Low light conditions alter genome-wide profiles of circular RNAs in rice grains during grain filling

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Abstract

In animals and plants, circRNAs regulate gene expression and act as sponges that inhibit the activity of microRNAs. This study aimed to determine how specific circRNAs are expressed in rice grains at different stages of grain filling, under normal and low light conditions. We extracted total RNA from rice grains under low and sufficient light conditions. Deep sequencing was performed using circRNA libraries, and bioinformatics tools were used to identify the circRNAs. In addition, we analyzed targeted messenger RNA functions using two databases to predict the processes involved in rice grain development, and we conducted real-time PCR on 15 of the circRNAs. During the grain development process, 8015 candidate circRNAs were isolated, among which the number of known circRNAs was 1661. We also found that the number of circRNAs changed with the time of development. Among them, six circRNAs acted as sponges that targeted more than two microRNAs at different stages of development, and these circRNAs showed a regulatory pattern consistent with the transcriptome sequencing results. However, no differential circRNA expression was found under different light treatments. These findings reveal a possible link between circRNA regulation and the expression of the functional genes associated with photosignal-mediated rice grain development.

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Keywords: *Oryza* , circular RNA, light,

1. INTRODUCTION

Light intensity is a key factor affecting plant growth. During the grain-filling period in rice, low light intensity affects the synthesis and transportation of photosynthetic products (Acreche, Ariel Briceño-Felix, Sánchez, & Slafer, 2009; Mauro, Occhipinti, Longo, & Mauromicale, 2011), such as sucrose, which is the main photosynthetic product in plants, the primary raw material for starch synthesis in grains of rice and other grain crops, and the most essential transport form in plants. Such effects of low light intensity influence the yield, seed setting rate, 1000-grain weight, and other yield components of rice. For example, the amylose content of rice grains decreases, and the degree of chalkiness and chalkiness rate increases under low light conditions, which severely affects rice quality (W. J. Ren, Yang, Fan, Zhu, & Xu, 2003; W.J. Ren, Yang, Xu, Fan, & Ma, 2003; L. Wang et al., 2016). Since Sichuan is the largest industrial province in southwestern China, air pollution reduces light availability, which has become one of the main constraints on rice and other crop production here (Deng et al., 2009; Li Wang, Deng, Ren, & Yang, 2013). With the increasing demand for high-quality rice, there is an urgent need to understand the molecular characteristics of the deterioration of rice quality under low light conditions and to provide a theoretical basis for rice breeding.

Circular RNAs (circRNAs) are a class of noncoding RNA that is highly conserved and not easily degraded. In the 1970s, Sanger et al. (1976) first discovered closed circRNA molecules in plant viruses. After the first discovery of circRNA in the roots of *Arabidopsis thaliana*(Linnaeus) Heynhold in 2014, multiple species of circRNA have been identified (Chen et al., 2018; Darbani, Noeparvar, & Borg, 2016; Z. Wang et al., 2017; Ye, Chen, Liu, Zhu, & Fan, 2015). The primary function of circRNA is to regulate microRNA (miRNA) using the sponge mechanism, and it also regulates variable shearing and transmits signals over long distances (Liu, Zhang, Chen, & Shi, 2017). CircRNA can be obtained by altering the chloroplast and mitochondrial genomes, indicating that circRNA is involved in the regulation of many important life processes, including photosynthesis and respiration (Darbani et al., 2016; Sun et al., 2016; Ye et al., 2015).

CircRNAs of different plants at different growth stages have specific expressions in space and time. During the lifespan of *Arabidopsis*leaves, circRNAs express differentially at the growth-to-maturation stage of four days and the maturation-to-senescence stage of 16 days (Liu et al., 2017). Analysis of the circRNA-miRNA-mRNA regulatory network has shown that circRNAs might be involved in plant hormone signal transduction and porphyrin and chlorophyll metabolism during leaf senescence (Liu et al., 2017), and in animals it has been found that circRNAs can function as a miRNA sponge (Lasda & Parker, 2014). In cold-treated tomatoes, 102 circRNAs have the potential to act as miRNA sponges based on the predicted miRNA-binding sites for 24 distinct mature miRNAs (Zuo, Wang, Zhu, Luo, & Gao, 2016). In rice, 2354 circRNAs have been found in different tissues, and they have no significant enrichment effect on miRNA targets. This suggests that circRNA and its linear form may be negative regulators of their parent genes (Lu et al., 2015).

Previous reports have shown that circRNAs express differentially under abiotic stresses, such as phosphate, light, chilling, drought, zinc, and iron stress (Chen et al., 2018; Darbani et al., 2016; Ye et al., 2015; Zuo et al., 2016). The expression level of circRNA in *Arabidopsis*leaves was also different under different light conditions and treatment times (Ye et al., 2015), and 163 circRNAs presented differential expression between the control and chilled plants. Most of the deregulated circRNA in the control plants deregulated in the frozen treatment (Zuo et al., 2016). The authors identified 62 candidate circRNAs that showed differential expression under drought-like stress, with 16 circRNAs that were upregulated and 46 that were downregulated under this condition. This work also indicated the sponge action of circRNAs by showing that 6 out of the 62 circRNAs have miRNA-binding sites that can potentially regulate 26 distinct wheat miRNAs (Y. Wang et al., 2017). In addition, 27 differentially expressed circRNAs were found under conditions of phosphate deficiency in rice, with six circRNAs that were upregulated and 21 circRNAs that were downregulated; moreover, several circRNAs were positively correlated with their parental genes (Ye et al., 2015).

