SDG711 is Involved in Rice Seed Development Through Regulation of Starch Metabolism Gene Expression in Coordination With Other Histone Modifications

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Abstract

SDG711 is a histone H3K27me2/3 transmethylases in rice, homolog of CLF in Arabidopsis, that plays key roles in regulating flowering time and panicle development. In this work, we investigated that the role of SDG711 in rice seed development. Overexpression and down-regulation of SDG711 lead to the decrease and increase of the expression level of genes related to starch accumulation, resulting in smaller seed or even seed abortion. ChiP assay showed that SDG711-mediated H3K27me3 changed significantly in genes related to endosperm development and SDG711 can directly bind to the gene body region of several starch synthesis genes and amylase genes. In addition, H3K4me3 and H3K9ac modifications also cooperate with H3K27me3 to regulate the development of endosperm. Our results suggested that the crosstalk of SDG711-mediated H3K27me3 with H3K4me3 and H3K9ac are involved in starch accumulation to control normal seed development.

Background

As one of the most important agricultural crops, rice provides food for more than one-half of population of the world. The main body of rice as food is the seed. Starch metabolism is a key process of seed development that directly affects grain yield and quality. Starch metabolism mainly includes starch synthesis and starch degradation.

The starch synthesis genes in the seed can be divided into 4 groups according to tissue- and developmental stage-specific: group I genes, which are expressed in very early period of in grain formation and are thought to be involved in the construction of fundamental cell machineries and initiation of starch granules; group II genes, highly expressed during endosperm development; group III genes, which transcript low at the onset but raise their expression level steeply at the start of starch synthesis in the endosperm and are presumed to play essential roles in endosperm starch synthesis; and group IV genes, mainly expressed at the onset of grain development with scanty expression, and might be involved in synthesis of starch in the pericarp (Odhani et al. 2005). The mechanism regulating starch biosynthesis in cereal seeds is not well understood. So far, genetic and functional genomics studies have only identified some key regulators of starch synthesis. For example, The MYC transcriptional factor OsBP-5 can interact with an ethylene-responsive element binding protein (EREBP), OsEBP-89, to enhance the transcription of OsGBSSI which belongs to group III starch synthesis gene. Knockdown of OsBP-5 expression results in reduced expression of OsGBSSI, leading to a reduction in the amylose content of mature seeds (Zhu et al. 2003). The FLO2 gene, encoding a nuclear-localized TPR-binding protein, has been shown to positively regulate expression of starch synthesis-associated genes by interacting with bHLHs transcription factors (She et al. 2010). The AP2/EREBP TF gene RSR1 negatively regulates starch synthesis genes including OsGBSSI, OsSSI and OsSSIla, In RSR1 mutants, the expression levels of these genes increased, while in RSR1 overexpression, the expression of these genes decreased (Fu and Xue 2010). It is shown that OsZIP58 encodes a key transcriptional regulator required for starch synthesis through direct binding to the promoters of OsAGPL3, OsGBSSI, OsSIIa, SBE1, OsBElb, and ISA2 to promote their expression. (Wang et al. 2013). During grain filling, starch biosynthesis genes such as OsGBSSI, OsSSI, OsSSIla, and OsAGPL2 are up-regulated in SERF1 knockout mutants. Moreover, SERF1 is a direct upstream regulator of GBSSI (Schmidt et al. 2014). In contrast to these positive and negative regulators controlling starch biosynthesis gene expression, FLO6 (a CBM domain containing protein) may act as a starch-binding protein involved in starch synthesis and compound starch grain formation through a direct interaction with isoamylase1 (OsiSIIA1) which belongs to the third group of starch synthase genes in developing rice seeds (Peng et al. 2014). In addition to the genetic approaches, a genome-wide DNA methylation analysis of a series of developmental stages of rice endosperm reveals that DNA methylation is involved in repression of genes involved in starch synthesis during seed development (Xing et al. 2015).

