OsDDM1b Controls Grain Size by Influencing Cell Cycling and Regulating Homeostasis and Signaling of Brassinosteroid in Rice

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Snf2 family proteins are the crucial subunits of chromatin-remodeling complexes (CRCs), which contributes to the biological processes of transcription, replication, and DNA repair using ATP as energy. Some CRC subunits have been confirmed to be the critical regulators in various aspects of plant growth and development and in epigenetic mechanisms such as histone modification, DNA methylation, and histone variants. However, the functions of Snf2 family genes in rice were poorly investigated. In this study, the relative expression profile of 40 members of Snf2 family in rice was studied at certain developmental stages of seed. Our results revealed that OsCHR741/OsDDM1b (Decrease in DNA methylation 1) was accumulated highly in the early developmental stage of seeds. We further analyzed the OsDDM1b T-DNA insertion loss-of-function mutant, which exhibited dwarfism, smaller organ size, and shorter and wider grain size than the wild type (Hwayoung, HY), yet no difference in 1,000-grain weight. Consistent with the grain size, the outer parenchyma cell layers of lemma in osddm1b developed more cells with decreased size. OsDDM1b encoded a nucleus, membrane-localized protein and was distributed predominately in young spikelets and seeds, asserting its role in grain size. Meanwhile, the osddm1b was less sensitive to brassinosteroids (BRs) while the endogenous BR levels increased. We detected changes in the expression levels of the BR signaling pathway and feedback-inhibited genes with and without exogenous BR application, and the alterations of expression were also observed in grain size-related genes in the osddm1b. Altogether, our results suggest that OsDDM1b plays a crucial role in grain size via influencing cell proliferation and regulating BR signaling and homeostasis.

Keywords: rice, Snf2 family, OsDDM1b, grain size, cell proliferation, brassinosteroid
INTRODUCTION

In eukaryotes, genomic DNA is wrapped around the histone octamer to form a nucleosome which is the subunit of chromatin. A highly organized chromatin structure is essential for gene expression in eukaryotes. Chromatin packages large quantities of genetic information into the nucleus and provides an efficient integrative platform that translates signals to regulate gene responses (Hu et al., 2013; Zhang et al., 2019). The mechanism of epigenetic regulation involves histone modification, histone variants, DNA methylation, and chromatin remodeling (Hu et al., 2013). A recent study reveals that many proteins have been identified to mediate these processes, among which Snf2 family proteins are responsible for chromatin remodeling to regulate gene expression using ATP energy (Cairns, 2005; Hu et al., 2013; Zhang et al., 2019). According to the helicase-like region, the Snf2 family proteins are classified into six groups with two conserved domains (SNF2_N and Helicase_C) (Andrew et al., 2006). Each group could be subdivided into 24 subfamilies, and some of these subfamilies are unique for specific organisms and others are ubiquitous (Andrew et al., 2006; Song et al., 2021).

Snf2 family proteins have been identified to contain 41, 45, and 40 members in Arabidopsis, tomato, and rice, respectively. Many of these proteins have been reported to play the essential roles in regulating plant development and stress responses (Kniezowski et al., 2008). For example, the loss-of-function of SPLAYED (SYD) causes shoot apical meristem (SAM) defects in Arabidopsis; SYD interacts with WUSCHEL (WUS), the central regulator in SAM (Kwon et al., 2005); mutations of BRM cause reduced root length and plant size (Farrona et al., 2004), curly leaves (Hurtado et al., 2006), sensitive to abscisic acid (ABA) (Han et al., 2012), and early flowering (Farrona et al., 2011). Indeed, BRM is always recruited and associated with other nuclear proteins, such as RELATIVE OF EARLY FLOWERING 6 (REF6) and FORGETTER1 (FGT1), to modulate gene transcription (Brzezinka et al., 2016; Li et al., 2016). BRM also interacts with TEOSINTE BRANCHED1 CYCLOIDEA AND PCF-CODING GENE (TCP4), ANGUSTIFOLIA3 (AN3), and BREVIPEDICELLUS (BP) to regulate leaf development and inflorescence architecture (Efroni et al., 2013; Vercruyssen et al., 2014) and PHY-INTERACTING FACTOR1 (PIF1) to modulate PROTOCHLOROPHYLLIDE OXIDOREDUCTASE C (PROC) expression for chlorophyll biosynthesis (Zhang et al., 2017). The plants overexpressing AICHRI2 showed growth arrest of primary buds and stem under drought and heat stresses (Mlynarova et al., 2007). Moreover, both DEFECTIVE IN RNA-DIRECTED DNA METHYLATION 1 (DRD1, a DRD1 subfamily member) and DECREASED DNA METHYLATION 1 (DDM1, an Lsh subfamily member) are involved in DNA methylation process (Jeddeloh et al., 1999; Kanno et al., 2004). In addition, the mutant of DRD1 and DDM1 exhibits the delay of leaf senescence (Cho et al., 2016). Furthermore, PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1 (PIE1, an Swr1 subfamily member) is responsible for H2A.Z deposition to regulate flowering and plant development (Choi et al., 2007), and PICKLE (a Mi-2 subfamily member) regulates the H3K27me3-enriched genes (Zhang et al., 2012) and the plant hormone signaling, such as brassinosteroid (BR), gibberellic acid (GA), and cytokinin (CK) (Kakimoto and Physiology, 2011; Zhang et al., 2014). CHROMATIN REMODELING 4 (CHR4) interacts with transcription factors and affects the expression of critical floral regulators to mediate the flowering response pathways of inflorescence meristem to promote floral identity (Sang et al., 2020).

In addition to Arabidopsis, several Snf2 family proteins have been identified and studied in other plant species. For example, constitutively, SICHR1 (Solyg01g079690) overexpression causes reduced plant growth (Volta et al., 2016). DRD1 and Snf2 subfamilies are involved in stress responses in tomato plants (Bargsten et al., 2013). In rice (Oryza sativa L.), the loss-of-function of CHR729 (a member of Mi-2 subfamily) results in dwarf, later flowering, less tiller, and narrow leaf by affecting contents of GA3 (Hu et al., 2012; Ma et al., 2015). OsALT1 (alkaline tolerance 1, OsCHR706), an Snf2 family chromatin remodeling ATPase, negatively regulates alkaline tolerance (Guo et al., 2014), and OsBRHIS1 plays a critical role in SA-independent disease resistance by suppressing innate immunity (Li et al., 2015).

