Uncovering ceRNA integrated networks that associate with fertility in a photoperiod and temperature sensitive male sterile wheat line

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ABSTRACT

The pollen fertility of photoperiod/temperature sensitive genic male sterile (P/TGMS) wheat is controlled by light and/or temperature. Circular RNA (circRNA) and long non-coding RNA (lncRNA) are known to participate in the development of anthers in plants, but their impact on male sterility in the P/TGMS line is not well understood. In this study, we carried out high-throughput sequencing to investigate the differential expression of lncRNAs and circRNAs and their biological functions in anthers of photo-thermo-sensitive genic male sterile (PTGMS) wheat line BS366-42L during the transition phase of male fertility under four different photoperiod and temperature treatments. Eight lncRNAs, 40 mRNAs and three circRNAs were screened out and thought as essential candidates that closely related to male sterility. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to predict the potential functions of differentially expressed RNAs. The results indicated that carbohydrate-related metabolism was important for male sterility in the wheat PTGMS line BS366-42L. lncRNA/circRNA-miRNA-miRNA (ceRNA) integrate networks were constructed to reflect their complex inner association with male sterility. Our study provides a systematic perspective on the potential function of RNAs in male fertility in PTGMS lines of wheat.

Introduction

Wheat (Triticum aestivum L.) is one of the most important grain and forage crops throughout the world, and it comprises one-fifth of the total energy consumed by humans and serves as the major food source for one-third of the global population [1]. Hybrid breeding is a potentially disruptive technology that is widely used in plant production to improve the yield per area, enhance yield stability and produce other favourable agronomic traits [2]. In recent years, hybrid breeding has had remarkable success in several allogamous species, such as maize, sunflower, sorghum, sugar beet and rye, but it has not been fully exploited in autogamous crops [3]. Remarkably, hybrid wheat currently accounts for less than 1% of the total wheat acreage planted in the world [4]. P/TGMS can respond to light and temperature owing to its fertility. The features of transformable fertility enable the P/TGMS lines to propagate via self-pollination under environmental conditions that restore male fertility and outcross with restorer lines for hybrid seed production under conditions that suppress male fertility [5, 6]. The discovery and successful utilization of P/TGMS greatly promotes the process of scale development of hybrid wheat [5].

Recently, non-coding RNA (ncRNA), including microRNA (miRNA), long non-coding RNA (lncRNA) and circular RNA (circRNA), have become a focus of research in plants and animals [7]. Many studies have shown that lncRNAs can regulate genes at the transcriptional and post-transcriptional levels by acting as
signals, decoys, scaffolds and guides [8, 9]. In addition, IncRNA as bait could bind miRNAs and block the interaction between miRNAs and their target genes. For example, in Brassica rapa, IncRNA IPS1 (INDUCED BY PHOSPHATE STARVATION)1 could competitively bind to miR399, resulting in up-regulated expression of its targets gene PHO2 [10]. Circular RNA (circRNA), another novel type of non-coding RNA, is produced from precursor mRNAs (pre-mRNAs) through back-splicing. circRNAs form covalently closed loop structures with a process in which a 3′ splicing acceptor site is joined to a 5′ splicing donor site. These closed loop structures enable them to avoid degradation by RNase R [11]. Recent studies have also shown that circRNAs participate in plant responses to biotic and abiotic stresses. For example, the circRNAs in Arabidopsis can respond to heat and low-light and high-light stresses [12].

The pollen sterility of the PTGMS line is controlled by temperature and/or photoperiod [6,7]. Recently, many studies have shown that non-coding RNAs are involved in plant fertility. For example, the overexpression of tae-miR167 in Arabidopsis, could negatively regulate target mRNAs AtARF6 and AtARF8, and cause abnormalities in anther, inducing male sterility phenotypes [13]. In addition, the photoperiod-sensitive male sterility (PSMS) gene pms3, cloned from a PSMS rice line (Nongken 58S), encodes a long non-coding RNA designated LDMAR that is required for normal male fertility of the rice plant under long-day condition[14]. However, there are few reports on the connection of IncRNA, miRNA and circRNA on male sterility in crops. Therefore, researching into the activity of IncRNA and circRNA may lead to significant developments in pollen and pollen sterility in wheat PTGMS lines.