On the other hand, starch degradation genes mainly contain α- and β-amylases (Asatsuama et al. 2006; Zhang et al. 2016). There are few studies on the regulation mechanism of amylases. It is shown that SERF1 negatively regulates germination by controlling RPBFB expression, which mediates the gibberellic acid (GA)-induced expression of RICE AMYLASE1A (RAMy1A), loss of SERF1 enhances RPBFB expression resulting in larger grains with increased starch content, while SERF1 overexpression represses RPBFB resulting in smaller grains (Schmidt et al. 2014). OsSRT1 mediates H3K9ac by directly binding to starch metabolism genes such as OsAmy3B, OsAmy3E, OsBmy9 and OsBmy9 to regulate the expression of these genes, down-regulation of OsSRT1 lead to abnormal seed development (Zhang et al. 2016). Therefore, the regulation of starch metabolism is crucial for determining rice yield and quality. However, the chromatin and epigenetic mechanisms that directly regulate rice starch metabolism are currently only sporadically described.

Histone acetylation and methylation are important chromatin modifications that regulate gene expression during plant development (He et al. 2011). Particularly, H3 lysine 9 (H3K9) acetylation and H3 lysine 4 trimethylation (H3K4me3) are tightly correlated with genes with high expression level and low tissue specificity, whereas histone H3 lysine 27 trimethylation (H3K27me3) is associated with genes with low expression level and high tissue specificity (Charron et al. 2009; Makarevitch et al. 2013; Zhang et al. 2009; Zhang et al. 2007). Polycomb-group (PcG) and Trithorax-group (TrxG) proteins catalyze H3K27me3 or H3K4me3, respectively (Steffen and Ringrose 2014). Several studies reveal that some development-related genes have bivalent modifications (H3K4me3-H3K27me3) to coordinately regulate their expression in plants (Berr et al. 2010; Sequeira-Mendes et al. 2014). Our previous study also suggested that the dynamic change of H3K27me3/H3K4me3 ratio of bivalent marking genes related to development during the SAM-to-IM transition was critical for genome-wide gene expression reprogramming in IM (Liu et al. 2015). Subsequently, readers (EBS and SHL) were identified as having the ability to recognize both H3K27me3 and H3K4me3 via its bromo-adjacent homology (BAH) and plant homeodomain (PHD) domains, which was further confirmed that the genes can be coordinately regulated by different histone modifications (Qian et al. 2018; Yang et al. 2018).

The Polycomb-repressive complex2 (PRC2), a subset of the PcG proteins, has four core proteins: ENHANCER OF ZESTE [E(z)], SUPPRESSOR OF ZESTE 12 [Su(z)12], EXTRA SEXCOMBS (ESC) and P55 (Schuettengruber and Cavalli 2009). The E(z) protein catalyzes the addition of H3K27 methylation (Czernin et al. 2002). There are three E(z) homologs also catalyzing the addition of H3K27me3 in Arabidopsis: CURLY LEAF (CLF), SWINGER (SWN), and MEDEA (MEA). MEA mainly plays an important role in gametogenesis and early seed development, whereas CLF/SWN are partially redundant and function primarily in vegetative and reproductive development (Hennig and Derkacheva 2009). The homologs of E(z) (OsCLF/SDG711 and OsE21/SDG718) have also been found in rice (Luo et al. 2009). They are required for H3K27me3 during rice flowering development and reproductive transition (Liu et al. 2014b; Liu et al. 2015). They may be involved in regulating seed dormancy, seedling growth, vegetative and reproductive development and seed development together with other PRC2
components in rice (Chen et al. 2017; Huang et al. 2016; Li et al. 2014; Liu et al. 2016; Nallamilli et al. 2013; Zhong et al. 2018). Previous studies revealed that during rice seed development, PRC2 complex mainly regulates the development of embryo, endosperm and seed coat by regulating transcription factors(Huang et al. 2016; Nallamilli et al. 2013).

In this work, we showed that both SDG711 RNAi plants and overexpression plants produced smaller seeds. To understand the molecular mechanism, we examined the expression and histone modification of starch related genes, and direct association of SDG711 to those genes by ChIP assay using anti-SDG711 antibody. Our results suggested that SDG711 directly repressed the expression of several starch synthesis genes and amylase genes through H3K27me3 modification, leading to impaired starch accumulation in developing seeds. In addition, H3K4me3 and H3K9ac enrichment also changed on these target genes. Our results suggested that H3K27me3 and H3K4me3 have antagonistic effects on starch synthesis genes and H3K27me3 and H3K9ac have antagonistic effects on amylase genes, respectively. The cooperation of SDG711-mediated H3K27me3 with H3K4me3 and H3K9ac is involved in starch accumulation to regulate normal seed development.