OsDDM1a and OsDDM1b, as the homologous genes to Arabidopsis DDM1, are involved in DNA methylation (Higo et al., 2012), which results in high methylation levels at CHG and CG contexts (Tan et al., 2016). The mutation of OsDDM1 decreases histone H3K9me2 and increases the small heterochromatic RNA and long non-coding RNA (Tan et al., 2018). However, the relative expression profile of Snf2 gene family in rice during reproductive development has not been systematically analyzed. Here, we deeply investigated the expression profiles of the rice Snf2 genes during different developmental stages of seed and characterized the phenotype of osddm1b. The mutant displayed dwarfism and shorter and wider grain size and was insensitive to BR treatments. Our results provide an in-depth knowledge for further exploration of the function of Snf2 family proteins in rice, which indicates that OsDDM1b helps to regulate organ size by influencing cell proliferation and involving in BR response regulation.

MATERIALS AND METHODS

Plant Material and Growth Condition
The OsDDM1b T-DNA insertion mutant (PFG_2B-60109) and wild-type cultivar Hwayoung were obtained from the Crop Biotech Institute, Department of Plant Systems Biotech, Kyung Hee University. The T-DNA information was obtained from SIGnAL database at http://signal.salk.edu/cgi-bin/RiceGE. The plants were grown in the greenhouse at 22–32°C and 80–90% humidity with a 14-h/10-h (light/dark) photoperiod. About 1, 3, 7, 10, and 25 days after fertilization (DAF) of seeds were harvested using micro-dissection needles, frozen in liquid nitrogen immediately, and stored at -80°C for total RNA extraction. A total of three biological replicates of each sample were collected for experimental analysis.

The T-DNA insertion in osddm1b was confirmed by PCR using the primers 2B-60109-Lp and 2B-60109-Rp.
and the T-DNA-specific primer Rb (2707). The full-length coding sequence of OsDDM1b was amplified from WT cDNA by PCR and cloned into the EcoRI/KpnI sites of the pCAMBIA2300 vector. The derived constructs were introduced into the osddm1b by Agrobacterium tumefaciens-mediated transformation. The relative expression levels of OsDDM1b were detected in 7-day-old seedling leaves of osddm1b and complementary mutants by the primers P1 and P2. The primers sequences are described in Supplementary Table 2 for genotyping identification. The gene structure of OsDDM1b was searched from Gene Structure Display Server.1

RNA Isolation and qRT-PCR Analysis
Total RNAs of all collected samples were extracted using Plant RNeasy Mini kit (Qiagen, Hilden, Germany). About 1 µg RNA was reverse-transcribed using the PrimeScript RT-PCR kit (Takara, Kyoto, Japan) according to the manufacturer’s instruction (Cai et al., 2019). The relative expression level was detected by qRT-PCR using the Bio-Rad qRT-PCR system (Foster City, CA, United States) and SYBR Premix Ex TaqII (TaKaRa Perfect Real Time) (Zhang et al., 2020). The qRT-PCR program was 95°C for 30 s; 40 cycles of annealing at 95°C for 5 s and extension at 60°C for 35 s; and 95°C for 15 s (Zhang et al., 2020; Zhao et al., 2021). The rice OsUBQ5 gene was used as an internal control. To evaluate the relative expression levels of the examined genes, we used the comparative C T method (Su et al., 2017). The gene-specific primers are listed in Supplementary Tables 3–6.

Observation of Pollen and Ovule in Rice
Several anthers were randomly selected before pollination and placed on microscopic slide for dissecting with the microdissection needles using a microscope. The pollen was stained with 1% I2-KI solution and observed under the microscope (Ren et al., 2019). For the analysis of the ovule fertility, the spikelets before pollination were fixed in the FAA solution (50% ethanol; acetic acid: formaldehyde = 89:6:5) and using the whole-mount eosin B-staining confocal laser scanning microscopy (WECLSM) to observe the ovule development (Zeng et al., 2018; Yu et al., 2020). The cell number and cell size in the outer parenchyma layer of the spikelet hulls were measured by ImageJ2 (Yu et al., 2020).

Promoter Fusion and GUS Staining
A 2,559-bp fragment upstream of OsDDM1b ATG codon sequence was amplified by PCR from DNA of rice leaf using the primers listed in Supplementary Table 3. Then, the products were constructed into a pENTER/D-TOPO vector (Invitrogen, CA, United States). After that, the positive clones were recombined with the pGWB533 vector by LR Clonase II enzyme (Invitrogen, CA, United States). The wild-type ZH11 (Zhonghua 11) callus was transformed using the Agrobacterium-mediated transformation using the pOsDDM1b:

1http://gsds.gao-lab.org/index.php

2https://imagej.nih.gov/ij/
contents were analyzed using the BR ELISA kit according to the manufacturer's protocol (SINOBESTBIO, Shanghai, China) by UV spectrophotometer in 450 nm.

RESULTS

The Comparative Expression Level of Snf2 Gene Family During Seed Development

The development of seeds is important to get high yield, and many genes highly regulate the developmental process. To explore the expression level of the Snf2 family genes during seed development, we collected the seeds from 1, 3, 7, 10, and 25 DAF. We checked the gene expression level shown in Figure 1 and Supplementary Figure 1. The results showed that OsCHR712 and OsCHR730 were predominantly expressed in early developmental stages of seeds (S1), and OsCHR703, OsCHR735, and OsCHR741 were preferentially expressed in both S1 and S5 stages (Figure 1A). In addition, OsCHR701, OsCHR707, OsCHR711, OsCHR739, and OsCHR745 were preferentially expressed in both S1 and S4 stages (Figure 1B). There were 23 Snf2 genes that showed similar expression pattern which were predominantly expressed in the S4 stage, such as OsCHR702, OsCHR704, OsCHR705, OsCHR706, OsCHR708, OsCHR709, OsCHR710, OsCHR713, OsCHR715, OsCHR719, OsCHR721, OsCHR722, OsCHR725, OsCHR727, OsCHR729, OsCHR731, OsCHR732, OsCHR736, OsCHR737, OsCHR742 (Figure 1C), OsCHR720, OsCHR726, and OsCHR740 (Supplementary Figure 1A). These results indicated that Snf2, a sizeable family that exhibits specific expression patterns, may have unique functions in different developmental stages of seed in rice.

Characterization of the OsDDM1b Mutant

The previous studies showed that two homologous genes in rice (Oryza sativa var. Nipponbare) offered 60% identity to DDM1 (AT5G66750) (Higo et al., 2012). The two genes, OsDDM1a (LOC_Os09g27060) and OsDDM1b (LOC_Os03g51230), share 93% amino acid identity to each other (Higo et al., 2012; Tan et al., 2016). Here, we identified a T-DNA insertion approximately 0.55 kb downstream of the translation start site ATG in osddm1b (2B-60109.L) from Hwayoung background (Figure 2A), which were identified in the SGNal database. The homozygous plants for the T-DNA insertion were identified and obtained by PCR (Figure 2H). In addition, the PCR products of homozygous plants were sequenced, and the sequencing results were aligned with the genome sequence (OsDDM1b) and vector sequence (2707), and then, we found the T-DNA insertion site (Supplementary Figure 3).