Studies have shown that abscisic acid (ABA) plays a vital role in the regulation of rice grain filling and is involved in multiple biological processes to regulate the resistance of rice to environmental stresses (MingHui,

Liu, Lu, Zhao, & Yang, 2009; T. Suzuki et al., 2008; Thoms & Rodriguez, 1994). Abscisic acid-deficient mutants, such as*Arabidopsis* aba1, aba2, aba3, and ABA-deficient mutants in tobacco, tomatoes, and corn mostly grow normally under normal growth conditions, but the plants are stunted. However, these ABA-deficient mutants were more likely to wither and die than wild-type plants under drought and high-salt treatments, while the *Arabidopsis*supersensitive mutant era1 was more resistant to drought stress, suggesting that ABA plays an essential role in plant stress tolerance (Zhu, 2002).

This study aimed to determine how specific circRNAs are expressed during rice grain filling at different stages of development and under low light conditions, what mechanisms and pathways circRNA uses to regulate grain filling and how this affects grain quality.

2. MATERIALS AND METHODS

2.1. Plant materials and stress treatments

Rice seeds, *Oryza sativa* Linnaeus, of the Shuhui 498 variety were placed in barrels that were 27 cm high and 33 cm in diameter, and whole plants were placed in a Conviron A1000PG artificial climate box in 2018 and 2019 with light quantity of 700 $mol.m^{-2}s^{-1}$ for a photoperiod of 14 h at a temperature of 29 and a temperature of 20 during the 10 h night period (humidity: 75%) when rice was at heading stage. At the beginning of the flowering period, the rice plants were exposed to a low light treatment, of 233 $mol.m^{-2}$ s^{-1} and the control remaining unchanged, with the other conditions remaining unchanged, and the glume was removed from the rice grains at the filling stage for RNA separation at 5, 10 and 15 days after treatment with low light.

2.2 Total RNA extraction, circRNA library construction, and sequencing

Total RNA was extracted from the control group and low light stress rice seeds using TRIzol reagent (Invitrogen Corporation, Carlsbad, CA, USA). The total RNA from each sample was used to prepare the circRNA sequencing library. After the RNA samples were qualified for detection, the rRNA was removed from the total RNA sample using the ribo-zero kit. Some long noncoding RNAs (lncRNAs) have the same polyA-tailed structure as mRNA, so the removal of rRNA can maximize the retention of lncRNAs containing polyA-tailed RNA. A fragmentation buffer was added to the enriched RNA to break the RNA into small fragments. Then, a library was constructed using the segmented RNA as a template, and Qubit 2.0 was used for initial quantification and dilution of the library. After this, Agilent 2100 was used to detect the inserted fragment size of the library. After it was established that the inserted fragment size met expectations, real-time PCR was used to accurately quantify the effective concentration of the library to ensure quality of the library. After successfully passing library detection, different libraries were pooled into flow cells according to the requirements for effective concentration and target disembarkation data volume. After cBOT clustering, the Illumina HiSeqX high-throughput sequencing platform was used for sequencing.

2.3. Identification of circular RNAs

The filtered transcriptome sequencing data from each sample were combined, and four software programs, Starchip, CIRI2, CIRCexplorer2, and CircRNA_finder, were independently used to predict the back-spliced junction trans-shear site. The predicted circRNA genome sequence (osa_predict_seq.fa, as the query sequence) was compared with the known circRNA database genome sequence or transcriptome sequence to complete the preliminary verification of the circRNA. The Plantcircbase database, for genome sequences, was used with the Blastn ratio and a filtering condition of subject coverage greater than 90%, and the Plantcircnet database, for transcriptome sequences, was also used to identify circRNA that was located in the intergenic region or single exon region (i.e., circRNA spans multiple exons) with filtering conditions of more than 90% similarity and lengths greater than 100.

2.4. Prediction of miRNA targets of circRNAs, mRNA targets of miRNA, and annotation of functions

The sequence in the middle of the circRNA's trans-shear site was extracted, and the reverse sequence was

spliced from the middle and extended by default by 15 nt. The circRNA-miRNA regulatory relationship was predicted using two software programs, RNAhybrid and TargetFinder, using sequence-based and free energy-based calculations. The RNAhybrid result filter parameter that was used was an energy cutoff of -20 and significant P-value of 0.05. The TargetFinder result filter parameter that was used was a score of three. Regulatory networks were constructed, for the control group and low light treatment for the period 5-10 d after flowering and for the control group for the period 10-15 d after flowering, using differential expression (DE) circRNA-DE miRNA and DE miRNA-DE mRNA, and enrichment analysis was conducted of the mRNAs in the network. The filtering conditions of DE circRNA-DE miRNA were predicted using at least one predictive software. The filtering condition for the pairs of DE miRNA-DE mRNA effects was that the P-value of the expression correlation coefficient was less than 0.05 and was predicted by at least one predictive software program (Supplemental Figures 1-3). An analysis of targeted messenger RNA functions was performed using the Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Wikipathway databases to predict the processes involved in rice grain development, such as photosynthesis, sugar and starch synthesis and metabolism, and the signaling of plant hormones, such as abscisic acid and auxin. Then the functional annotation results of the GO, KEGG, and Wikipathway databases were used to analyze the enrichment of differentially expressed mRNAs in the network.

