Euphytica (2021) 217:38
https://doi.org/10.1007/s10681-021-02766-3

Identification and fine mapping of a multi-tillering semi-dwarf gene in rice

Mingyue Wu · Junyu Chen · Dongqing Dai · Chengxing Du · Huali Zhang · Liangyong Ma

Received: 6 July 2020 / Accepted: 12 January 2021 / Published online: 11 February 2021

Abstract Plant height is one of the most important agronomic traits of rice. So far, more than 80 genes related to dwarf mutants had been cloned in rice, but most of them cause severe dwarf and other adverse phenotypes, which is difficult to apply in rice breeding. Here, we identified a novel multi-tillering semi-dwarf line *sde*, a near-isogenic line of ZX5T. Compared with ZX5T, *sde* performed proportionally shortened internodes. Thus, *sde* is a semi-dwarf of "dn" type. The longitudinal sections of stem showed that the decrease of cell number should be the major mechanism for *sde* semi-dwarfism. Moreover, *sde* was insensitive to exogenous GA_3 and GR24. Genetic analysis revealed that *sde* was controlled by single recessive nuclear gene. To isolate *SDE* gene, a map-based cloning method was employed using F_2 recessive plants derived from a cross between *sde* and NJ6. Finally, the target *SDE* gene was located to a 58 Kb region on the short arm of chromosome 6. There were 9 predicted opening reading frames located in this region, but only one nucleotide substitution (C to T) has been detected in the first exon of *Os06g0154200* between *sde* and ZX5T, which result in a substitution of amino acid (R to W). Additionally, expression of *Os06g0154200* in the culm and panicle of the *sde* was significantly increased compared to ZX5T. Interestingly, *SDE* shared the common locus with tillering dwarf mutant *DWARF3* (*D3*) gene, suggesting *sde* may be a novel weak allelic of *D3*. Collectively, we here identified a novel multi-tillering semi-dwarf line *sde*, which would provide novel dwarf source and improving the genetic diversity for important agronomic traits of rice and the main component of plant architecture.

Keywords Rice (*Oryza sativa* L.) · Dwarf · Gene mapping · *Sde*

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10681-021-02766-3.

M. Wu · J. Chen · D. Dai · C. Du · H. Zhang (✉) · L. Ma (✉)
State Key Laboratory of Rice Biology and Chinese National Center for Rice Improvement, National Rice Research Institute, Hangzhou 310006, China
e-mail: zhanghuali621@126.com

L. Ma
e-mail: ricemaly@163.com

Introduction

Plant height is one of the most important agronomic traits of rice. IR8, a high-yielding rice variety with semi-dwarf gene *sd1*, bring about great promotion of rice production in southeast Asia, which was called the "green revolution" of rice (Hedden 2003). Therefore, semi-dwarf gene *sd1* was known as the "green revolution gene". So far, more than 80 genes related to dwarf mutants had been cloned in rice (http://www.

gramene.org/rice_mutant/). However, most of cloned dwarf genes perform severe dwarf and other adverse phenotypes such as more tillers, small grain, brittle stems, which is difficult to apply in rice breeding and unconducive to expanding the genetic diversity (Hargrove and Cabanilla 1979; Piao et al. 2014).

Most of the dwarf mutants in rice have been identified to be defective in biosynthesis or signal transduction of plant hormones such as gibberellins, brassinosteroids, strigolactone (SL) and auxin (Qi et al. 2008; Sazuka et al. 2009; Song et al. 2009). SL is a kind of plant hormone which can inhibit the growth and development of plant branches (Gomez-Roldan et al. 2008; Umehara et al. 2008). At present, several genes related to high tillers and dwarf phenotype have been reported to be involved in the regulation of the SL pathway, including biosynthesis and signal transduction genes, such as *HTD2*, *D10*, *D27*, *MIT3*, *HTD1* (Liu et al. 2009, 2018; Arite et al. 2007; Lin et al. 2009; Zou et al. 2005) and *D14*, *D53*, *D3*, *THIS1* (Kameoka and Kyozuka 2015; Jiang et al. 2013; Zhao et al. 2014; Liu et al. 2013). Plant height and tillers are main factors of architecture (Wang et al. 2018). The uniformity of stem height and tillers in rice directly affects yield (Ma et al. 2009). Studies of *IPA1* and *OsSHI1* have revealed insight into regulation of balance between plant architecture and yield in rice (Wang et al. 2018; Duan et al. 2019). Therefore, discovering and utilizing new dwarf genes can further elucidate the molecular mechanism of plant height and tillers, which is of great significance to rice production.

In the present study, we identified a multi-tillering semi-dwarf rice line, *sde*, which is a near-isogenic line (NIL) of ZX5T derived from double dwarf local variety Te'ai (Ma et al. 2003). The phenotypic observation, genetic analysis, cytological observation and gene mapping of *sde* were conducted. The results indicated that the multi-tillering semi-dwarf line *sde* was probably a novel weak allelic of *D3*. Our results proved new understanding of regulatory network of plant height in rice.

Materials and methods

Plant materials and agronomic traits analysis

In our previous study, we selected a variety Xinte'ai, which possessing new semi-dwarf gene *sd-e(t)* from double dwarf Te'ai, a local variety of Zhejiang (Ma et al. 2003). To create near-isogenic line containing *sd-e(t)* gene, semi-dwarf plants in F_2 population of Xinte'ai/Zhongxuan 5 (ZX5T) were selected to backcrossed with ZX5T, then with ZX5T as recurrent parent, Xinte'ai as donor line, a near-isogenic line with *sd-e(t)* gene, was constructed by consecutive backcrosses and selections. For each generation, multi-tillering semi-dwarf individuals were selected for further backcrossing until BC_6F_8. After four times self-cross, one stable multi-tillering semi-dwarf plant was used for research and denoted as NIL-*sde* (simply for *sde*).

