Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Identification and characterization of circular RNAs in rapeseed (Brassica napus L.) under abiotic stress

Xiaoyan Zhou
Hainan University
Lianjin Zeng
Hainan University
Jie Liang
Hainan University
Run Li
Hainan University
hao Deng
Hainan University
Chunyan Li
Hainan University
Wanli Zhang
Hainan University
Pengfei Wang
Chinese Academy of Tropical Agriculture Sciences
Xianming Zhou
xm.zhou@hainanu.edu.cn

Hainan University

Research Article

Keywords: Brassica napus, circular RNAs, abiotic stress, ceRNA network

Posted Date: November 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11449878/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Abiotic stress poses a serious threat to rapeseed (*Brassica napus* L.) production. Circular RNAs (circRNAs) play crucial roles in multiple plant developmental processes. To elucidate the mechanism underlying the response to abiotic stress in rapeseed, high-throughput transcriptome sequencing was performed to identify and characterize circRNAs in rapeseed plants subjected to chilling stress (CS), drought stress (DS), salt stress (SS), and control (CK) conditions. A total of 1,597 circRNAs were identified and found to be unevenly distributed across 19 chromosomes, of which 102 circRNAs were differentially expressed between the CK and abiotic stress groups. Enrichment analysis revealed that the parental genes of differentially expressed circRNAs (DECs) participated in multiple biological processes and pathways related to abiotic stress response. Construction of a competing endogenous RNA (ceRNA) network elucidated that circRNAs may participate in abiotic stress response as miRNA sponges. Taken together, our study provides significant insights into the regulation of abiotic stress response by circRNAs and lays the foundation for functional verification of candidate circRNAs.

Introduction

Circular RNAs (circRNAs) are a class of endogenous non-coding RNAs that are ubiquitously present in eukaryotes and are characterized by a closed-loop structure [1, 2]. Unlike linear RNAs, circRNAs are formed by covalent bonds linking the 5’ cap and 3’ tail. Therefore, circRNAs are more stable than linear RNAs and can resist degradation by Ribonuclease R (RNase R) [3–5]. The mechanism of plant circRNA biogenesis remains largely unknown. The majority of circRNAs are derived from the circularization of single or multiple exons, and in animals, this circularization is mediated by flanking intronic complementary sequences [6]. However, repetitive elements and reverse complementary sequences have been observed in a fraction of circRNAs in plants [7, 8], indicating the existence of alternative mechanisms for circularization of plant circRNAs. circRNAs can regulate gene expression at both transcriptional and post-transcriptional levels. Exon-intron circRNAs (EIciRNAs) have been reported to positively regulate parental genes by interacting with U1 snRNP in humans [9]. Owing to their stability and the presence of microRNA (miRNA) binding sites, circRNAs can also inhibit miRNA activity and regulate gene expression, a mechanism known as miRNA sponges or competing endogenous RNAs (ceRNAs) [2, 10, 11].

Although circRNAs were first reported in the 1990s, they were initially regarded as by-products of transcription resulting from scrambled splicing [12–15]. The prevalence and significance of circRNAs were not recognized until the advent of high-throughput sequencing [16]. Numerous studies of circRNAs have been conducted in humans [17, 18], mice [10, 19], zebrafish [20, 21], and *Caenorhabditis elegans* [22, 23]. In recent years, circRNA studies have rapidly emerged in various plant species. Plant circRNAs have been reported to be involved in multiple biological processes, including development [24–26], biotic stress response [27–30], and abiotic stress response [31–34]. However, only a fraction of plant circRNAs have been functionally validated. A total of 2,932 circRNAs were identified in rice under *Magnaporthe oryzae*-infected or uninfected conditions, and overexpression of circR5g05160 enhanced resistance to

Magnaporthe oryzae in rice [29]. In addition, 475 DECs were identified in grape under cold stress, and overexpression of Vv-circATS1 in *A. thaliana* improved cold tolerance [32]. These studies highlight the significance of circRNAs in genetic regulation, yet the functional relevance of circRNAs in abiotic stress response remains largely undetermined.

Brassica napus (2n = 38, AnAnCnCn) is an allotetraploid formed by interspecific hybridization between *Brassica rapa* (2n = 20, ArAr) and *Brassica oleracea* (2n = 19, CoCo) approximately 7,500 years ago [35]. Rapeseed (*Brassica napus*) is a globally important oil crop and serves as protein-rich fodder for animals. However, its production is threatened by serious abiotic stress [36]. Drought, salt, and temperature stresses are frequently occurring abiotic stresses that impair rapeseed productivity [37–40]. Previous studies have shown that circRNAs play vital roles under these abiotic stresses in other plant species, such as maize [31], cucumber [41], and grapevine [32]. However, no comprehensive study has investigated circRNAs in response to various abiotic stresses in rapeseed. To comprehensively unravel the mechanisms by which circRNAs are involved in various abiotic stresses, high-throughput RNA sequencing was performed to identify and characterize circRNAs from rapeseed leaves under chilling stress, drought stress, salt stress, and control conditions. Furthermore, enrichment analysis of the parental genes of differentially expressed circRNAs (DECs) and construction of a circRNA-miRNA-mRNA ceRNA network were conducted to explore the potential functions of circRNAs under abiotic stresses in rapeseed.

Materials and methods

Plant material and treatments

The experimental material, ZS11, is an elite semi-winter rapeseed accession widely cultivated in the Yangtze River basin [42]. Initially, all plants used in this study were cultivated under a comprehensive speed breeding (CSB) environment [43] for 45 days. Subsequently, the plants were transferred to illumination incubators with a temperature of 25/22°C (light/dark, 16h/8h), a light intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a relative humidity of 50%. After preincubation for 3 days in the illumination incubators, the plants were divided into four groups: control (CK) group, chilling stress (CS) group, drought stress (DS) group, and salt stress (SS) group. For the CS group, each plant was irrigated with 150 mL water and the temperature was lowered to 4°C; for the DS group, each plant was irrigated with 150 mL 20% polyethylene glycol 6000 (PEG-6000) solution to simulate drought stress; for the SS group, each plant was irrigated with 150 mL 300 $mmol \cdot L^{-1}$ NaCl solution; and for the CK group, each plant was irrigated with 150 mL water (Supplementary Table 1). After 24 hours, young leaves were collected from three plants of each group for RNA extraction. The samples were immediately frozen in liquid nitrogen and subsequently stored in a -80°C refrigerator.

RNA extraction and circRNA sequencing

Total RNA was extracted from the leaves of each plant using a Plant Total RNA Extraction Kit (Tiangen Biochemical Technology Co. Ltd., Beijing, China). Then, the total RNA was treated using a Ribo-Zero Magnetic Kit (Illumina, San Diego, CA, USA) to deplete ribosomal RNA (rRNA). The rRNA-depleted RNA was incubated at 37°C with RNase R Reaction Buffer and Ribonuclease R to degrade linear RNA. After linear RNA degradation, a circRNA library was constructed using the NEB Next Ultra Directional RNA Library Prep Kit (Illumina) according to the manufacturer's instructions. Finally, circRNA sequencing was performed on an Illumina NovaSeq 6000 platform with a paired-end 150 bp model (Biomarker Technologies Co., Ltd., Beijing, China).