2.5. Validation of differentially expressed circular RNAs

Quantitative real-time PCR and Sanger sequencing techniques were used to verify the circular structure and expression patterns of circRNAs identified using RNA sequences. During validation, 15 differentially expressed circRNAs were used, of which six were predicted to be sponges for more than two miRNA, and nine were selected randomly from differentially expressed circRNAs. Two micrograms of total RNA were used before real-time quantitative PCR with DNase I (2270 a, Takara, Japan).Primers were designed to ensure that the circular template was amplified (Ting, Miao, Gang, & Ting, 2015). The sequence of primers is shown in Table 1. The expression of circRNAs was quantified on an ABI StepOnePlus system (USA) using an SYBR green master mixture (Applied Biosystems, Foster City, CA, USA). The relative expression rate $(^{C}t)ofeachcircRNAwascalculatedusingthe2^{-t}methodandexpressedaslog_2ofthevalue,whereCtistheperiodicthresholdvalueoftheamplifiedtargetorrefe$

2.6. Quantification of sucrose and ABA

Approximately 100 mg of developing caryopses was used to quantify the ABA and sucrose content at the grain-filling stage 5, 10, and 15 days after treatment in both the light stress and the control groups, using liquid chromatography-tandem mass spectrometry as described previously (Mikiko et al., 2009).

3. RESULTS AND DISCUSSION

3.1. Sucrose content at different growth stages under different light conditions

The synthesis and transportation of sucrose were affected to some extent under low light conditions. The sucrose content in the grains gradually decreased during the grain development process. Under low light conditions, the content of sucrose in the rice grains was higher than the control before 10 d, it was lower than the control at 15 d, and higher than the control at maturity (Figure 1a). This result might indicate that, under low light conditions, the synthesis and transportation of sucrose, the primary photosynthetic product in plants, follows a dynamic process.

Sucrose enters the starch synthesis pathway after being transported to the rice grain through vascular bundles, and it regulates changes in sugar signals, inducing changes to various metabolic cycles in the grain. Studies have shown that sugar starvation may induce the expression of an amylase synthesis gene and improve its activity. Reductions in starch synthesis result in a decrease in starch accumulation and rice quality (L. Wang et al., 2016; Li Wang et al., 2013). Abscisic acid plays an essential role in the response of plants to various environmental stresses. In the developing grains in the control group, the ABA content first increased, then it reached a peak at 15 days, and then it gradually decreased (Figure 1b). After the shading treatment, the ABA concentration in the grain was significantly higher than the control at the beginning, it gradually decreased with time, and it was lower than the control after 15 days. These findings are similar to other

research that found that the ABA concentration in firm and weak rice grains showed a decreasing trend during grain filling, but the ABA concentration decreased faster in the firm grains than in the weak grains at the same grain filling stage (Ang, Liang, & Lin, 2003; J. Yang, Zhang, Wang, Liu, & Wang, 2005). In a study on wheat, 9-14 days of spraying low concentrations of exogenous ABA was found to improve wheat grain sucrose synthetase (SuSase), soluble starch synthase, and ADP glucose focal phosphorylase activity (J. Yang, Zhang, Wang, Xu, & Zhu, 2004). Among these, SuSase activity is closely related to library strength and is considered an indicator of library strength. This finding infers that ABA promotes grain filling by increasing the reservoir strength by regulating the critical enzyme activity of sucrose-starch metabolism in grains (F. Wang, Sanz, Brenner, & Smith, 1993).

3.2. Overview of the circular RNAs in rice grains

Although there have been some reports on circRNAs in rice (Chen et al., 2018; Liu et al., 2017; Y. Wang et al., 2017; Z. Wang et al., 2017; Ye et al., 2015; Zuo et al., 2016), research on changes in circRNAs during the development of rice grains under different light treatments is limited. Circular RNAs are characterized by the occurrence of 3'-5' connections in splicing reactions of individual RNA molecules. Based on the sequence reads, a total of 8015 circRNA candidates were identified by the Starchip, CIRCexplorer2, CIRI2, and CircRNA_finder software from a total of 18 samples from the two treatments of rice seeds (Figure 2a). This is 240.48% more than previously found in rice (Lu et al., 2015). After filtering and comparing the data from the two databases, Plantcircbase and Plantcircnet, 1661 known circRNAs were obtained (Supplemental Table 1). A total of 7270 of the sources of circRNA formation were concentrated in coding RNA, with 5507 in the exon region, making up 85.73% of all sources in the exon region, 373 sources were found in other regions, with 306 in the exon region making up 4.76% of all exon regions, 367 were from lincRNA, with 238 in the exon region making up 3.70% of all exon regions, and only five were from miRNA from other regions (Figure 2b).

