Gibberellins orchestrate panicle architecture mediated by DELLAX-KNOX signalling in rice

Su Su1, Jun Hong1, Xiaofei Chen1, Changquan Zhang2, Mingjiao Chen1, Zhijing Luo1, Shuwei Chang1, Shaoxing Bai1, Wanqi Liang1, Qiaoquan Liu2* and Dabing Zhang1,3,5*  

1Joint International Research Laboratory of Metabolic & Developmental Sciences, State Key Laboratory of Hybrid Rice, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China  
2Key Laboratory of Plant Functional Genomics of the Ministry of Education, College of Agriculture, Yangzhou University, Yangzhou, China  
3School of Agriculture, Food and Wine, University of Adelaide, Umbrue, SA, Australia  

Introduction

Rice (Oryza sativa) is one of the most important crops in the world, comprising the staple diet for over half of the world’s population. For decades, research has focused on improving rice yield to meet the demands of a rapidly growing population. A key determinant of grain yield in rice is spikelet numbers per panicle, which is directly regulated by inflorescence architecture; inflorescence meristems at the shoot apex differentiate into primary branch meristems attached to a central rachis, which then form several secondary branch meristems that bear spikelets (Zhang and Yuan, 2014). Several genes involved in inflorescence meristem development have been characterized, such as aberrant spikelet and panicle1 (ASP1), DROUGHT AND SALT TOLERANCE (DST), TAWAWA1 (TAW1), and ABERRANT PANICLE1 and 2 (AP1 and AP2), whose loss of function result in change of primary/secondary branch number, grain number or spikelet length (Ikeda-Kawakatsu et al., 2012; Ikeda-Kawakatsu et al., 2009; Li et al., 2013; Yoshida et al., 2012; Yoshida et al., 2013). Researchers have also identified dozens of quantitative trait loci (QTL) that contribute to panicle morphology and grain yield. In japonica rice varieties, some examples include the following: Dense and Erect Panicle1 (DEP1), which encodes the γ-subunit of a heterotrimeric G-protein complex that regulates meristem activity (Huang et al., 2009); and OsSPL14, also known as Ideal Plant Architecture 1 (IPA1), a member of the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) family of transcription factors that directly bind promoters of hormone synthesis and signalling-related genes such as SLENDER RICE 1 (SLR1) and LONELY GUY (LOG) (Lu et al., 2013). In indica varieties, NARROW LEAF1 (NAL1) increases spikelet number and has become a useful breeding tool in indica-growing regions such as South and Southeast Asia (Zhang et al., 2014). Recently, some of the genes in these QTLs have been identified through sequencing, bioinformatics and natural allelic diversity analysis (Zhang and Yuan, 2014), but further characterization of the genetic control of rice panicle shape is required to advance our fundamental understanding of biology to produce breeding innovation.  

Gibberellic acid (GA) phytohormones are known to affect different plant developmental processes such as stem elongation, flowering, pollen maturation and seed germination (Cheng et al., 2004; King and Evans, 2003; Kuroha et al., 2018; Tyler et al., 2004). Four genes encoding GA 20-oxidases (OsGA20ox1–4) have been thought to play a role in the penultimate step of GA biosynthesis (Spilmeyer et al., 2002). SEMI-DWARF 1 (SD1), known as the ‘Green Revolution’ gene, encodes GA 20-oxidase 2, which has been applied in rice breeding for decades. Recently, SD1 has been reported to modulate rice growth and grain yield by regulating nitrogen and carbon metabolism (Li et al., 2018). Moreover, Grain Number per Panicle (GNP1) has been isolated in rice that encodes a GA 20-oxidase (Wu et al., 2016). Genetic variations in its promoter region affect transcript expression and
panicle grain number, revealing a new role for GAs in rice inflorescence meristem development (Wu et al., 2016).

DELLA proteins are highly conserved plant-specific GRAS family transcription regulators that usually act as negative regulators in the GA signalling pathway (Zentella et al., 2007). The DELLAlN-terminal domain binds with the GA receptor GIBBERELLIN INSENSITIVE 1 (GID1) to sense GA signalling (Daviere and Achard, 2016). DELLAs lack DNA-binding elements, but interact with a diverse range of regulatory proteins – transcription factors, transcriptional regulators, chromatin remodelling complexes and co-chaperones – to regulate downstream processes (Van De Veld et al., 2017). A single DELLAlproteins, SLR1/OsGA1, has been reported in rice (Ikeda et al., 2001; Ogawa et al., 2000), which interacts directly with NAC transcription factors to inhibit a NAC–MYB–CESA signalling cascade that regulates secondary cell wall cellulose synthesis (Huang et al., 2015). SLR1 can also disrupt nitrogen metabolism, by disrupting the interaction between the transcription activator GRF4 and its co-factor GIG, which promotes nitrogen uptake and assimilation (Li et al., 2018). Class 1 KNOX subfamily proteins are three-amino acid loop extension (TALE) homeodomain (HD) transcription factors involved in establishment and maintenance of the shoot apical meristem (SAM) (Scofield et al., 2007; Sentoku et al., 1999; Vollbrecht et al., 1991). Five functional class 1 KNOX genes (OSH1, OSH6, OSH15, OSH43 and OSH71) have been identified in rice (Sato et al., 1999; Sentoku et al., 1999). KNOX class 1 proteins can homo- or heterodimerize with other HD proteins to affect development and growth (Bellaoui et al., 2001; Bhatt et al., 2004; Muller et al., 2001), and directly regulate phytohormone biosynthesis and metabolism to create the correct hormone balance to form and maintain the SAM (Sakamoto et al., 2006). OSH1 is required for SAM maintenance after germination, whereas double osh1 osh15 mutants lack a SAM during embryogenesis and regeneration (Tsuda et al., 2011).

In this study, we have identified a QTL, qPA1 (QTL for panicle architecture on chromosome 1), which regulates rice panicle architecture. Through map-based cloning and complementation tests, we show that qPA1 is identical to SD1, which positively regulates rice panicle length and spikelet number via the DELLAlclass 1 KNOX pathway. This work provides insight into rice panicle development and provides new breeding targets for panicle architecture refinement. In addition, combining these two QTLs (GPN1 and SD1) provides new breeding targets for panicle architecture refinement.

Results

Map-based cloning of qPA1 reveals the underlying SD1 gene

Among 136 chromosome segment substitution lines (CSSLs) derived from two inbred parents – Nipponbare (receptor parent) and 9311 (donor parent) – only CSSL-9 exhibited short stature and small panicle size with reduced primary and secondary branch numbers compared with the receptor parent (Figure 1a–f, Figure S1; Zhang et al., 2011). The number of primary and secondary branches per panicle of CSSL-9 decreased by ~17% and ~20%, respectively, compared with the Nipponbare parent (Figure 1d, e), which resulted in a ~23% reduction in grain number in the main panicle (Figure 1f). CSSL-9 was found to harbour an intronsequence segment from 9311 on chromosome 1 between two markers R1M147 and RP145 (Figure 1g).

To determine the gene(s) underlying the CSSL-9 phenotype, an F2 segregation population derived from CSSL-9 and Nipponbare was constructed for fine mapping. Short stature and small panicle size were observed to co-segregate, suggesting that these two traits are controlled by a single QTL, designated qPA1. Panicle size of the F2 population segregated as small:large 263:838 ($\chi^2 = 0.669 < \chi^2_{0.05} = 3.841$, $P > 0.05$), indicating that qPA1 is a single locus that segregates in a Mendelian ratio. Using markers, the candidate region was narrowed down to the region flanked by M16 and M17, which contains 43 genes based on the Rice Genome Annotation Project Database (http://rice.plantbiology.msu.edu/) (Figure 1g). Of these, LOC_Os01g66100, also known as SD1, encodes a putative GA 20-oxidase 2 with a known semi-dwarf and yield-related effect, and was thus a strong candidate gene for qPA1.