Identification of circRNAs

Raw data were filtered using Trimmomatic (version 0.36) [44] with the parameters "ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 SLIDINGWINDOW:5:20 LEADING:5 TRAILING:5 MINLEN:50" to remove adaptors, low-quality sequences, and reads shorter than 50 bp. The clean data were used to identify circRNAs with CIRI2 (version 2.0.6) [45, 46] and CIRCexplorer2 (version 2.3) [47]. For the CIRI2 pipeline, short reads were aligned to the ZS11 reference genome [48] using the BWA MEM program (version 0.7.17) [49] with the parameter "-T 19". Then, the output file was used for circRNA identification by CIRI2. For the CIRCexplorer2 pipeline, short reads were aligned to the ZS11 reference genome using STAR (version 2.73a) [50] with the parameter "-chimSegmentMin 10", and circRNAs were identified by CIRCexplorer2. To obtain high-confidence circRNAs, the intersection of results generated by the two software programs was used for subsequent analysis.

Characterization and conservation analysis of circRNAs

In-house scripts were used to calculate the length distribution, genomic origin distribution, and chromosome distribution of the identified circRNAs. For conservation analysis, the identified circRNAs were aligned against a local PlantCircBase database (release v4, http://ibi.zju.edu.cn/plantcircbase/) [51] using BLASTn software (v2.9.0). Then, an in-house Python script was used to summarize the conservation status.

Differentially expressed circRNAs (DECs) analysis

Back-splicing junction reads were used to quantify the expression level of circRNAs for each sample. DECs analysis between the CK group and abiotic stress-treated group (CK vs CS, CK vs DS, and CK vs SS) was conducted using the R package edgeR (version 3.20.0) [52]. circRNAs with an absolute value of log2-transformed fold change greater than 1, a *p*-value less than 0.05, and a minimum of 2 back-splicing junction reads were considered as DECs.

Enrichment analysis

To uncover the potential functions of DECs between the CK group and abiotic stress group, GO (Gene Ontology) [53] and KEGG (Kyoto Encyclopedia of Genes and Genomes) [54] enrichment analyses were conducted using the parental genes of DECs. GO and KEGG enrichment analysis were conducted using TBtools (version 0.6731) [55] with a threshold of "*p*-value < 0.05".

Construction of the "circRNA-miRNA-mRNA" interaction network

Rapeseed miRNAs were downloaded from the miRBase database; then the miRNA binding sites of circRNAs identified in this study were predicted using miRanda (version 3.3a) [56] and TargetScan (version 7.0) [57]. An online tool, psRNATarget (http://plantgrn.noble.org/psRNATarget/), was adopted to identify the target mRNAs of miRNAs. The "circRNA-miRNA-mRNA" interaction network was analyzed and visualized using Cytoscape (version 3.7.2) [58].

Results

Identification and characterization of rapeseed circRNAs

To identify circRNAs involved in the abiotic stress response in rapeseed, a circRNA sequencing strategy was employed. After quality control and filtering, a total of 118 gigabytes (Gb) of clean data was obtained from the CK, CS, DS, and SS groups, with three biological replicates per group (Supplementary Table 2). More than 100 million paired reads per group and a Q30 base percentage greater than 96% for each sample conferred the reliability of sequencing data for identifying circRNAs. The CIRI2 and CIRCexplorer2 pipelines were adopted to identify circRNAs, and the intersection of circRNAs identified from the two pipelines was considered the final result. A total of 1,597 high-quality circRNAs were identified across the four groups, with 836 (52.34%) on the negative strand and 761 (47.66%) on the positive strand (Fig. 1A, Supplementary Fig. 1, Supplementary Table 3). The CS group had the most circRNAs identified (643), while the CK group had the fewest (362), and only 31 circRNAs were identified in all groups (Fig. 1B; Supplementary Table 4). Comparison of circRNA numbers between different groups revealed that circRNAs may be specifically induced by different abiotic stresses.

Among the identified circRNAs, 714 (44.71%) were exonic circRNAs, 709 (44.40%) were intronic circRNAs, and only 174 (10.89%) were intergenic circRNAs (Fig. 1C). The exonic and intronic circRNAs identified in the CS group were more abundant than those identified in the other groups, accounting for the largest proportion of circRNAs in the CS group (Supplementary Fig. 2). The average length of circRNAs was 731 nucleotides (nt). More than 98.31% of circRNAs were shorter than 2,000 nt, and most of them were distributed between 200 and 1,000 nt (Fig. 1D). circRNAs identified from the four groups had a similar distribution among different chromosomes. Chromosome A04 had the fewest circRNAs, while C03 had the most circRNAs (Supplementary Fig. 1A). The distribution of junction reads on different chromosomes was also similar among the four groups, but most junction reads were located on chromosome C08 (Supplementary Fig. 1B).

Conservation analysis was performed by aligning the identified rapeseed circRNAs to a local PlantCircBase database using BLASTn software [51]. Only 595 (37.25%) of the rapeseed circRNAs were homologous to circRNAs in PlantCircBase, but 569 (95.63% of 595) of the conserved circRNAs were homologous to circRNAs from *Arabidopsis thaliana* (Fig. 1E). This result revealed that circRNAs are more conserved between rapeseed and *Arabidopsis thaliana*.

Differential expression analysis of circRNAs

To identify circRNAs involved in abiotic stress response, DECs between the CK group and abiotic stress group were identified, and a total of 102 DECs were obtained across the three comparisons (Fig. 2A). The CS comparison group (CS vs CK) had 68 DECs, which was the most among the three comparisons (Supplementary Table 5); the DS comparison group (DS vs CK) had 52 DECs, which was the fewest among the three comparisons (Supplementary Table 6), and the SS comparison group (SS vs CK) had 56 DECs (Supplementary Table 7). Overall, there were more down-regulated DECs among the three comparisons (Fig. 2A). Additionally, 31 DECs were identified in all three comparisons, accounting for 45.59%, 59.62%, and 55.36% of DECs between CK and CS, DS, and SS groups, respectively (Fig. 2B). This result revealed that there may be a potential common mechanism linked by circRNAs that modulates abiotic stresses response in rapeseed.

Functional enrichment analysis of parental genes of DECs

To elucidate the potential functions of circRNAs responding to abiotic stress, GO and KEGG enrichment analyses were conducted to analyze the parental genes of DECs (p-value < 0.05). In the CS comparison group, 43 DECs had identifiable parental genes, while 25 DECs did not have parental genes (Supplementary Table 5). GO enrichment analysis revealed that the cellular component category mainly contained “peroxisome” (GO:0005777), “chloroplast part” (GO:0044434), and “cytoplasm” (GO:0005737); the molecular function category mainly contained “NAD + ADP-ribosyltransferase activity” (GO:0003950), “squalene monooxygenase activity” (GO:0004506), and “ADP-ribose pyrophosphohydrolase activity” (GO:0080041); the biological process category mainly contained “thiamine diphosphate biosynthetic process” (GO:0009229), “response to abiotic stimulus” (GO:0009628), “negative regulation of gene expression” (GO:0010629), and “regulation of gibberellin biosynthetic process” (GO:0010371) (Fig. 3A, Supplementary Table 8). KEGG enrichment identified 16 significant pathways, including “Carbon metabolism” (map01200), “RNA transport” (map03013), “TCA cycle” (map00020), and “Photosynthesis - antenna proteins” (map00196) (Fig. 3B, Supplementary Table 9).