In osddm1b, the dwarfism was observed in the seedlings and mature plants (Figures 2B,1 and Supplementary Figure 2A and Table 1). The length of first internode was similar to the wild type. However, the second, third, and fourth internodes of osddm1b were shortened, which suggests that abnormal internode elongation leads to dwarf phenotype (Figure 2J and Supplementary Figure 2B). The reproductive organs of the osddm1b, such as spikelets, anthers, and pistils, were much smaller than those of wild type (Figures 2C–F). The panicle morphology in osddm1b exhibited a significantly reduced growth of 11.03% than the wild type at mature stage (Figures 2G,K). Then, we compared male and female gametophyte morphology in the osddm1b and wild-type plants. The staining of pollen with iodine potassium iodide (I2-KI) solution and the observation of ovule with eosin B by confocal showed that the male (Supplementary Figures 2C–D) and female gametophytes (Supplementary Figures 2E,F) have no noticeable difference compared to the wild type. However, the seed setting in osddm1b was decreased to 84.39% (Supplementary Figure 2G).

To further confirm the effect of OsDDM1b on grain size, we examined the grain length, width, thickness, and 1,000-grain weight. The results showed that osddm1b had significantly reduced grain length of 8.15% than the wild type (Figures 3A,D, Supplementary Figure 2J), but it increased 17.59% in grain width (Figures 3B,E and Supplementary Figures 2K,M) and 10.67% in grain thickness, respectively (Figures 3C,F and Supplementary Figures 2L,N). Even though there was a change in the grain size, there was no noticeable change in the 1,000-grain weight (Supplementary Figure 2H). To verify the role of OsDDM1b in determining grain size in rice, we performed a genetic complementation test. An overexpression plasmid containing full-length coding sequence was inserted by the transformation into the osddm1b. Then, we got two positive complementation lines. The overexpression of OsDDM1b in osddm1b could partially complement the grain size defect of mutant (Figures 3A–C). The grain size (length, width, and thickness) was significantly different between the complementary lines and osddm1b (Figures 3D–F). Particularly, the grain width in the complementary lines was similar to wild type (Figure 3E). The OsDDM1b expression level was dramatically reduced in 7-day-old young leaves in osddm1b but increased in the complementary lines compared with those of the wild type (Figure 3G). The other defention phenotypes could be partially rescued in the complementation lines. This result indicated that the mutation in OsDDM1b was responsible for the phenotype of grain size exhibited in osddm1b.

OsDDM1b Regulates Grain Size by Affecting Cell Proliferation

The spikelet hullin osddm1b became shorter and more comprehensive compared with wild type before fertilization (Figure 4A). Since the cell division and/or cell expansion are responsible for the alteration in the final spikelet hull size and grain size, we carefully examined the cross-section of the central part of lemma and palea in mature spikelets and compared the epidermal cells of osddm1b and HY by scanning electron microscope. The hull cross-section of the spikelet hullin osddm1b...

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3http://signal.salk.edu/cgi-bin/RiceGE
revealed a significant increase of 27.84% in the length of the total cells and 8.15% in cell number of outer parenchyma in the osddm1b. In comparison, the cell width was decreased by 18.07% in the outer parenchyma cells (Figures 4B–F). It also changed the number of rows of specialized cells with a rigid wall in the upper epidermis (Supplementary Figures 4A–B).

The data suggested that the cell division was significantly increased in a transverse direction in the osddm1b spikelet hulls. We also investigated the expression of genes involved in the cell cycle, such as CYCA2.2, CYClZm, E2F2, CYCB2, MCM3, MCM4, and MCM5. We found that these genes were upregulated in young panicles of osddm1b, which suggests that the increased cell number might have resulted from the elevated expression of genes that promote cell proliferation (Supplementary Figure 4C). In addition, we observed the average length and width of longitudinal epidermal cells in outer and inner glumes of wild type and osddm1b by scanning electron microscopy. An apparent 58.43% decrease in cell width was observed in the outer glum, but 10.41% in cell width was increased in the inner glum. Then, there is no significant difference in outer and inner glumes’ cell length in osddm1b (Figures 4G–I). These observations suggest that OsDDM1b might promote latitudinal growth by increasing cell proliferation.
OsDDM1b Localization and Expression Pattern

To investigate the subcellular localization of OsDDM1b, we performed a transient transformation assay. When OsDDM1b-green fluorescent protein (GFP) was transformed into tobacco leaves, the signals mainly targeted the nucleus and cell membrane (Figure 5A).

To further examine the expression profile of OsDDM1b, a construct was produced in which the OsDDM1b promoter (approximately 2.5 kb upstream of the ATG site) was fused to the β-glucuronidase gene (GUS) to transform wild-type (ZH11) plants. GUS signals were detected in the vegetative organs, such as root tips, young and mature leaf blades, stem, and internodes (Supplementary Figures 5A–D). We also detected at different developmental stages of the reproductive organs, such as spikelets, stamens, pistils, and seeds. GUS expression was visualized during the developmental stage of spikelet hulls except for the mature stage (Figure 5B). The developmental period of the spikelet can provide a reference for the pistil and stamen. Then, we estimated the developmental stage of pistil and stamen according to the length of the spikelet. Filaments of early stamens and the stamens of 4–5 mm and mature spikelets had the GUS
OsDDM1b Mutant Is Less Sensitive to BRs

The phenotype of osddm1b, shown, such as erect and dark green leaves, dwarfism, and small grains, is typical BR response phenotypes in rice (Yamamuro et al., 2000; Hong et al., 2003). So, we hypothesize that OsDDM1b is involved in the BR response. We evaluated the sensitivity of osddm1b to 24-epibrassinolide (24-epiBL) using a lamina joint assay. As shown in the results, the second lamina of the wild type exhibited a 24-epiBL dosage-dependent inclination. By contrast, osddm1b did not show a blatant response (Figures 6A,B and Supplementary Figure 6A). The previous studies suggested that BR promoting coleoptile growth was an another indicator of a BR-responsive phenotype (Yamamuro et al., 2000; Li et al., 2002; Je et al., 2010). To analyze the response of coleoptiles to BR, we also performed a coleoptile elongation assay for BR sensitivity (Yamamuro et al., 2000). The wild-type and osddm1b seeds were germinated and grown under dark conditions in half MS medium containing different concentrations of 24-epiBL. The results showed that the relative elongation of coleoptile treatments was much weaker in osddm1b than in the wild type under BR treatment (Figure 6C), which suggests that osddm1b was less sensitive to BRs than the wild type.