To examine whether SD1 is responsible for the qPA1 phenotype, the Nipponbare genomic SD1 sequence was introduced into CSSL-9. All 20 T1 transformants (complemented lines) showed significantly longer panicle length, higher plant height, increased primary and secondary branch numbers, and total grain number per panicle than CSSL-9 lines (Figure 1a–f), confirming that SD1 is the causal gene for qPA1.

Small panicle size is caused by a non-functional SD1 allele

To find the genetic cause of smaller panicle size in CSSL-9, we sequenced SD1 (LOC_Os01g66100) in both parents. Genomic analysis of SD1 in CSSL-9/9311 and Nipponbare lines revealed 74 single nucleotide polymorphisms (SNPs) in the 2 kb promoter region, 41 SNPs in introns and four SNPs in exons: 2 mis-sense mutations at +299 and +1019 bp, one silent mutation at +654 bp and a non-sense mutation at +1026 bp that truncates the protein by 47 amino acids (Figure 1g).

To determine the functions of Nipponbare and 9311 alleles, we transformed CSSL-9 lines with overexpression constructs encoding different parental alleles under control of the CaMV 35S promoter (Figure 2a). SD1 expression levels in all transgenic plants were higher than in the CSSL-9 control line (Figure 2b). Transgenic lines expressing the Nipponbare allele (SD1Nip) were taller and had significantly increased panicle length, primary and secondary branch numbers, and total grain number than the CSSL-9 line (Figure 2a, b, d–g). In contrast, no obvious change in panicle traits was observed in transgenic lines overexpressing the 9311 allele (SD19311), suggesting that the SD19311 allele may be non-functional (Figure 2d–g).

According to the previous research, SD1Nip is an SD1-EQ-type allele with a weaker phenotype than a SD1-GR allele present in other rice varieties such as Kasalath (Asano et al., 2011). By comparing coding sequences of SD1 in Kasalath (SD1k), with SD19311, we found two SNPs: the same silent mutation at +654 bp as for SD1Nip, and the non-sense mutation at +1026 bp (Figure S2). The two nucleotide changes at +299 and +1019 bp, which caused mis-sense mutations between SD19311 and SD1Nip, were identical in SD1Kas and SD19311. These codons define the stronger SD1-GR allele SD1k, indicating that these mutations do not compromise protein function. Thus, the only difference between SD19311 and SD1Nip that could lead to a non-functional SD19311 allele was the non-sense mutation at +1026 bp that led to premature truncation of the protein. This genetic variation is rare in natural accessions, with only 21 varieties in the 3K genome data having this variation (Table S2) (Mansuetto et al., 2017),
which does not appear advantageous for rice growth and development under selection pressure.

To examine the effects of SNPs in the promoter region, we examined $SD1$ expression in CSSL-9 and Nipponbare. Gene expression was higher in CSSL-9 than in Nipponbare plants (Figure S3a), supported by higher observed transcriptional activity of the $SD1_{9311}$ promoter as measured by a dual-luciferase assay in Nicotiana benthamiana (Figure S3b). Thus, differences in gene expression due to divergent promoter sequences cannot explain the lack of function of $SD1$ in CSSL-9, further supporting our conclusion that small panicle size in CSSL-9 is caused by the single non-sense mutation in the $SD1$ coding region.

**$SD1$ play a crucial role in panicle development**

To determine the effects of $SD1$ in different backgrounds, CRISPR/Cas9 was applied to knockout $SD1$ in Nipponbare and Kasalath lines (Figure 2a, c; Figure S4). Plant height was decreased in both backgrounds, shown in Kasalath due to

decreases in length of all internodes (Figure 2a; Figure S4c). The panicle length of $sd1$ homozygous mutants was reduced by 8–21%, the primary branch number by 18–26%, the secondary branch number by 34–46% and the total grain number per main panicle by 29–41% (Figure 2d–g; Figure S4d–g). These results indicate the loss of $SD1$ function resulted in smaller, less branched panicles, resulting in decreased yield in two backgrounds. Even $SD1$ in Nipponbare is a weak allele, it still has an indispensable role in panicle shape and plant height. Combining these with overexpression $SD1_{Nip}$ transgenic plants produced a larger panicle (Figure 2), $SD1$ likely plays a positive role in panicle architecture.

$SD1$ has previously been shown to be expressed globally throughout plant development, especially in vegetative organs (Monna et al., 2002; Sakamoto et al., 2004), in line with our qRT-PCR results showing expression in leaf, leaf sheath, roots and panicle (Figure 3a).

Figure 1 Phenotype of parental, CSSL-9 and complemented plants, and map-based cloning of qPA1. (a) Plant architecture of Nipponbare (Nip), CSSL-9 and complemented plants. Bar = 30 cm. (b) Panicle architecture of Nip, CSSL-9 and complemented plants. Bar = 10 cm. (c–f) Panicle traits of Nip, CSSL-9 and complemented plants, showing (c) panicle length; number of (d) primary and (e) secondary branches per panicle; and (f) number of grains in the main panicle. Mean ± SE, n = 20. (g) Map-based cloning of the plant height QTL on chromosome 1 in the CSSL-9 × Nipponbare F$_2$ population, showing the position and result of the 4 SNPs. Differences in wild-type plants indicated *$P$ < 0.05 and **$P$ < 0.01, t-test.

Figure 2 Phenotype of $SD1$ transformants. (a) Plant architecture of overexpression lines, $sd1$ mutants, and control plants. Nip, Nipponbare; OE, overexpression lines in CSSL-9; $sd1$, $SD1$ knockout lines. For plant architecture, Bar = 30cm; For panicle architecture, Bar = 10cm. (b) Relative expression of $SD1$ in transgenic inflorescence primordia. Mean ± SE, n = 3. (c) Target sequences of CRISPR/Cas9-mediated $sd1$ knockout lines. (d–g) Panicle traits of overexpression lines, $sd1$ mutants, and control plants, showing (d) panicle length; number of (e) primary and (f) secondary branches per panicle; and (g) number of grains in the main panicle. Mean ± SE, n = 20. Differences in CSSL-9 plants indicated *$P$ < 0.05, **$P$ < 0.01, t-test.
Gibberellins determines rice panicle patterning

- (a) Comparison of CSSL-9 and p35S::SD1<sup>911</sup>/CSSL-9
- (b) Relative expression of SD1
- (c) SD1 gene structure
- (d) Panicle length comparison
- (e) Number of primary branches
- (f) Number of secondary branches
- (g) Number of grains per panicle
during panicle development. SD1 was detected in rachis meristems, elongated primary and secondary branch meristems, and spikelet meristems (Figure 3b–e). Similarly, GFP localization under control of the 3-kb SD1 promoter in transgenic plants was observed in these tissues of rachis meristem, early primary branch meristem, elongated primary branch meristem and secondary branch meristem (Figure 3f–i). These results indicate that SD1 expresses during early panicle development.

Divergent functions of SD1 and GNP1 are due to changes in promoter activity

Phylogenetic analysis of GA 20-oxidases from five monocotyledons and one dicotyledon revealed that OsGA20ox1/GNP1 and OsGA20ox3 clustered in a large clade that contained most Arabidopsis proteins, while OsGA20ox2/SD1 and OsGA20ox4 belonged to a separate clade (Figure S5). Each OsGA20ox parologue falls in the same clade with GA20oxs from other monocotyledon species.