In the DS comparison group, 30 DECs had identifiable parental genes, while 22 DECs had no annotated parental genes (Supplementary Table 6). GO enrichment analysis revealed that the cellular component category mainly contained “cytoplasm” (GO:0005737), “ribonucleoprotein complex” (GO:1990904), and “cytoplasmic mRNA processing body” (GO:0000932); the molecular function category mainly contained “3-hydroxyisobutyryl-CoA hydrolase activity” (GO:0003860) and “translation initiation factor binding” (GO:0031369); the biological process category mainly contained “regulation of gibberellin biosynthetic process” (GO:0010371) and “terpenoid metabolic process” (GO:0006721) (Fig. 3A, Supplementary

Table 10). KEGG enrichment identified 12 significant pathways, including “Nitrogen metabolism” (map00910), “Purine metabolism” (map00230), “Glyoxylate and dicarboxylate metabolism” (map00630), and “Photosynthesis - antenna proteins” (map00196) (Fig. 3B, Supplementary Table 11).

In the SS comparison group, 33 DECs had identifiable parental genes, while 23 DECs had no annotated parental genes (Supplementary Table 7). GO enrichment analysis revealed that the cellular component category mainly contained “peroxisome” (GO:0005777), “cytoplasmic stress granule” (GO:0010494), and “cytoplasm” (GO:0005737); the biological process category mainly contained “positive regulation of lipid biosynthetic process” (GO:0046889), “response to metal ion” (GO:0010038), “isoprenoid metabolic process” (GO:0006720), and “regulation of gibberellin biosynthetic process” (GO:0010371) (Fig. 3A, Supplementary Table 12). KEGG enrichment identified 16 significant pathways, including “Carbon metabolism” (map01200), “Arginine biosynthesis” (map00220), “Phenylalanine metabolism” (map00360), “Photosynthesis” (map00195), and “Photosynthesis - antenna proteins” (map00196) (Fig. 3B, Supplementary Table 13).

Construction of circRNA-miRNA-mRNA networks

To unravel the mechanisms by which circRNAs are involved in post-transcriptional modification by serving as miRNA sponges, miRNA binding sites were identified to construct a circRNA-miRNA-mRNA ceRNA network in abiotic stress response in rapeseed. A total of 519 (32.50% of 1,597) circRNAs had potential binding sites for 92 miRNAs (Supplementary Table 14). Among them, 344 (66.28% of 519) circRNAs had multiple predicted miRNA binding sites and 21 (4.05% of 519) circRNAs had more than 10 miRNA binding sites, while 90 (97.83% of 92) miRNAs could bind to multiple circRNAs (Supplementary Table 14). There were 1,867 edges and 612 nodes within the circRNA-miRNA network (Fig. 4A). Then, 6,126 potential target mRNAs of the 92 miRNAs were identified, and a comprehensive circRNA-miRNA-mRNA ceRNA network was constructed (Supplementary Table 15). This result revealed that circRNAs may widely involved in gene post-transcriptional regulation as miRNA sponges.

Furthermore, the DECs were used to construct a ceRNA sub-network represented by 45 circRNAs, 59 miRNAs, and 4,173 mRNAs (Fig. 4B). Among these miRNAs, several abiotic stress-related miRNA families, such as miR159, miR164, miR168, miR169, miR171 and miR390, were predicted to bind to 14 circRNAs (Fig. 4B; Supplementary Table 7). Notably, many mRNAs involved in the response to stimuli were present in the ceRNA sub-network. Taken together, these results suggest that rapeseed circRNAs may participate in abiotic stress responses by acting as miRNA sponges, providing a mechanistic hypothesis for the post-transcriptional regulation of stress-related genes that warrants further functional validation.

Discussion

CircRNAs are a class of non-coding RNAs (ncRNAs) widely present in plants and play vital roles in various biological processes [2]. Previous studies have revealed that circRNAs respond to abiotic stress in many crops, such as soybean [33], tomato [59], and grapevine [32]. Here, we present a comprehensive

study on the genome-wide identification of circRNAs and their potential functional roles in rapeseed under chilling stress, drought stress, and salt stress.

In this study, 1,597 circRNAs were systematically identified in rapeseed, and their expression exhibited clear specificity under different stress conditions. Only 31 circRNAs were detected in all treatment groups, and the significant difference in circRNA numbers between the CS group and CK group indicated that circRNA production has obvious stress-induced specificity. This finding is consistent with the existing knowledge that plant circRNA expression exhibits tissue, developmental stage, and environmental specificity [60, 61]. In addition, among all circRNAs, exonic circRNAs (714, 44.71%) and intronic circRNAs (709, 44.40%) were dominant (Fig. 1C), similar to reports in common bean, tomato, and *Salvia miltiorrhiza* [59, 62, 63]. The average length of circRNAs was 731 nt, with more than 98% shorter than 2,000 nt and enriched in the 200-1,000 nt range in rapeseed. This length distribution characteristic is consistent with the results of studies in Chinese cabbage [64], suggesting that the length distribution of plant circRNAs is conserved across species.

The numbers of DECs under the three stress treatments were 68 in the CS comparison group, 52 in the DS comparison group, and 56 in the SS comparison group, among which 31 DECs were identified in all three comparisons, accounting for a relatively high proportion (45.59%-59.62%) of the total DECs. This finding indicates the existence of a common regulatory mechanism mediated by circRNAs when rapeseed responds to different abiotic stresses. From the enrichment analysis, the “Photosynthesis - antenna proteins” (map00196) pathway and “regulation of gibberellin biosynthetic process” pathway were enriched under all three stresses (CS, DS, and SS), suggesting that they may be common regulatory nodes for rapeseed responding to multiple abiotic stresses. In addition, “peroxisome” (GO:0005777) and “Carbon metabolism” (map01200) were commonly enriched in the CS and SS comparison groups; these overlapping pathways may represent potential targets through which circRNAs coordinate multiple stress responses.

Meanwhile, stress-specific responses were also evident. The enrichment of “thiamine diphosphate biosynthetic process” and “TCA cycle” specific to the CS comparison group, as well as the “Nitrogen metabolism” and “Purine metabolism” pathways specific to the DS comparison group, reflected different perturbation patterns of cellular metabolic networks by different stress types. The enrichment of “peroxisome” and “response to metal ion” in the SS comparison group was consistent with the characteristics of ion toxicity and oxidative stress caused by salt stress. This regulatory pattern of specificity within commonality indicates that circRNAs may achieve fine-tuned stress adaptation by combining shared core stress response modules (such as photosynthetic system protection and hormone signaling regulation) with stress-specific metabolic reprogramming.

In the present study, 519 circRNAs were predicted to have miRNA binding sites, and a ceRNA sub-network containing 45 DECs, 59 miRNAs, and 4,173 mRNAs was constructed. Among them, bna-miR169 was predicted to bind to multiple circRNAs, and the miR169n-NF-YA8 module has been proven to play a negative regulatory role in drought resistance in rapeseed, with overexpression of its target gene *NF-YA8*

enhancing osmotic stress tolerance [65]. In this study, multiple circRNAs were predicted to act as sponges for miR169, suggesting that they may positively regulate drought resistance by competitively binding to miR169 and relieving its inhibition of *NF-YA8*. This prediction provides a mechanistic hypothesis for the involvement of circRNAs in drought stress response in rapeseed.