The wild-type Nanjing 6 (NJ6) and ZX5T were preserved by our lab. All plants were grown in experimental fields of China National Rice Research Institute in Hangzhou, China (120° 12′ E, 30° 30′ N), or Lingshui, China (109° 57′ E, 18° 35′ N).

All plants were grown followed normal agricultural field management. At maturity stage, the agronomic traits of plant height, tilliering number, panicle length, grain width, grain length, grain number per panicle, and 1000-grain weight were investigated for *sde* and ZX5T, respetively. The statistical significant difference determined by the t-test.

Microscopic observations

Microscopic observations method followed by previous methods with minor modification (Wang et al. 2014). The internodes of ZX5T and *sde* at the mature stage were fixed in the 70 % FAA solution, dehydrated with graded concentrations of ethanol (70 %, 83 %, 95 % and 100 %) successively, transparented with different concentrations of xylene, embedded in paraffin, and sectioned by Leica RM2235 (5 μm thick). Then the prepared microtome sections were stained with Safranin O-Fast Green and investigated under the fluorescence microscope.

GA_3 and SL treatments

GA_3 and SL treatment was conducted as previous study with minor modification (Li et al. 2010; Umehara et al. 2008). 20 seeds of *sde* and ZX5T were sterilized in 75 % ethanol for 5 min, disinfected with 25 % NaClO for 30 minutes, then washed with sterile distilled water for three times, finally immersioned into the sterile water in dark circumstance at 28 °C for

3 days. The conformity germinating seeds were selected to grown in the Yoshida solution containing different concentrations of GA_3 or GR24 at 30 °C, 16 h light/8 h dark cycle (Yoshida et al. 1976). Seedling height or tiller numbers was measured after 7 days of GA_3 treatment or SL treatment.

Assay of α-amylase activity

The assays of α-amylase activities were following the previous methods (Piao et al. 2014; Yamaguchi et al. 1999). The embryoless half seeds were sterilized with 75 % alcohol for 5 min, disinfected with 20% sodium hypochlorite for 20 min, and then rinsed with sterile distilled water for 5 times. Then the embryoless half seeds were placed vertically on 2% agar plates containing 0.2% soluble potato starch, 10 mM sodium acetate and 2 mM $CaCl_2$ at pH 5.3. In addition, 1 μM GA_3 was added to the experimental group. After 4 days at 30°C in dark environment incubated, the plates were smoked with iodine vapor until the plate turned blue-purple.

Quantitative real-time PCR analysis

Total RNA were isolated from leaf, sheath, stem and spike of *sde* and ZX5T at the mature Stage using AxyPrep total RNA preparation kit. cDNAs were synthesized by ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO). Real-time PCR were performed with SYBR Green Realtime PCR Master Mix (TOYOBO) on 7900HT Fast Real-time PCR System. Real-time PCR was conducted in a 10 μL volume containing 0.4 μL of each primer, 1 μL of cDNA, 5 μL of SYBR Green Realtime PCR Master Mix (TOYOBO) and 7.7 μL sterile water. The rice *OsActin* gene was used as the internal control. The primers used for real-time PCR are as follows: 5′-GAGTTGCAACACCGGCTACA-3′ and 5′-AACTAAAGCAGCTTTCAATC-3′ for *D3* and 5′-TGGACAGGTTATCACCATTGGT-3′ and 5′-CCGCAGCTTCCATTCCTATG-3′ for *OsActin*. Four biological replications were implemented and the $2^{-\Delta\Delta CT}$ method was used to calculate relative expression levels (Livak and Schmittgen 2001).

Gene mapping

To map *SDE*, a cross was made between *sde* and NJ6. Plants with multi-tillering and semi-dwarf phenotypes were selected from the F_2 populations for mapping. Chi-square (χ^2) test was conducted to analyze the hereditary rule. Bulked segregant analysis was used to seek markers linked to *SDE* gene. The semi-dwarf and wild type DNA pool were constructed with 20 multi-tillering and semi-dwarf plants and 20 wild type plants randomly selected from the F_2 population respectively. Total DNA was extracted from young leaves with TPS method following Lei et al. (2014). A total of 622 SSR markers were used to preliminary genetic mapping. The PCR reaction was conducted according to Fu et al. (2016). Then the products were separated on 8 % non-denaturing polyacrylamide gels and visualized with silver staining following (Zhang et al. 2011). To fine map the *SDE* gene, 3 Indel and 4 dCAPS polymorphic markers were developed according to the available sequence difference between NJ6 and *sde*.

Candidate gene analysis

Candidate genes were predicted according to the available sequence annotation databases (http://rapdb.dna.affrc.go.jp/). New markers (Table S1) were designed based on the reference sequences of putative genes in the mapping region. The related sequences were amplified from wild-type ZX5T and *sde* for sequencing analysis.

Results

Phenotype analysis of the *sde*

The multi-tillering semi-dwarf line *sde* was the NIL of ZX5T and selected from double dwarf local variety Te'ai in our previous study (Ma et al. 2003). Compared to ZX5T, the *sde* was no difference at seedling stage, while showed significantly dwarfism and multi-tillering at mature stage, with an average plant height of 88.23 cm, 62 % of that in ZX5T (Fig. 1; Table 1). Additionally, panicle and internode of *sde* were significantly shortened in relative to ZX5T (Fig. 1). Each internode length of *sde* was reduced in almost equal proportion to that of the wild type ZX5T

(Fig. 1). Therefore, it could be inferred that *sde* was a dwarf mutant of "dn" type, kind of with normal internode proportion feature. In addition to dwarfism, compared with the wild-type ZX5T, the *sde* also showed increased tiller number, and the decreased grain number per panicle (Table 1).

