grains was compared with those of *indica ZF802*, *japonica Xiushui11* and glutinous rice *QFN* grains. As shown in Fig. 1A, the completely dried *du1* grain exhibits an intermediate degree of translucence in the cross-section, showing a higher similarity to glutinous rice *QFN* than to the grains of *indica ZF802* or *japonica* WT. When stained with $I_2$-KI, the *du1* grain looks dark blue, distinctive from the brown red of *QFN* (Fig. 1B). The AC of *du1* grains is dramatically decreased compared to those of the wild-type *Xiushui11* or *ZF802*

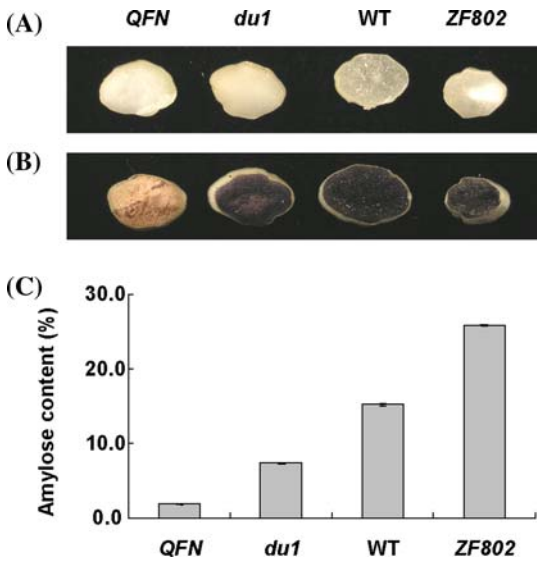


**Fig. 1** Phenotypes and amylose contents of the wild-type and *du1* mutant. (**A**) Cross-sections of *QFN*, *du1*, wild-type, and *ZF802* grains, showing that the *du1* is semi-translucent, different from the opaque *QFN* and transparent wild-type grains. (**B**) Cross-sections stained with $I_2$-KI, showing that the *du1*, wild-type and *ZF802* are dark blue, a sharp contrast to the brown color of *QFN*. (**C**) The amylose contents of *QFN*, *du1*, wild-type, and *ZF802*. Data are means ± SE from at least three independent measurements

grains, but was still significantly higher than that of glutinous varieties (Fig. 1C).

## Map-based cloning of *Du1*

To clone the *Du1* gene through a map-based approach, we generated a large $F_2$ mapping population derived from a cross between *du1* and *Minghui63*, in which a total of 1,936 segregants showed the *du1* mutant phenotype. Based on the previous mapping result that *du1* is located on Chromosome 10 (Eguchi et al. 1998), a set of PCR-based molecular markers were developed and *Du1* was placed on the genome segment between the two markers, P2 and P3, with a genetic distance of 1.6 and 1.1 cM, respectively (Fig. 2A, Supplemental Table 1). Between P2 and P3 markers, additional nine molecular markers (P7–P15) (Supplemental Table 1) were developed and *Du1* was fine mapped in an interval of ~65-kb region between markers P9 and P10 (Fig. 2B, C). To find the mutation lesion of *du1*, the 65-kb genomic DNA segments from wild-type and mutant plants were sequenced and compared. A mutation was identified in the predicted *OSJNBa0017E08.20* gene in the *du1* genome. Comparison of the *OSJNBa0017E08.20* cDNA sequences between *du1* and wild-type plants showed that the mutation in *du1* results from a substitution of G by A (G1742 → A1742) in exon 1, leading to a non-synonymous change from serine to asparagine (Fig. 2D).