In addition, multiple abiotic stress-related miRNA families were also predicted to bind to the circRNAs identified in this study. miR159 regulates proline and putrescine accumulation by targeting *SlMYB33* under drought stress in tomato, thereby enhancing osmotic protection [66]; in poplar, miR159a overexpression significantly enhances drought resistance by reducing stomatal aperture, improving water use efficiency, and increasing reactive oxygen species scavenging capacity [67]. The function of miR164 has been reported in both salt stress and drought stress. In *Tamarix*, the miR164-TcNAC1 module participates in salt stress response through negative regulation of NAC transcription factors [68]; in foxtail millet, the miR164b-SiNAC015 module regulates drought tolerance by scavenging reactive oxygen species [69]; and miR164d in barley enhances salt tolerance by inhibiting the HvNAC92-HvHKT1;5 module [70]. miR168-mediated regulation of *AGO1* homeostasis plays a key role in ABA signaling and drought response in *Arabidopsis* [71]. The function of miR171 has been verified in multiple species: mno-miR171a in mulberry enhances salt and drought tolerance by regulating *SCL* target genes [72]; overexpression of Osa-miR171a in rice significantly improves drought resistance by reducing reactive oxygen species accumulation, and this function is conserved in *Arabidopsis* [73]. miR390 negatively regulates salt tolerance by targeting *GhCEPR2* in cotton [74], and miR390 is up-regulated in soybean roots under water stress [75]. These lines of cross-species functional evidence indicate that the circRNA-miRNA binding relationships predicted in this study are not accidental. If circRNAs indeed act as sponges for these conserved stress-responsive miRNAs, their expression changes may participate in post-transcriptional regulation of stress signaling by competitively binding to miRNAs and releasing the inhibition of downstream target genes.

This study provides a genome-wide expression map of circRNAs in rapeseed under multiple abiotic stresses; however, the ceRNA network in this study is mainly based on bioinformatic prediction, lacking experimental verification, and the false positive rate is unknown. Therefore, the actual biological significance of these circRNAs in abiotic stress resistance in rapeseed remains unclear. In the future, we will select key candidate circRNAs from the ceRNA network and validate their functions by combining CRISPR-based knockout and overexpression approaches, followed by evaluation of their effects on abiotic stress resistance in rapeseed under stress conditions.

Declarations

Competing interests

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate

not applicable.

Consent for publication

Not applicable.

Funding

This work was supported by the National Natural Science Foundation of China (32501930), the Hainan Provincial Natural Science Foundation of China (323QN192), and the Scientific Research Startup Foundation of Hainan University (KYQD(ZR)-22103).The funders had no role in the design of the study, data collection, analysis, interpretation of data, or writing of the manuscript.

Author Contribution

Xiaoyan Zhou, Lianjin Zeng: Methodology, Investigation, Data curation, Visualization, Writing-Original Draft. Jie Liang, Run Li, Hao Deng, Chunyan Li, Wanli Zhang: Investigation, Writing-review & editing. Pengfei Wang: Formal analysis, Data curation, Writing-review & editing. Xianming Zhou: Conceptualization, Supervision, Writing-review & editing, Funding acquisition. All authors read and approved the paper.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (32501930), the Hainan Provincial Natural Science Foundation of China (323QN192), and the Scientific Research Startup Foundation of Hainan University (KYQD(ZR)-22103).

Data Availability

The datasets generated and analysed in this study are available in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1550963 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1550963).

References

1. Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, Sun W, Dou K, Li H. Circular RNA: A new star of noncoding RNAs, Cancer letters, 365 (2015) 141–148.

2. Zhang P, Li S, Chen M. Characterization and Function of Circular RNAs in Plants. Front Mol Biosci. 2020;7:91.
3. Suzuki H, Zuo Y, Wang J, Zhang MQ, Malhotra A, Mayeda A. Characterization of RNase R-digested cellular RNA source that consists of lariat and circular RNAs from pre-mRNA splicing. Nucleic Acids Res. 2006;34:e63.
4. Ashwal-Fluss R, Meyer M, Pamudurti NR, Ivanov A, Bartok O, Hanan M, Evantal N, Memczak S, Rajewsky N, Kadener S. circRNA biogenesis competes with pre-mRNA splicing, Molecular cell, 56 (2014) 55–66.
5. Chen L-L. The biogenesis and emerging roles of circular RNAs. Nat Rev Mol Cell Biol. 2016;17:205–11.
6. Zhang XO, Wang HB, Zhang Y, Lu X, Chen LL, Yang L. Complementary sequence-mediated exon circularization. Cell. 2014;159:134–47.
7. Lu T, Cui L, Zhou Y, Zhu C, Fan D, Gong H, Zhao Q, Zhou C, Zhao Y, Lu D, Luo J, Wang Y, Tian Q, Feng Q, Huang T, Han B. Transcriptome-wide investigation of circular RNAs in rice, RNA (New York, N.Y.), 21 (2015) 2076–87.
8. Ye CY, Chen L, Liu C, Zhu QH, Fan L. Widespread noncoding circular RNAs in plants, The New phytologist, 208 (2015) 88–95.
9. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, Zhu P, Chang Z, Wu Q, Zhao Y, Jia Y, Xu P, Liu H, Shan G. Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol. 2015;22:256–64.
10. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. Nature. 2013;495:384–8.
11. Liang Y, Liu N, Yang L, Tang J, Wang Y, Mei M. A Brief Review of circRNA Biogenesis, Detection, and Function, Current genomics, 22 (2021) 485–95.
12. Nigro JM, Cho KR, Fearon ER, Kern SE, Ruppert JM, Oliner JD, Kinzler KW, Vogelstein B. Scrambled exons Cell. 1991;64:607–13.
13. Cocquerelle C, Daubersies P, Majérus MA, Kerckaert JP, Bailleul B. Splicing with inverted order of exons occurs proximal to large introns. EMBO J. 1992;11:1095–8.
14. Capel B, Swain A, Nicolis S, Hacker A, Walter M, Koopman P, Goodfellow P. Lovell-Badge, Circular transcripts of the testis-determining gene Sry in adult mouse testis. Cell. 1993;73:1019–30.
15. Cocquerelle C, Mascrez B, Hétuin D, Bailleul B. Mis-splicing yields circular RNA molecules, FASEB journal: official publication of the Federation of American Societies for Experimental Biology, 7 (1993) 155–60.
16. Huang Y, Li Y, Lin W, Fan S, Chen H, Xia J, Pi J, Xu JF. Promising Roles of Circular RNAs as Biomarkers and Targets for Potential Diagnosis and Therapy of Tuberculosis, Biomolecules, 12 (2022).

17. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. PLoS ONE. 2012;7:e30733.
18. Szabo L, Morey R, Palpant NJ, Wang PL, Afari N, Jiang C, Parast MM, Murry CE, Laurent LC, Salzman J. Statistically based splicing detection reveals neural enrichment and tissue-specific induction of circular RNA during human fetal development. Genome Biol. 2015;16:126.
19. Wu HJ, Zhang CY, Zhang S, Chang M, Wang HY. Microarray Expression Profile of Circular RNAs in Heart Tissue of Mice with Myocardial Infarction-Induced Heart Failure, Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology, 39 (2016) 205–16.
20. Shen Y, Guo X, Wang W. Identification and characterization of circular RNAs in zebrafish. FEBS Lett. 2017;591:213–20.
21. Özdemir S, Arslan H. circRNA-based biomarker candidates for acute cypermethrin and chlorpyrifos toxication in the brain of Zebrafish (Danio rerio), Chemosphere, 298 (2022) 134330.
22. Cortés-López M, Gruner MR, Cooper DA, Gruner HN, Voda AI, van der Linden AM, Miura P. Global accumulation of circRNAs during aging in Caenorhabditis elegans. BMC Genomics. 2018;19:8.
23. Kumar L, Shamsuzzama P, Jadiya R, Haque S, Shukla A, Nazir. Functional Characterization of Novel Circular RNA Molecule, circzip-2 and Its Synthesizing Gene zip-2 in C. elegans Model of Parkinson's Disease, Molecular neurobiology, 55 (2018) 6914–6926.
24. Cheng J, Zhang Y, Li Z, Wang T, Zhang X, Zheng B. A lariat-derived circular RNA is required for plant development in Arabidopsis. Sci China Life Sci. 2018;61:204–13.
25. Zhang G, Diao S, Zhang T, Chen D, He C, Zhang J. Identification and characterization of circular RNAs during the sea buckthorn fruit development. RNA Biol. 2019;16:354–61.
26. Shi F, Pang Z, Liu C, Zhou L, Tan C, Ren J, Ye X, Feng H, Liu Z. Whole-transcriptome analysis and construction of an anther development-related ceRNA network in Chinese cabbage (Brassica campestris L. ssp. pekinensis). Sci Rep. 2022;12:2667.
27. Wang Z, Liu Y, Li D, Li L, Zhang Q, Wang S, Huang H. Identification of Circular RNAs in Kiwifruit and Their Species-Specific Response to Bacterial Canker Pathogen Invasion. Front Plant Sci. 2017;8:413.
28. Yuan J, Wang Z, Xing J, Yang Q, Chen XL. Genome-wide Identification and characterization of circular RNAs in the rice blast fungus Magnaporthe oryzae. Sci Rep. 2018;8:6757.
29. Fan J, Quan W, Li GB, Hu XH, Wang Q, Wang H, Li XP, Luo X, Feng Q, Hu ZJ, Feng H, Pu M, Zhao JQ, Huang YY, Li Y, Zhang Y, Wang WM. circRNAs Are Involved in the Rice-Magnaporthe oryzae Interaction. Plant Physiol. 2020;182:272–86.
30. Liu H, Nwafor CC, Piao Y, Li X, Zhan Z, Piao Z. Identification and Characterization of Circular RNAs in Brassica rapa in Response to Plasmodiophora brassicae. Int J Mol Sci, 23 (2022).
31. Zhang P, Fan Y, Sun X, Chen L, Terzaghi W, Bucher E, Li L, Dai M. A large-scale circular RNA profiling reveals universal molecular mechanisms responsive to drought stress in maize and Arabidopsis. Plant journal: cell Mol biology. 2019;98:697–713.

32. Gao Z, Li J, Luo M, Li H, Chen Q, Wang L, Song S, Zhao L, Xu W, Zhang C, Wang S, Ma C. Characterization and Cloning of Grape Circular RNAs Identified the Cold Resistance-Related Vv-circATS1. Plant Physiol. 2019;180:966–85.
33. Wang X, Chang X, Jing Y, Zhao J, Fang Q, Sun M, Zhang Y, Li W, Li Y. Identification and functional prediction of soybean CircRNAs involved in low-temperature responses. J Plant Physiol. 2020;250:153188.
34. Yang Z, Zhu P, Kang H, Liu L, Cao Q, Sun J, Dong T, Zhu M, Li Z, Xu T. High-throughput deep sequencing reveals the important role that microRNAs play in the salt response in sweet potato (Ipomoea batatas L). BMC Genomics. 2020;21:164.
35. Chalhoub B, Denoeud F, Liu S, Parkin IA, Tang H, Wang X, Chiquet J, Belcram H, Tong C, Samans B, Corréa M, Da Silva C, Just J, Falentin C, Koh CS, Le Clainche I, Bernard M, Bento P, Noel B, Labadie K, Alberti A, Charles M, Arnaud D, Guo H, Daviaud C, Alamery S, Jabbari K, Zhao M, Edger PP, Chelaifa H, Tack D, Lassalle G, Mestiri I, Schnel N, Le Paslier MC, Fan G, Renault V, Bayer PE, Golicz AA, Manoli S, Lee TH, Thi VH, Chalabi S, Hu Q, Fan C, Tollenaere R, Lu Y, Battail C, Shen J, Sidebottom CH, Wang X, Canaguier A, Chauveau A, Bérard A, Deniot G, Guan M, Liu Z, Sun F, Lim YP, Lyons E, Town CD, Bancroft I, Wang X, Meng J, Ma J, Pires JC, King GJ, Brunel D, Delourme R, Renard M, Aury JM, Adams KL, Batley J, Snowdon RJ, Tost J, Edwards D, Zhou Y, Hua W, Sharpe AG, Paterson AH, Guan C. P. Wincker, Plant genetics. Early allopolyploid evolution in the post-Neolithic Brassica napus oilseed genome, Science, 345 (2014) 950–953.
36. Raza A, Razzaq A, Mehmood SS, Hussain MA, Wei S, He H, Zaman QU, Xuekun Z, Hasanuzzaman M. Omics: The way forward to enhance abiotic stress tolerance in Brassica napus L, GM crops & food, 12 (2021) 251–281.
37. Long W, Zou X, Zhang X. Transcriptome analysis of canola (Brassica napus) under salt stress at the germination stage. PLoS ONE. 2015;10:e0116217.
38. Kumar M, Choi JY, Kumari N, Pareek A, Kim SR. Molecular breeding in Brassica for salt tolerance: importance of microsatellite (SSR) markers for molecular breeding in Brassica. Front Plant Sci. 2015;6:688.
39. Wang J, Mao L, Li Y, Lu K, Qu C, Tang Z, Li J, Liu L. Natural variation in BnaA9.NF-YA7 contributes to drought tolerance in Brassica napus L, Nat Commun, 15 (2024) 2082.
40. Huang Z, Zhao N, Qin M, Xu A. Mapping of quantitative trait loci related to cold resistance in Brassica napus L. J Plant Physiol. 2018;231:147–54.
41. Zhu YX, Jia JH, Yang L, Xia YC, Zhang HL, Jia JB, Zhou R, Nie PY, Yin JL, Ma DF, Liu LC. Identification of cucumber circular RNAs responsive to salt stress. BMC Plant Biol. 2019;19:164.
42. Zhou X, Dai L, Wang P, Liu Y, Xie Z, Zhang H, Xin Q, Wan L, Yang L, Yang G, Hong D. Mining favorable alleles for five agronomic traits from the elite rapeseed cultivar Zhongshuang 11 by QTL mapping and integration. Crop J. 2021;9:1449–59.
43. Song Y, Duan X, Wang P, Li X, Yuan X, Wang Z, Wan L, Yang G, Hong D. Comprehensive speed breeding: a high-throughput and rapid generation system for long-day crops. Plant Biotechnol J.

2022;20:13–5.

44. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.
45. Gao Y, Wang J, Zhao F. CIRI: an efficient and unbiased algorithm for de novo circular RNA identification. Genome Biol. 2015;16:4.
46. Gao Y, Zhang J, Zhao F. Circular RNA identification based on multiple seed matching. Brief Bioinform. 2018;19:803–10.
47. Zhang XO, Dong R, Zhang Y, Zhang JL, Luo Z, Zhang J, Chen LL, Yang L. Diverse alternative back-splicing and alternative splicing landscape of circular RNAs. Genome Res. 2016;26:1277–87.
48. Song JM, Guan Z, Hu J, Guo C, Yang Z, Wang S, Liu D, Wang B, Lu S, Zhou R, Xie WZ, Cheng Y, Zhang Y, Liu K, Yang QY, Chen LL, Guo L. Eight high-quality genomes reveal pan-genome architecture and ecotype differentiation of Brassica napus. Nat plants. 2020;6:34–45.
49. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754–60.
50. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29:15–21.
51. Chu Q, Zhang X, Zhu X, Liu C, Mao L, Ye C, Zhu QH, Fan L. PlantcircBase: A Database for Plant Circular RNAs, Mol Plant, 10 (2017) 1126–1128.
52. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010;26:139–40.
53. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM. G. Sherlock, Gene ontology: tool for the unification of biology. The Gene Ontology Consortium, Nat Genet, 25 (2000) 25–29.
54. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28:27–30.
55. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. Mol Plant. 2020;13:1194–202.
56. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. MicroRNA targets Drosophila Genome biology. 2003;5:R1.
57. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;120:15–20.
58. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498–504.
59. Yang X, Liu Y, Zhang H, Wang J, Zinta G, Xie S, Zhu W, Nie WF. Genome-Wide Identification of Circular RNAs in Response to Low-Temperature Stress in Tomato Leaves. Front Genet. 2020;11:591806.

60. Zhang P, Dai M. CircRNA: a rising star in plant biology. J Genet genomics = Yi chuan xue bao. 2022;49:1081–92.
61. Zhang D, Ma Y, Naz M, Ahmed N, Zhang L, Zhou JJ, Yang D, Chen Z. Advances in CircRNAs in the Past Decade: Review of CircRNAs Biogenesis, Regulatory Mechanisms, and Functions in Plants. Genes (Basel), 15 (2024).
62. Wu Z, Huang W, Qin E, Liu S, Liu H, Grennan AK, Liu H, Qin R. Comprehensive Identification and Expression Profiling of Circular RNAs During Nodule Development in Phaseolus vulgaris. Front Plant Sci. 2020;11:587185.
63. Jiang M, Chen H, Du Q, Wang L, Liu X, Liu C. Genome-Wide Identification of Circular RNAs Potentially Involved in the Biosynthesis of Secondary Metabolites in Salvia miltiorrhiza. Front Genet. 2021;12:645115.
64. Wang W, Wang J, Wei Q, Li B, Zhong X, Hu T, Hu H, Bao C. Transcriptome-Wide Identification and Characterization of Circular RNAs in Leaves of Chinese Cabbage (Brassica rapa L. ssp. pekinensis) in Response to Calcium Deficiency-Induced Tip-burn. Sci Rep. 2019;9:14544.
65. Li J, Duan Y, Sun N, Wang L, Feng S, Fang Y, Wang Y. The miR169n-NF-YA8 regulation module involved in drought resistance in Brassica napus L. Plant science: Int J experimental plant biology. 2021;313:111062.
66. Lopez-Galiano MJ, Garcia-Robles I, Gonzalez-Hernandez AI, Camanes G, Vicedo B, Real MD, Rausell C. Expression of miR159 Is Altered in Tomato Plants Undergoing Drought Stress, Plants (Basel), 8 (2019).
67. Fu T, Wang C, Yang Y, Yang X, Wang J, Zhang L, Wang Z, Wang Y. Function identification of miR159a, a positive regulator during poplar resistance to drought stress. Hortic Res. 2023;10:uhad221.
68. Zheng Y, Wang J, Xu M, Cao M, Jiang S, Wang J, Xu LA. Tamarisk miRNA target genes TcSPL5 and TcNAC1 mediate the post-transcriptional responses of salt stress. Plant science: Int J experimental plant biology. 2026;364:112915.
69. Xiao T, Ma L, Zhang Y, Zhang L, Song S, Diao X, Yu J. The miR164b-SiNAC015 Module Regulates Drought Tolerance by Scavenging Reactive Oxygen Species in Foxtail Millet. Plant Biotechnol J. 2025;24:1804–21.
70. Kuang L, Zhou H, Zhang T, Gao F, Yan T, Chen ZH, Shen Q, Zhang G, Li L, Wu D. MicroRNA164d suppresses the HvNAC92-HvHKT1;5 module to enhance salinity tolerance in barley. Proc Natl Acad Sci USA. 2025;122:e2514555122.
71. Li W, Cui X, Meng Z, Huang X, Xie Q, Wu H, Jin H, Zhang D, Liang W. Transcriptional regulation of Arabidopsis MIR168a and argonaute1 homeostasis in abscisic acid and abiotic stress responses. Plant Physiol. 2012;158:1279–92.
72. Sun Z, Kumar RMS, Li J, Yang G, Xie Y. In Silico search and biological validation of MicroR171 family related to abiotic stress response in mulberry(Morus alba). J Horticulture: Engl Ed, (2022) 008.
73. Zhang J, Wang L, Xue M, Gan L, Pei X. MicroRNA171a regulates plant development and enhances drought stress tolerance. Environ Exp Bot, 219 (2024).

74. Chu Y, Bai W, Wang P, Li F, Zhan J, Ge X. The mir390-GhCEPR2 module confers salt tolerance in cotton and Arabidopsis. Ind Crops Prod, 190 (2022).
75. Zheng Y, Hivrale V, Zhang X, Valliyodan B, Lelandais-Briere C, Farmer AD, May GD, Crespi M, Nguyen HT, Sunkar R. Small RNA profiles in soybean primary root tips under water deficit. BMC Syst Biol. 2016;10:126.