Microscopic observation of *sde*

The internode length was proved to be connected with cell division and cell elongation in the interstitial meristem in rice (Kitano and Futsuhara 1981). To identify the factors in responsible for shorten internode in *sde*, paraffin sections of the first internode were observed at maturity stages in the wild type and *sde*. The results showed that in longitudinal sections of the stem, cell in wild type and *sde* were similar, both regular and oblong (Fig. 2). Fewer cells were observed in *sde* than wide type ZX5T in the longitudinal sections of the first internode, while there was no significantly change in cell length compared with the wild type (Fig. 2). Therefore, these results indicated that the decreased number of cells was the interpretation of the shortened internode of *sde*.

Table 1 Comparison of agronomic traits between *sde* and ZX5T

Traits	ZX5T	*sde*
Plant height (cm)	142.20 ± 5.03	88.23 ± 2.47**
No. of tillers per plant	12.34 ± 0.87	34.42 ± 1.34**
1000-grain weight (g)	27.78 ± 1.21	27.49 ± 0.28
Panicle length (cm)	27.83 ± 2.86	19.45 ± 2.38**
Grain length (mm)	7.61 ± 0.36	7.82 ± 0.33
Grain width (mm)	3.22 ± 0.25	3.07 ± 0.25
Grain number per panicle	130.25 ± 3.12	55.47 ± 5.09**

*Significantly difference at $P = 0.05$; **Significantly difference at $P = 0.01$

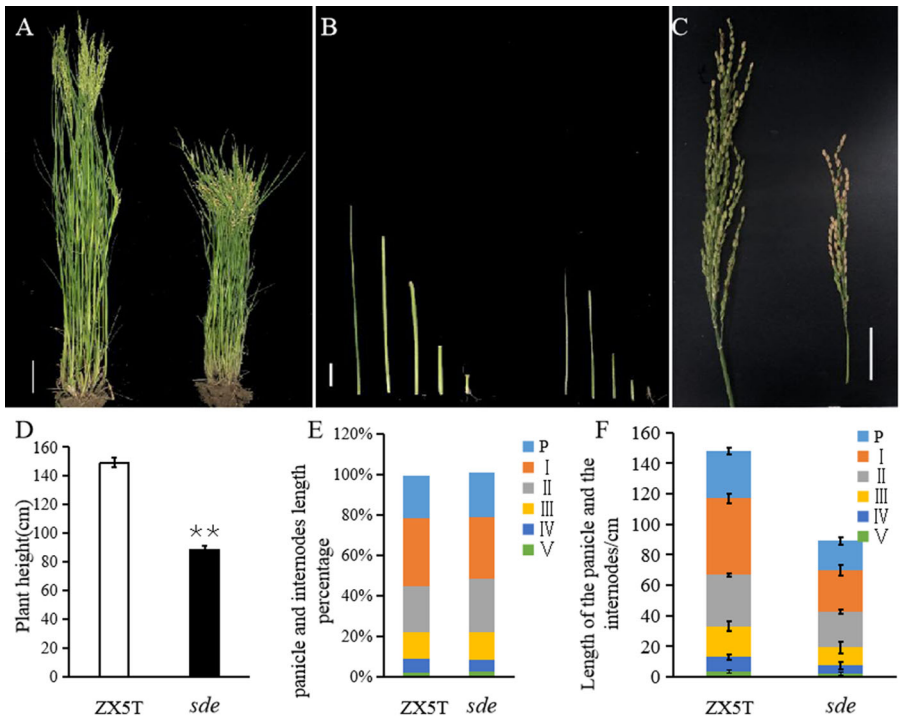

Fig. 1 Morphological comparison between *sde* and ZX5T. **a** The plant architecture at mature stage (left: ZX5T; right: *sde*) (Bar = 10 cm); **b** Different internodal length at mature stage (left: ZX5T; right: *sde*) (Bar = 5 cm); **c** Panicles (left: ZX5T; right: *sde*) (Bar = 5 cm); **d** The comparisons of plant height between ZX5T and *sde*; **e**, **f** Length of the panicles and internodes of ZX5T and *sde* at the mature stage, P indicates the panicle, and I, II, III, IV, V indicate five internodes, respectively (n = 10). **Significantly difference at $P = 0.01$