3.3. Identification of differentially expressed circular RNAs

At different developmental stages, the number and expression of circRNAs were different. As fertility progressed, the amount of circRNA increased gradually under the control and low light conditions (Figure 2a). Overall, there was a small difference between the quantities detected under low light conditions (1455, 2032, 2360) and the control (1448, 2141, 2748) after 5, 10, and 15 days, respectively. There were 582 circRNAs detected at different times and under different light treatments, accounting for 7.26% of the total number of circRNAs. This result shows that a certain number of circRNAs show stable expression during the grain development process regardless of the environment, and this plays an important role in grain-filling development (Figure 2a).

Different types of circRNAs play different roles during different stages of seed development, and the number of circRNAs involved at different stages varies greatly. For example, 59 were detected in both the control and treatment after 5 d of shading, 36 after 10 d, and 295 after 15 d. Forty-three circRNAs were detected at both 10 d and 5 d, and 272 were detected at both 10 d and 15 d. However, in the control group, only 11 circRNAs were detected after each time period (Figure 2b). The number of upregulated expressions of circRNA at 10 d compared with 5 d under low light conditions was 96, which is significantly higher than that of 16 in the control group (Figure 2c, Supplymental Table 2). Such circRNAs may play different roles in grain development under low light conditions.

In this study, there was no differential expression of circRNA under different light treatment conditions, while in previous studies, there was more differential expression, which may be related to the different tissues that were examined. Previous studies mainly used leaves and roots (Lu et al., 2015; Ye et al., 2015), which express carbon and nitrogen metabolism during plant development, such as photosynthesis and nutrients. However, we only selected grains in rice, which are mainly concerned with the development and accumulation of embryos and endosperm. On the other hand, we also found that although there was no significant difference in the expression of circRNAs under different light treatments, the types of circRNA changed substantially.

3.4. Putative functions of the regulations of rice circRNAs acting as miRNA sponges

The RNAhybrid software predicted 612,874 targeted miRNA, while TargetFinder predicted 3,527, and 1,398 were predicted by both prediction software programs, making the combined number of predicted targeted miRNA 615003. The six circRNAs predicted to be miRNA sponges showed positive amplification from the expected corresponding circular template, while the six circRNAs showed expression differences consistent with the transcriptome sequencing results. In addition, of the 15 circRNA candidate genes that were tested, all were verified to be circular, and 13 showed expression patterns consistent with the RNA sequence results (Figure 3b).

Of the circRNAs that showed differences in light processing and development, 40 circRNAs have been reported as having known miRNA targets, including MiRNA164, miRNA398, miRNA167, and various isomers (Figure 3a). MiRNA164 and MiRNA167 can influence the formation of root caps, lateral root development, and adventitious root development through auxin response factors (Guo, Xie, Fei, & Chua, 2005; Meng et al., 2009), and they can also be induced by light conditions and participate in phyb-mediated signaling pathways (N. Suzuki et al., 2015). MiRNA167 can further influence the development of pollen mother cells to pollen grains by cutting the auxin response factors*LOC_Os10g33940, LOC_Os02g06910, LOC_Os04g57610, LOC_Os06g46410,* and *LOC_Os12g41950* (Peng et al., 2012). MiRNA164e was used as an *OsDBH* (DEAD Box Helicase) gene to encode helicase under simulated salt stress, and adapted to salt stress by regulating expression of helicase related genes (Macovei & Tuteja, 2012). The decreased expression level of miR398a and 398b under aluminum stress can cause the up-regulation of the target genes *AtSOD1* and*AtSOD2* (Superoxide dismutase1/2), while SOD is an important enzyme that promotes the conversion of superoxide free radicals into hydrogen peroxide and oxygen to reduce cell damage (Lima, Arenhart, Margis-Pinheiro, & Margis, 2011). In addition, many circRNAs in this study have not been discovered previously, which also indicates that more needs to be understood about the regulation of targeted miRNA by circRNA.

The results showed that the functions of these known targeted miRNAs are not directly related to the grain development and filling processes. There are two main reasons for this. Firstly, during the development of grains under different light conditions, circRNA might be regulated by a sponge mechanism targeting miRNA. In previous studies, it has also been suggested that during plant growth, circRNA might also regulate the entire biological process by regulating the expression level of the parent gene (Lu et al., 2015). Secondly, there is little evidence of miRNAs in plants playing a role in light regulation (Kumari, Rastoqi, Shukla, Srivastava, & Yaday, 2018), and there are a large number of undiscovered miRNAs in this study. These unknown miRNAs might act on grain filling and be targeted by circRNAs.

3.5. Functional categorization of predicted mRNAs

The differences in the biological processes in the control group from 5-10 d were mainly concentrated in carbohydrate metabolic processes (GO: 0005975), embryo development (GO: 00097990), lipid transport (GO: 0006869), and microtubule-based movement (GO: 0007018). These are essential metabolic and biological development processes in the development of grain embryos and endosperms. In terms of cell structure, monolayer-surrounded lipid storage bodies (GO: 0012511), cell walls (GO: 0005618), membranes (GO: 0016020), and extracellular regions (GO: 0005576) were identified. In terms of molecular function, 698 GO pathways were found to be involved. These were mainly related to hydrolase activity (GO: 000453, GO: 0016788) enzyme inhibitor activity (GO: 0004857), ion channel inhibitor activity (GO: 0008200), lipid binding (GO: 0008289), and carbohydrate metabolism and transport. The enrichment analysis that was conducted using the KEGG database showed significant differences in the expression of starch and sucrose metabolism (ma00500), phenylpropanoid biosynthesis (map00940), and glycerolipid metabolism (map00561). Analyses using the Wikipathway database found differences in seed development (WP2199), photosynthetic carbon reduction (WP1461), abscisic acid biosynthesis (WP626), sucrose metabolism (WP2623), and the ethylene signaling pathway (WP2851).