Materials And Methods

Plant materials and growing conditions

Rice (Oryza sativa ssp japonica) material used in this study were from the 'DongJin' (DJ) background, including wild-type, SDG711 (OsCLF) overexpression, and RNAi lines (Liu et al. 2014b). The germinated rice seedlings of all genotypes were transplanted in field at the beginning of May and grown till the mid of August in Wuhan. For analyzing developmental seeds, spikelet samples were collected daily from 1 DAP to 7DAP (Day After Pollination). The flowering spikelets were tagged with a pen mark on the lemmas. About 300 developing seeds from 30 rice plants of each line were collected, and three biological repeats were performed.

Microscopy analysis of endosperm structure

The collected spikelet samples were immediately fixed in 5%(v/v) formaldehyde, 5% (v/v) acetic acid, 45% (v/v) ethanol, and 45%(v/v) distilled, deionized water at 4°C, followed by vacuum infiltration until the samples sank to the bottom of container. Fixed samples were embedded with Technovit 7100 ( Heraeus Kulzer), then cut to semi-thin sections of 1–5 µm thickness with a Leica RM2265 microtome for imaging.

Gene expression analysis

Total RNA was extracted from seeds of 3DAP using Trizol reagent (TransGen Biotech) according to the manufacturer's protocol. Two micrograms of total RNA were reverse-transcribed to obtain cDNA by using cDNA enzyme and M-MLV Reverse transcriptase (Invitrogen) according to the manufacturer's instruction.

Synthesized first strand cDNA was used as template for qRT-PCR. The qRT-PCR was performed on ABI 7900 instrument. The reactions were performed at 95°C for 10 s, 45 cycles of 95°C for 5 s, and 60°C for 40 s. Disassociation curve analysis was performed as follows: 95°C for 15 s, 60°C for 20 s, and 95°C for 15 s. Data were collected using the ABI 7900 sequence detection system following the instruction manual. The relative expression levels were analyzed using the 2-△△CT method (Livak and Schmittgen 2001). The rice ACTIN1 gene was used as the internal control. Primers in Table S1 were designed with PRIMER EXPRESS 2.0 software (PE Applied Biosystems) to amplify 80–250 bp products.

ChIP assay

ChIP analysis was performed as previous described (Hu et al. 2012). Briefly, chromatin isolated from 4 g seeds of 3 DAP was incubated with antibody coated beads (Life technology, 10001D) overnight. After wash and elution, products were reverse crosslinked. Then the products were treated with protease K (9034, Takara), recovered, and used as template for real-time PCR with primers listed in Table S1. Antibodies for histone modifications are anti-H3K4me3 (ab8580, Abcam), anti-H3K27me3 (07-449, Millipore) and anti-H3K9ac (07-352, Millipore) respectively. Antibody of SDG711 was produced by immunizing rabbits with E. coli produced full-length SDG711 protein (Liu et al. 2012).

Yeast two hybrid assay

Constructs for yeast two-hybrid analysis were generated using the Matchmaker® Gold Yeast Two-Hybrid System (Clontech) vectors pGBKKT7 and pGADT7, which express protein fusions to the GAL4 DNA-binding domain or transcriptional-activation domain, respectively. Full length of cDNA inserts encoding OsCLF (SDG711) and OsIZE1 (SDG718) were introduced into pGADT7, full length of cDNA inserts encoding OsIZE1, OsFIE1, OsFIE2 and OsEMF2b were introduced into pGBKKT7. The analysis was performed in strain AH109 carrying HIS3 and MEL1 reporters for reconstituted GAL4 activity.

Starch Contents, protein content and 100-Grain Weight Determination

Fully filled grains of 30 DAF were used for measuring grain quality and yield traits. Embryo and pericarp were removed from the dehulled grains, and the endosperm were ground to powder. The AAC (Apparent Amylose Content) of the sample was measured by the iodine colorimetric method (Juliano 1971). To determine the total starch content, 50 mg of powder was washed two to three times by using 80%(v/v) ethanol and then extracted with 9.2 and 4.6 M perchloric acid in order. The supernatant was collected and diluted to 50 mL with water. An aliquot of this solution was analyzed for starch content by the anthrone method (Turner and Turner 1960). Seed storage protein content was measured using near-infrared reflectance with XDS Rapid liquid Analyze (FOSS Tecator AB, Sweden). All samples were determined with 3 replications (Ge et al. 2007) The 1,000-grain weight was determined by counting 10 replicates of 100-grain samples independently on an electronic balance. Data are shown as means ± SD.