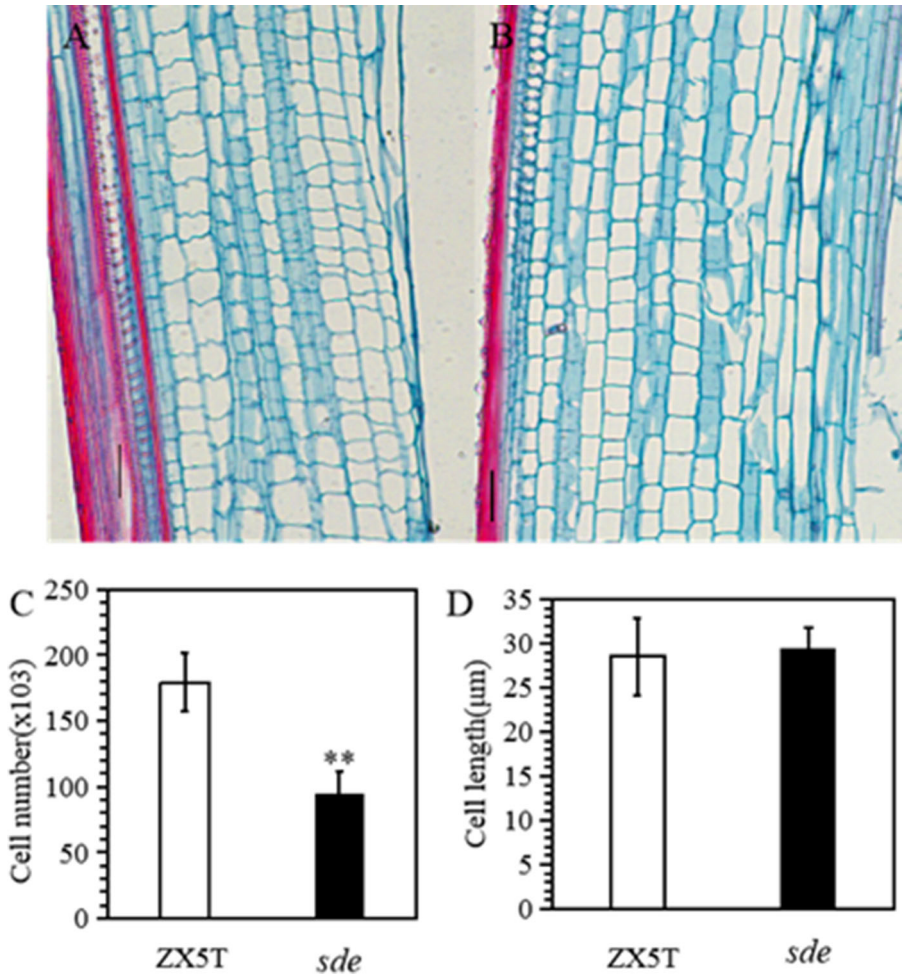

Fig. 2 Longitunal section of the 1st internode in the wild type and *sde*. **a, b** Longitunal section of the first internode of the wild type and *sde*, (**a** ZX5T; **b** *sde*) Bar 50 μm; **c** Cell numbers of the first internode; **d** Cell length of the first internode. **Significantly difference at $P = 0.01$

sde was insensitive to GA_3

To clarify the effects of *SDE* mutation on GA_3 sensitivity, both of seedlings height and α-amylase activity were detected in seedlings treated with different concentrations of exogenous GA_3 (Fig. 3). Compared to ZX5T, *sde* showed significantly decreased plant heights and amylase activities within the investigated concentrations of exogenous (Fig. 3). However, no significant difference was detected in seedling height of *sde* for varied GA_3 concentrations, whereas *sde* showed relatively weaker activities of α-amylase than ZX5T (Fig. 3). The results indicated that *sde* was less sensitive to exogenous GA_3 than ZX5T (Fig. 3).

sde was insensitive to GR24

Most high tillering and dwarf mutants are associated with defects in SLs biosynthesis or signal transduction (Liu et al. 2009, 2018; Arite et al. 2007; Lin et al. 2009; Zou et al. 2005). Previous studies have revealed that the tiller number of the SL-deficient mutant *d10* or *d27* was significantly decreased under GR24 treatment while *d3* mutants show no significant change of phenotype (Arite et al. 2007; Lin et al. 2009; Zhao et al. 2014). Thus, to identify whether the *sde* was related to SLs, seedlings of wild-type ZX5T and *sde* were treated with GR24, a synthetic SL analogue. The results showed that the tiller number of *sde* was not

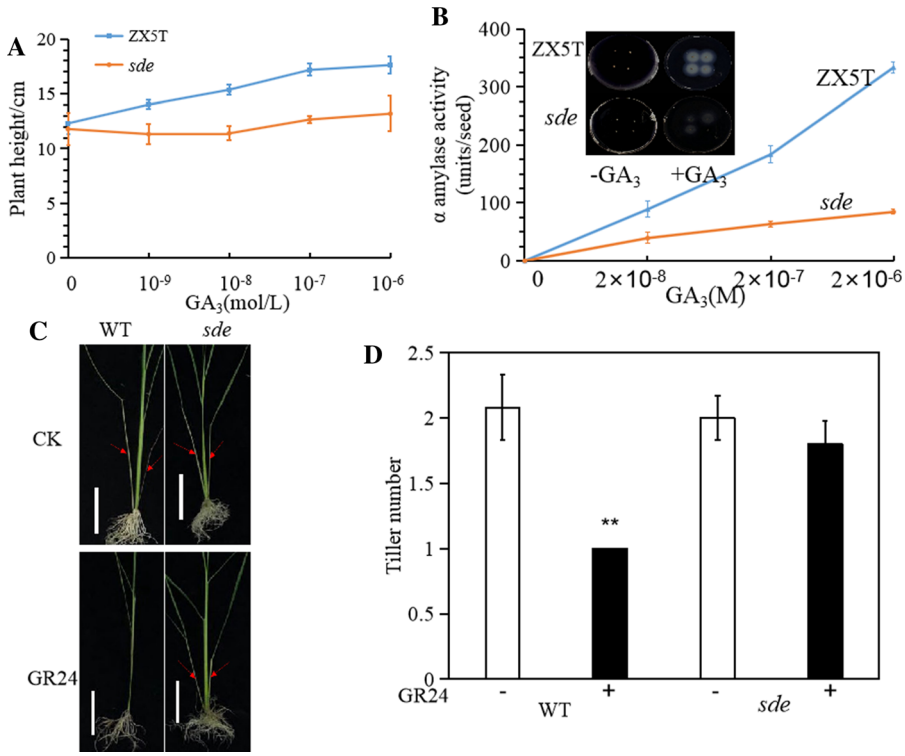

Fig. 3 Resposes of ZX5T and ***sde*** to gibberellic acid (GA_3) and GR24 treatment. **a** Plant height of ZX5T and *sde* with varying concentrations of GA_3 trestment; Data are mean ± SD (n = 10); **b** α-amylase activity induced by gibberellic acid (GA_3) in ZX5T and *sde*. **c** The tiller phenotype of WT (ZX5T) and *sde* after four weeks treatment with 1 μmol/L GR24; CK, Without GR24; Arrows indicate the tiller buds. Bars 5 cm. **d** Tiller numbers after 4 weeks treatment with GR24. Tillers (> 2 mm) were counted (values are mean ± SD, n = 8)

significantly decreased under GR24 treatment (Fig. 3), suggesting that *sde* was insensitive to GR24.

Genetic analysis and mapping of the *SDE* gene

To reveal the genetic characteristic of the *sde*, one F_2 mapping populations was developed from the cross of *sde* and NJ6. The plant heights of F_1 generation performed the similar wild-type phenotype to that of NJ6. Furthermore, there were two segregated phenotypes showed in F_2 population, with one type was in accordance with *sde*, and the other was similar to NJ6. In the F_2 population of 324 plants, the separation ratio of phenotype of wild-type to dwarf was 250:74, corresponding to the separation ratio of 3:1 ($\chi^2_{0.05} = 0.70 < \chi^2_{0.05} = 3.84$), indicating that the traits of the multi-tillering semi-dwarf *sde* was controlled by a one pair of recessive genes.