Figures

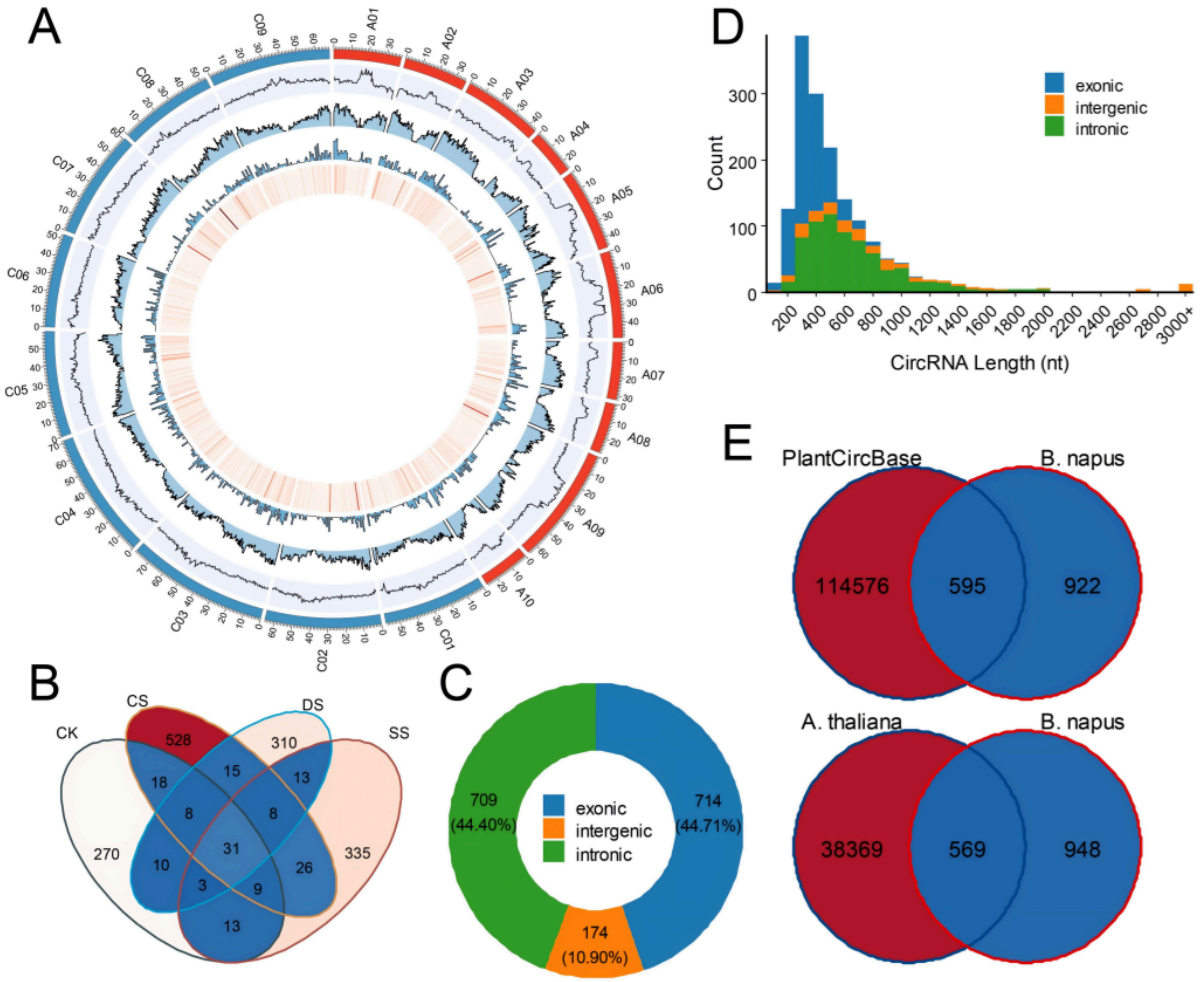


Figure 1

Identification of *B. napus* circRNAs. (A) Distribution of circRNAs and junction reads. (B) Venn diagram of circRNAs identified in each group. (C) The circRNAs number generated from exonic, intronic and intergenic regions. (D) The length distribution of circRNAs. (E) Conservation of *B. napus* circRNAs, top is venn diagram of circRNAs identified in this study and those of PlantcircBase database, bottom is venn diagram of circRNAs identified in this study and *A. thaliana* circRNAs in PlantcircBase database.

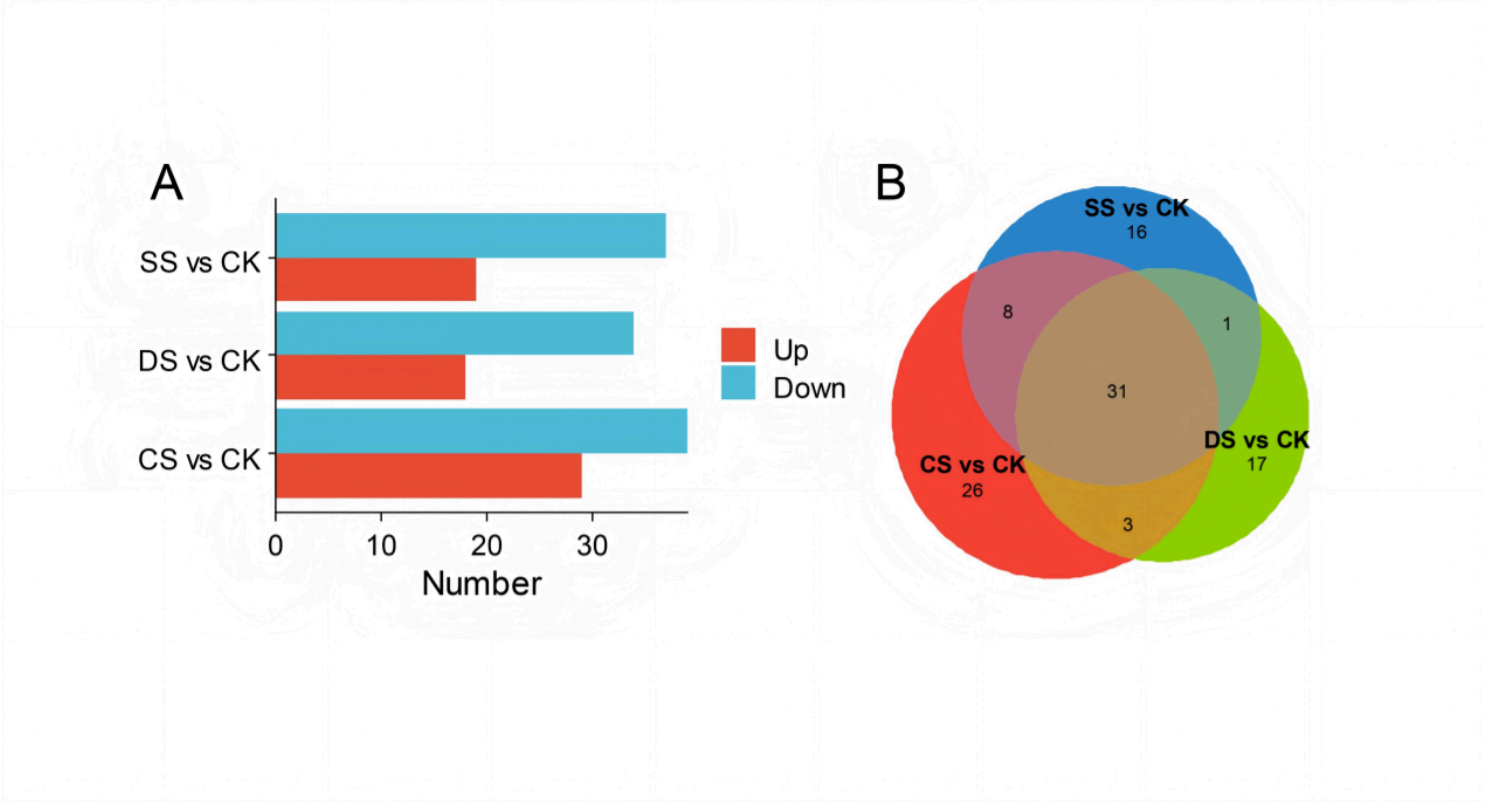


Figure 2

Differential expression analysis of circRNAs between CK and abiotic stress groups. (A) Up- and down-regulated DECs numbers of each comparison. (B) Venn diagram of DECs of each comparison.