Results
Knockdown and overexpression of the SDG711 gene leads to abnormal seed development in rice

Our previous studies characterized transgenic and mutant plants for the rice E(Z) gene SDG711 and showed that SDG711 regulated rice flowering time and inflorescence meristem activity (Liu et al. 2014b; Liu et al. 2015). We also observed that SDG711 expression levels affect rice seed development. Seed setting rate was reduced by 71–89% compared to wild type (WT) in both overexpression and RNAi plants (figure S1). Through the investigation of the structure of the flowers, it is showed that the stigmas and anthers are not significantly different from WT in the external structure, whether they are in overexpression plants or RNAi plants. Observation of the morphology of seeds at different days after fertilization shows that seeds of overexpression plants significantly developed more slowly than WT seeds, and most of them do not develop into full seeds normally (figure S1). Subsequently, the agronomic traits of overexpression, RNAi and WT plants in full mature seeds were measured. Compared with WT, the grain width and 100-grain weight of overexpression and RNAi plants were significantly decreased (figure S1). This indicates that SDG711 may be involved in very complicated regulation pathway of seed development process.

SDG711 affects starch accumulation in endosperm cells at the early stage of seed development

Next, we examined the development of the internal structure of the seeds by semi-thin-sections. The cross sections of 3 and 4 DAP seeds showed that the number of starch granules in the endosperm of overexpression plants and RNAi plants is much less than in WT. In addition, the process of starch accumulation is hindered, the starch granules are loosely distributed and cannot completely fill the cavity in the middle of the endosperm (figure 2A). In order to identify the mechanism related to decreased seed quality, we measured the total starch content and apparent amyllose content (AAC) in mature seeds of overexpression, RNAi and WT plants. And the measurement showed that the starch content of seeds in overexpression and RNAi plants were significantly reduced by 16–19% compared to that in WT, while the ACC contents were reduced by 40–52% compared to that in WT (figure S2B). Meanwhile, we also tested the content of the four main storage proteins (albumin, globulin, prolamin and glutelin), and observed that the storage protein content in seeds of overexpression and RNAi plants did not change significantly compared with those in WT (figure S2). The above results provide the clues that SDG711 may regulate seed development by playing vital roles in starch accumulation and distribution but not in protein storage in endosperm.

Starch metabolism related genes were differentially expressed in overexpression and RNAi lines

The accumulation of starch in seed endosperm is directly related to starch synthesis and starch degradation. In order to find out the causes of the low starch content in SDG711 overexpression and RNAi lines, we analyzed the expression levels of starch metabolism-related genes in the 3 DAP seeds, including regulators that affect starch metabolism, starch synthesis-related genes, and starch degradation-related genes. First, we tested the typical regulator genes RSR1, OsbZIP58, FLO2, FLO6, SERF1 and OsBP-5 those have been found to regulate starch metabolism, but yet there were no obvious difference between SDG711 overexpression, RNAi lines and WT (figure S3). Next, we examined 24 genes of four groups of starch synthase (OsSSI, OsSSIIa, OsSSIIb, OsSSIIIa, OsSSIbb, OsSSIva, OsSSIVb, OsAGPS1, OsAGPS2a, OsAGPS2b, OsAGP1, OsAGP1L, OsAGP2, OsAGP3, OsGBSSI, OsGBSSII, OsSSIIa, OsBEI, OsBEI, OsBEI, OsBEI, OsISA1, OsISA2, OsISA3 and OsPUL), 20 starch degradation genes including 10 α-amylase genes (OsAmy1A, OsAmy1B, OsAmy1C, OsAmy2A, OsAmy3A, OsAmy3B, OsAmy3D, OsAmy3E and OsAmy5A) and 10 β-amylase genes (OsBmy1, OsBmy2, OsBmy3, OsBmy4, OsBmy5, OsBmy6, OsBmy7, OsBmy8, OsBmy9 and OsBmy10). The expression of 14 starch synthesis genes (OsSSI, OsSSIa, OsSSIb, OsSSIva, OsSSIIa, OsSSIVa, OsSSIVb, OsAGPS2b, OsAGP2L, OsAGP3, OsGBSSI, OsBEI, OsBEI, OsBEI, OsBEI, OsISA1, OsISA2 and OsPUL) was higher in SDG711 RNAi than WT seeds, and lower in in SDG711 overexpression than WT seeds. And the degree of decrease in overexpression lines is greater than the degree of increase in RNAi lines (figure S3A). The expression of 4 α-amylase genes (OsAmy1C, OsAmy2B, OsAmy3E and OsAmy5A) and 6 β-amylase genes (OsBmy3, OsBmy4, OsBmy6, OsBmy7, OsBmy9 and OsBmy10) was also higher in SDG711 RNAi than WT seeds, and lower in in SDG711 overexpression than WT seeds. However, the degree of decrease in overexpression lines is lower than the degree of increase in RNAi lines (figure S3B). Similar expression levels of other genes were detected in the overexpression, RNAi and WT seeds (figure S4A). This result suggested that in the process of starch accumulation, starch synthesis genes reduced expression to a greater extent than amylase genes so that the starch synthesis genes may play a leading role in SDG711 overexpressing lines, while the starch amylase genes can increase the expression more than starch synthesis genes so that the starch amylase genes may play a leading role in SDG711 RNAi lines.