A total of 622 SSR markers distributed evenly on 12 chromosomes were employed to map the *SDE* gene using the aforementioned F_2 population. The *SDE* gene was initially located between RM469 and RM3805 on the short arm of chromosomes 6 by bulked sergeant analysis (BSA) with 74 F_2 recessive individuals (Fig. S1). Based on the coarse mapping, 7 additional polymorphic markers were developed and used to narrow the interval with 358 more recessive individuals identified from F_2 population (Table. S1). And finally, the multi-tillering semi-dwarf gene *SDE* was located in a 58Kb region between Indel marker DE2 and dCAPS marker ME2 (Fig. 4).

There were 9 annotated genes located in the target region (http://rapdb.dna.affrc.go.jp/) (Table 2). To identify the candidate gene of *SDE*, the entire sequence of located region were sequenced. Sequence comparison revealed that only one bp substitution (C-T) was found in the first exon of *Os06g0154200* between wild type ZX5T and *sde*, which causing amino acid arginine (R) mutates into tryptophan (W) at the 702th amino acids (Fig. 4). Besides, not any

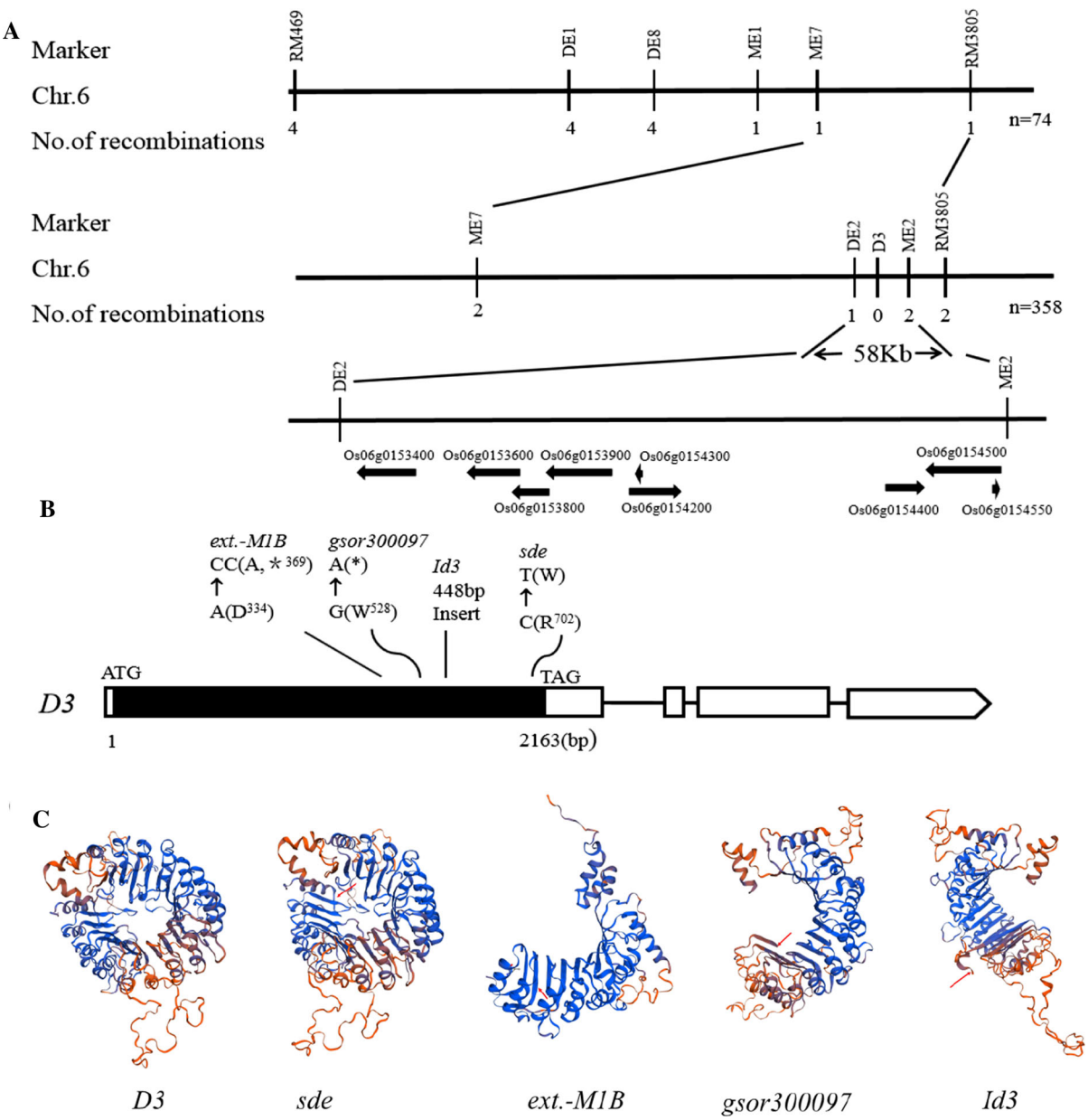

Fig. 4 Molecular mapping of *SDE* gene in rice. **a** Fine mapping of the *D3* gene. Location of the *SDE* locus was narrowed down to a 58 kb region between DE2 and ME2 on chromosome 6. The number below the corresponding markers indicates the numbers of recombinants between the markers and *sde*. **b** Structure of the *D3* gene and its mutation sites in the three alleles. Nucleotide substitutions and inserts in the three mutants are indicated. *sde* has one nucleotide C_{2104} substitution in the exon. Black boxes indicate exons, white boxes indicate UTRs and lines indicate introns. **c** Predicted structures of three-dimensional model of D3 protein in WT, *sde* and other three mutations

other differences were observed in the remaining eight genes sequences between ZX5T and *sde* were observed. Interestingly, *SDE* shared the common locus of the previously reported *DWARF3* (*D3*) gene, encoding an F-box protein with leucine-rich repeats and essential for SL signal transduction (Zhao et al. 2014). Dwarf and multi-tillering characters of the *sde* were similar to that of *d3*. These results indicate that the *sde* is probably a novel allelic to *d3*.