Materials and methods

Plant materials, growth conditions and sample collection

The wheat (Triticum aestivum L.) PTGMS line BS366-42L was used in this study. All the plants were planted and managed until the four-leaf stage as described by Bai et al. (2017) [7]. The plants were randomly transferred to four light and temperature treatments in artificial climate incubators: 12°C (10L/14D and 14L/10D, L: light; D: dark) and 20°C (10L/14D and 14L/10D, L: light; D: dark) at a relative humidity of 60~80% for the entire reproductive period of 10 d. Anthers from more than 50 different plants, including those from the whole process of meiosis, were collected from each treatment. All the samples were rapidly frozen in liquid nitrogen and stored in at −80°C until the RNA was extracted. The seed setting rate was calculated as described by Yuan et al. (2020) 15:

RNA sequencing and transcript assembly

Sequencing libraries were generated using ribosomal RNA (rRNA)-depleted RNA according to the manufacturer’s instructions. The constructed libraries were then sequenced on an Illumina HiSeq™ 2500 (125 bp PE). The genome of high quality reads was mapped using HISAT (v. 2.0.6) undefined. Filtered clean reads were processed to map to the wheat reference genome (IWGSC RefSeq v. 1.0) undefined. Cufflinks was used to assemble the transcript assembly and estimate the abundance undefined [18]. The data reported in this article have been deposited in the National Genomics Data Center (NGDC) Genome Sequence Archive (GSA) database under the GSA accession no CRA003350 (https://bigd.big.ac.cn).

Prediction of IncRNA and circRNA and analysis of function

The novel transcripts (>200 bp) were processed to identify long noncoding RNAs (lncRNAs) based on four computational approaches, including the Coding Potential Calculator (CPC, score <0) [19], Coding-Non-Coding Index (CNCI score <0) [20], Coding Potential Assessment Tool (CPAT score <0) [21] and Pfam (E value < 0.01). The remaining transcripts were considered to be reliably expressed IncRNAs. CIRI software [22] was used to identify the circRNA. The levels of expression of circRNAs were reflected by mapped back-splicing junction reads per million mapped reads (RPM).

To understand the function and interactions among these RNAs, Gene Ontology (GO) terms and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed on DEGs, parent genes of circRNA and targets of IncRNA using the DAVID tool undefined [23]. P values and q values were used to test the reliability of the analysis.

Construction of the lncRNA/circRNA-miRNA-mRNA network

It was reported that mRNAs, IncRNA and circRNA could regulate the same miRNA to play important roles on biological processes in plant development [24]. To understand the connections among miRNAs, mRNAs, IncRNA and circRNA and explain the function of these non-coding RNAs in fertility conversion for a wheat PTGMS line in more detail, we performed interactions analysis between miRNAs and the differentially
expressed mRNAs/genes (DEGs), IncRNAs (DELs) and circRNAs (DECs) using miRanda software [25]. The mRNA/miRNA-IncRNA-circRNA network maps were visualized using Cytoscape software (v. 3.6.1) (http://cytoscape.org/).

**Quantitative real-time PCR validation**

Quantitative real-time PCR (qRT-PCR) was performed using a SYBR Premix Ex Taq™ Kit (TaKaRa, Dalian, Japan) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA), following the manufacturer’s instructions. The protocol used to perform qPCRs involved pre-denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 30 s. The results were analyzed using the 2-ΔΔCt method [26], and the mean values with standard errors (±SE) of three biological replicates are presented. Wheat 18S gene served as the reference control. The primers used in this study are listed in Supplemental Table S1. All the experiments were performed in triplicate.