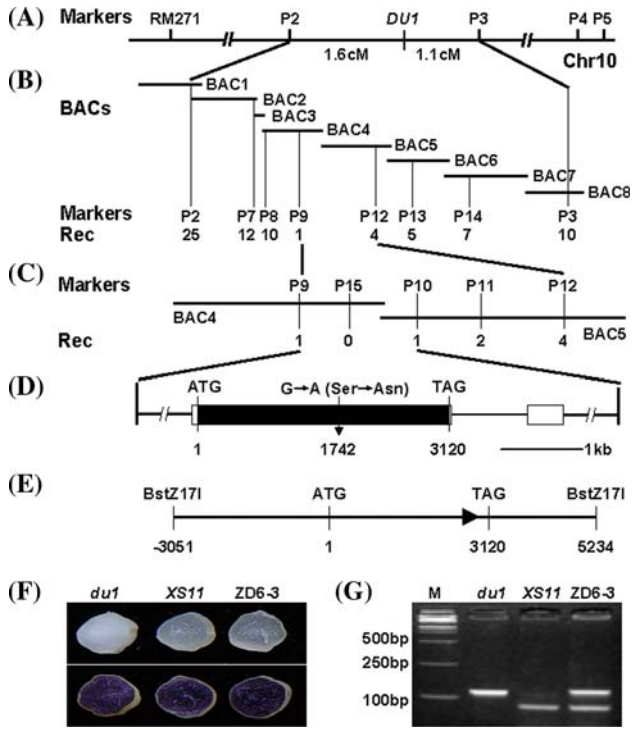


**Fig. 2** Cloning and confirmation of the *Du1* gene. (**A**) The *Du1* locus was mapped to a region between markers P2 and P3 on chromosome 10. (**B**) A BAC contig covering the *Du1* locus. The numerals indicate the number of recombinants identified from 1,936 *du1* $F_2$ plants. BAC1, OSJNBa0051D19; BAC2, OSJNBa0041P03; BAC3, OSJNBa0066I08; BAC4, OSJNBa0017E08; BAC5, OSJNBa0078O01; BAC6, OSJNBb0073N24; BAC7, OSJNBa0062C05; BAC8, OSJNBb0015K05. (**C**) Fine mapping of the *Du1* locus. The *Du1* locus was narrowed to a ~65-kb region between markers P9 and P10 and cosegregated with marker P15. (**D**) Structure of *Du1*, showing the *du1* mutation site. The start (ATG) and stop codon (TAG) are indicated. Closed boxes stand for exons, open boxes for the 5′ and 3′ UTRs, and lines for introns. (**E**) The complementation construct *p1300Du1* containing the entire *Du1* gene. (**F**) The phenotypes of the transgenic line *ZD6-3* and its controls. (**G**) Identification of transgenic plants with the dCAPS marker dudc

To confirm that the mutation of *Du1* is responsible for the mutant phenotype, we carried out a genetic complementation experiment. The plasmid *p1300Du1* containing the entire wild-type *Du1* gene and its regulatory sequences, which consist of a 3,120-bp coding sequence, a 3,051-bp upstream sequence including the regulatory region and untranslated region (UTR), and a 2,114-bp downstream region (Fig. 2E), was introduced into the mutant plants through *Agrobacterium*-mediated transformation (Li et al. 2003b). All the eight independent transgenic lines carrying the *Du1* transgene showed a complete complementation of the *du1* phenotype (Fig. 2F), whereas all the six lines transformed by *pCAMBIA1300* failed to rescue the mutant phenotype (data not shown). The authentic transgenic plants were identified with a dCAPS marker dudc (Fig. 2G), which could distinguish the transformants from *du1* plants by digestion of the amplified transgene DNA with *AcuI* (Supplemental Table 1).

## *Du1* encodes a Prp1 protein

Sequence analysis of 5′- and 3′-RACE cDNA products indicated that the *Du1* full-length cDNA consists of two exons, including a 3,120-bp coding sequence, a 71-bp 5′ UTR, and a 479-bp 3′ UTR (Fig. 2D). Alignment analysis of the *Du1* sequences from four *indica* and four *japonica* varieties showed no variation at the amino acid level between *indica* and *japonica*, though four synonymous substitutes of bases were found (Supplemental Fig. 1), suggesting that *Du1* may play a very conserved role in rice.

BlastP search against SwissProt and GenBank databases found two Du1 homologs with 44.7% identity to Prp1p/Zer1p of *Schizosaccharomyces pombe* (Urushiyama et al. 1997) and 54.8% to *Homo sapiens* U5-102kD (Makarov et al. 2000). Previous studies have shown that the Prp1p/Zer1p protein involves in pre-mRNA splicing and the U5-102kD protein is a component of the U4/U6 snRNP required for spliceosome assembly. Therefore, it is likely that Du1 may function in mRNA maturation. Structural analysis revealed that Du1 has all the conserved features of the Prp1 family (Lamb et al. 1995), including a highly conserved N-terminal domain and a 19-repeat of tetratric

peptide repeats (TPR) at the C-terminal (Fig. 3A). Since the TPR domain functions in protein–protein interactions (Chung et al. 1999; Lockhart and Rymond 1994; Makarov et al. 2000), the substitution of serine by asparagines ($S_{581} \rightarrow N_{581}$) in the *du1* TPR7 motif may affect its interaction with other proteins, leading to the functional deficiency.