The difference between 10-15 d and 5-10 d after flowering in the control group was differences in electron carrier activity (GO: 0009055), protein heterodimerization activity (GO: 0046982), and the fructose 6-phosphate metabolic process (GP: 0005975). The main difference in enrichment found from the KEGG database was related to carbon metabolism (map01200), whereas the Wikipathway enrichment analysis found changes in

photosynthetic carbon reduction (mapWP1461), glycolysis (mapWP2862), and the tricarboxylic acid cycle (mapWP2624).

When comparing the low light treatment with the control group at 5-10 d after flowering, there was specific differential expression of biological processes such as the abiotic stress response and photosynthesis, and when looking at cell structure, there were differences in nucleosomes (GO: 0000786) and cell wall structure development (GO: 0005618). The expression of the cell wall invertase gene decreases during the development of vulnerable granules with multiple consequences: inhibiting the development of vulnerable granules, delaying the sucrose conversion rate and synthesis rate of vulnerable granules, forming a stagnant period of filling of vulnerable granules, and playing a role in the unloading of assimilating effects (E. Wang et al., 2008). Under low light conditions, the grains of rice also showed similar weaknesses in development, which were similar to grout lag. The photosystem I reaction center (GO: 0009538, 7/2) also showed differential expression. Previous studies have shown that during rice grain filling, some photosynthesis still occurs in the grain. However, under low light conditions, photosynthetic products, such as sucrose, are reduced in the photosynthetically active tissues and thus the supply of these products to the grains is affected. Some photosynthetic cells in the rice grains appeared to be more active to make up for the inadequate synthesis of starch. In terms of molecular functions, protein heterodimerization activity (GO: 0046982), serine type endopeptidase inhibitor activity (GO: 0004867), and peroxiredoxin activity, which play important roles in the accumulation of nutrients, showed differences in activity. For the development of heterodimeric proteins, NAM, ATAF, and CUC (NAC) transcription factors are composed of two chains forming a stable heterogeneous complex. During the process of protein translation in the organization of new polypeptides, synthesis can lag behind and the wrong protein molecules bind (Wiedmann, Sakai, Davis, & Wiedmann, 1994). They are a dynamic component of the ribosome export channel, protect the formation of nascent polypeptides, and prevent inappropriate protein interactions (Rospert, Dubaquie, & Gautschi, 2002). After treatment with high-salt stress, the expression of the *OsBTF3* gene in rice is significantly inhibited, and transgenic T2 plants that overexpress *OsBTF3* have an increased resistance to high salt and cold stress, while resistance to RNAi transgenic lines is weakened, indicating that *OsBFT3* may be present in rice (Li, Chen, Wu, & He, 2012). It plays a very important role in the regulation of high salt and low temperature stress. After the overexpression of Saβ-NAC in *Arabidopsis thaliana* , the transgenic *Arabidopsis thaliana* does not grow normally, but it has relatively strong resistance to coastal conditions and drought, and under conditions of abiotic stress and the overexpression of Saβ-NAC, the chlorophyll content and proline content of NAC transgenic*Arabidopsis* plants is increased and the ion dynamic balance is also enhanced. When *Spartina alterniflora* are exposed to salt damage, drought, cold stress, or a specified concentration of ABA, the expression levels of Saβ-NAC in leaves and roots changes to varying degrees (Karan & Subudhi, 2012). Compared with the control at 5-10 d after flowering, the enrichment analysis conducted using the KEGG database showed differential expression of photosynthesis antenna-proteins (map00196), flavonoid biosynthesis (map00941, 48/8), and starch and sucrose metabolism (map00500).

In the low light treatment for the period 5-10 d after flowering, analysis of the Wikipathway database compared with the control group mainly showed changes related to sucrose metabolism (mapWP2623), ABA synthesis (mapWP626), and the chloroplast electron transport chain (mapWP2861). The content of endogenous ABA in grains under low light in this study was significantly higher than that of the control group at 5 and 10 d after flowering. Abscisic acid regulates sugar metabolism during stress, and α-amylase breaks down starch into fructose and glucose, which has a particular negative regulation effect on the decomposition of sucrose (Hakata et al., 2012). At present, it is believed that the mechanism of ABA regulation under adverse stress conditions, such as drought, high salt or low temperature, might start with adverse stress conditions promoting the accumulation of abscisic acid in plants, which induces the gene expression of ABA response elements and generates resistance to adverse stress. From the point of stimulation by stress to the plant response there are a series of complex information transmission processes, including three links: the cell or tissue must sense the original environmental stimulus and respond by producing an intercellular signal, the intercellular messenger must be transferred between cells or tissues and reach the active site of the receptor cells, and the receptor cells must accept, transduce and respond to the intracellular messenger,

which makes the optimal combination of physiological, biochemical, and other functions in the receptor tissues, and finally reflects the adaptation or resistance of plants to environmental stimulation or adversity (Anderson, Ward, & Schroeder, 1994; Knetsch, Wang, Snaar-Jagalska, & Heimovaara-Dijkstra, 1996; Lee et al., 1996; Schwartz, Wu, Tucker, & Assmann, 1994; Stone & Walker, 1995).