In rice, OsGA20ox1/GNP1 and OsGA20ox2/SD1 have been found to control rice plant height and yield (Li et al., 2018; Wu et al., 2016). Both proteins were observed to localize to the cytoplasm (Figure S6). To study possible redundant functions of GA20ox1 and GA20ox2, a gnp1 mutation was generated in Nipponbare and CSSL-9 backgrounds via CRISPR/Cas9 technology to create a gnp1 single mutant and a gnp1 sd1 double mutant, respectively (Figure 4). Compared with Nipponbare, gnp1 single mutants showed a decrease of ~15% and ~30%, respectively, in primary and secondary branch numbers and ~23% reduction in grain number per panicle (Figure 4c–e). The double mutant sd1gnp1 showed a more severe reduction: ~30%, ~65% and ~54% reduction in primary branch, secondary branches and total grain numbers per main panicle compared with Nipponbare (Figure 4c–e). These data suggest that GNP1 and SD1 may function independently to regulate panicle development, and cause a dosage effect of GA to influence plant development.

In Arabidopsis, AtGA20oxs exhibit partial redundancy, mainly due to differences in expression patterns (Plackett et al., 2012; Rieu et al., 2008). According to our qRT-PCR results and the RiceXPro database (Sato et al., 2013), both GNP1 and SD1 are expressed throughout plant development, including during panicle development (Figure S7; Wu et al., 2016), but the GNP1 construct had little effect on the CSSL-9 phenotype, the pSD1:GNP1 construct could rescue the sd1 phenotype (Figure 4g–k). This result indicates that their spatiotemporal patterns of expression are associated with their functional divergence.

GAs may regulate other genes known to be involved in panicle development

GA 20-oxidases participate in the GA biosynthetic process by converting GA13 to GA20 via GA53 and GA19, or by converting GA12 to GA3 via GA15 and GA24 (Olzewski et al., 2002). Quantification of GAs in young panicles showed that levels of GA intermediates produced by OsGA20oxs were lower in sd1 plants: levels of GA15, GA20 and GA30 in the GA3 pathway, and GA19 and GA28 in the GA1 pathway decreased significantly (Figure 5a), indicating that loss of SD1 function impacted GA synthesis. However, bioactive GA8 was not detected in either wild-type and sd1 plants, while levels of bioactive GA7 and GA2 did not change (Figure S9), whereas the expression of GA catabolic enzymes OsGA2ox2, OsGA2ox5 and OsGA2ox6 was decreased.
significantly in sd1 mutants (Figure S10). Therefore, we speculated that the similar level of GA1 and GA3 between wide-type and sd1 mutants was caused by down-regulation of these catabolic genes in the sd1 mutant.

The expression of known regulators of panicle development was examined in young panicle of sd1 and wild-type Nipponbare to assess whether their transcription altered in response to different levels of GA intermediates. Some genes, such as D2...
observed that KNOX class 1 proteins (OSH1, OSH6, OSH15, OSH43 and OSH71) could directly interact with SLR1 in Y2H system, and confirmed this result using split-luciferase and bimolecular fluorescence complementation (BiFC) assays (Figure 5b). KNOX class 1 genes have been implicated in inflorescence formation. Genes encoding these five KNOX class 1 proteins are expressed in shoot apical meristem, inflorescence meristem and floral meristem (Harrop et al., 2016; Sato et al., 1999; Sentoku et al., 1999), and their expression is induced by GAs (Wu et al., 2016). OSH1, OSH6, OSH43, OSH15 and OSH71 expressions were lower in sd1 compared with wild-type plants (Figure S13). As osh1 mutants have a smaller panicle than wild-type plants (Tsuda et al., 2011), we selected OSH1 for further protein domain analysis. OSH1 proteins are predicted to have four domains – a KNOX 1 domain, two ELK domains and a HOX domain – while DELLA proteins have two predicted domains, an N-terminal DELLA domain and a C-terminal GRAS domain (Figure 6e; http://smart.embl-heidelberg.de/). In OSH1, the KNOX 1 and ELK domains are required for suppression of target gene expression, while the HOX domain is important for homodimerization and binding to target sequence (Nagasaki et al., 2001). Domains in both proteins were systematically deleted, singly and in combinations, to identify interacting domains (Figure 6e). The truncation interaction experiments in yeast showed that either the HOX or the KNOX 1 domain of

Figure 5 SD1 allele affects GA biosynthesis and expression of panicle-related genes. (a) GA content in sd1 and wild-type lines (Nipponbare) of eight GA biosynthesis intermediates during early panicle development (~1 cm). Mean ± SE, n = 3. F.W., fresh weight. (b) Relative expression of genes involved in panicle development in panicle branch primordia of wild-type and sd1 mutant plants. Mean ± SE, n = 3. Difference in wild-type plants indicated: *P < 0.05 and **P < 0.01, t-test.
OSH1 was required to mediate interaction with the SLR1 GRAS domain (Figure 6f).

To examine whether SLR1 can repress gene activation by OSH1, we used reporter genes driven by the OsREL2/ASP1 promoter; ASP1 has been reported to regulate panicle architecture and contains putative OSH1 binding motifs in its promoter (Eric et al., 1995; Bolduc and Hake, 2009; Kwon et al., 2012; Nagasaki et al., 2001; Sakamoto et al., 2004; Tsuda et al., 2011; Tsuda et al., 2014; Yoshida et al., 2012). Yeast 1-hybrid (Y1H) and luciferase assays confirmed that OSH1 could directly bind the ASP1 promoter (Figure 7a) and that co-expression of SLR1 with OSH1 reduced ASP1 expression (Figure 7b–d).

Our results indicate that a DELLA protein (SLR1) can disrupt KNOX class 1 protein (OSH1) activation of downstream genes via physical interaction. When loss of function of GA 20-oxidase 2/SD1 accumulated, SLR1 interacts with KNOX class 1 proteins to represses KNOX class 1-mediated activation of downstream genes. When SD1 has normal function, DELLA proteins are decreased and cannot interact with KNOX class 1 proteins, promoting downstream gene expression to direct GA-regulated panicle development (Figure 7e).

Discussion

**SD1 acts as a positive regulator determining panicle architecture**

Here, we have used map-based cloning of CSSLs and complementation experiments to identify the qPA1 QTL as the SD1 gene known to affect plant height and panicle development (Figure 1). The CSSL-9 phenotype could be rescued by overexpression of the SD1Nip, but not the SD19311, protein suggesting that SD19311 in CSSL-9 was not functional. Sequence comparison of the two parental alleles (japonica Nipponbare and indica 9311) revealed several SNPs in the promoter, introns and coding region (Figure 1g). The coding region contained two missense SNPs (at +299 bp and +1019 bp) that are known to produce two functional alleles of different ‘strengths’ (Asano et al., 2011), so the mutation that caused SD19311 loss of function was the premature termination that truncated the protein by 47 amino acids. Combining these with overexpression and knockout transgenic results, it indicates that SD1 acts as a positive factor to determine panicle architecture.

SD1 catalyses conversion of GA53 and GA12 into GA20 and GA9, respectively, by multistep reactions; these GA intermediates are finally converted into functional GA1 and GA4, respectively, by a GA 3-oxidase (Itoh et al., 2001; Kuroha et al., 2018). GA1 is predominant in vegetative tissues, while GA 4 levels peak in anthers during reproductive development (Kuroha et al., 2018; Kobayashi et al., 1988; Zhu et al., 2006). However, the details of how GAs finely regulate rice panicle development remain unknown. In this study, we did not detect any changes in levels of bioactive GAs (GA1,G A3 and GA4) in sd1 and wild-type inflorescences (Figure S9); however, GA intermediates were significantly reduced in sd1 compared with wild-type plants (Figure 5a). Similarly, Rieu et al., (2008) found similar levels of GA1 and GA4 in wild-type and ga20ox1 Arabidopsis plants,
although the double ga20ox1 ga20ox2 mutant had significantly lower levels of GA1 and GA4. They reasoned that even minor changes in hormone levels could cause distinct changes in plant growth or that specific spatiotemporal changes in GA levels were not detected. However, the study of Wu et al. (2016) showed that the expression of OsGA2oxs could affect final GA contents. In our study, OsGA2ox2, OsGA2ox5 and OsGA2ox6 were decreased obviously in sd1 mutants (Figure S10). It may indicate that down-regulation of these catabolic genes results in similar GA1 and GA3 level between wide-type and sd1 mutants.