**Results**

**Phenotypic characteristics of fertility of PTGMS wheat line BS366**

Iodine stain tests were performed to characterize the features of fertility in BS366 under four conditions: 12 °C (10L/14D and 14L/10D, L: light; D: dark) and 20 °C (10L/14D and 14L/10D). The pollen grains were stained fully black at 20 °C (14L/10D), whereas all the wrinkled, inadequately stained and completely aborted characteristics at 12 °C (10L/14D) and partial black staining at 12 °C (14L/10D) indicated that the changes in environment could induce the conversion of male fertility. The rate of pollen iodine staining subjected to the four conditions (12 °C 10L/14D, 12 °C 14L/10D, 20 °C 10L/14D and 20 °C 14L/10D) were 4%, 24%, 81% and 90%, respectively (Figure 1A-D). Moreover, the seed setting rates were also studied. The averaged seed setting rate from these four conditions were 7.76%, 21.96%, 71.66% and 75.11%, respectively, which was consistent with the results of iodine staining of the pollen (Figure 1E, Supplemental Table S2). These results indicated that light and temperature might play important roles in the transformation of fertility.

**Predictions and properties of IncRNAs and circRNAs**

Given the crucial roles of IncRNAs and circRNAs in response to temperature and light changes in plants, Illumina sequencing was performed, which aimed to find essential candidates. After removing the redundant and low quality reads (Supplemental Table S3), a total of 2,629 IncRNAs and 1,043 circRNAs (Supplemental Table S4, Figure 2B and D) were identified from all the chromosomes (Supplemental Table S5, Figure 2A, E). Eight types, including antisense/sense genic exonic, genic intronic, intergenic downstream and intergenic upstream IncRNAs (Figure 2C), and four kinds of circRNAs including intergenic type, intronic type, antisense type and exonic type (Figure 2D) were identified. The minority of IncRNAs (4.11%) were antisense genic exonic type, and other types ranged from 10.45% to 16.54% (Figure 2C). The majority of circRNAs

![Figure 1. Phenotypic characteristics of pollen fertility and seed setting rate in PTGMS wheat line BS366. (A-D): Ki–I2 staining of pollen under four conditions including 12 °C 10L/14D, 12 °C 14L/10D, 20 °C 10L/14D and 20 °C 14L/10D. Scale bars = 1 mm; (E): Statistics of iodine staining rates and seed setting rates.](image-url)
were intergenic type (54%), followed by intronic circRNAs (41%), and the minority (4.11%) were antisense genic exonic type (Figure D). Besides, the majority (31.28%) of lncRNAs ranged from 200 to 400 bp, and approximately a quarter of the identified circRNAs (28.95%) were more than 2,000 bp (Figure 2B), which corresponds with the features of lncRNAs and circRNAs in other species [27].

**Analysis of differentially expressed mRNAs, lncRNAs and circRNAs**

To investigate the function of the isolated lncRNAs and circRNAs, which could respond to light and temperature, during the conversion of fertility in the PTGMS line BS366, DEGs, DELs and DECs were screened out and analyzed subsequently. A total of 2,341, 3,883, 6,426 and 6,694 DEGs (Figure 3A); 113, 131, 204 and 215 DELs (Figure 3B); and 94, 150, 152 and 84 DECs (Figure 3C) were identified in 12 °C 14 L vs. 10 L, 10 L 20 °C vs. 12 °C, 14 L 20 °C vs. 12 °C and 20 °C 14 L vs. 10 L comparable groups, respectively. In order to understand the different effects between light period and temperature more deeply, two group comparison analyses were performed. In total, 378 DEGs, 24 DELs and 15 DECs were considered as photoperiod-related candidates (Figure 3 D-F), and 4,789 DEGs, 137 DELs and 48 DECs were thought as thermo-related...
candidates (Figure 3 G-I). There were barely detectable differences in the numbers between up- and down-regulated photoperiod-related DEGs, DELs and DECs (Figure 3 D-F), particularly in the photoperiod-related DELs and DECs. Down-regulated DEGs and DELs comprised a larger amount than that of the downregulated ones in the thermo-related DEGs and DELs (Figure 3G and H), while downregulated DECs comprised less than half of those that were upregulated (Figure 3I).