The ABA biosynthesis-related gene expression of the low light treatment was significantly different from the control group at 10 days after flowering. Abscisic acid can participate in a series of processes in the adversity stress reaction of plants, it can improve the free proline and soluble sugar, sucrose osmotic regulation substances, and enhance the capacity of osmotic regulation. In this study, the ABA content increased at the same time as the sucrose content, indicating that grain under weak light can exhibit regulation of endogenous ABA to the detriment of resistance to abiotic stress. When the drought stress was postponed to reduce the water content of sesame leaves, the content of MDA increased significantly, and the content of soluble sugar increased first and then decreased (Yan et al., 2008). This result is consistent with our results (Figure 1).

Abscisic acid reduces the activity of ATPase, reduces the transporting force of H+ across the plasma membrane, and then affects the H+ / sucrose co-transport pathway (W., 1980). During the grain-filling period, the lower ABA levels in the early stage and the higher ABA level in the middle stage were not conducive to rice grain filling. The application of exogenous ABA at an appropriate concentration in the early stage of grain filling can promote the development of grain embryos, assimilate transport, promote grain filling, and increase the seed setting rate and yield of rice (Dewdney & Mcwha, 1979; Tang, Xie, Lu, & Liang, 2011). Zhang et al. (1996) used the method of tracer dynamics analysis to apply ABA to paddy rice ears. This can inhibit the formation of temporarily non-exportable substances in the flag leaves, promote the formation of transmissible substances, and increase the output rate of photosynthetic products. After this, it has an inhibitory effect on structural substances and respiratory consumption, and it has a promotion effect on the formation of injectable substances. Therefore, although photosynthesis was inhibited at the early stage of grain filling in this study, the endogenous ABA in the grains did not decrease, the sucrose content was still maintained at a certain level, and the dry weight of the entire ear was not significantly inhibited.

Yang et al. (2006) showed that the regulation effect of ABA on grain filling showed a dose effect: the concentration was promoted, but a high concentration was inhibited as ethylene could reduce the critical enzyme activity of the sucrose-starch metabolism pathway in the grain and inhibit grain filling. The interaction of ABA and ethylene regulates grain filling. Therefore, appropriately increasing the ABA level and the ratio of ABA to ethylene using methods such as moderate soil drought can promote grain filling in rice and wheat (Figure 4) (JiangChang Yang & Zhang, 2018).

6. CONCLUSIONS

There were significant differences in circRNA expression in rice grains at different stages of rice development, and there were also changes in circRNA styles. Through enrichment analysis of different databases, it was found that circRNA had a certain degree of influence on hormone synthesis factors, helicase and sod-related enzymes, and ABA metabolism, which further affected sucrose decomposition, starch synthesis and metabolism, and finally affected grain filling and grain quality.

7. CONFLICT OF INTRREST

The authors declare no conflict of interest.

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Table 1. Primers in the RT-PCR assay for validating the expressions of circRNAs

Gene ID	RT-PCR name	Primer	Sequence(5'-3')	Tm()	product size(bp
Chr7:6283218:6283551:-	circRNA-1	1-Forward	TCTTCCTGACGGCGACAATGG	65.4	195
		1-Reverse	GGTCCTTGGGCACCACGC	64.0	