BlastP analysis identified a *Du1*-like (*Du1L*) gene located in Chromosome 1, which shares 73.9% identity to Du1 (Supplemental Fig. 2). Phylogenetic analysis showed that both Du1 and Du1L belong to a higher plant subfamily, but Du1 has a closer relationship to barley and maize than Du1L (Fig. 3B). The Prp1 family in cereal and dicots forms a monophyletic clade with 99% bootstrap support, suggests that the genes in monocots and dicots diverged from a common ancestral species. Phylogenetic analysis also demonstrated that Prp1 may serve as a molecular marker to determine the phylogenetic relation.

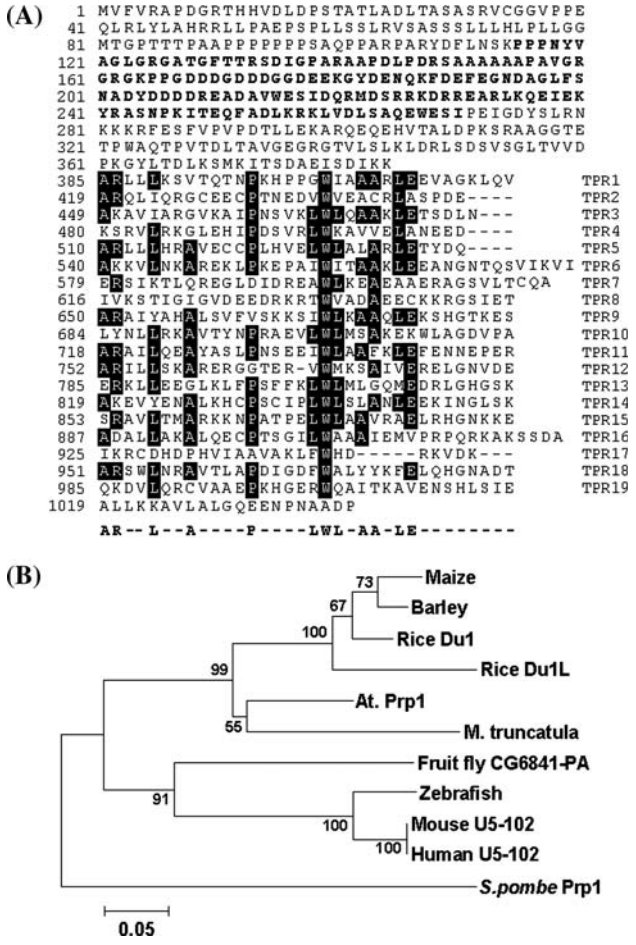


**Fig. 3** Structural features and phylogenetic analysis of Du1. (**A**) The predicted Du1 amino acid sequence and its 19 conserved TPR motifs, which are aligned with the consensus sequence of the *S. pombe* Prp1p/Zer1p and listed on the right. Bold letters indicate the specific N-terminal domain. (**B**) Phylogenetic relationship of Du1 and Du1L to other Prp1 proteins. Accessions included in the list are maize (*Zea mays* L., CD_439474), barley (*Hordeum vulgare* L., TC150206), rice Du1 (*Oryza sativa* L., NP_922169) and Du1L (NP_912872), AtPrp1 (*Arabidopsis thaliana*, NP_192252), *M. truncatula* (ABE92650), fruit fly CG6841-PA (*Drosophila melanogaster*, NP_649073), zebrafish (*Danio rerio*, AHH_56710), mouse U5-102 (*Mus musculus*, NP_598462), human U5-102 (O94906), and *S. pombe* Prp1 (CAA_17050). The number at each node represents the bootstrap support (percentage). The scale bar is an indicator of genetic distance based on the branch length

## Expression patterns of *Du1* and *Du1L*

The expression patterns of *Du1* and *Du1L* (Fig. 4A) were assayed with gene specific primers, pdu1 and pdu1L (Supplemental Table 1). As shown in Fig. 4B, *Du1* is expressed in all the five examined organs including panicles, blades, sheathes, culms and roots, with a highest expression in panicles (4-fold higher than in roots), showing a well-correlation to the starch biosynthesis in the organ (Fig. 4B). In contrast, *Du1L* has lower but relatively constant expression levels in all five organs, just ~10% of that in the *Du1* panicles (Fig. 4B). However, the expression of *Du1* or *Du1L* displayed no significant difference between the *du1* and wild-type plants (Fig. 4B).