SDG711 regulates the enrichment of H3K27me3 on genes related to starch metabolism

Our previous study confirmed that SDG711 is a histone methyltransferase, can catalyze the addition of H3K27me3 in overexpression, RNAi and WT seeds at 3 DAP. Given that H3K27me3 was enriched within gene bodies, most of 5’ end of the in rice genes, we analyzed the ChIP by real-time PCR using two primer sets, one corresponding to the 5’ transcripational start site (TSS), the other to 5’ end of the gene body (Fig. 4). Among these genes, 6 starch synthesis genes (OsSSIa, OsSSIIb, OsAGP1L, OsGBSSI, OsBEI and OsISA2) and 4 starch amylase genes (OsAmy1C, OsAmy3B, OsBmy4 and OsBmy9) displayed H3K27me3 in the gene body region, suggesting that regulation of these genes might involve PRC2 function. H3K27me3 on these genes was clearly reduced in the RNAi lines but increased in the overexpression lines (Fig. 4), which reversely correlated with their expression changes in the transgenic plants. Although the expression levels of other genes in the corresponding transgenic plants have changed, the enrichment of H3K27me3 has not changed significantly (figure S5). This may support an indirect effect of SDG711 on their expression. These data suggested that SDG711-mediated H3K27me3 was involved in the regulation of starch synthesis and degradation related genes during seed development.
SDG711 directly binds to starch synthesis genes and amylase genes

To further assess the function of SDG711 on regulation the starch synthesis genes and amylase genes, we performed anti-SDG711 ChIP assays and analyzed by real-time PCR using the same primer sets as for the histone methylation ChIP. Non-immunized serum was used as control. The analysis revealed that SDG711 was obviously enriched in the gene body of 6 starch synthesis genes (OsSSI, OsSSIla, OsAGPL3, OsGBSSI, OsBEI and OsIS2A) and 4 starch amylase genes (OsAmy1C, OsAmy3B, OsBmy4 and OsBmy9) compared with the control (Fig. 5). These results suggested that SDG711 may directly target to these genes.

H3K27me3 can affect H3K4me3 and H3K9ac on some starch synthesis genes and amylase genes