As SD1 is one of the OsGA20ox enzymes, its eventual products are GA9 and GA20. We suggest another possibility that precursors of known bioactive GAs may have some biological function during rice panicle development. We found that levels of GA20 and GA9, the ultimate products of SD1, were most affected in sd1 lines, decreasing by ~58% and ~100%, respectively (Figure 5a).

GA9 has been shown to be more effective than GA3 or GA1 in promoting interaction between SLR1 and the GA receptor (Ueguchi-Tanaka et al., 2007); and more active than GA1 and GA4 in promoting stem growth in Thlaspi arvense L. (Metzger, 1990). However, more research will be required to provide evidence for our theory that GA intermediates play a critical role during rice panicle development.

GNP1 and SD1 are functionally divergent due to different patterns of gene expression

GA 20-oxidases have been reported to mediate GA-regulated developmental processes in plants, affecting plant height, flowering and fertility (Ashikari et al., 2002; King and Evans, 2003; Rieu et al., 2008; Spielmeyer et al., 2002; Tyler et al., 2004). Phylogenetic analysis of GA 20-oxidases from five cereal crops revealed that the GA20ox paralogues group together (Figure S5), consistent with the result of Plackett et al. (2012) who suggested that GA20ox gene duplication happened before the divergence. Arabidopsis GA20ox proteins were more similar to each other than to their respective cereal paralogues, suggesting considerable divergence between dicot and monocot proteins.

In rice, previous studies of OsGA20ox1 and OsGA20ox2 have identified effects on plant height and yield (Li et al., 2018; Oikawa et al., 2004; Sasaki et al., 2002; Wu et al., 2016). We have shown that sd1 (osga20ox2) and gnp1 (osga20ox1) single mutants both have significant decreased plant height and panicle traits, while the double mutants exhibited even more severe phenotypes (Figure 4a–e). Thus, these two genes also show similar but complementary roles in rice development. Functional differences between paralogues are most likely caused by three differences based on previous studies. Firstly, different subcellular
localizations may lead to different functions, such as between the tonoplast-located OsNRT1.1A and plasma membrane-located OsNRT1.1B in nitrogen utilization (Wang et al., 2018). However, both SD1/OsGA20ox2 and GNP1/OsGA20ox1 were observed to localize in the cytoplasm (Figure S6). Secondly, different spatiotemporal patterns of the two genes may lead to their functional variation, such as KNOX Class 1 genes and KNOX class 2 genes (Kerstetter et al., 1994). OsGA20ox1/SD1 and OsGA20ox2/SD1 had partially overlapping expression patterns throughout development: SD1 was expressed more highly in young leaves and roots, while GNP1 was expressed more highly in panicle organs (Figure S7). In sd1 mutant, GNP1 expression was not significantly changed (Figure S11). In addition, the results of Wu et al., (2016) showed that SD1 expression was not changed between NIL-GNP1T and NIL-GNP1TQ. These showed that SD1 and GNP1 may not show the antagonistic expression. It is likely that SD1 and GNP1 have differential roles for panicle, which remains to be investigated. While GNP1 expressed under its own promoter could not rescue the sd1 phenotype in CSSL-9 plants, GNP1 expression under an SD1 promoter could (Figure 4F–K). These results suggest that differences in SD1 and GNP1 function are caused by divergent expression patterns, although our analyses indicate that both genes were expressed in the inflorescence meristem, albeit at different levels (Figure S7; Wu et al., 2016). A finer approach, such as single-cell transcriptomics, will be required to unravel whether spatiotemporal differences in gene expression are responsible for different effects of these genes on panicle development. Finally, functional differences may be caused by different catalytic activities or products, such as AtGA20ox5 and other AtGA20oxs as the former only catalyses conversion of GA12 to GA3 (Plackett et al., 2012). Previous research suggests that GNP1/OsGA20ox1 may have higher affinities for GA12 than for GA12 (GA1 pathway), while SD1/OsGA20ox2 has higher affinities for GA12 than for GA12 (GA4 pathway, Kuroha et al., 2018; Toyomasu et al., 1997). Slightly different activities may explain the dose effects of GA in single mutants and double mutants involved in panicle development (Figure 4A–E).

SLR1–KNOX class 1 protein interaction may mediate GA-regulated panicle development

Previous studies have shown that KNOX class 1 proteins repressed the abundance of GA by promoting expression of GA metabolic genes or repressing expression of GA synthetic genes (Bolduc and Hake, 2009; Jasinski et al., 2005). However, GNP1/OsGA20ox1 has also been shown to induce KNOX class 1 gene expression to regulate panicle meristem activity (Wu et al., 2016). It is still uncertain on how KNOX class 1 proteins participate in GA-regulated panicle development in rice. In our study, SD1 transcripts were detected in the branch primordium (Figure 3), which was overlapping with KNOX class 1 genes (i.e. OSH1, OSH15 and OSH6) (Harrop et al., 2016). This expression pattern indicates that SD1 plays an essential role in regulating panicle development and may have some connection with KNOX class 1 genes.

DELLA proteins usually act as negative regulators in the GA signalling pathway (Zentella et al., 2007), and participate in GA-induced plant growth and development by interaction several key proteins (Chen et al., 2017; Daviere and Achard, 2016; Huang et al., 2015). Although SD1 has reported to improve rice yield by DELLA-GRF4 interaction to regulate nitrogen and carbon metabolism (Li et al., 2018), the specific mechanisms by how GA modulates panicle development are still poorly understood. In our sd1 mutants, SLR1 was accumulated in young inflorescence (Figure 6A), which may indicate its potential role on panicle development. Meanwhile, SLR1 was found to directly interact with KNOX class 1 proteins OSH1, OSH6, OSH15, OSH43 and OSH71, in our Y2H, BiFC and split-luciferase assays (Figure 6; Figure S12). The N-terminal domain of DELLA proteins is required for GA signal recognition by interaction with GA receptor GID1 protein (Itoh et al., 2002; Ueguchi-Tanaka et al., 2007; Willige et al., 2007), and the C-terminal domain is used to interact with other proteins to participate in plant development or target the DELLA for degradation (Dill et al., 2001; Dill et al., 2004; Gomi et al., 2004; Li et al., 2016; Van De Velde et al., 2017). Protein domain analysis revealed that the KNOX 1 and HOX domains of OSH1 could interact with the C-terminal GRAS domain of SLR1 (Figure 6); interaction of the KNOX 1 domain with SLR1 impacted the ability of OSH1 to bind to promoters of downstream genes, known to be crucial for SAM formation and maintenance (Figure 7; Nagasaki et al., 2001; Tsuda et al., 2011).

We have detected expression of panicle-related genes in the sd1 mutant, and observed several genes were reduced distinctly such as IPA1, but others have no obvious change such as TAW1 (Figure 5; Figure S11). According to public Chip-seq data (Tsuda et al., 2014), OSH1 might not directly bind to IPA1, so we speculated that SLR1–OSH1 interaction would not affect IPA1 expression. It is likely that other KNOX class 1 proteins or other SLR interaction proteins regulates IPA1 expression, which remains to be investigated.

Based on these findings, we have proposed a model for GA regulation of panicle development in sd1 mutants and wild-type plants. During the panicle development, in wild type DELLA (SLR1) is degraded and KNOX class 1 gene expression is increased, thus promoting the expression of OSH1-activated downstream effector genes such as ASP1 to promote panicle development. Conversely, in sd1 mutants, non-functional SD1 leads to SLR1 accumulation and sequestration of functional OSH1 to reduce activation of genes involved in panicle development (Figure 7E).