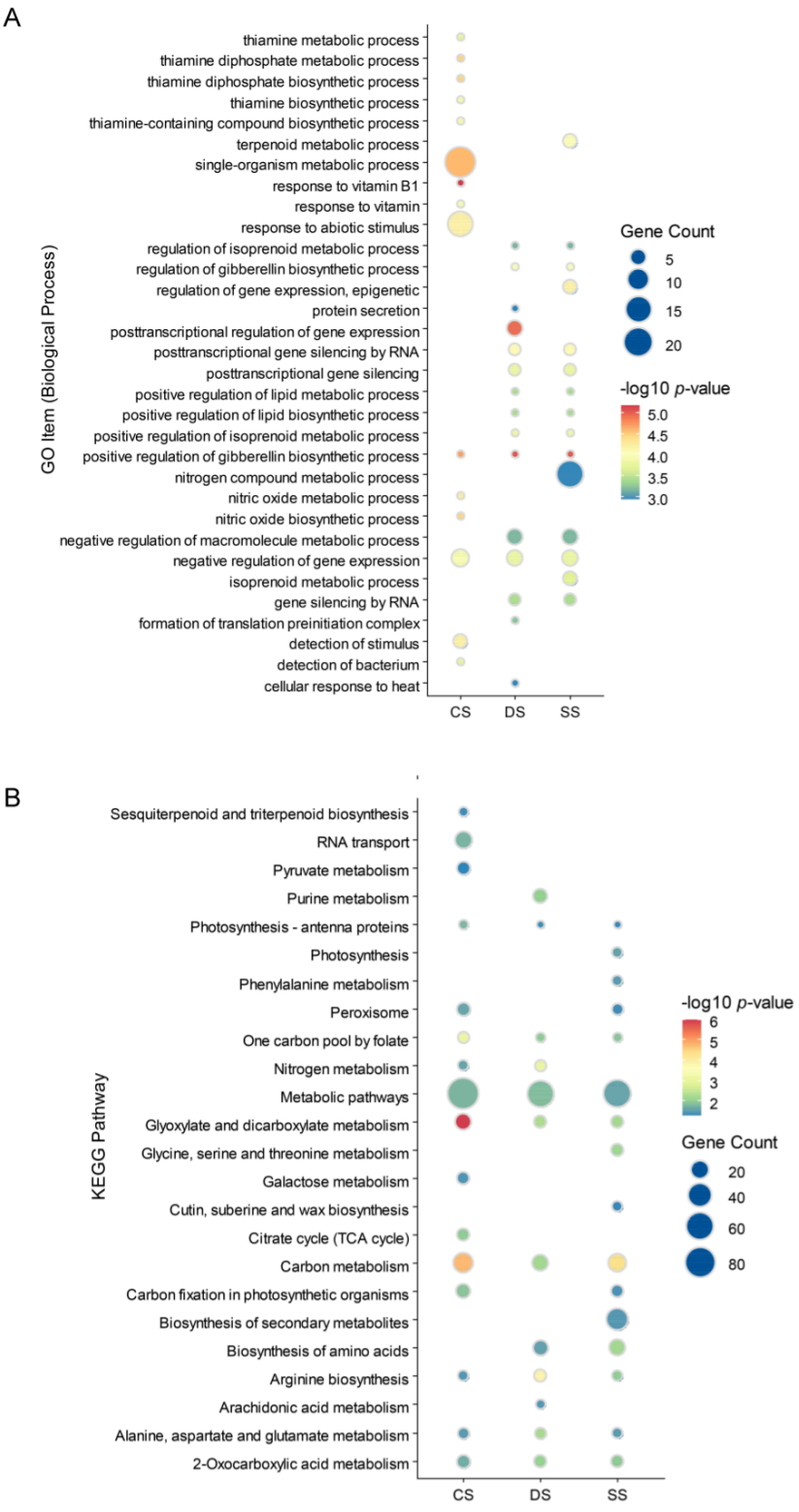


Figure 3

GO and KEGG enrichment of DECs' parental genes. (A) Bubbleplot of GO biological process enrichment. (B) Bubble plot of KEGG pathway enrichment.

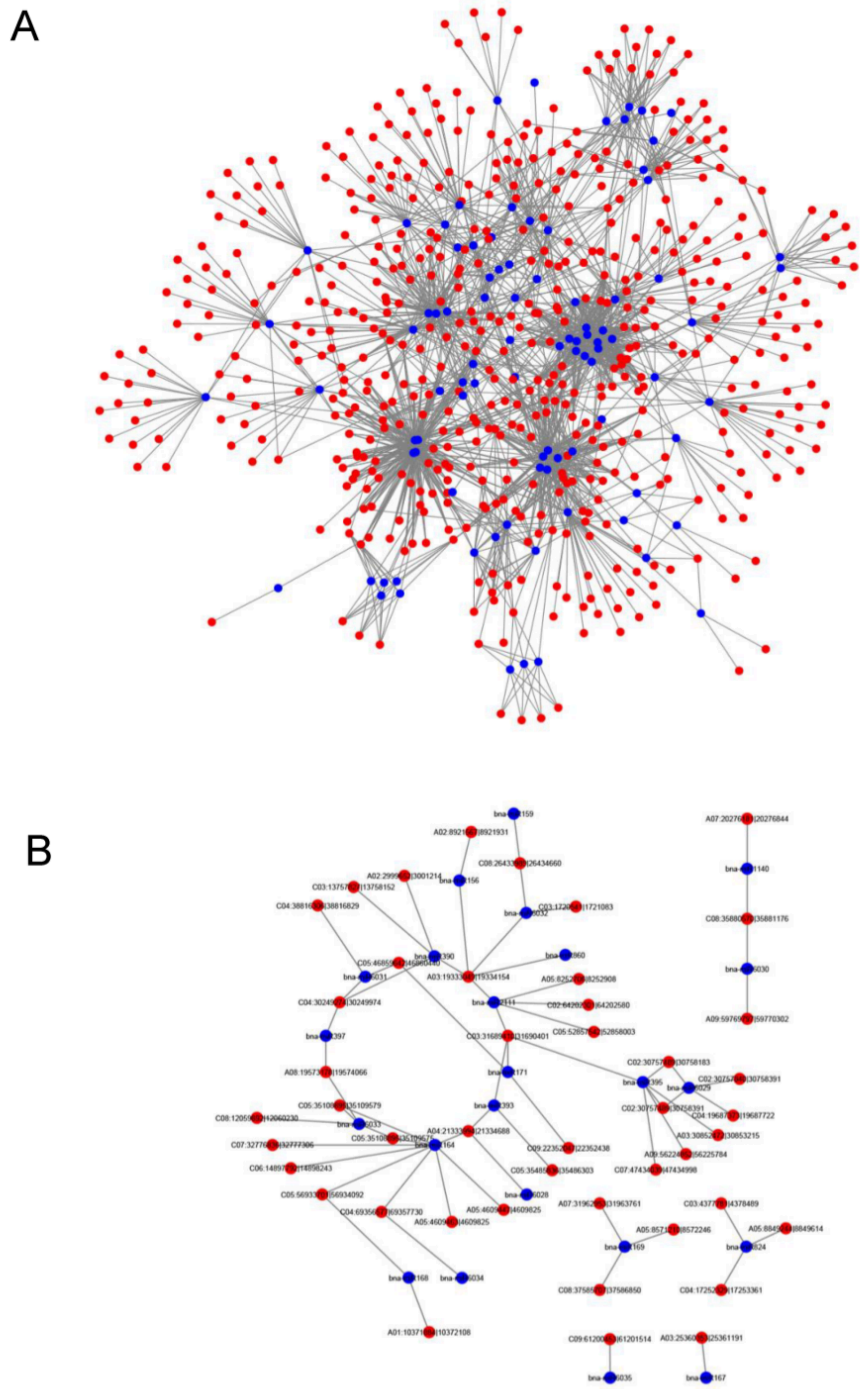


Figure 4

ceRNA networks of all circRNAs and DECs between CK and abiotic stress groups. (A) ceRNA network of all circRNAs identified in this study. (B) ceRNA network of DECs between CK and abiotic stress groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable.xlsx