**Functional analysis of differentially expressed mRNAs, IncRNAs and circRNAs**

Based on the expression profiles analysis, we found that highly significant patterns of expression were apparent in photoperiod- (Figure 4A-C) and thermo-induced (Figure 5A-C) male sterility-related DEGs, DELs and DECs. To obtain a better understanding of the mechanisms involved in light- and temperature-induced male sterility, we performed GO enrichment and KEGG pathway analyses for photoperiod- and thermo-induced male sterility-related DEGs, targets of DELs and hosts of DECs, respectively. The top significantly enriched GO terms are shown in Figure 4D. The most obvious photoperiod-induced male sterility-related DEGs that were associated with biological processes were response to hydrogen peroxide, followed by response to reactive oxygen species. The red or far-red light signalling pathway and regulation of long-day photoperiodis were also enriched in the GO terms for DEGs. For
photoperiod-induced male sterility-related targets of DEls, enriched GO terms included 3'-UTR-mediated mRNA destabilization, protein lipoylation, reductive pentose-phosphate cycle, photorespiration, cell cycle and cell division. For photoperiod-induced male-sterility related host of DECs were associated with the regulation of reactive oxygen species, metabolic process response to superoxide, programmed cell death, response to ozone, response to ethylene, response to osmotic stress and nitric oxide biosynthetic process.
Figure 5. Hierarchical cluster and functional analysis of thermo-induced overlapped DEGs, DELs and DECs. Heatmap of overlapping DEGs (A), DELs (B) and DECs (C). Enriched biological process of DEGs (D), DELs (E) and DECs (F). Gene expression is represented by colours, with brighter red for higher values and brighter blue for lower values.
In addition, it was found that the GO terms including anther morphogenesis, anther wall tapetum cell differentiation, pollen exine formation and mitotic sister chromatid separation were enriched in thermo-induced male sterility-related DEGs (Figure 5D). The targets of DELs for thermo-induced male sterility-related that were enriched included the auxin biosynthetic process, cellular response to water deprivation, response to auxin, regulation of gibberellin biosynthetic process, gibberellin catabolic process and regulation of pollen tube growth (Figure 5E). The carbohydrate metabolic process, photosynthesis, reductive pentose-phosphate cycle and ethylene biosynthetic process were enriched for the

Figure 6. Hierarchical cluster and functional analysis of photo-thermo responsive DEGs, DELs and DECs. Venn analysis showing male sterility-related differentially expressed mRNAs (A), lncRNAs (B) and circRNAs (C). The cluster heat map of male sterility-related differentially expressed mRNAs (D), lncRNAs (F) and circRNAs (H), red colour represents upregulated, blue colour represents downregulated genes, and heavier colour represents higher fold change. GO analysis of male sterility-related differentially expressed mRNAs (E), lncRNAs (G).
thermo-induced male sterility-related hosts of DELs (Figure 5F).

The KEGG pathway of photoperiod-induced male sterility-related DEGs, targets of DELs and hosts of DECs were enriched in glucose-related biosynthesis and metabolism, including carbon metabolism, galactose metabolism, fructose and mannose metabolism and glycolysis/gluconeogenesis (Supplemental Figure S1). Unlike the enriched KEGG pathway of photoperiod-induced male sterility-related DEGs, the targets of DELs and hosts of DECs, thermo-induced male sterility-related DEGs, targets of DELs and hosts of DECs were primarily enriched in cell proliferation and differentiation and cell signal transduction-related pathways, such as the Wnt signalling pathway, p53 signalling pathway, mitogen-activated protein kinase (MAPK) signalling pathway, oocyte meiosis and cell cycle (Supplemental Figure S2).