Table 2 Annotated genes and their putative functions in the candidate region

Locus gene	Gene annotation
Os06g0153400	Expressed protein
Os06g0153600	Expressed protein
Os06g0153800	Beta 5 subunit of 20S proteasome
Os06g0153900	Similar to Thiol methyltransferase 2
Os06g0154200	F-box component of the SKP-Cullin-F box (SCF) E3 ubiquitin ligase complex, Strigolactone (SL) signal perception
Os06g0154300	Hypothetical protein
Os06g0154400	Domain of unknown function DUF676, lipase-like domain containing protein
Os06g0154500	Mitogen-activated protein kinase
Os06g0154550	Hypothetical gene

Expression patterns of *SDE*

The real-time RT-PCR was employed to detect the expression patterns of the candidate gene *Os06g0154200 (D3)* in different tissues of ZX5T and *sde*. The results showed that *D3* was expressed in all tissues (Fig. 5). Nonetheless, compared with ZX5T, the expression of *D3* gene in the culm and panicle of the *sde* was significantly increased (Fig. 5).

Discussion

SL is a kind of plant hormone, which can inhibit the growth and development of plant branches (Gomez-Roldan et al. 2008; Umehara et al. 2008). At present, *D3*, *D14*, and *D53* has been proved to play various roles in SL signaling pathway (Zhao et al. 2014; Kameoka and Kyozuka 2015; Jiang et al. 2013). *D3* encodes an F-box leucine-rich repeat protein, which is assembled into complex SCF^{D3} with SCF (Zhao et al. 2014). SL signaling is positive regulated by *D3*, which can inhibit mesocotyl elongation through degrade the OsGSK2-phosphorylated CYC U2 (Sun et al. 2018). *D14* encodes α/β-fold hydrolase protein, which inhibits rice branch elongation together with SCF^{D3} (Kameoka and Kyozuka 2015). *D53* is the substrate of SCF^{D3} ubiquitination complex, forming complexes with D14 and D3 to inhibit the SL signaling pathway (Jiang et al. 2013). *OsMADS57*, together with *OsTB1*, target *D14* to control tillers in SL signaling pathway (Guo et al. 2013). Here, the treatments of exogenous SL (GR24) indicated that *sde* is insensitive to GR24, indicating that *sde* may have a defect in SL pathway and *SDE* may control tiller development through SL signaling pathway.

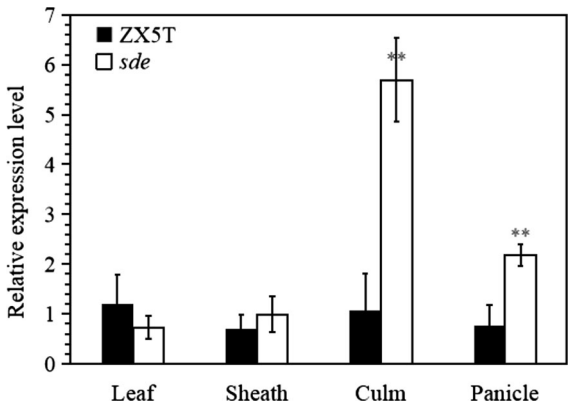

Fig. 5 Expression pattern of the *D3* gene in wild type ZX5T and *sde*

In this study, we characterized a new allelic mutant of *D3*, *sde*, which showed typical multi-tillering and semi-dwarf phenotypes. This was consistent with three reported allelic *D3* mutants, i.e. *Id3*, *gsor300097* and *ext.-M1B*. The *Id3* mutant contained a 448 bp insertion in the *D3* gene, which included a hypothesized transposon at the 154th amino acid (Fig. 4), resulting in alteration of amino acid sequences and a premature stop mutation (Ishikawa et al. 2005). This mutation in *Id3* caused suppression of tiller bud growth, thus more than 45 tillers were noticed in *Id3* mutant (Ishikawa et al. 2005). Another high-tillering dwarf *D3* mutant, *gsor300097*, had a single-base mutation from G to A at the 1583th position of *D3*, converted amino acid from lysine to a premature stop codon (Fig. 4) (Zhao et al. 2014). The mutation from A to CC at the 1000th position of *D3* (Fig. 4), caused frameshift mutation and a premature stop codon, is responsible for the 42.21 cm height and nearly 121 tillers phenotype of *ext.-M1B* allelic mutant (Liang et al. 2017).

However, although exhibiting similar phenotypes, *sde* was a novel and never-reported mutant. One nucleotide substitution (C-T) at the 1583th position of *D3* first exon was responsible for *sde*. This mutation only result in a substitution of amino acid (R-W) at 702th amino acids (Fig. 4). Compared to the dwarf mutant *Id3, gsor300097* and *ext.-M1B*, the semi-dwarf *sde* could still remain higher height (88.23 cm) and fewer tillers (34.42) (Table 1). This may be attributed

to the maintenance of full length of 720 amino acids in *sde* (Fig. 4), whereas only 528, 564, 369 amino acids existed in *Id3, gsor300097* and *ext.-M1B* respectively. Obviously, the mutation in *Id3, gsor300097* and *ext.-M1B* caused defect changes in D3 protein (Fig. 4), which would lead to severe phenotypes. Nonetheless, the mutation of *SDE* showed a relatively little impact on the D3 function, is a weakest mutation of *D3*. The results of qRT-PCR showed that *D3* was expressed in various tissues in wild type (Fig. 5), which was similar with previous research (Zhao et al. 2014; Liang et al. 2017). The premature stop codon would hindered the *D3* expression in *Id3* mutant (Ishikawa et al. 2005), while the increased expression of *D3* in *sde* probably due to the negative feedback regulation. The weak allelic mutant of *sde* probably explained the less-tillering and higher height performance in *sde* than *Id3, gsor300097* and *ext.-M1B*. Moreover, appropriate height and tiller number in rice play an important role in yield composition (Ma et al. 2009; Wang et al. 2018; Duan et al. 2019). Hence, less-tillering and higher height may provide more possibility application for *sde* in rice breeding.