In this study, the mechanisms on how SD1 regulates panicle development are further elucidated, and thus provide a basis for high-yield rice breeding. In addition, combining these two QTLs (GNP1 and SD1) provides new breeding targets for panicle architecture refinement.

Experimental procedures

Plant materials and growth conditions

Chromosome segment substitution lines (CSSLs) bred from a O. sativa var. Nipponbare (japonica) × 9311 (indica) cross were received from Yang Zhou University (Zhang et al., 2011). All transgenic plants in this paper were sown during late spring and grown during summer in paddy fields at Shanghai (31.03°N, 121.45°E), China, during 2015–2020.

For QTL mapping, CSSL-9 plants were back-crossed to Nipponbare to generate the F2 mapping population, grown in Hainan province (18.1°N, 109.3°E). Molecular markers were designed according to sequence differences between the parent lines (Nipponbare and 9311) in the Rice SNP-Seek Database (Mansueto et al., 2017).

Construction of transgenic lines

For complementation, a whole genomic fragment of SD1, containing 2.7 kb coding sequences, 2.0 kb upstream promoter

© 2021 The Authors. Plant Biotechnology Journal published by Society for Experimental Biology and The Association of Applied Biologists and John Wiley & Sons Ltd., 19, 2304-2318.
region and 0.7 kb downstream region, was amplified from Nipponbare genomic DNA, cloned into pCAMBIA1301 (In-Fusion HD Cloning Kit; Takara), and the resulting pSD1:SD1 was transformed into Agrobacterium tumefaciens EHA105, and infiltrated into CSSL-9 calli, as previously described (Li et al., 2006). The pGPN1:GPN1 construct, containing a GPN1 3.0 kb promoter region and 1.1 kb coding sequence in pCAMBIA1301, was similarly generated and infiltrated into CSSL-9 calli. The pSD1:GPN1 construct contained the 3.0 kb SD1 promoter upstream of the 1.1 kb GPN1 coding sequence in pCAMBIA1301, again infiltrated into CSSL-9 calli. All primers are listed in Table S1. To generate p35S:SD1nip and p35S:SD1311 overexpression constructs, a full-length coding sequence (open reading frame) of SD1 was amplified from each parent (SD1lep and SD1331) using cDNA from rice primordia (see below), and cloned into pCAMBIA1301 under control of the 35S promoter (Table S1). To generate the SD1 knockout lines, a sequence from the first exon (106–127 bp; TGAGGATGGGAGCCAAAGATCC) was amplified with primers and cloned into pBI-NG-R-Cas9-OsU3 vector (Table S1) (Biswas et al., 2020). To generate the GPN1 knockout lines, two target sequences from the exon (24–43 bp, GCAAGAGGTGTGTTGCGAC; 347–366 bp, GCATTACGACGAGCTTCACG) were amplified with primers and cloned into pRGE32 vector for CRISPR/Cas9-mediated mutation (Table S1) (Xie et al., 2015). The homozygous mutants were obtained by segregation and sequencing of the T1 generation. To generate GFP expression constructs, the 2.0 kb promoter region from Nipponbare was amplified and cloned into a pCAMBIA1301:GFP vector containing the enhanced GFP (eGFP) coding sequence (He et al., 2016; Table S1). Primary and secondary branch primordia (panicle length was less than 1 mm) were collected from transformed Nipponbare plants for GFP analysis. CSSL-9, Nipponbare (Nip), and Kasalath were used as the recipients for Agrobacterium-mediated transformation to generate the transgenic rice.

Expression analysis

For quantitative reverse-transcription PCR (qRT-PCR), total RNA from seedling leaves, mature leaves, root, panicle and leaf sheath was extracted from rice tissues with TRizol reagent (Invitrogen). For each sample, 1 μg of RNA was used to synthesize cDNA using the PrimeScriptRT reagent kit with gDNA eraser (Takara), according to the manufacturer’s instructions. qRT-PCR was performed using SYBR Premix Ex Taq (Takara), according to the manufacturer’s instructions with the Bio-Rad Real-Time PCR System. Rice actin gene was used as an internal control to normalize the data. Measurements were obtained via the relative quantification method. Each experiment was repeated with three independent biological samples and three technical replicates. Primers of OsGA2oxs were referred to the study of Wu et al., (2016). Other primers are listed in Table S1.

For in situ hybridization, rice young panicle samples (<1 mm) were fixed in FAA solution (10:50:5 formaldehyde: ethanol: acetic acid in water) for 24 h at 4 °C, then dehydrated with a graded ethanol solution, and embedded in paraffin according to Li et al., (2006). 8-μm-thick sections were cut using a Leica microtome (RM2235). Deparaffinization, probe hybridization and immunological detection of digoxigenin were performed as previously described (Kouchi and Hata, 1993). All probes in this paper were expressed under the T7 promoter using the DIG RNA labelling kit (Roche). For subsequent cloning of gene coding sequences, cDNA was generated from RNA collected from young panicle tissues.

Yeast two-hybrid assays

The full-length and truncated coding sequences of SLR1 from Nipponbare were amplified and cloned into EcoRI and BamHI restriction sites of pGADT7 (Takara), which encodes the GAL4 activation domain. The coding sequence of KNOX genes (OSH1, OSH6, OSH15, OSH42 and OSH71) and truncated OSH1 were amplified and cloned into EcoRI and BamHI restriction sites of pGBKTK7 (Takara), which encodes the GAL4 binding domain. To detect protein–protein interactions, recombinant pGBKTK7-KNOX class 1 and pGADT7-SLR1 plasmids were co-transformed into Saccharomyces cerevisiae strain AH109, according to the manufacturer’s instructions (Takara). Transformsants were selected on SD medium lacking SD/Gal-Trp/Leu-His/-Ade.

Bimolecular fluorescence complementation assays

The full-length coding sequence of SLR1 and KNOX class 1 were amplified and cloned into pXY104-cYFP and pXY106-nYFP plasmids, respectively. SLR1-cYFP and KNOX 1-nYFP plasmids were introduced into A. tumefaciens GV3101, which were grown in LB medium with 50 μg/mL kanamycin and 25 μg/mL rifampicin, resuspended in infection solution (10 mM MES and 200 μM acetylsyringone) and co-infiltrated into 3-week-old N. benthamiana leaves. The BIFC assay was performed as previously described (Zhang et al., 2020). After 48-h incubation, fluorescent eYFP signals were detected at excitation 514 nm and emission 522–555 nm using a Leica SP8 confocal microscope.

Yeast one-hybrid assays

The full-length coding sequence of OSH1 was amplified and cloned into p842AD vector (Ma et al., 2018). The ASP1 promoter was cloned into pLacZ vector (Ma et al., 2018). The recombinant pl842AD-OSH1 and placZ-ASP1 plasmids were co-transformed into yeast strain EGY48 according to the Clonectechn transformation procedure. Transformsants were grown on the medium lacking Ura/Trp and tested on SD/-Trp/-Ura plates with X-gal, as described as Ma et al., (2018).

Western blot analysis

Young panicles (<1 mm) from wild-type and sd1 plants were collected and ground into powder in liquid nitrogen. Proteins were extracted with TRizol reagent (Invitrogen) based on Joy et al., (2018). The extraction protein was kept in 0.3M guanidine hydrochloride. Proteins were separated on an SDS-PAGE gel and immunodetected with anti-SLR1 antibody (kindly provided by Professor Donglei Yang from Nanjing Agricultural University, 1:5000 dilution) and then anti-tubulin antibody (Sigma, 1:5000 dilution).