**Analysis of photo-thermo respond DEGs, DELs and DECs**

In order to further identify and characterize the male sterility-related genes, DEGs, DELs and DECs from photoperiod- and thermo-induced male sterility were combined and analyzed. A total of 40 DEGs, eight DELs and three DECs were identified from four conditions (Figure 6A-C). Highly significant patterns of expression were also apparent in these RNAs (Figure 6D, F and H), particularly in the DECs. Three DECs annotated as intergenic genes revealed highly significant patterns of expression in four conditions (Figure 6H). GO analysis was also performed on these RNAs. The results showed that the enriched GO terms on these DEGs were closely associated with male sterility, including UDP-galactose transmembrane transporter activity, starch biosynthetic process and the auxin-activated signalling pathway (Figure 6E).

**Figure 7.** Network analysis of male sterility-related lncRNA-miRNA-mRNA (A) and circRNA-miRNA-mRNA (B). Validation of the expression of the male sterility-related ceRNA network (TraesCS5A02G109300, TraesCS5A02G521500, TraesCS5D02G121500, TraesCSU02G145900, circRNA_0479, circRNA_0476, TCONS_00011114 and tae-miR10518) by qPCR (C). (D): The correlation between the transcriptome data and the expression levels detected by qPCR for the selected genes.
enriched GO terms for the targets of DELs were primarily associated with chaperone binding, octanoyl transferase activity and lipoic (octanoyl) transferase activity in molecular function, photosynthesis cell division and cell cycle in molecular function (Figure 6G).

Construction of three IncRNA/circRNA-miRNA-mRNA regulatory networks

In this study, we constructed male sterility-related ceRNA networks from the genes that were regulated by both photoperiod and temperature to clarify the functions of DELs and DECs that were identified. The results showed that one IncRNA could regulate many genes in different ways, and one gene could be regulated by many IncRNAs. For example, IncRNA (TCONS_00028200) could regulate four mRNAs (TraesCS4B02G217300, TraesCS4B02G217310, TraesCS4B02G217310, TraesCS4B02G217310) by tae-miR531 (Figure 7A). Similarly, one circRNA can ‘sponge’ many miRNAs and then regulate mRNAs. In this study, circRNA_0875 (Chr7A:47534636_47536054-) attracted tae-miR9673-5p, tae-miR10520, tae-miR9652-3p and tae-miR10518, thus, regulating their associated mRNAs (Figure 7B).
To investigate the function of circRNAs and IncRNAs during the transformation to fertility in more detail, the genes enriched in GO:0000302 (response to reactive oxygen species) from photoperiod-induced DEGs and GO:0048657 (anther wall tapetum cell differentiation) from thermo-induced DEGs were selected to construct IncRNA/circRNA-miRNA-mRNA regulatory networks. As shown in Figure 8A, IncRNA TCONS_00042550 was a ceRNA of one miRNA (tae-miR10516) that targeted five mRNAs (TraesCS3D02G114900, TraesCS4D02G145500, TraesCS3D02G114700, TraesCS3B02G131000 and TraesCS3A02G113000) (Figure 8A). In GO:0000302, circRNA-miRNA-mRNA was simple; one circRNA bound to one miRNA and regulated one mRNA (Figure 8C). In GO:0048657, 19 IncRNAs, five miRNAs and two mRNAs were involved in this IncRNA-miRNA-mRNA ceRNA network (Figure 8E). In addition, eight circRNAs, seven miRNAs and two mRNAs were involved in the circRNA-miRNA-mRNA that was related in the differentiation of anther wall tapetum cell (GO:0048657) (Figure 8G). The expression of these RNAs is shown in Figure 8 B, D, F and H. It was found that these RNAs in GO:0000302 were downregulated in low temperature conditions of photoperiod-induced male sterility conditions (Figure 8B, C).