Similar situation were also reported about multiple allele mutations in previous research. Three *d11* mutants, *d11-1*, *d11-4*, and *d11-2*, have typical dwarf phenotype. The mutations in *d11-1*, *d11-4*, and *d11-2* generated premature stop codon and caused truncates protein. While another *d11* weak mutant *d11-3* only has a single-base mutation from C to T at exon 4, which cause an amino acid substitution (Thr to Ile). Thus, *d11-3* shows a relatively mild dwarfing phenotype compared with *d11-1*, *d11-4*, or *d11-2* (Tanabe et al. 2005). *cyp2-1* contained a nucleotide mutation that resulted in a premature stop codon and a short protein, while *cyp2-2* had a nucleotide mutation that changed an amino acid from Gly (72) to Ala (72) in the *OsCYP2 gene*. In contrast to *cyp2-1*, *cyp2-2* showed a weaker phenotype with the ability to inhibit lateral root development (Kang et al. 2013). Two *eg1* alleles mutant (*eg1-1* and *eg1-2*) exhibited a wide variety of spikelet developmental defects. The strong allelic mutant *eg1-1* produces a premature stop codon and causes a short protein, resulting in severe effect on phenotype. While the weak allelic mutant *eg1-2* only cause amino acid exchange in *EG1* gene, and result in weak effect on phenotype (Li et al. 2009).

Generally speaking, the variation of strong allele may lead to early termination of protein translation or affect the important structural and functional domains, and the variation of weak allele may only leads the substitution of single amino acid. The identification of weak allelic mutants of important functional genes would provide new insight for their application in rice breeding. The *sde* in this study, as a new weak allelic of *D3*, have moderate plant height and tiller number and may play an important role in new dwarf varieties creation and application in the future.

Acknowledgements This study was financially supported by the Open Project Program (20190110) of State Key Laboratory of Rice Biology, National Program of National Natural Science Foundation of China (31501288), Zhejiang Province Natural Science Foundation of China (LQ16C130002), and the Chinese 863 Program (2014AA10A604-15). We also thank Dr. Ruiqing Li (Anhui Agricultural University) for his critical comments to help improving the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Arite T, Iwata H, Ohshima K, Maekawa M, Nakajima M, Kojima M, Sakakibara H, Kyozuka J (2007) DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. Plant J 51(6):1019–1029

Duan E, Wang Y, Li X, Lin Q, Zhang T, Wang Y, Zhou C, Zhang H, Jiang L, Wang J, Lei C, Zhang X, Guo X, Wang H, Wan J (2019) OsSHI1 regulates plant architecture through modulating the transcriptional activity of IPA1 in rice. Plant Cell 31(5):1026–1042

Fu B, Zhong Y, Yong J, Chen F, Zhang C, Li Y (2016) Construction of PCR amplification using cucumber leaf directly. Mol Plant Breed 14(7):1691–1697

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Bécard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. Nature 455(7210):189–194

Guo S, Xu Y, Liu H, Mao Z, Zhang C, Ma Y, Zhang Q, Meng Z, Chong K (2013) The interaction between OsMADS57 and OsTB1 modulates rice tillering via DWARF14. Nat Commun 4(1):1566

Hargrove TR, Cabanilla VL (1979) The impact of Semidwarf varieties on Asian rice-breeding programs. Bioscience 29(12):731–735

Hedden P (2003) The genes of the Green Revolution. Trends Genet 19(1):5–9

Ishikawa S, Maekawa M, Arite T, Onishi K, Takamure I, Kyozuka J (2005) Suppression of tiller bud activity in

tillering dwarf mutants of rice. Plant Cell Physiol 46(1):79–86

Jiang L, Liu X, Xiong G, Liu H, Chen F, Wang L, Meng X, Liu G, Yu H, Yuan Y, Yi W, Zhao L, Ma H, He Y, Wu Z, Melcher K, Qian Q, Xu HE, Wang Y, Li J (2013) DWARF 53 acts as a repressor of strigolactone signalling in rice. Nature 504(7480):401–405

Kameoka H, Kyozuka J (2015) Downregulation of rice DWARF 14 LIKE suppress mesocotyl elongation via a strigolactone independent pathway in the dark. J Genet Genomics 42(3):119–124

Kang B, Zhang Z, Wang L, Zheng L, Mao W, Li M, Wu Y, Wu P, Mo X (2013) OsCYP2, a chaperone involved in degradation of auxin-responsive proteins, plays crucial roles in rice lateral root initiation. Plant J 74(1):86–97

Kitano H, Futsuhara Y (1981) Character expression of induced dwarf mutants in rice: I. Effects of temperature on culm elongation in the dwarf mutant Line, Fukei No 71. Jpn J Breed 31(1):9–18

Lei W, Zhao X, Xu M, Feng S, Yan R, Li S, Shi Y, Mei Y, Wang C (2014) Optimization of rapid DNA extraction methods from peanut leaves. Shandong Agric Sci 46(7):11–14

Li H, Xue D, Gao Z, Yan M, Xu W, Xing Z, Huang D, Qian Q, Xue Y (2009) A putative lipase gene *EXTRA GLUME1* regulates both empty-glume fate and spikelet development in rice. Plant J 57(4):593–605

Li W, Wu J, Weng S, Zhang Y, Zhang D, Shi C (2010) Identification and characterization of *dwarf 62*, a loss-of-function mutation in DLT/OsGRAS-32 affecting gibberellin metabolism in rice. Planta 232(6):1383–1396