OsDDM1b Influences BR Homeostasis and Signaling

According to the above results, loss-of-function of OsDDM1b decreased the response to BR treatments, which suggests that OsDDM1b may involve in BR signaling. Then, we analyzed the expression levels of BR-related genes, which include OsBAK1, OsBRI1, OsBU1, OsDLT, OsIL11, OsLIC, OsMDP1, OsBZR1, OsD2, and OsGSK2 in the wild type and osddm1b (Yamamuro et al., 2000; Hong et al., 2003; Ito et al., 2005; Duan et al., 2006; Bai et al., 2007; Koh et al., 2007; Wang et al., 2008; Tong et al., 2009, 2012; Zhang et al., 2009). In roots, the OsBAK1, OsBRI1, OsIL11, and OsBZR1 were slightly upregulated in osddm1b (Figure 6D). However, the expression level of OsBU1 and OsDLT, positive regulator of BR signaling, was significantly lower in osddm1b than signals (Supplementary Figure 5E). It was detected in the basal of pistils during the early stage (≤5 mm spikelet) and stigma of the mature stage (Supplementary Figure 5F). The GUS staining in seeds showed that it was highly expressed in the early stage [such as 5 h after fertilization (HAF), 1 DAF, 3 DAF, and 10 DAF] and mature seeds (Figure 5C), consistent with the results of the qRT-PCR. This expression profile indicated that OsDDM1b functioned in different young tissues, in line with the results presented above that OsDDM1b regulated cell division and proliferation rate.
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**FIGURE 4** | Histological analysis of spikelet hulls. (A) Young spikelet hulls of wild type (Hwayoung, HY) and osddm1b. The white line indicates the position of the cross-section. Bar = 1 mm. (B,C) Cross-sections of spikelet hulls from wild type (HY) and osddm1b, respectively. Bar = 500 µm. (D,E) Magnified view of the cross-section area boxed in panels (B,C), respectively. Bar = 50 µm. (F) Statistical data of the total length, cell number, and length in the outer parenchyma layer (n = 12). (G,H) Scanning electron microscope (SEM) analysis of the outer and inner surfaces of glumes. Bar = 60 µm. (I) Statistical analysis of cell width and length in outer and inner glumes (n = 40, 60, respectively). Data are given as means ± SD. ***p < 0.001 compared with wild type (HY) using Student’s t-test.

in the wild type. The expression level of OsLIC, OsMDP1, and OsGSK2, as the negative regulator of BR, was also upregulated in osddm1b. OsD2, as a representative BR biosynthetic gene, was slightly upregulated in osddm1b. These results suggested that OsDDM1b influenced BR homeostasis and signaling.

It had been reported that the regulation of feedback-inhibited genes in BR biosynthesis usually defects mainly in BR signaling in mutants (Bai et al., 2007; Chen et al., 2013). To confirm this, we analyzed the expression of OsBRI1, OsCPD, OsD11, and OsDWARF (Yamamuro et al., 2000; Hong et al., 2002; Tanabe et al., 2005) genes in the wild type and osddm1b in the absence or presence of 24-epiBL. In osddm1b, BRs inhibition of these feedback-inhibited genes was released, and they were all strongly suppressed in the mutant than wild type (Figure 6E). To find the essential answers of OsDDM1b in regulating BR biosynthesis, the amounts of endogenous BRs were measured in the mutants and wild type (Supplementary Figure 6B). The contents of endogenous BRs were increased by 36.01% in osddm1b compared with wild type, which indicated that OsDDM1b was required for the feedback inhibition of BR biosynthetic and signaling genes.

**The Effect of Osddm1b on Grain Size-Related Genes**

Grain size includes length, width, and thickness, as a quantitative trait locus (QTL), which is vital for grain yield and quality. Thus far, multiple QTLs have been cloned and studied, such as BG1, GL3.1, GL6, GL7, GLW7, GS2, GS5, GS9, GS1, MKKK10,
FIGURE 5 | The expression profile of OsDDM1b. (A) Subcellular localization of OsDDM1b protein was shown to target the cytomembrane and nuclear by transient expression of 35:S:OsDDM1b-GFP in tobacco. The upper row is the 35S:GFP as the control. Bar = 50 µm. (B,C) Expression pattern detected in transgenic plants carrying the pOsDDM1b:GUS vector in ZH11 background. Developing spikelets in turn 1–2, 2–3, 3–4, 4–5, 5–6, 6–7 cm, and mature spikelet (B); The seeds of 5 HAF and 1, 3, 7, 10, 15, and 25 DAF, respectively (C). Bar = 1 mm. HAF, hours after fertilization; DAF, days after fertilization.

qLGY3, qTGW3, GW2, GW5, GW8, DEP1, RGB1, and XIAO, which contributed to rice yield (Song et al., 2007; Huang et al., 2009; Li et al., 2011; Jiang et al., 2012; Qi et al., 2012; Wang et al., 2012, 2015, 2019; Hu et al., 2015, 2018; Liu L. C. et al., 2015; Si et al., 2016; Liu et al., 2017, 2018; Guo et al., 2018; Xu et al., 2018; Ying et al., 2018; Zhao et al., 2018). Snf2 family genes, as the chromatin remodeling factors, regulate the transcription of the genes, and our data showed that loss-of-function of OsDDM1b resulted in abnormal grain size. To analyze the expression level of grain size-related genes in osddm1b, we detected several genes in inflorescence (length 8 cm) and seeds (1 DAF) in wild type and osddm1b using qRT-PCR. The results showed that GL3.1, GL7, GS2, GS5, GSN1, M KK K10, GW8, DEP1, RGB1, and XIAO were upregulated in osddm1b compared with wild type in inflorescence or seeds (Figure 7), and GL3.1, GL7, GS2, and DEP1 were significantly upregulated. It indicates that OsDDM1b negatively regulates their expression level, which affects the grain length (Figure 7A). The loss-of-function of GSN1, M KK K10, and XIAO caused small grain size, which was highly expressed in inflorescence and seeds in osddm1b, which suggests that OsDDM1b might negatively regulate the expression of these genes (Figures 7A,B). The expression of GS5 and GW2, which affects the milk-filling rate in rice, increased significantly in 1 DAF seeds of osddm1b. However, in-depth study is required for identifying that the loss-of-function of OsDDM1b influences milk-filling rate in rice.