Verification of transcripts and identification of male sterility-related IncRNAs and circRNAs

From the male sterility-related IncRNAs/circRNAs-mRNAs networks, we selected one IncRNA, two circRNAs and four mRNAs that were regulated by the same miRNA (tae-miR10518) to verify the level of expression (Figure 7C) and validate the reliability of our transcriptome data (Figure 7D). TraesCS5A02G109300, TraesCS5A02G521500 and TraesCSU02G145900 were upregulated at 12 °C 14 L but downregulated at 20 °C 14 L. However, circRNAs, IncRNAs and mRNAs showed opposite profiles of expression (Figure 7C). In addition, the Pearson’s correlation coefficient between the data generated from the two platforms was high ($R^2 = 0.9131$), indicating that the expression of RNA selected was consistent with the expression observed from sequencing (Figure 7D).

Discussion

The control of male fertility is central to the hybrid seed production for monoclinous crops, including rice and wheat. PTGMS wheat lines are important materials in two-line hybrid systems because of their fertility features. The plants appear to be almost entirely sterile when they are planted in a sterile environment, but they become fertile when planted in a fertile environment [15]. In a previous study, we found that the fertility of PTGMS wheat line BS366 was controlled by temperature and light period [7]. Recently, many studies have indicated that some non-coding RNAs regulate anther development and have some relationship to male sterility. Ding et al. [14] found that sufficient amounts of a long-day-specific male-fertility-associated IncRNA (LDMAR) transcript is required for the normal development of pollen of plants grown under long-day conditions in photoperiod-sensitive male sterility (PSMS) rice. Conversely, a low expression of LDMAR will cause premature programmed cell death (PCD) in developing anthers, causing PSMS [14]. Bai et al. [7] proposed a possible regulatory model by miRNAs on signalling pathways during the transition to fertility in wheat PTGMS line BS366. MiRNAs as negative regulators regulated their targets, affecting the transition to fertility in the PTGMS line [7]. CircRNA is a new hot spot of research and has been widely studied in humans but not in plants; systematic studies on plants are just beginning [28]. It has been confirmed that the circRNA functioned primarily through a miRNA ‘sponge’ and their correlated mRNAs. Previous studies for circRNAs primarily focussed on their roles in anti-stress processes in plants [29]. The fertility conversion for the PTGMS line is a very complex processes, which involves thousands of genes and multiple layers of regulation. To our knowledge, this study is the first to compare the difference in the profiles of expressions of miRNAs, IncRNAs and circRNAs in light- and temperature-induced male sterility in wheat PTGMS line BS366 to identify the key factors involved in the conversion to pollen fertility. Additionally, a potential ncRNA-miRNA-mRNA regulatory network was constructed to provide new insights into the molecular mechanism of the conversion to pollen fertility in wheat PTGMS.

Light is one of the key environmental regulators of multiple developmental processes, including plant morphogenesis, growth, development, physiological metabolism and male sterility [30]. In this study, we found that photoperiod-induced male sterility-related genes were primarily involved in some male sterility-related GO terms, such as the response to reactive oxygen species, response to hydrogen peroxide, regulation of auxin mediated signalling pathway, cell division and programmed cell death. Many studies showed that male sterility is associated with reactive oxygen species (ROS) [31], presumably because...
excessive ROS can cause serious damage to cells, including protein denaturation, lipid peroxidation, DNA mutation and PCD [32]. Mitochondria provide energy to all of the activities that occur in cells via respiration. Moreover, they generate ROS, including the superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2). The superfluous accumulation of ROS disturb the redox state balance in plant cells and can disrupt the normal function of mitochondria, resulting in damage to proteins and nucleic acids, lipid peroxidation, necrocytosis, which are deleterious to the growth of plant cells [33]. In addition, respiration-associated metabolism, such as ATP synthesis, is also affected. In this study, in addition to the GO terms, such as the response to hydrogen peroxide, regulation of reactive oxygen species metabolic process and response to superoxide directly associated with ROS, there are many other GO terms, such as ATP synthesis-coupled electron transport and response to stresses in photoperiod-induced male sterility GO analytical terms (Figure 4), that were consistent with those described in previous studies [34]. We also constructed a lncRNA/circRNA-miRNA-mRNA regulatory network that was associated with the response to reactive oxygen species (GO:0000302) from photoperiod-induced male sterility-related genes (Figure 8A and B). Five heat shock protein-related genes and TCONS_00042550 were regulated by tae-miR164 (Figure 8A). Many studies showed that heat shock proteins (Hsps) are involved in anther and pollen development [38]. For example, TMS1, an Hsp40 protein, is required for normal pollen tube growth under heat shock environments in Arabidopsis. In addition, studies showed that the absence of phytochromes could rapidly induce the levels of expression of HSPs [36]. In this study, we also found that the red or far red light signalling pathway, regulation of long-day photoperiodism, flowering and response to light stimulus were eliminated in enriched GO terms, indicating that phytochromes are involved in male sterility. Phytochromes, including phytochrome A (PHYA), PHYB and PHYC, serve as important photoreceptors that can respond to red and far-red light to regulate morphogenesis [37]. In rice, the phyA/phyB/phyC triple mutant and phyA/phyB double mutant were less fertile than the wild-type (WT), while the single mutants displayed normal fertility [38].