Luciferase assays

For dual-luciferase assays, the 2.0 kb SD1 promoter region from Nipponbare and 9311 was amplified and cloned into pGREENII 0800 vector. For luciferase reporter assays, the effector was generated by inserting the coding sequence of OSH1 into pGREEN II 000 plasmid (kindly provided by Hao Yu, National University of Singapore, Singapore) under control of the 35S promoter. The reporter was generated by inserting the 3.0-kb promoter sequence of ASP1 upstream of the LUC reporter gene in pGREENII 0800 vector (provided by Hao Yu; see Table S1 for primers).

For split-luciferase assays, the coding sequences of KNOX class 1 genes from Nipponbare were cloned into the pCAMBIA 1300-
nLuc plasmid, which encodes the N-terminal luciferase domain. The coding sequence of SLR1 from Nipponbare was cloned into pCAMBIA 1300-cluc plasmids, which encodes the C-terminal luciferase domain (see Table S1 for primers).

All constructs above were introduced into tobacco leaves via Agrobacterium-mediated transformation as described above. After 36-h incubation in the dark, pictures were captured by a cooled CCD imaging apparatus (Tanon S200). Tobacco leaves were ground on liquid nitrogen, and luciferase and Renilla activities were measured with a dual-luciferase reporter assay kit (Promega), according to the manufacturer’s instructions.

Subcellular localization assays
The coding regions of SD1 and GNP1 were cloned into pCAMBIA1301-eGFP. We used 12-day-old rice leaves to generate protoplasts, and transform them with vectors as described by Bart et al., (2006). Fluorescence was analysed by a Leica SP8 confocal microscope (Leica TCS SP8 X) at excitation 488–507 nm for eGFP signal, and 597–648 nm for mCherry of endoplasmic reticulum marker.

Phylogenetic analysis
Homologous and paralogous amino acid sequences of SD1 from different species were obtained from National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/), and aligned with MEGA 5 (Tamura et al., 2011). The aligned sequences were used to construct a phylogenetic tree with neighbour-joining method using MEGA 5 with the following parameters: Poisson correction, pairwise deletion and 1000 bootstrap replicates.

Quantification of endogenous GA
For GA quantification, young inflorescences (<1 cm) of sd1 mutants (sd1-T1) and Nipponbare plants were harvested into liquid nitrogen. Approximately 500 mg inflorescences were collected for each of three biological samples. GAs were measured by an ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) according to Xin et al., (2020).

Statistical analysis
Data analysis was carried out by Student’s t-test of Microsoft Excel software.

Acknowledgments
We thank Pro. Donglei Yang from Nanjing Agricultural University for providing SLR1 antibody, and Prof. Kabling Xie from the Huazhong Agricultural University for the CRISPR/Cas9 vector, Prof. Hao Yu from the National University of Singapore for kindly providing the pGreen vectors, and Dr. Natalie Betts for editing this manuscript, and thank Jinfang Chu from the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences of Beijing, for their technical assistance with quantification of endogenous gibberelin. This work was supported by the National Key Research and Development Program of China (2016YFD0100804 and 2016YFD0100903), the National Natural Science Foundation of China (31861163002 and 31700276), the Innovative Research Team, Ministry of Education, and 111 Project (B14016).

Competing interests
The authors declare no competing interests and approved the paper.

Author contributions
D. Z. directed project. S. S. discussed the experiments. D. Z., J. H., S. S. and W. L. conceived and designed the research. X. C. and M. C. provided the transgenic technology. Z. L. conducted the fieldwork. S. C. and S. B. participated in the experiments. C. Z. and Q. L. created CSSL materials. S. S., J. H. and D. Z. wrote the paper.

References
Asano, K., Yamazaki, M., Takuno, S., Miura, K., Katagiri, S., Ito, T., Doi, K. et al. (2011) Artificial selection for a green revolution gene during japonica rice domestication. Proc. Natl Acad Sci. USA, 108, 11034–11039.
Ashikari, M., Sasaki, A., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A., Datta, S., Ishiyama, K. et al. (2002) Loss-of-function of a rice gibberelin biosynthetic gene, GA20 oxidase (GA20ox-2), led to the rice ‘green revolution’. Breeding Sci. 52(2), 143–150.
Bart, R., Chern, M., Park, C.J., Bartley, L. and Ronald, P.C. (2006) A novel system for gene silencing using siRNAs in rice leaf and stem-derived protoplasts. Plant Methods, 2, 1–9.
Belaou, H., Pulkowich, M.S., Samach, A., Kushalappa, K., Kohalmi, S.E., Modruncan, Z., Crosby, W.L. and et al. (2001) The Arabidopsis BEL1 and KNOX TALE homeodomain proteins interact through a domain conserved between plants and animals. Plant Cell, 13, 2455–2470.
Bertolinio, E., Reimund, B., Wildt-Perinicz, D. and clerq, R.G. (1995) A novel homeobox protein which recognizes a TGT core and functionally interferes with a retinoid-responsive motif. J. Biol. Chem., 270, 31178–31188.
Bhatt, A.M., Etchells, J.P., Canales, C., Lagodienko, A. and Dickinson, H. (2004) VAAMANA—a BEL1-like homeodomain protein, interacts with KNOX proteins BP and STM and regulates inflorescence stem growth in Arabidopsis. Gene, 328, 103–111.
Biwas, S., Tian, J., Li, R., Chen, X., Luo, Z., Chen, M., Zhao, X. et al. (2020) Investigation of CRISPR/Cas9-induced SD1 rice mutants highlights the importance of molecular characterization in plant molecular breeding. J. Genet. Genomics, 47, 273–280.
Bolduc, N. and Hake, S. (2009) The maize transcription factor KNOTTED1 directly regulates the gibberellin catalabolism gene ga2ox1. Plant Cell, 21, 1647–1658.
Chen, J., Gao, H., Zheng, X.M., Jin, M.N., Weng, J.F., Ma, J., Ren, Y.L. et al. (2015) An evolutionarily conserved gene, FUWA, plays a role in determining panicle architecture, grain shape and grain weight in rice. Plant J. 83, 427–438.
Chen, L., Xiang, S., Chen, Y., Li, D. and Yu, D. (2017) Arabidopsis WRKY45 interacts with the DELLA protein RGL1 to positively regulate age-triggered leaf senescence. Mol. Plant, 10, 1174–1189.
Chen, Y., Xu, Y., Luo, W., Li, W., Chen, N., Zhang, D. and Chong, K. (2013) The F-box protein OsFBK12 targets OsSAM5 for degradation and affects pleiotropic phenotypes, including leaf senescence, in rice. Plant Physiol. 163, 1673–1685.
Cheng, H., Qin, L., Lee, S., Fu, X., Richards, D.E., Cao, D., Luo, D. et al. (2004) Gibberelin regulates Arabidopsis floral development via suppression of DELLA protein function. Development, 131, 1055–1064.
Daviere, J.M. and Achard, P. (2016) A Pivotal Role of DELlas in Regulating Multiple Hormone Signals. Mol Plant, 9(1), 10–20.
Daviere, J.M., Wild, M., Regnault, T., Baumberger, N., Eisl, H., Geschick, P. and Achard, P. (2014) Class I TCP-DELLA interactions in inflorescence shoot apex determine plant height. Curr. Biol. 24, 1923–1928.
Dill, A., Jung, H.S. and Sun, T.P. (2001) The DELLA motif is essential for gibberellin-induced degradation of RGA. Proc. Natl Acad. Sci. USA, 98, 14162–14167.
Dill, A., Thomas, S.G., Hu, J., Steber, C.M. and Sun, T.-P. (2004) The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell, 16, 1392–1405.

Fang, N., Xu, R., Huang, L., Zhang, B., Duan, P., Li, N., Luo, Y. et al. (2016) SMALL GRAIL 11 controls grain size, grain number and grain yield in rice. *Nature*, 539, 64.

Gao, X.C., Liang, W.Q., Yin, C.S., Ji, S.M., Wang, H.M., Su, X.A., Guo, C.C. et al. (2010) The SEPALLATA-like gene OsMADS34 is required for rice inflorescence and spikelet development. *Plant Physiol.* 153, 728–740.