**DISCUSSION**

**Snf2 Gene Family in Rice**

Snf2, as a sizeable gene family, has conserved domains (SNF2_N, DEAD and Helicase_C, HELICs) as in animal counterparts, which suggests that they may have a similar function to regulate
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FIGURE 6 | The OsDDM1b mutant is less sensitive to brassinolide (BL). Response to BR treatments, (A) Lamina joints of wild type (HY) and osddm1b. Bar = 2 mm. (B) Measurements of the lamina joint angle of wild type (HY) and osddm1b after various BR treatments. (C) Effect of BR on the coleoptile in wild-type (HY) and osddm1b seedling. (D) The expression level of BR signaling pathway-related genes and assayed using RNA from roots of 7-day-old seedlings cultured in half-strength MS medium. (E) Quantitative RT-PCR analysis of feedback-inhibited genes of BR biosynthesis. Data are given as means ± SD. **p < 0.01, *p < 0.05 compared with wild type (HY) using Student’s t-test.

FIGURE 7 | Comparative expression of the genes to determine grain size between wild-type (HY) and osddm1b plants. (A) The expression level of the genes for the determination of grain size by qRT-PCR using 8 cm panicle. (B) The relative expression of grain size determination genes in the seeds of one day after fertilization. Data are given as means ± SD. *p < 0.05, **p < 0.01 compared with wild type (HY) using Student’s t-test.

the gene expression. There are 42 proteins in chromDB database that belong to Snf2 family in rice. The proteins of OsCHR723 and OsCHR744 (homologs of MOM1/CHR15 in Arabidopsis) do not have the Helicase_C domain, which is not the member of Snf2 family (Hu et al., 2013). Many genes exhibit specific expression patterns at the different developmental stages of various reproductive tissues (inflorescence, stamens, pistils, and seeds). In addition, some genes have been reported to regulate...
the abiotic or biotic stress responses, such as OsALT1 (alkaline tolerance 1, OsCHR706) (Guo et al., 2014) and OsBRH51 (Li et al., 2015). Here, we report the comparative expression profile of 40 Snf2 genes during the reproductive development to enrich the expression patterns and insights into the functions of this family. However, the responses of the Snf2 gene family under abiotic or biotic stresses have not been systematically analyzed.

The Function of DDM1 Is Conserved in Arabidopsis and Rice

DDM1 is a nucleosome remodeling ATPase and regulates the DNA damage that is critical influence for the chromatin structure in Arabidopsis. The RNAi mutation of DDM1 has a typical phenotype of demethylation (Shaked et al., 2006). The repeated sequence, even a single-copy sequence, is induced toward hypomethylation after repeated self-pollination, which activates the transposons (Kakutani et al., 1996). The mutation of DDM1 showed reduced fertility only after repeated self-pollination and produced several kinds of developmental abnormalities. DDM1 is conserved in regulating DNA methylation. For example, Lsh (lymphocyte-specific helicase), a homolog gene of DDM1, causes genomic DNA hypomethylation in the mouse (Dennis et al., 2001). The antisense OsDDM1 lines exhibit dwarf phenotypes, and some sublines were observed to be progressive loss of fertility even complete infertility. In addition, methylation is severely reduced in antisense OsDDM1 lines, and reduction is evident in later generations but not in the genetic transformation process. It is still unknown whether the demethylation reduction was mediated by the repression of the OsDDM1a/OsCHR746, OsDDM1b/OsCHR741, or both of them. Our data showed that these two homologous genes, OsDDM1a/OsCHR746 and OsDDM1b/OsCHR741, had similar expression patterns and slight differences during seed development, which indicates that the two genes might have diverse functions.

The Phenotype of Osddm1b on Regulating the Grain Size

The dwarfism phenotype was also observed in the T-DNA insertion mutation of OsDDM1b (Figures 2B,H,I and Supplementary Figures 2B,C). The male and female gametophyte formation was found to be normal as expected. However, the seed setting rate was partially decreased to 84.39%, which suggests that OsDDM1b might play a role in pollen tube growth or embryo development after fertilization (Supplementary Figure 2D–H). The dwarfism and partial fertility are similar to antisense OsDDM1 lines. The single mutation of OsDDM1a or OsDDM1b showed a normal phenotype during vegetative or reproductive stages, even in several generations (Tan et al., 2016). However, our data showed that osddm1b exhibited dwarfism and grain size variations. The T-DNA insertion in Tan’s mutation of OsDDM1b was in the third intron. However, in our experiment, the mutation was in the second exon (Figure 2A and Supplementary Figure 2A, see footnote 3), which suggests that this difference may be due to the position of T-DNA insertion. OsDDM1a and OsDDM1b, as the homologous genes, have a significantly similar genome and amino acid sequences. The primers of qRT-PCR were designed by aligning the CDS of two genes and finding the different bases among T-DNA insertion positions to improve primers’ specificity and obtain more accurate expression data (Figure 2A).

As an important agronomic trait, grain size is essential for crop genetics and breeding, which includes length, width, and thickness. Several genes have been reported to play the critical roles in regulating grain size. Cell proliferation and expansion are coordinately significant in the spikelet hull, which controls the final grain size. The expression levels of both OsDDM1 are more enriched in proliferating young tissues (Hu et al., 2013), which is consistent with the expression pattern of OsDDM1b by GUS staining (Figures 5B,C and Supplementary Figure 5). The results of the paraffin section and SEM suggested that OsDDM1b might promote latitudinal growth by increasing cell proliferation to contribute to the grain size (Figure 4 and Supplementary Figure 4). Many regulators are involved in controlling grain size, such as G protein signaling (DEP1, RGB1) (Huang et al., 2009; Zhang et al., 2021), the ubiquitin–proteasome pathway (GW2) (Song et al., 2007), the MAPK signaling pathway (GSN1, MKKK10, and MAPK6) (Liu S. Y. et al., 2015; Guo et al., 2018; Xu et al., 2018), phytohormone signaling (BG1, GL3,1, and G55) (Li et al., 2011; Qi et al., 2012; Liu L. C. et al., 2015), transcriptional regulation (GL7, GLW7/OsSPL13, GSW/OsGRF4, GSW, GSW/OsSPL16, and qLGY3/OsMADS1) (Wang et al., 2012, 2015; Hu et al., 2015; Si et al., 2016; Liu et al., 2018; Zhao et al., 2018), and other regulators such as protein kinase (qTGW3/OsGSK5) (Hu et al., 2018). The creation of insertion mutations is a valuable tool for active transposons to analyze the function of endogenous genes (Hirochika, 2001). The abnormal grain size was caused by the defection of OsDDM1b, which affected the expression of grain size regulators.