Liang Y, Wang S, Huang X, Wang H, Liu F, Li S, Zhu J, Deng Q, Liu H, Zheng A, Wang L, Li P (2017) Characterization of a new allelic mutant of DWARF3 in rice and analysing its function and stability in the presence of strigolactone. Mol Breed 37(3):39

Lin H, Wang R, Qian Q, Yan M, Meng X, Fu Z, Yan C, Jiang B, Su Z, Li J, Wang Y (2009) DWARF27, an iron-containing protein required for the biosynthesis of Strigolactones, regulates rice tiller bud outgrowth. Plant Cell 21(5):1512–1525

Liu W, Wu C, Fu Y, Hu G, Si H, Zhu L, Luan W, He Z, Sun Z (2009) Identification and characterization of HTD2: a novel gene negatively regulating tiller bud outgrowth in rice. Planta 230(4):649–658

Liu W, Zhang D, Tang M, Li D, Zhu Y, Zhu L, Chen C (2013) THIS1 is a putative lipase that regulates tillering, plant height, and spikelet fertility in rice. J Exp Bot 64(14):4389–4402

Liu L, Xie T, Peng P, Qiu H, Zhao J, Fang J, Patil SB, Wang Y, Fang S, Chu J, Yuan S, Zhang W, Li X (2018) Mutations in the *MIT3* gene encoding a caroteniod isomerase lead to increased tiller number in rice. Plant Sci 267:1–10

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 – ΔΔCT method. Methods 25(4):402–408

Ma L, Zhu X, Li X, Yang C, Zhuang J, Min S, Xia Y (2003) Genetic and allelic analysis of new dwarf genes in two double-dwarf germplasms. Chin J Rice Sci 17(4):291–296

Ma L, Bao J, Guo L, Zeng D, Li X, Ji Z, Xia Y, Yang C, Qian Q (2009) Quantitative trait loci for panicle layer uniformity identified in doubled haploid lines of rice in two environments. J Integr Plant Biol 51(9):818–824

Piao R, Chu SH, Jiang W, Yu Y, Jin Y, Woo MO, Lee J, Kim S, Koh HJ (2014) Isolation and characterization of a dominant dwarf gene, *d-h*, in rice. PLoS One 9(2):e86210

Qi J, Qian Q, Bu Q, Li S, Chen Q, Sun J, Liang W, Zhou Y, Chu C, Li X, Ren F, Palme K, Zhao B, Chen J, Chen M, Li C (2008) Mutation of the rice *Narrow leaf1* gene, which encodes a novel protein, affects vein patterning and polar auxin transport. Plant Physiol 147(4):1947–1959

Sazuka T, Kamiya N, Nishimura T, Ohmae K, Sato Y, Imamura K, Nagato Y, Koshiba T, Nagamura Y, Ashikari M, Kitano H, Matsuoka M (2009) A rice tryptophan deficient dwarf mutant, *tdd1*, contains a reduced level of indole acetic acid and develops abnormal flowers and organless embryos. Plant J 60(2):227–241

Song Y, You J, Xiong L (2009) Characterization of *OsIAA1* gene, a member of rice Aux/IAA family involved in auxin and brassinosteroid hormone responses and plant morphogenesis. Plant Mol Biol 70(3):297–309

Sun SY, Wang T, Wang LL, Li XM, Jia YC, Liu C, Huang XH, Xie WB, Wang XL (2018) Natural selection of a GSK3 determines rice mesocotyl domestication by coordinating strigolactone and brassinosteroid signaling. Nat Commun 9:2523

Tanabe S, Ashikari M, Fujioka S, Takatsuto S, Yoshida S, Yano M, Yoshimura A, Kitano H, Matsuoka M, Fujisawa Y, Kato H, Iwasaki Y (2005) A novel cytochrome P450 is implicated in Brassinosteroid biosynthesis via the characterization of a rice dwarf mutant, *dwarf11*, with reduced seed length. Plant Cell 17(3):776–790

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. Nature 455(7210):195–200

Wang W, Li G, Zhao J, Chu H, Lin W, Zhang D, Wang Z, Liang W (2014) Dwarf Tiller1, a Wuschel-related homeobox transcription factor, is required for tiller growth in rice. PLoS Genet 10(3):e1004154

Wang J, Zhou L, Shi H, Chern M, Yu H, Yi H, He M, Yin J, Zhu X, Li Y, Li W, Liu J, Wang J, Chen X, Qing H, Wang Y, Liu G, Wang W, Li P, Wu X, Zhu L, Zhou J-M, Ronald PC, Li S, Li J, Chen X (2018) A single transcription factor promotes both yield and immunity in rice. Science 361(6406):1026–1028

Yamaguchi J, Itoh S, Saitoh T, Ikeda A, Tashiro T, Nagato Y (1999) Characterization of β-amylase and its deficiency in various cultivars. Theor Appl Genet 98:32–38

Yoshida S, Forno D, Cock J, Gomez K (1976) Laboratory manual for physiological studies of rice. The International Rice Research Institute, Los Baños

Zhang F, Quan JL, Shan WX (2011) Improvement of polyacrylamide gel electrophorus in *Phytophthora infeatans* SSR marker. J Anhui Agric Sci 39(27):16606–16607

Zhao J, Wang T, Wang M, Liu Y, Yuan S, Gao Y, Yin L, Sun W, Peng L, Zhang W, Wan J, Li X (2014) DWARF3 participates in an SCF complex and associates with DWARF14 to suppress rice shoot branching. Plant Cell Physiol 55(6):1096–1109

Zou J, Chen Z, Zhang S, Zhang W, Jiang G, Zhao X, Zhai W, Pan X, Zhu L (2005) Characterizations and fine mapping of a mutant gene for high tillering and dwarf in rice (*Oryza sativa* L.). Planta 222(4):604–612