Gomi, K., Sasaki, A., Itoh, H., Ueguchi-Tanaka, M., Ashikari, M., Kidono, H. and Matsuoka, M. (2004) GID2, an F-box subunit of the SCF E3 complex, specifically interacts with phosphorylated SLR1 protein and regulates the gibberellin-dependent degradation of SLR1 in rice. *Plant J.* 37, 626–634.

He, Y., Wang, C., Higgins, J.D., Yu, J.P., Zong, J., Lu, P.L., Zhang, D.B. and Liang, W.Q. (2016) MEIOTIC F-BOX is essential for male meiotic DNA double-strand break repair in rice. *Plant Cell*, 28, 1879–1893. https://doi.org/10.1105/tpc.16-00108

Harrop, T.W., Ud Din, I., Gregis, V., Osnato, M., Jouannic, S., Adam, H. and Kouchi, H. and Hata, S. (1993) Isolation and characterization of novel nodulin genes. *Plant J.* 3, 27–33.

Kobayashi, M., Yamaguchi, I., Murofushi, N., Ota, Y. and Takahashi, N. (1988) Gibberellins and flowering of grasses and cereal crops. *Annu. Rev. Plant Physiol.* 39, 347–375.

Li, S., Zhang, D.S., Liu, H.S., Yin, C.S., Li, X.X., Liang, W.Q., Yuan, Z. et al. (2006) The rice tapetum degeneration retardation gene is required for tapetum degradation and anther development. *Plant Cell*, 18, 2999–3014.

Luan, W., Liu, Y., Zhang, F., Song, Y., Wang, Z., Peng, Y. and Sun, Z. (2011) OsCD1 encodes a putative member of the cytosol synthase-like D sub-family and is essential for rice plant architecture and growth. *Plant Sci.* 180, 513–524.

Li, S., Zhao, B., Yuan, D., Duan, M., Qiang, C., Tang, L., Wang, B. et al. (2013) Zinc rice finger protein DST enhances grain production through controlling *Gln1>OsDCK2* expression. *Proc. Natl Acad. Sci. USA.*, 110, 3167–3172.

Lyu, Z.F., Yu, H., Xiong, G.S., Wang, J., Jao, Y.Q., Liu, G.F., Jing, Y.H. et al. (2013) Genome-wide binding analysis of the transcription activator IDEAL PLANT ARCHITECTURE1 reveals a complex network regulating rice plant architecture. *Plant Cell*, 25, 3743–3759.

Luan, W., Liu, Y., Zhang, F., Song, Y., Wang, Z., Peng, Y. and Sun, Z. (2011) OsCD1 encodes a putative member of the cytosol synthase-like D sub-family and is essential for rice plant architecture and growth. *Plant Sci.* 180, 513–524.

Matsuoka, M. (2001) Cloning and functional analysis of two gibberellin 3 beta-hydroxylase genes that are differently expressed during the growth of early rice inflorescences by laser microdissection. *Plant J.* 27, 1681–1696.

Matsuoka, M. (2001) Cloning and functional analysis of two gibberellin 3 beta-hydroxylase genes that are differently expressed during the growth of early rice inflorescences by laser microdissection. *Plant J.* 27, 1681–1696.

Nagasaki, H., Sakamoto, T., Sato, Y. and Matsuoka, M. (2001) Functional analysis of the conserved domains of a rice KNOX homeodomain protein, OSH15. *Plant Cell*, 13, 2085–2098.

Ogawa, M., Kusano, T., Katsumi, M. and Sano, H. (2000) Rice gibberellin-insensitive gene homolog, OsGAI encodes a nuclear-localized protein capable of gene activation at transcriptional level. *Gene*, 245(1), 21–29.

Okawa, T., Koshioka, M., Kojima, K., Yoshida, H. and Kawata, M. (2004) A role of OsGA20ox1, encoding an isoform of gibberellin 20-oxidase, for regulation of plant stature in rice. *Plant Mol. Biol.* 55, 687–700.

Olszewski, N., Sun, T.P. and Gubler, F. (2002) Gibberelin signaling: biosynthesis, catabolism, and response pathways. *Plant Cell*, 14, 561.

Plackett, A.R.G., Powers, S.J., Fernandez-Garcia, N., Urbanova, T., Takebayashi, Y., Seo, M., Ikumaru, Y. et al. (2012) Analysis of the developmental roles of the Arabidopsis gibberellic 20-oxidases demonstrates that GA20ox1 is the dominant paralog. *Plant Cell*, 14, 1653–1665.

Rieu, I., Ruiz-Rivero, O., Fernandez-Garcia, N., Griffiths, J., Powers, S.J., Gong, F., Linhartova, T. et al. (2008) The gibberellic biosynthetic genes AtGA20ox1 and AtGA20ox2 act partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. *Plant J.* 53, 488–504.

Sakamoto, T., Monna, L., Kitazawa, N., Yoshino, R., Suzuki, J., Masuda, H., Maehara, Y., Sakamoto, T., Sakakibara, H., Kojima, M., Yamamoto, Y., Nagasaki, H., Inukai, T., Rieu, I., Ruiz-Rivero, O., Fernandez-Garcia, N., Griffiths, J., Powers, S.J., Gong, F., Linhartova, T. et al. (2008) The gibberellic biosynthetic genes AtGA20ox1 and AtGA20ox2 act partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. *Plant J.* 53, 488–504.

Shi, Y., Zhao, B., Yuan, D., Duan, M., Qiang, C., Tang, L., Wang, B. et al. (2013) Zinc rice finger protein DST enhances grain production through controlling *Gln1>OsDCK2* expression. *Proc. Natl Acad. Sci. USA.*, 110, 3167–3172.

Su, S., Itoh, H., Ueguchi-Tanaka, M., Ashikari, M., Kidono, H. and Matsuoka, M. (2004) GID2, an F-box subunit of the SCF E3 complex, specifically interacts with phosphorylated SLR1 protein and regulates the gibberellin-dependent degradation of SLR1 in rice. *Plant J.* 37, 626–634.
Sasaki, A., Ashikari, M., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A., Swapan, D., Ishiyama, K. et al. (2002) Green revolution: a mutant gibberellin-synthesis gene in rice. *Nature*, 416, 701–702.

Sato, Y., Sentoku, N., Miura, Y., Hirochika, H., Kitano, H. and Matsuoka, M. (1999) Loss-of-function mutations in the rice homeobox gene OSH15 affect the architecture of internodes resulting in dwarf plants. *EMBO J.*, 18, 992–1002.

Sato, Y., Takehisa, H., Kamatsuki, M., Minami, H., Namiki, N., Ikawa, H., Ohyanagi, H. et al. (2013) RiceXPro Version 3.0: expanding the informatics resource for rice transcriptome. *Nucleic Acids Res.*, 41, D1206–D1213.

Scorfield, S., Dewitte, W. and Murray, J.A.H. (2007) The KNOX gene SHOOT MERISTEMLESS is required for the development of reproductive meristematic tissues in Arabidopsis. *Plant J.*, 50, 767–781.

Sentoku, N., Sato, Y., Kurata, N., Ito, Y., Kitano, H. and Matsuoka, M. (1999) Regional expression of the rice KN1-type homeobox gene family during embryo, shoot, and flower development. *Plant Cell*, 11, 1651–1663.

Spelmeyer, W., Ellis, M.H. and Chandler, P.M. (2002) Semidwarf (sd-1), “green revolution” rice, contains a defective gibberellin 20-oxidase gene. *Proc. Natl Acad. Sci. USA*, 99, 9043–9048.