There may be a balance mechanism in plants when the mutation of OsDDM1b displayed decreased grain length. In contrast, grain width and thickness were increased due to the compensation mechanism or regulated grain filling rate to balance the 1,000-grain weight with wild type. The higher expression levels of G55 produced more comprehensive grains and increased grain filling rate. Then, G55 was upregulated in the inflorescence (length 8 cm) and seeds (1 DAF) of osddm1b, which suggests that OsDDM1b might negatively regulate G55 to affect the grain width. However, whether OsDDM1b can influence the grain filling rate is still unknown.

A Proposed Model for OsDDM1b Regulation on Grain Size

Above all, OsDDM1b (decrease in DNA methylation) contributes to grain size by regulating latitudinal cell proliferation and cell width to enhance the grain width and thickness, which decreases the grain length reversely. In addition, OsDDM1b, as a chromatin remodeling regulator, influences BR homeostasis and signaling pathway, the expression levels of BR, and grain size–related genes (Supplementary Figure 7). DNA methylation is involved in embryo and endosperm development and is required to determine the methylation pattern in osddm1b. The relationship between grain size and dynamic DNA methylation is necessary to be revealed with the future studies.
This helps to understand the mechanism of epigenetic regulation in controlling grain size in rice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

YQ and HZ conceived the initial screening and research plans and designed the experiments. MG, WZ, ZH, ZS, MY, CS, LL, AW, and JL performed most of the experiments. ZS, MY, CS, LL, and AW extracted the RNA. MM, MG, and WZ performed the qRT-PCR and analyzed the data. ZH and DT performed the paraffin section and SEM. YQ, HZ, and MG wrote the article with the contributions of all the authors. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.873993/full#supplementary-material

REFERENCES

Andrew, F., Martin, D., Barton, G. J., and Tom, O. H. (2006). Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. Nucleic Acids Res. 34, 2887–2905. doi: 10.1093/nar/gkl295
Bai, M. Y., Zhang, L. Y., Gampala, S. S., Zhu, S. W., Song, W. Y., Chong, K., et al. (2013). Functions of OsBZR1 and 14-3-3 proteins in brassinosteroid signaling in rice. Proc. Natl. Acad. Sci. U.S.A. 104, 13839–13844. doi: 10.1073/pnas.0706386104
Bargsten, J. W., Folta, A., Mlynarova, L., and Nap, J. P. (2013). Snf2 family gene distribution in higher plant genomes reveals DRD1 expansion and diversification in the tomato genome. PLoS One 8:e81147. doi: 10.1371/journal.pone.0081147
Brzezinka, K., Allmann, S., Czesnick, H., Nicolas, P., Gorka, M., Benke, E., et al. (2016). Arabidopsis FORGETTER1 mediates stress-induced chromatin memory through nucleosome remodeling. Elife 5:e17061. doi: 10.7554/eLife.17061

Supplementary Figure 1 | The relative expression level of Snf2 gene family in the wild-type (ZH11) seeds. (A) Highly expressed OsChR714 in the S3 (7 DAF) stage, while OsChR728 is just lowly expressed in the S5 (25 DAF) stage. (B) Preferentially expressed OsChR720, 726, and 740 in the S4 (10 DAF) stage. (C) Predominantly expressed OsChR717, 724, 743, and 746 in the S4 (10 DAF) or S5 (25 DAF) stage. S1, the seeds of 1 DAF; S2, the seeds of 3 DAF; S3, the seeds of 7 DAF; S4, the seeds of 10 DAF; S5, the seeds of 25 DAF.

Supplementary Figure 2 | Genotype and phenotype identification of OsDDM1b mutant. (A) The young seedlings of wild type (HY) and osddm1b in 10 days. Bar = 4 cm. (B) The internode length of wild type (HY) and osddm1b. Bar = 5 cm. (C,D) Viability of mature pollen grains in wild type (G), HY and osddm1b (D) as assessed by I2-KI staining. Bar = 200 µm. (E,F) Mature ovules in the wild type (E), HY and osddm1b (F). Bar = 25 µm. (G) The seed setting rate of wild type (HY) and osddm1b (n = 10). (H) The 1,000-grain weight of wild type (HY) and osddm1b (n = 27). (I–L) Comparision on grain length (I,J), width (K,M), and thickness (L,N) of wild type (HY) and osddm1b. (G–H) Bar = 5 mm. (I–L) Bar = 2 mm. Data are given as means ± SD. t-test with Student’s t-test.

Supplementary Figure 3 | Identification of T-DNA insertion site. A segment was amplified from osddm1b homozygous mutants DNA using the 28-60109-Lp and Rb (2707) primers. The PCR fragments were sequenced and aligned with the genome of OsDDM1b and 2707 vector sequence by the SnapGene software, respectively.

Supplementary Figure 4 | Histological analysis of spikelet hulls and the effect of OsDDM1b on the expression of genes involved in the cell cycle. (A,B) Magnified views of the cross-section of wild type (HY) and osddm1b. The black and blue arrows indicate the rows of specialized cells with lower epidermis cells and rigid walls, respectively. Bar = 50 µm. (C) The expression levels of cell cycle-related genes in the 8-cm panicles. Data are given as means ± SD. **p < 0.01, *p < 0.05 compared with wild type (HY) using Student’s t-test.

Supplementary Figure 5 | The native OsDDM1b promoter drives GUS expression. (A–D) GUS signal was detected in young root tips (A), young leaf (B), mature leaf (C), stem node (D). Bar = 1 mm (A), Bar = 2 mm (B–D). (E–F) The stamen (E) and pistil (F) from 1–2, 2–3, 3–4, 4–5, 5–6, 6–7 cm, and mature spikelet, respectively. Bar = 500 µm.

Supplementary Figure 6 | The phenotype of wild type (HY) and osddm1b in response to BR treatment. (A) Lamina joint of the wild type (HY) and osddm1b in response to various concentrations of BR. Bar = 2 mm. (B) The contents of endogenous BR in the wild type (HY) and osddm1b. Data are given as means ± SD. **p < 0.01 compared with wild type (HY) using Student’s t-test.

Supplementary Figure 7 | A working model on OsDDM1b regulation on grain size. As an epigenetic regulator, OsDDM1b is involved in DNA methylation, which participates in multiple biological processes. For example, OsDDM1b affected the expression of key genes determining cell cycle, BR signal, and grain size. Abnormal cell proliferation can influence the character of spikelets resulting in grain size defect.