Because H3K27me3 is antagonistic to H3K4me3 on gene activity and H3K9ac has also been reported to be enriched in these genes, we therefore analyzed whether alteration of H3K27me3 affected H3K4me3 and H3K9ac on the starch synthesis genes and amylase genes in the transgenic plants. We performed anti-H3K4me3 and H3K9ac ChIP assays and analyzed by real-time PCR using the same primer sets as for H3K27me3 ChIP. Enriched H3K4me3 and H3K9ac are near the 5’ transcriptional start site (TSS), and the P1 primer meets the requirements. The analysis revealed on OsAGPL3, OsBEI and OsIS2A, relatively higher levels of H3K4me3 in SDG711 RNAi plants than those in WT, and relatively lower levels of H3K4me3 in SDG711 overexpression plants than those in WT (Fig. 6A), which conversely correlated to that of H3K27me3 and suggested that SDG711-mediated H3K27me3 might affect H3K4me3 in these 3 loci. Similarly, H3K9ac on OsSSI, OsSSIla, OsBmy4 and OsBmy9 was increased in SDG711 RNAi plants but decreased in SDG711 overexpression plants (Fig. 6B), which also conversely correlated to that of H3K27me3 and suggested that SDG711-mediated H3K27me3 might affect H3K9ac in these five loci. Although the alteration of H3K27me3 is relatively obvious in other genes, the enrichment of H3K4me3 or H3K9ac on these genes has not changed significantly in the transgenic plants. These results indicated that both single and multiple modifications can regulate gene expression, and multiple modifications should achieve balance in those starch synthesis genes and amylase genes. Once this balance is broken, it will cause abnormal gene expression (Fig. 7).

Discussion

In Arabidopsis, at least three PRC2-like complexes: the EMBRYONIC FLOWER (EMF), VERNALIZATION (VRN) and FERTILISATIONINDEPENDENT SEED (FIS) complexes play critical roles in different development stages. The EMF complex (CLF/SWN, EMF2, FIE and MSII) mainly promotes vegetative development of the plant, and delays reproduction. The VRN complex (CLF/SWN, VRN2, FIE and MSII) establishes epigenetic silencing of FLC after vernalization and enables flowering. The FIS complex (MEA, SWN, FIS2, FIE and MSI1) prevents seed development in the absence of fertilization and is required for normal seed development (Hennig and Derkacheva 2009). Although there have been some advances in few studies of the components of the PRC2 complex in rice (Chen et al. 2017; Folsom et al. 2014; Huang et al. 2016; Li et al. 2014; Liu et al. 2016; Liu et al. 2014b; Liu et al. 2019; Nallamilli et al. 2013; Zhong et al. 2018), the specific composition and diversity of the PRC2 complex have not been clearly described. Combining previous research and our current study on the relationship between some key components (figureS6), we propose that during the flowering stage of rice, there are two PRC2 complexes: OsCLF (SDG711)-complex and OsIZE1 (SDG718)-complex, regulating the flowering time under long day light and short day light, respectively; during the other development stages, OsFIE1-complex mainly plays roles in seed development and OsFIE2-complex play essential roles in the regulation of rice vegetative and reproductive development (figureS7).

MEF and FIS2 are the two core components of PRC2, revealed by previous studies that they are imprinted genes and only transcribed from the maternal allele in endosperm and interact directly with each other to regulate endosperm formation by controlling the activity of a number of imprinted genes in the endosperm in Arabidopsis (Kinoshita et al. 1999; Luo et al. 2000; Spillane et al. 2000; Baroux et al. 2006). In rice, OsFIE1, the homologs of FIS2 expressed only in endosperm, is a maternally expressed imprinting gene, other five PRC2 genes (OsFIE2, OsEMF1, OsEMF2, OsCLF and OsIZE1) are expressed in a wide range of tissues and are not imprinted (Lu et al. 2009). However, recent research showed that except for OsCLF (SDG711) being a non-imprinted gene, other four genes those previous studies considered as non-imprinted genes showed the characteristics of imprinted genes at different stages of endosperm development in rice (Kuang et al. 2019). In addition, our resent research suggested that the CLF homologous gene OSCLF (SDG711) regulates endosperm development by directly binding to the gene body region of several starch synthesis genes and amylase genes to mediate H3K27me3 enrichment on them in rice. Meanwhile, we tested the expression of some imprinted genes surveyed previously (Luo et al. 2011; Rodrigues et al. 2013; Yuan et al. 2017) in SDG711 overexpression plants. In overexpression of SDG711, the expression of these genes increased or decreased, which indicated that SDG711 may also be able to regulate the development of endosperm by affecting the expression of these imprinted genes (figureS8). Overall, although the PRC2 genes are very conservative and play an important role in the entire growth and development stage of plants, the components, and functions of PRC2 are very different between Arabidopsis and rice such as the imprinting effect and their roles in regulating endosperm development.