## *Du1* affects maturation of the $Wx^b$ pre-mRNA

The findings that *Du1* encodes a protein highly homologous to pre-mRNA splicing proteins and that the AC of *du1*

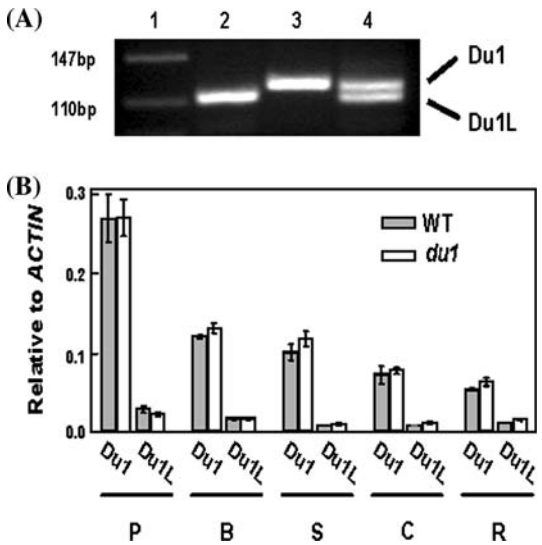


**Fig. 4** Expression analysis of *Du1* and *Du1L*. (**A**) *Du1* and *Du1L* are specifically amplified from the total RNA. Lane 1, DNA size markers; Lanes 2, *Du1*; Lanes 3, *Du1L*; Lane 4, *Du1* and *Du1L*. (**B**) The quantitative real-time RT-PCR analysis of *Du1* and *Du1L* expressed in different organs of panicles (P), blades (B), sheaths (S), culms (C) and roots (R) of *du1* and wild-type plants. The relative amounts of transcripts were expressed by the ratio of *Du1* and *Du1L* to Actin, respectively. Data are means ± SE from at least four independent measurements

grains is significantly decreased compared to that of the wild-type suggest that Du1 may function in splicing of pre-mRNAs involved in the grain starch biosynthesis. We therefore systematically examined the splicing efficiencies of the pre-mRNA transcripts of *Du1* and other 18 genes encoding starch biosynthesis enzymes including Wx, three AGPases, GBSSII, eight SSSs, three SBEs and two DBEs. As shown in Fig. 5A, the splicing efficiency of $Wx^b$ pre-mRNA transcripts in *du1* was remarkably decreased to ~30% of the wild-type, but the mRNA maturation of all the other starch biosynthesis genes examined was unaffected. These results strongly suggest that *Du1* may play a specific role in the maturation of $Wx^b$ pre-mRNAs.

The regulation of *Du1* on *Wx* was further confirmed by phenotypic comparison among *du1* (a homozygous $Wx^b$), *QFN* (a homozygous *wx*), and their double mutant *du1wx*. The grains of $du1Wx^b$, *Du1wx* and *du1wx* were all opaque (Fig. 5B), but their $I_2$-KI stained grain colors were apparently different: *du1wx* was brown red, similar to *Du1wx* but different from the dark blue of $du1Wx^b$ (Fig. 5C). Moreover, the amylose content of *du1dx* is almost the same as that of *Du1wx*, which is much lower than that of $du1Wx^b$ (Fig. 5D). These findings demonstrated that the phenotype of the double mutant *du1wx* was the same as that of the *Du1wx* mutant, indicating that *Du1* acts upstream of *Wx* in the starch biosynthetic pathway.

## Effect of *du1* on the expression of other starch biosynthetic pathway genes

The expression levels of many starch biosynthesis genes were also affected by the mutation of *Du1* (Fig. 6). Compared to the wild type, the transcripts of all the three genes encoding AGPase (*OsAGPiso*, *OsAGPL1* and *OsAGPS1*) were decreased by 26, 26 and 35%, respectively, but those of *ALK*, *OsSSIIIa* and *OsSSIVb* were increased in the mutant. As for genes encoding starch branching enzymes, the expression level of *OsSBEIIb* was increased in the *du1* mutant grains, but that of *OsSBEIIa* decreased. In addition, we also detected a significant increase in *OsPUL* transcripts in *du1* (Fig. 6). These results suggest that *Du1* may regulate the expression of the starch biosynthesis genes through an unidentified mechanism.