Cai, H. Y., Zhang, M., Chai, M. N., He, Q., Huang, X. Y., Zhao, L. H., et al. (2019). Epigenetic regulation of anthocyanin biosynthesis by an antagonistic interaction between H2A.Z and H3K4me3. New Phytol. 221, 295–308. doi: 10.1111/nph.15306
Cairns, B. R. (2005). Chromatin remodeling complexes: strength in diversity, precision through specialization. Curr. Opin. Genet. Dev. 15, 185–190. doi: 10.1016/j.gde.2005.01.003
Chen, L. S., Xiong, G. S., Cui, X., Yan, M. X., Xu, T., Qian, Q., et al. (2013). OsGRAS19 may be a novel component involved in the brassinosteroid signaling pathway in rice. Mol. Plant 6, 988–991. doi: 10.1093/mp/ss307
Cho, E. J., Choi, S. H., Kim, J. H., Kim, J. E., Lee, M. H., Chung, B. Y., et al. (2016). A mutation in plant-specific SWI2/SNF2-like chromatin-remodeling proteins, DRD1 and DDM1, delays leaf senescence in Arabidopsis thaliana. PLoS One 11:e0146826. doi: 10.1371/journal.pone.0146826
Choi, K., Park, C., Lee, J., Oh, M., Noh, B., and Lee, I. (2007). Arabidopsis homologs of components of the SWR1 complex regulate flowering and
Hu, Z., Liu, S.-J., Wang, M.-J., He, H., Sun, L., Wang, H., et al. (2018). A novel QTL QGW3 encodes the GSK3/SHAGGY-like kinase OsGSK3/OsSK41 that interacts with OsARF4 to negatively regulate grain size and weight in rice. Mol. Plant 11, 736–749. doi: 10.1016/j.molp.2018.03.005

Huang, X. Z., Qian, Q., Liu, Z. B., Sun, H. Y., He, S. Y., Luo, D., et al. (2009). Natural variation at the DEPI locus enhances grain yield in rice. Nat. Genet. 41, 494–497. doi: 10.1038/ng.352

Hurtado, L., Farrona, S., and Reyes, J. C. (2006). The putative SWI/SNF complex subunit BRAHMA activates flower homeotic genes in Arabidopsis thaliana. Planta Mol. Biol. 62, 291–304. doi: 10.1007/s11103-006-9021-2

Ito, Y., Takaya, K., and Kurata, N. (2005). Expression of SRF family receptor-like protein kinase genes in rice. Biochem. Biophys. Acta Gene Struct. Expr. 1730, 253–258. doi: 10.1016/j.bbabop.2005.06.007

Je, B. I., Hai, L. P., Park, S. J., Park, S. H., Kim, C. M., Xuan, Y. H., et al. (2010). RAV-Like1 maintains brassinosteroid homeostasis via the coordinated activation of BRI1 and biosynthetic genes in rice. Plant Cell 22, 1777–1791. doi: 10.1105/tpc.109.09575

Jeddeloh, J. A., Stokes, T. L., and Richards, E. J. (1999). Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat. Genet. 22, 94–97. doi: 10.1038/3803

Jefferson, R. A., Kavanagh, T. A., and Bevan, M. W. (1987). GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. 6, 3901–3907. doi: 10.1046/j.1469-1820.1987.tb02730.x

Jiang, Y. H., Bao, L., Jeong, S. Y., Kim, S. K., Xu, C. G., Li, X. H., et al. (2012). XIAO is involved in the control of organ size by contributing to the regulation of signaling and homeostasis of brassinosteroids and cell cycling in rice. Plant Cell 70, 398–408. doi: 10.1111/j.1365-3131.2011.04877.x

Kakimoto, T. J., and Physiology, C. (2011). The CKH2/PKL chromatin remodeling factor negatively regulates cytokinin responses in Arabidopsis. Plant Cell 23, 952–965. doi: 10.1007/s11192-010-9468-0

Kanam, T., Mette, M. F., Kreil, D. P., Aufsatz, W., Matzke, M., and Matzke, A. J. M. (2004). Involvement of putative SNF2 chromatin remodeling protein DRD1 in RNA-directed DNA methylation. Curr. Biol. 14, 801–805. doi: 10.1016/j.cub.2004.03.037

Kniewski, L., Ginalska, K., and Jerzmanowski, A. (2008). Snf2 proteins in plants: gene silencing and beyond. Trends Plant Sci. 13, 557–565. doi: 10.1016/j.tplants.2008.08.004

Koh, S., Lee, S. C., Kim, M. K., Koh, J. H., Lee, S., An, G., et al. (2007). T-DNA tagged knockout mutation of rice OsGSK1, an orthologue of Arabidopsis BIN2, with enhanced tolerance to various abiotic stresses. Plant Mol. Biol. 65, 453–466. doi: 10.1007/s11103-007-9213-4

Kwon, C. S., Chen, C. B., and Wagner, D. (2005). WUSCHEL is a primary target for transcriptional regulation by SPL14 in dynamic control of stem cell fate in Arabidopsis. Genes Dev. 19, 992–1003. doi: 10.1101/gad.1276305

Li, C. L., Lu, I. F., Gao, L., Chen, C., Wei, C. Q., Qiu, Q., et al. (2016). Concerted genomic targeting of H3K27 demethylase REF6 and chromatin-remodeling ATPase BRM in Arabidopsis. Nat. Genet. 48, 687–693. doi: 10.1038/ng.3555

Li, J., Wen, J., Q., Lease, K. A., Doke, J. T., Tax, F. E., and Walker, J. C. (2002). BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell 110, 213–222. doi: 10.1016/s0092-8674(02)00812-7

Li, X., Jiang, Y., Ji, Z., Liu, Y., and Zhang, Q. (2015). BRHIS1 suppresses rice innate immunity through binding to monoubiquitinated H2A and H2B variants. EMBO Rep. 16, 1192–1202. doi: 10.15252/embr.201440000

Li, Y. B., Fan, C. C., Xing, Y. Z., Jiang, Y. H., Luo, J. S., Lin, L., et al. (2011). Natural variation in GSK5 plays an important role in regulating grain size and yield in rice. Nat. Genet. 43, 1266–1269. doi: 10.1038/ng.977

Liu, J., Chen, J., Zheng, X., Wu, F., Lin, Q., Heng, Y., et al. (2017). GW5 acts in the brassinosteroid signalling pathway to regulate grain width and weight in rice. Nat. Plants 3:17043. doi: 10.1038/nplants.2017.43

Liu, L. C., Tong, H. N., Xiao, Y. H., Che, R. H., Xu, F., Hu, B., et al. (2015). Activation of big Grain1 significantly improves grain size by regulating auxin transport in rice. Proc. Natl. Acad. Sci. U.S.A. 112, 11102,2015.