Additionally, carbohydrate metabolism and photosynthesis-related genes were significantly affected in the phyA phyB double mutant that is involved in male sterility [38]. In this study, a KEGG pathway analysis showed that a large number of genes were enriched in carbon- and sugar-related metabolism, particularly in photoperiod period-induced DEGs, DELs and DECs, such as starch and sucrose metabolism, carbon metabolism and carbon fixation in photosynthetic organisms (Supplemental Figures S1 and S2). Sugars play the key roles in the development of anthers. Studies have shown that starch is the most abundant sugar in the endothecium at the early stage of rice anther development [39]. It then decreases after meiosis. Additionally, the contents of reducing sugars will increase, while those of non-reducing sugars decrease during the maturation of rice anthers [39] [39]. It has also been found that a water deficit has a significant inhibitory effect during later stages of anther development. Thus, this could be the reason why many DEGs, DELs and DECs were enriched in GO terms, including cellular response to water deprivation and response to osmotic stress (Figures 4 and 5). Previous studies revealed that male sterility induced by abiotic stress treatments was caused by impaired carbohydrate metabolism in many plant species. Thus, we deduced that the photoperiod treatments that were abnormal led to a functional deficiency for phytochromes, which dissipated a large amount of carbohydrates and energy, and the male sterility of the PTGMS line resulted from the imbalance in carbohydrate metabolism. The anther wall is an important place for sugar synthesis, secretion, storage, mobilization and regulation [40]. In addition, it was reported that glycosyltransferases are involved in primexine formation and exine patterning in pollen walls [41]. Primexine can provide a substrate on the developing microspore for sporopollenin deposition, polymerization and patterning in pollen wall formation [42,43]. In this study, anther wall tapetum cell differentiation-related DEGs (GO:0048657) were observed to be enriched in thermo-induced male sterility DEGs (Figures 4A and 8C and D). The lncRNA/circRNA-miRNA-mRNA showed that tae-miR164 was involved in both the lncRNA and circRNA network. Bai et al. (2017) revealed that tae-miR164 with its target provided new insights into the genetic basis of male sterility in a wheat PTGMS line [7].

Conclusions

We compared the structural and expressional features of mRNAs, lncRNAs and circRNAs in the photoperiod and thermo-induced male sterility in wheat PTGMS line BS366. The results showed that ncRNAs were involved in male sterility by modulating lncRNA- and circRNA-associated ceRNA networks. This study provides new insights into the genetic basis of male sterility in a wheat PTGMS line, and additional research
is merited to validate the ceRNA mechanisms of ncRNAs and mRNAs.

**Disclosure statement**
The authors declare that they have no conflict of interest.

**Ethical standards**
We declare that these experiments comply with the ethical standards in China.

**Data availability**
All data that support the findings reported in this study are available from the corresponding author upon reasonable request.

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