To understand whether the expressional alteration of the starch biosynthesis genes in the *du1* plant affects other grain properties besides AC, we compared the grain weight, alkali digestibility and gel consistency between the wild-type and *du1* grains. As shown in Table 1, the grain

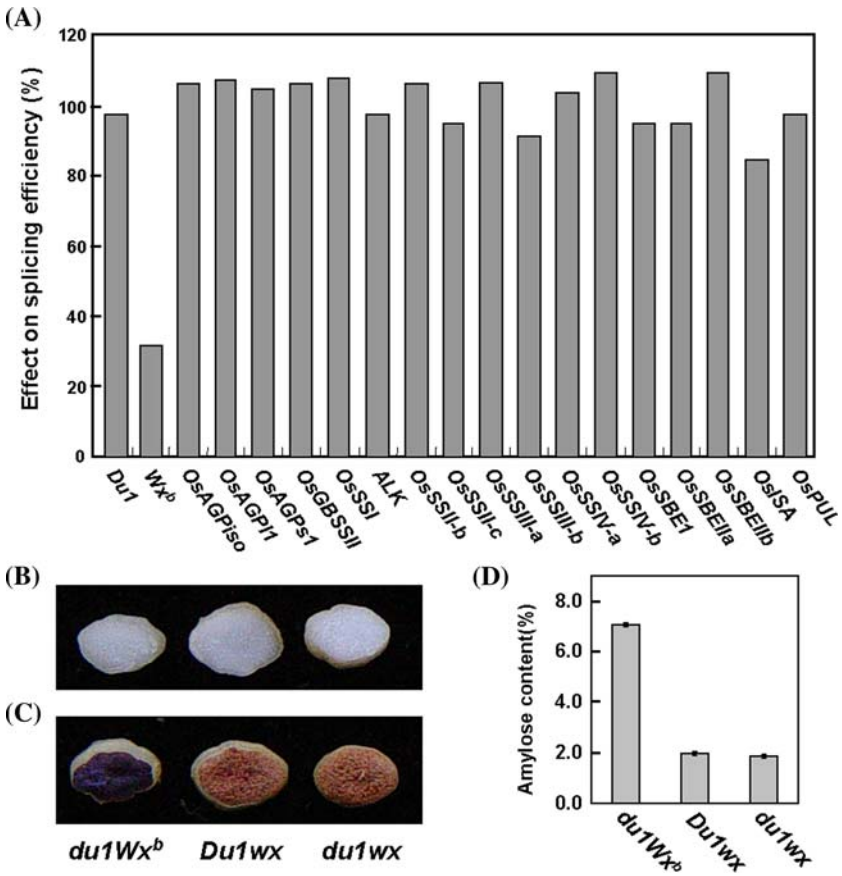


**Fig. 5** Effects of the mutation in *Du1* on the splicing of $Wx^b$ pre-mRNAs and the grain AC. (**A**) Effect on the splicing of $Wx^b$ pre-mRNAs in panicle. The value is the ratios of splicing efficiency of *du1* to wild type, showing that splicing efficiency of $Wx^b$ in *dul* is only ~30% of that in wild type. (**B**) The cross-sections of $du1Wx^b$, *Du1wx* and *du1wx*. (**C**) The cross-sections stained with $I_2$/KI in $du1Wx^b$, *Du1wx* and *du1wx*. (**D**) The ACs of $du1Wx^b$, *Du1wx* and *du1wx*

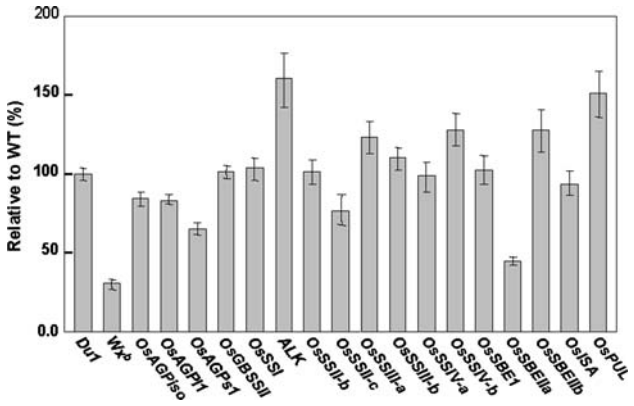


**Fig. 6** Effect of the mutation in *Du1* on the expression of starch biosynthesis genes in panicles. The value represents the relative abundance of mature transcripts in *du1* to that of in the wild type. Data are means ± SE from at least three independent measurements