Liu, Q., Han, B., Wu, K., Zhang, J., Ye, Y., Wang, S., et al. (2018). G-protein βγ subunits determine grain size through interaction with MADS-domain transcription factors in rice. Nat. Commun. 9:852. doi: 10.1038/s41467-018-03047-9
Si, L. Z., Chen, J. Y., Huang, X. H., Gong, H., Luo, J. H., Hou, Q. Q., et al. (2021). Chromatin remodeling factors regulate Shaked, H., Avivi-Ragolsky, N., and Levy, A. A. (2006). Involvement of the Ren, D. Y., Yu, H. P., Rao, Y. C., Xu, Q. K., Zhou, T. T., Hu, J., et al. (2019). AH2 Tong, H. N., Jin, Y., Liu, W. B., Li, F., Fang, J., Yin, Y. H., et al. (2009). DWARF Guo et al. Wang, L., Xu, Y., Zhang, C., Ma, Q., Joo, S. H., Kim, S. K., et al. (2008). OsLIC, a novel CCCH-Type zinc finger protein with transcription activation, mediates rice architecture via brassinosteroid signaling. PLoS One 3:e3521. doi: 10.1371/njournal.pone.0003521 Wang, S., Wu, K., Yuan, Q., Liu, X., Liu, Z., Lin, X., et al. (2012). Control of grain size, shape and quality by OsSPL16 in rice. Nat. Genet. 44, 950–954. doi: 10.1038/ng.2327 Wang, Y. X., Xiong, G. S., Hu, J., Jiang, L., Yu, H., Xu, J., et al. (2015). Copy number variation at the GL7 locus contributes to grain size diversity in rice. Nat. Genet. 47, 944–948. doi: 10.1038/ng.3346 Xu, R., Duan, P. G., Yu, H. Y., Zhou, Z. K., Zhang, B. L., Wang, R. C., et al. (2018). Control of grain size and weight by the OsMKK10-OsMKK4-OsMMP6 signaling pathway in rice. Mol. Plant 11, 860–873. doi: 10.1016/j.molp.2018.04.004 Yamamura, C., Ihara, Y., Wu, X., Noguchi, T., Fujisaka, S., Ashikari, M., et al. (2000). Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. Plant Cell 12, 1591–1606. doi: 10.1105/tpc.12.9.1591 Ying, J.-Z., Ma, M., Bai, C., Huang, X.-H., Liu, J.-L., Fan, Y.-Y., et al. (2018). TGW3, a major QTL that negatively modulates grain length and weight in rice. Mol. Plant 11, 750–753. doi: 10.1016/j.molp.2018.03.007 Yu, X. Q., Xia, S. S., Xu, Q. K., Cui, Y. J., Geng, M., Zeng, D. L., et al. (2020). ABNORMAL FLOWER AND GRAIN 1 encodes OsMADS6 and determines palea identity and affects rice grain yield and quality. Sci. China Life Sci. 63, 228–238. doi: 10.1007/s11427-019-1593-0 Zeng, Y. X., Hu, C. Y., Lu, Y. G., Li, J. Q., and Liu, X. D. (2007). Diversity of abnormal embryo sacs in indica/japonica hybrids in rice demonstrated by confocal microscopy of ovaries. Plant Breed. 126, 574–580. doi: 10.1111/j.1439-0236.2007.01380.x Zeng, Y. X., Hu, C. Y., Lu, Y. G., Li, J. Q., and Liu, X. D. (2009). Abnormalities occurring during female gametophyte development result in the diversity of abnormal embryo sacs and leads to abnormal fertilization in indica/japonica hybrids in rice. J. Integr. Plant Biol. 51, 3–12. doi: 10.1111/j.1744-7909.2008.00733.x Zhang, D., Gao, S., Yang, P., Yang, J., Yang, S., and Wu, K. (2019). Identification and expression analysis of Snf2 family proteins in tomato (Solanum lycopersicum). Int. J. Genomics 2019:5089035. doi: 10.1155/2019/5089035 Zhang, D., Jing, Y., Jiang, Z., and Lin, R. (2014). The chromatin-remodelling Factor PICKLE integrates brassinosteroid and gibberellin signaling during skotomorphogenic growth in Arabidopsis. Plant Cell 26:2472. doi: 10.1105/tpc.113.121848 Zhang, D., Li, Y. H., Zhang, X. Y., Zha, P., and Lin, R. C. (2017). The SW2/SNF2 chromatin-remodelling ATPase BRAHMA regulates chlorophyll biosynthesis in Arabidopsis. Mol. Plant 10, 155–167. doi: 10.1016/j.molp.2016.11.003 Zhang, D., Zhang, M., and Liang, J. (2021). RBG1 regulates grain development and starch accumulation through its effect on OsYUC11-mediated auxin biosynthesis in rice endosperm cells. Front. Plant Sci. 12:585174. doi: 10.3389/fpls.2021.585174 Zhang, H., Bishop, B., Ringenberg, W., Muir, W. M., and Ogas, J. (2012). The CHD3 Remodeler PICKLE associates with genes enriched for Trimethylation of Histone H3 lysine 27 in plants. Plant Physiol. 159, 418–432. doi: 10.1104/pp.112.194878 Zhang, L. Y., Bai, M. Y., Wu, J. X., Zhu, J. Y., Wang, H., Zhang, Z. G., et al. (2009). Antagonistic HLH/HLH transcription factors mediate brassinosteroid regulation of cell elongation and plant development in rice and Arabidopsis. Plant Cell 21, 3767–3780. doi: 10.1105/tpc.109.074411 Zhang, M., Liu, Y. H., Cai, H. Y., Guo, M. L., Chai, M. N., She, Z. Y., et al. (2012). The bZIP Transcription Factor GmZIP1 negatively regulates salt- and drought-stress responses in soybean. Int. J. Mol. Sci. 21:7778. doi: 10.3390/ijms21207778 Zhao, D. S., Li, Q. F., Zhang, C. Q., Zhang, C., Yang, Q. Q., Pan, L. X., et al. (2018). GSA9 acts as a transcriptional activator to regulate rice grain shape and appearance quality. Nat. Commun. 9:1240. doi: 10.1038/s41467-018-03616-y Zhao, H. M., Guo, M. L., Yan, M. K., Cheng, H., Liu, Y. H., She, Z. Y., et al. (2020). Comparative expression profiling reveals genes involved in Megasporogenesis. Plant Physiol. 182, 2096–2024. doi: 10.1104/pp.19.01254 Zhao, H. M., Maokai, Y., Cheng, H., Guo, M. L., Liu, Y. H., Wang, L. L., et al. (2021). Characterization of auxin transporter AUX, PIN and PL5 genes families in pineapple and evaluation of expression profiles during reproductive
development and under abiotic stresses. PeerJ 9:e11410. doi: 10.7717/peerj.11410

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