Sun, H., Qian, Q., Wu, K., Luo, J., Wang, S., Zhang, C., Ma, Y. et al. (2014) Heterothrix, a GA metabolic enzyme, regulates nutrient-use efficiency in rice. *Nat. Genet.*, 46, 65–62.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kuma, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, 28, 2731–2739.

Toyomasu, T., Kawaide, H., Sekimoto, H., vonNumerus, C., Phillips, A.L., Hedden, P. and Kamiya, Y. (1997) Cloning and characterization of a cDNA encoding gibberelin 20-oxidase from rice (Oryza sativa) seedlings. *Physiol. Plant.*, 99, 111–118. https://doi.org/10.1111/j.1399-3054.1997.t003438.x

Tsuda, K., Ito, Y., Sato, Y. and Kurata, N. (2011) Positive autoregulation of a KNOX gene is essential for shoot apical meristem maintenance in rice. *Plant Cell*, 23, 4368–4381.

Tsuda, K., Kurata, N., Ohyanagi, H. and Hake, S. (2014) Genome-wide study of KNOX regulatory network reveals brassinosteroid catabolic genes important for shoot meristem function in rice. *Plant Cell*, 26, 3488–3500.

Tyler, L., Thomas, G.S., Hu, J., Dill, A., Alonso, J.M., Ecker, J.R. and Sun, T.P. (2012) A tailored high-throughput genotyping of substitution lines carrying the chromosome segments of indica 9311 in the background of japonica Nipponbare. *J. Genet. Genom.*, 38, 603–611.

Zhang, X., Zhao, G.C., Tan, Q., Yuan, H., Betts, N., Zhu, L., Zhang, D.B. and Liang, W.Q. (2020) Rice pollen aperture formation is regulated by the transcriptional co-repressor, is involved in the regulation of meristem fate and plant development. *Plant Physiol.*, 183, 651–663.

Yang, C., Ma, Y. and Li, J. (2016) The rice YABBY4 gene regulates plant growth and development through modulating the gibberellin pathway. *J. Exp. Bot.*, 67, 5545–5556.

Yoshida, A., Ohmori, Y., Kitano, H., Taguchi-Shiobara, F. and Hirano, H.Y. (2012) Aberrant spikelet and panicle1, encoding a TOPLESS-related transcriptional co-repressor, is involved in the regulation of meristem fate in rice. *Plant J.*, 70, 327–339.

Zentella, R., Zhang, Z.L., Park, M., Thomas, S.G., Endo, A., Murase, K., Fleet, C.M. et al. (2007) Global analysis of delta direct targets in early gibberelin signaling in Arabidopsis. *Plant Cell*, 19, 3037–3057.

Zhang, D. and Yuan, Z. (2014) Molecular control of grass inflorescence development. *Annu. Rev. Plant Biol.*, 65, 553–578.

Zhang, G.H., Li, S.Y., Wang, L., Ye, W.J., Zeng, D.L., Rao, Y.C., Peng, Y.L. et al. (2014) LSHL4 from Japonica Cultivar, Which Is Allelic to NAL1, Increases Yield of Indica Super Rice 93–11. *Mol. Plant.*, 7, 1350–1364.

Zhang, H., Zhao, Q., Sun, Z.Z., Zhang, C.Q., Feng, Q., Tang, S.Z., Liang, G.H. et al. (2017) Development and high-throughput genotyping of substitution lines carrying the chromosome segments of indica 9311 in the background of japonica Nipponbare. *J. Genet. Genom.*, 40, 11–22.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Plant height of 136 CSSLs plus 2 parent lines grown at Shanghai in 2015. The red arrows show the position of CSSL-9, SD1, Nipponbare (Nip), and 9311.

**Figure S2.** Alignment of coding sequences of *SD1* and *SD1* isoforms. Two SNPs at positions +654 bp and +1026 bp are boxed in red. Nucleotides at +299 bp and +1019 bp (blue boxes) that had mis-sense SNPs between *SD1* and *SD1* were the same in *SD1* and *SD1*.

**Figure S3.** Transcriptional activity of different SD1 promoter variants in vivo and in vitro. (a) *SD1* expression in young panicle in CSSL-9 and Nipponbare. Mean ± SE, n = 3. (b) LUC/REN ratio of different SD1 promoter variants used in dual-luciferase reporter assays in *N. benthamiana*. Mean ± SE, n = 3. Differences in wild-type plant indicated **P < 0.01**, t-test.

**Figure S4.** Knock out of *SD1* in cultivar Kasalath. (a) Plant and panicle architecture of of *sd1* mutants in the Kasalath background. Bar = 20 cm. (b) Target sequence of CRISPR/Cas9-mediated *sd1* knockout line. (c–g) Agronomic and panicle traits of wild type Kasalath (Kas) and *sd1* plants, showing (c) plant height; (d) panicle length, number of (e) primary and (f) secondary branches per panicle; and (g) number of grains in the main panicle. Mean ± SE, n = 20. Differences to wild type indicated **P < 0.01**, t-test.

**Figure S5.** Phylogenetic tree of *GAR2* oxidase proteins in six species. *Oz*, *Oryza sativa*; *At*, *Arabidopsis thaliana*; *Ta*, *Triticum aestivum*; *Hv*, * Hordeum vulgare*; *Sb*, *Sorghum bicolor*; *Zm*, *Zea mays*.© 2021 The Authors. *Plant Biotechnology Journal* published by Society for Experimental Biology and The Association of Applied Biologists and John Wiley & Sons Ltd., 19, 2304-2318
**Figure S6.** Localization of GNP1 and SD1 proteins in rice protoplasts. SD1-eGFP and GNP1-eGFP were driven by the 35S promoter. Bar = 5 μm. mCherry is the endoplasmic reticulum marker.

**Figure S7.** SD1 and GNP1 expression levels during rice growth. ml, mature leaf; yl, young leaf; yl sh, young leaf sheath; pbp, primary branch primordium; sbp, secondary branch primordium; el, elongated stem; pl, panicle length. Mean ± SE, n = 3. Differences between tissue pairs indicated: **P < 0.01, t-test.

**Figure S8.** GNP1 expression level under pGNP1:GNP1 transgenic plants. Mean ± SE, n = 3. Differences between tissue pairs indicated: **P < 0.01, t-test.

**Figure S9.** Content of two bioactive GAs in young sd1 and wild type (Nipponbare) inflorescences. Mean ± SE, n = 3. No differences were observed.

**Figure S10.** Expression of GA catabolic enzymes in Nipponbare (Nip) and sd1 mutants. Mean ± SE, n = 3. Differences between tissue pairs indicated: *P < 0.05, **P < 0.01, t-test.

**Figure S11.** Relative expression of genes involved in panicle development in wild type and sd1 panicle branch primordia. Mean ± SE, n = 3. No differences were observed.

**Figure S12.** Other KNOX class 1 proteins can directly interact with SLR1. (a) KNOX class 1 proteins interact with SLR1 in Y2H assays. KNOX class 1 proteins were fused to the GAL4 binding domain (BD); SLR1 was fused to the GAL4 activation domain (AD). (b) KNOX class 1 proteins interact with SLR1 in a BiFC assay. KNOX class 1 proteins were fused to nYFP; SLR1 was fused to cYFP. nYFP, N-terminal yellow fluorescent protein; cYFP, C-terminal yellow fluorescent protein. (c) Split-luciferase assays between KNOX class 1 proteins and SLR1 with controls in tobacco leaves. cLuc, C-terminal luciferase; nLuc, N-terminal luciferase.

**Figure S13.** Relative expression of KNOX class 1 genes involved in panicle development in wild type and sd1 panicle branch primordia. Mean ± SE, n = 3. Differences to wild type plants indicated: *P < 0.05, **P < 0.01, t-test.

**Table S1.** Primers used in this study.

**Table S2.** Variation of 3K varieties at genomic position of 38,385,064 bp (encoding position at 1026 bp).