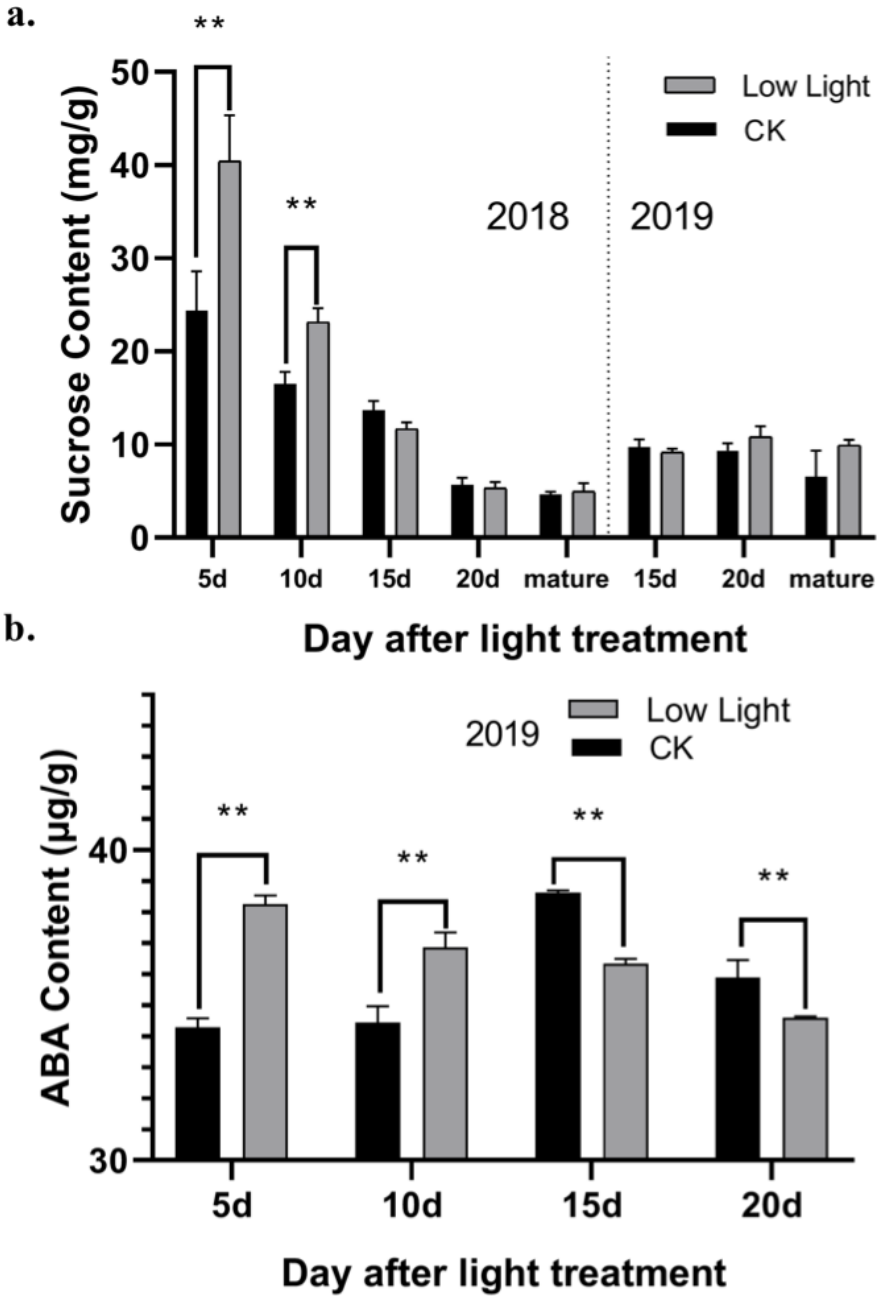
Gene ID	RT-PCR name	Primer	Sequence(5'-3')	Tm()	product size(bp
Chr10:19072854:19073418:-	circRNA-2	2-Forward	CGCTGATGGTGGTGTGGTC	63.9	295
		2-Reverse	CGTTCCTCCTCCCGAGATGCT	65.2	
Chr11:24659480:24659777:+	circRNA-3	3-Forward	ATTGGTCGTGGTGGTGCT	56.0	104
		3-Reverse	CCAGGAGGAGGAAGAGGC	56.4	
Chr11:24659620:24659976:+	circRNA-4	4-Forward	CATTGGTCGTGGTGGTGC	57.8	222
		4-Reverse	ACAACTACGGCGGCGAGA	59.7	
Chr5:21035563:21036008:-	circRNA-5	5-Forward	ATGTTTCTTGTTCGCCTTC	54.6	364
		5-Reverse	GTCGTCATCGCTGTTTGG	54.6	
Chr11:24660313:24660600:-	circRNA-6	6-Forward	CAAGAAGCAGTGCCGGTGGG	65.9	102
		6-Reverse	TCGTCGTCGTCGTCGTCCTC	64.0	
Chr1:18638726:18643834:+	circRNA-7	7-Forward	TGTGGGTATGCTCTGTTT	48.1	204
		7-Reverse	GACTTATCCCTTCTTCTTTT	47.5	
Chr9:14897093:14899808:-	circRNA-8	8-Forward	GGCGATATGGCGGATGAC	59.1	213
		8-Reverse	TATGGGCAGAGGCGAACC	58.9	
Chr1:33198033:33198346:-	circRNA-9	9-Forward	ATCCAAACCGTTAGAGCA	50.7	156
		9-Reverse	GGAGAAATTGTCCGTGTC	49.6	
Chr3:9751501:9751817:-	circRNA-10	10-Forward	GCCGACGACAAGAAGAAGA	55.8	132
		10-Reverse	GATGATCGGATTGCAGAGG	55.6	
Chr5:2778912:2779175:-	circRNA-11	11-Forward	CCACCTCCACGCCGAAAT	61.2	128
		11-Reverse	CGGTGAGCGAAACCCAGAG	61.2	
Chr1:39935271:39940971:+	circRNA-12	12-Forward	AATGTCAGGCTGGATAAG	47.1	206
		12-Reverse	GTCGGTTCACTGGTTAGA	47.4	
Chr3:12031348:12032481:-	circRNA-13	13-Forward	TGAGACAGGCGAGACAAC	50.9	223
		13-Reverse	TCTTCTGGAAAGAGTGGG	49.6	
Chr3:14409438:14413031:-	circRNA-14	14-Forward	GCTAGTGTTAGCGAGGTT	47.2	226
		14-Reverse	AAGAGGTGCTTGCTGTAT	47.0	
Chr2:36606976:36608842:-	circRNA-15	15-Forward	GGTCCAATAAACACCCTA	47.2	236
		15-Reverse	TTCAATTTCTCCCATCTC	46.7	
	OsActin	Forward	GACTCTGGTGATGGTGTCAGC	60.67	332
		Reverse	GGCTGGAAGAGGACCTCAGG	61.62	