weight of brown rice was decreased significantly in *du1*. The remarkable decrease in ASV (Supplemental Fig. 3) of the *du1* grains may reflect the significant increase in the expression of *ALK* (Fig. 6) that encodes OsSSIIa and controls the ASV in rice (Gao et al. 2003). In addition, the GC of *du1* grains is also increased compared to that of the wild type.

## Discussion

In this work, we report the genetic and molecular characterization of the classical rice mutant *du1*. Our results showed that *Du1* encodes a protein that is highly homologous to *S. pombe* Prp1 family members and involves in the splicing of the $Wx^b$ pre-mRNAs and the regulation of the expression of rice starch biosynthesis genes.

Pre-mRNA splicing in higher plants is believed to be regulated by mechanisms similar to those in mammals and *Drosophila* (Simpson and Filipowicz 1996). In mammals, the U5-102kD Prp1 protein interacts within the tri-snRNP with U4/U6 snRNPs and bridges the two particles through the contained TPR elements (Makarov et al. 2000). Structural features of Du1 suggest that it is a member of the Prp1 family and may function in the splicing of pre-mRNA processing. Based on the fact that the *du1* mutant grain has a lower AC, we therefore systematically compared the splicing efficiencies of the starch biosynthesis genes between *du1* and wild-type plants. The results clearly demonstrated that the mutation in *du1* specifically affects the splicing of $Wx^b$ pre-mRNAs (Fig. 5A), resulting in a low level of accumulation of mature $Wx^b$ transcripts, which in turn causes a dramatic decrease of AC in the *du1* mutant grains (Fig. 1C). This is consistent with the previous finding that an inefficient processing of pre-mRNA transcripts of $Wx^b$ will result in a lower AC grains in rice (Hirano et al. 1998; Wang et al. 1995). However, the question why the mutation of *Du1* specifically affects the splicing of the $Wx^b$ pre-mRNA remains to be answered.

*Du1* may also play a regulatory role in the starch biosynthetic pathways. Comparison of the expression profiles of all the genes required for the grain starch biosynthesis between *du1* and the wild type strongly suggests that *Du1* may regulate the starch biosynthesis by affecting the expression of some key genes in the pathway, for example *ALK* and *OsPUL* (Fig. 6). ALK controls the gelatilization temperature or AVS of rice grains (Gao et al. 2003), OsPUL facilitates the assembly of amylopectin (Fujita et al. 2003; Kubo et al. 1999), and OsDBEs contribute to the accumulation of amylopectin by clearing the improper branches in starch synthesis (Nakamura 2002). Therefore, it appears that *Du1* pleiotropically affects rice grain quality through regulating the gene expression of the starch biosynthetic pathways by two molecular mechanisms: post-transcriptional regulation of $Wx^b$ pre-mRNA transcripts and transcriptional regulation of some other key genes.

The yield of rice has been increased dramatically in the last century, but the poor cooking and eating quality of high-yielding cultivars and hybrid rice represents a major problem for rice production (Liu et al. 2003). The improvement of rice quality is increasingly demanded by consumers and has become a priority for rice breeders and genetists. Since AC is a pivotal factor in rice quality, many researchers have been trying to modify it by manipulating the expression levels of *Wx* transcripts via RNA inference or overexpression, and promising results have been reported (Itoh et al. 2003; Liu et al. 2003; Terada et al. 2000). Therefore, the cloning and molecular characterization of *Du1* will facilitate the molecular genetic engineering of rice.

**Table 1** Comparison of grain traits between the wild-type and *Du1*

| | Grains weight[a] (g) | Alkali digestibility (grade) | Gel consistency (mm) |
|---|---|---|---|
| Wild-type | 21.12 ± 0.128 | 6.94 ± 0.063 | 83.7 ± 0.760 |
| *du1* | 19.69 ± 0.200** | 3.75 ± 0.250** | 91.2 ± 1.241** |

**, Significance at 0.01

[a] 1,000-grain weight for brown rice and data are means ± SE

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