The N-terminal tails of the core histones were shown to be subject to multiple covalent modifications. The histone code hypothesis suggests that multiple histone modifications act in a combinatorial manner to affect gene transcription (Strahl and Allis 2000; Schreiber and Bernstein 2002). As the combination of dense marks in short clusters, situated at strategic locations in the histones, the ‘modification cassette’ was proposed to clarify the mechanism that may control the biological reading of different modification patterns (Fischle et al. 2003). Modification cassette also indicated that there were multiple modifications on the same nucleosome to coordinately regulate the expression of the same gene. For example, the crosstalk between serine phosphorylation and lysine methylation of the mitochondrial protein DAM1 and the transcription factor p53 has been described in human cancer cells (Fischle et al. 2003; Zhang and Dent 2005). In Arabidopsis, the regulation of the FLC locus provides a plant model of how multiple chromatin-modifying systems have emerged as important components in the control of a major developmental switch, the transition to flowering. H3K4me3 and histone acetylation are associated with active FLC expression, whereas histone deacetylation and H3K9me2 and H3K27me3 are involved in FLC repression (He and Amasino 2005).
Subsequently, the regions harbor both repressive and active chromatin modification were defined as bivalent domains (Bernstein et al. 2006). The bivalent domain (BD) marked by H3K27me3 and H3K4me3 which are catalyzed by specific PcG and TrxG complexes, respectively, was first discovered and characterized in mouse embryonic stem cells (ESCs) (Bernstein et al. 2006). The co-occurrence of H3K4me3 and H3K27me3 are often found in promoter regions of developmentally expressed TFs and developmental genes to be rapidly switched on during differentiation in specific cell types in mouse ESCs and human ESCs (Zhao et al. 2007; Bernstein et al. 2006; Xiang et al. 2020). In addition to the bivalent promoter described above, there is another class of bivalent region called bivalent enhancer (Blanco et al. 2020). It has been shown that co-occurrence of H3K4me1 and H3K27me3 marks the presence of bivalent enhancers in hESCs and mESCs. They likely play a key role during differentiation, similarly to bivalent promoters (Rada-Iglesias et al. 2011; Zentner et al. 2011). Recent studies have shown that DNA methylation can affect the H3K27me3 / H3K4me3 ratio of the bivalent promoter in different cell types, and DNA hypermethylation of the bivalent promoter in cancer is related to the H3K27me3 / H3K4me3 ratio in embryonic stem cells (Dunican et al. 2020). In plants, there are also a few studies describing the existence of bivalent modifications (H3K4me3/ H3K27me3) during the development process and stress treatment (Berr et al. 2010; Liu et al. 2014a; Sequeira-Mendes et al. 2014; Zeng et al. 2019).

Modification cassette and bivalent modification indicate that histone modifications have very important role in the development of organisms, and these modifications have balanced relationships. When the balance of these modification is broken, the growth and development of individuals may be seriously affected. This study also pointed out that during the development of rice endosperm starch, some key genes may be coordinately regulated by multiple histone modifications. For example, some starch metabolism related genes can be regulated by H3K27me3 and H3K4me3, and some can be regulated by H3K27me3 and H3K9ac. Under normal circumstances, these modifications will be in a balanced state. Once a certain modification changes, the balance will be broken and the gene expression will be abnormal (Fig. 7). This makes us realize that gene expression is regulated by multiple modifications in many cases, and this regulation may be more precise.

Conclusions
Collectively, our results show that overexpression and down-regulation of SDG711 lead to the decrease and increase of the expression level of genes related to starch accumulation, resulting in smaller seed or even seed abortion. ChiP assay showed that SDG711-mediated H3K27me3 changed significantly in genes related to endosperm development and SDG711 can directly bind to the gene body region of several starch synthesis genes and amylase genes. In addition, H3K4me3 and H3K9ac modifications also cooperate with H3K27me3 to regulate the development of endosperm. Our results suggested that the crosstalk of SDG711-mediated H3K27me3 with H3K4me3 and H3K9ac are involved in starch accumulation to control normal seed development. Our work provides new insights into the regulation of endosperm development.

Abbreviations
H3K27me3
H3 lysine 27 trimethylation; H3K4me3: H3 lysine 27 trimethylation; H3K9ac: H3 lysine 9 acetylation; PcG: polycomb group; OsAmy: α-amylase; OsBmy: β-amylase; ds: RNAi plants of SDG711; OX: overexpression plants of SDG711; WT: wildtype; DAP: Day After Pollination; ACC: Apparent Amylase Content; ChIP: Chromatin immunoprecipitation assay; qRT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction.