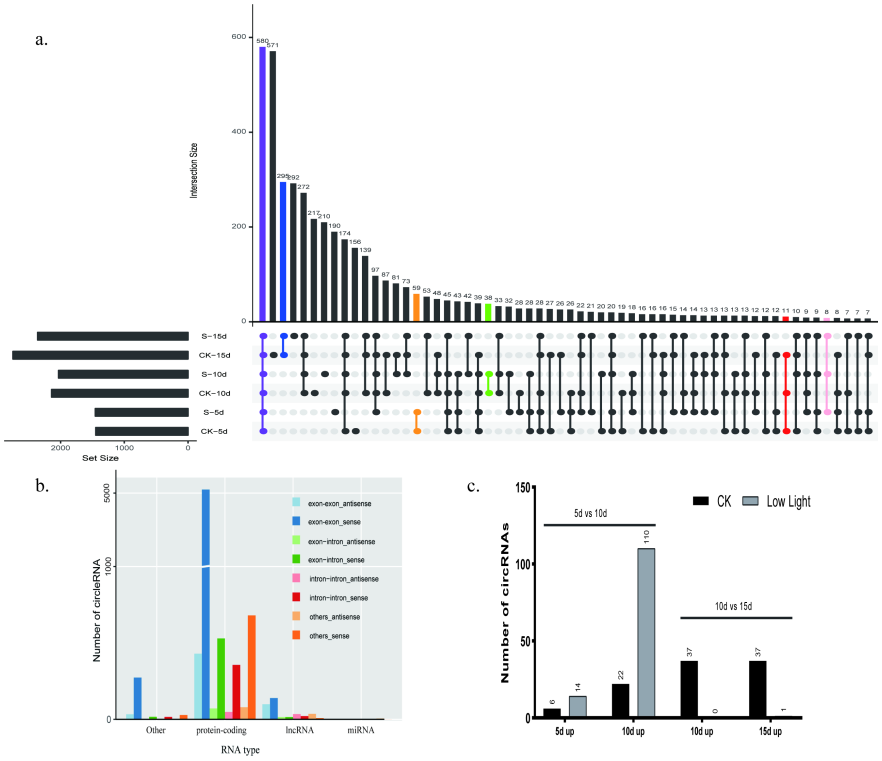
Figure 1 a. Sucrose content in rice grains at 5d, 10d, 15d, 20d and the mature stage after light treatment in 2018 and 2019;**b.**Contents of ABA in rice grains at 5d, 10d, 15d, and 20d after light treatment in 2019.

Figure 2 a. The number of circRNAs shared between different light treatments and different development times: all developmental stages (purple), the treatment and control at 5 d (orange), treatment and control at 10 d (green), three periods of control (red), and three periods of low light (pink). **b** . The sequences of all circRNAs constitute a specific source. **c.** The numbers of differentially expressed circRNAs were compared in the control at 5d and 10d, and at 5d and 10d after exposure to low light.

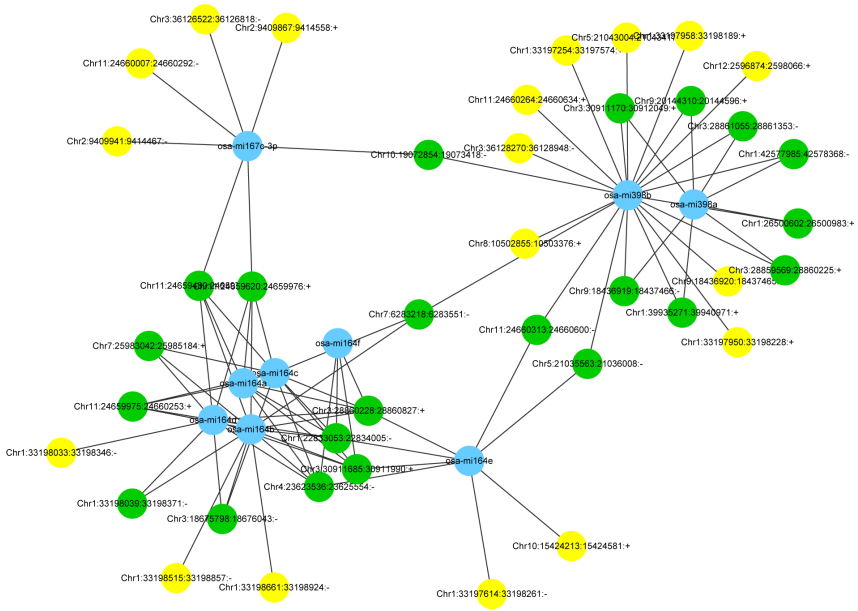
Figure 3 a. Networked view of the differentially expressed circRNAs targeting miRNAs. Blue represents miRNA, green represents circRNA that targets more than two miRNAs, and yellow represents less than two circRNAs. **b.** Validation of the differentially expressed circRNAs by real-time PCR assay. See Table 1 for the circRNA names. Means of samples denoted with the "*" are significantly different at $P < 0.05$ according to the t-test compared to the control after 5 d.

Figure 4 Possible regulatory mechanism involving differentially expressed circRNAs and their target genes in rice grains under weak light.





a.



b.