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# Deciphering the Regulatory Network of Some Key Drought-responsive Genes and microRNAs in Canola

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**ABSTRACT**

Drought is a major abiotic stress that constrains the growth and yield of Canola. This study was conducted to obtain a greater insight into the drought-related hub genes, their regulatory network, and relative expression patterns in tolerant and susceptible genotypes of Canola under drought stress. In the present study, we sought to find some of the key genes and their regulatory network involved in drought stress in Canola. We investigated gene network, functional pathways, and regulatory microRNAs (miRNAs) based on RNA sequencing data analysis and compared the relative expression pattern of hub genes in tolerant and susceptible genotypes by Real-time PCR technique. A total of 5275 differentially expressed genes were identified, with 3794 up-regulated and 1481 down-regulated genes under drought stress. The result showed that the most significant biological process of up-regulated and down-regulated genes enriched in response to water deprivation and light stimulus, respectively. The result demonstrated that the *ACP4*, *RCA*, *FNR1*, *HCEF1*, *PRK*, *GDC*, and *MDH* were some of the hub genes in drought stress. The hub genes were regulated by vital drought-responsive miRNAs such as miR9558, miR854, miR172, miR834, miR390, and miR167. The relative expression pattern of investigated hub genes was different in tolerant and susceptible genotypes of Canola. The identified drought-responsive hub genes appear to play an essential role in the regulation of carbon metabolism, activation of stress signaling, and the regulation of the stromal NADP(H) redox state in response to drought stress. They are regulated by important miRNAs in a complex regulatory network that worth being considered in genetic engineering programs of Canola.



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## Introduction

Canola (*Brassica napus*) is an important crop cultivated worldwide for its edible oil and production of biodiesel fuel. Drought stress is a major abiotic stress that constrains the growth and yield of Canola. Many changes in the biochemical and molecular levels of plants occur under drought stress (Moradyar *et al*., 2023; Morsi *et al*., 2023). The differentially expressed genes (DEGs) during drought stress encode the essential enzymes involved in osmotic adjustment and stress-responsive mechanisms (Shahriari *et al*., 2022). RNA Sequencing (RNA-Seq) approach is a high-throughput transcriptomic technology for a clear understanding of DEGs and the complex

networks of environmental stress responses under drought stress (Mahdavi Mashaki *et al*., 2018). RNA-Seq analysis has been conducted for investigation of gene families in different plants under drought stress conditions (Zhang *et al*., 2019). The drought stress response is complex and includes many genes, microRNAs (miRNAs), and other regulatory factors under drought stress conditions (Singroha *et al*., 2021) miRNAs play essential roles in gene expression regulation. Therefore, understanding the regulatory network of drought-responsive genes with miRNAs will facilitate the study of the regulation mechanism and the putative functions of hub genes (Tang *et al*., 2023).

MicroRNAs are small non-coding RNAs that have vital roles in biotic and abiotic stress as post-transcriptional regulators in plants (Singroha *et al*., 2021). The miRNAs regulate expression levels of target genes via cleavage and translation mechanisms that regulate the physiological process and response to stresses (Nadarajah and Kumar, 2019). Analysis of available RNA-Seq data is a precise and cost-effective way to decipher the novel drought response mechanisms by discovering associated hub genes and their regulatory network (Mahdavi Mashaki *et al*., 2018). Although some studies have been conducted on drought stress in Canola, there is little information about genes responding to drought stress and their regulation by miRNAs under drought stress.

This study was conducted to obtain a greater insight into the drought-responsive hub genes and their regulatory network by miRNAs in Canola under drought stress based on RNA-Seq data analysis and comparing relative expression patterns of hub genes in tolerant and susceptible genotypes of Canola.

## Materials and Methods

### RNA-Seq data analysis

The paired-end raw data of non-treated (SRR14321748, SRR14321747, SRR14321736) and drought-treated (SRR14321707, SRR14321745, SRR14321746) samples of Canola were obtained from NCBI SRA (https://www.ncbi.nlm.nih.gov/sra) by SRA toolkit (v2.3.5). The 7th-leaf age of wild-type Canola samples was treated with drought stress for 14 days. The non-treated samples were used as control samples. FastQC (v0.11.9) tool (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was applied to the quality control of FastQ read files. Raw data pre-processing by trimming low-quality reads, adapter and ploy-N bases was conducted using Trimmomatic (v0.32) (http://www.usadellab.org). The high-quality reads were mapped to the NCBI annotated Canola reference genome with HISAT2 (v2.1.0). The HTSeq (v0.6.1) (https://htseq.readthedocs.io) was used to count the read numbers mapped to each gene.

### Differential expression gene analysis

Identification of DEGs was done by R packages limma, edgeR, and DESeq2 (https://bioconductor.org). Genes with $|\log(FC)| \geq 3$ and a p-value $< 0.05$ were assigned as DEGs. The assembled transcripts were annotated by similarity in sequence against *Arabidopsis thaliana* model plant by the g: Profiler tool (https://biit.cs.ut.ee/gprofiler/). Then, a volcano plot was constructed to visually display the DEGs using the SRplot tool (https://www.bioinformatics.com.cn/en).