Declarations
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Authors' Contributions
XL and CZ designed the research; XL, JL, TL, HY, and PW conducted the experiments; JL, LS, YZ and CB analyzed the data; XL wrote the article; CZ supervised and complemented the manuscript. The authors read and approved the manuscript.

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Availability of Data and Materials
The data sets supporting the conclusions of this article are included within the article and its additional files.

Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
Not applicable.
Competing Interests

The authors declare that they have no competing interests.

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Figures
Figure 1

Phenotypic analysis of SDG711 transgenic seeds. A. Smaller mature seeds of SDG711 RNAi and overexpression transgenic plants. Bars =0.5cm. ds indicates independent lines of SDG711 RNAi transgenic plants; OX indicates independent lines of SDG711 overexpression transgenic plants. B. The 100-grain weight of SDG711 RNAi, overexpression transgenic plants and wild type. *Indicates means that differ significantly (p<0.01). Values are means ± SD. ds3, ds-7 and ds-9 indicate independent lines of SDG711 RNAi transgenic plants; OX-2, OX-4 and OX-5 indicate independent lines of SDG711 overexpression transgenic plants.

Figure 2

The endosperm starch granules and starch content of SDG711 transgenic plants. A. Cross sections of seeds at 3 DAP and 4 DAP. ds indicates independent lines of SDG711 RNAi transgenic plants; OX indicates independent lines of SDG711 overexpression transgenic plants. B. Total starch content in endosperm of SDG711 transgenic plants. C. AAC in endosperm of SDG711 transgenic plants. *Indicates means that differ significantly (p<0.01). Values are means ± SD. ds3, ds-7 and ds-9 indicate independent lines of SDG711 RNAi transgenic plants; OX-2, OX-4 and OX-5 indicate independent lines of SDG711 overexpression transgenic plants.
Figure 3

The expression level of genes associated with starch metabolism in WT and SDG711 transgenic seeds at 3 DAP. Real-time PCR detection of transcript levels of starch synthase genes (A) and amylase genes (B) in WT and SDG711 transgenic seeds at 3 DAP. RNAi indicates lines of SDG711 RNAi transgenic plants and OX indicates lines of SDG711 overexpression transgenic plants. Each qRT-PCR assay was repeated three times. Values are means ± SD. Values are shown as relative to ACTIN transcript levels.

Figure 4

SDG711 function in H3K27me3 of starch metabolism genes. Chromatin immunoprecipitation (ChIP) analysis of H3K27me3 of starch synthase genes (A) and amylase genes (B) in WT and SDG711 transgenic seeds at 3 DAP. H3K27me3 enrichments on the 5' transcriptional start site (TSS) (P1) and 5' end of the gene body (P2) were detected by quantitative PCR. Each q-PCR assay was repeated three times. Bars = mean ± SD from three technical repeats.
Figure 5

Direct association of SDG711 protein with genes of starch metabolism. SDG711 protein enrichment of the starch metabolism genes in WT seeds at 3 DAP tested by ChIP with anti-SDG711. Non-immunized serum (IgG) was used as control. SDG711 protein enrichments on the 5' transcriptional start site (TSS) (P1) and 5' end of the gene body (P2) were detected by quantitative PCR. Each q-PCR assay was repeated three times. Values are means ± SD from three technical repeats.
Figure 6

H3K4me3 and H3K9ac enrichments of starch metabolism genes. Chromatin immunoprecipitation (ChIP) analysis of H3K4me3 (A) and H3K9ac (B) of starch synthase genes and amylase genes in WT and SDG711 transgenic seeds at 3 DAP. H3K4me3 and H3K9ac enrichments on the 5' transcriptional start site (TSS) (P1) and 5' end of the gene body (P2) were detected by quantitative PCR. Each qPCR assay was repeated three times. Bars = mean ± SD from three technical repeats.
Balance of multiple modifications on starch metabolism genes. Under normal circumstances, H3K27me3/H3K4me3 and H3K27me/H3K9ac on starch metabolism genes are in a balanced state. Once a certain modification changes, the balance will be broken.

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