### Functional enrichment analysis of DEGs

Gene ontology (GO) enrichment terms, including biological process (BP), molecular function (MF) and cellular component (CC), and KEGG pathways analyses of up-regulated and down-regulated genes, were conducted by the DAVID database (https://david.ncifcrf.gov/) with a p-value less than 0.05. The resulting GO terms and KEGG pathways analyses were plotted and visualized by the SRplot tool (https://www.bioinformatics.com.cn/en).

### PPI network analysis and identification of hub genes

The protein-protein interaction (PPI) network analysis of DEGs was performed by Cytoscape software (v3.9.1). The candidate hub genes in the PPI network were identified by the MCC (Maximal Clique Centrality), MNC (Maximum Neighborhood Component), degree, and Closeness algorithms in the Cytohubba plugin of Cytoscape software (v3.9.1). Commonly identified nodes by the algorithms were considered as hub genes. A Chord plot was used

to visually display the functional enrichment of hub genes based on DAVID database (https://david.ncifcrf.gov/) with a p-value less than 0.05 using the SRplot tool (https://www.bioinformatics.com.cn/en).

**Prediction of potential miRNAs of hub genes**

The mRNA sequences of hub genes in Canola were obtained from NCBI (https://www.ncbi.nlm.nih.gov/). The potential miRNAs of hub genes were predicted based on all identified miRNAs in *Arabidopsis thaliana*, *Brassica napus*, *Brassica rapa*, and *Brassica oleracea* using the psRNATarget web server (https://www.zhaolab.org/psRNATarget/). The network of miRNAs-hub genes was constructed by Cytoscape software (v3.9.1).

**Investigation of relative expression patterns of hub genes**

The tolerant (SLM046) and susceptible (Hayola308) genotypes of Canola were used based on previous studies to compare the relative expression patterns of hub genes in these genotypes (Pasandideh Arjmand *et al*., 2023b). Plants were grown in pots under 23 ± 1 °C for a 12 h photoperiod in the greenhouse. Drought stress treatment was induced by withholding to reduce soil moisture to 25% FC. The drought treatment and control samples (100% FC) of two genotypes were collected at the 5 to 7-leaf stage. Total RNAs were extracted from the leaf tissues using the super RNA extraction kit (AnaCell, Iran). After treating total RNA by DNaseI (CinnaGen, Iran), a NanoDrop spectrophotometer (BioTek Epoch™ 2, USA) and 1% agarose gel electrophoresis were used for the concentration and quality control. The cDNA synthesis was performed by the cDNA synthesis kit (AnaCell, Iran). The relative expression of some hub genes was determined by the Real-time PCR (qRT-PCR) technique using a SYBR Green Master Mix (CinnaGen, Iran) in Roche LightCycler® 96 instrument with three biological and technical replicates (Schmittgen and Livak 2008). Primer pairs for the Real-time PCR technique were designed using the Primer3 program (Kõressaar *et al*. 2018). *Actin* gene was used as an internal control of genes. The primers used for qRT-PCR analysis were listed in Supplement 1. The relative expression rate was considered double (Mohsenzadeh Golfazani *et al*., 2022).

## Results

**Identification of differentially expression genes**

After the quality control and trimming, approximately 264 million pair-end reads were generated. On average, 92.21% of reads aligned to the Canola reference genome. A total of 5275 DEGs between non-treated and drought-treated samples was identified with 3794 up-regulated and 1481 down-regulated genes (Fig. 1).

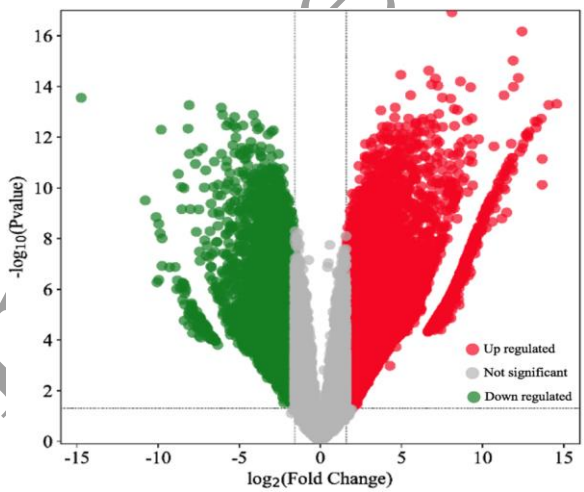


**Fig. 1.** Volcano plot of significant DEGs under drought stress: The red and green dots represent the up-regulated and down-regulated genes, respectively. The gray dots indicate the expression of the gene without significant differences with $|\log FC| \geq 3$ at a p-value less than 0.05.

**Functional enrichment of DEGs**

Gene ontology enrichment analysis showed that the most significant GO term of up-regulated genes enriched in response to water deprivation (GO:0009414), cytosol (GO:0005829), and protein binding (GO:0005515) (Fig. 2A, Supplement 2). The result showed that the most significant GO term of down-regulated genes was a response to light stimulus (GO:0009416), chloroplast stroma (GO:0009570), and mRNA binding (GO:0003729) (Fig. 2B, Supplement 2). The result illustrated that the most significant pathways of up-regulated and down-regulated genes were metabolic pathways (ath01100) and carbon fixation in photosynthetic organisms (ath00710), respectively (Fig. 2C).

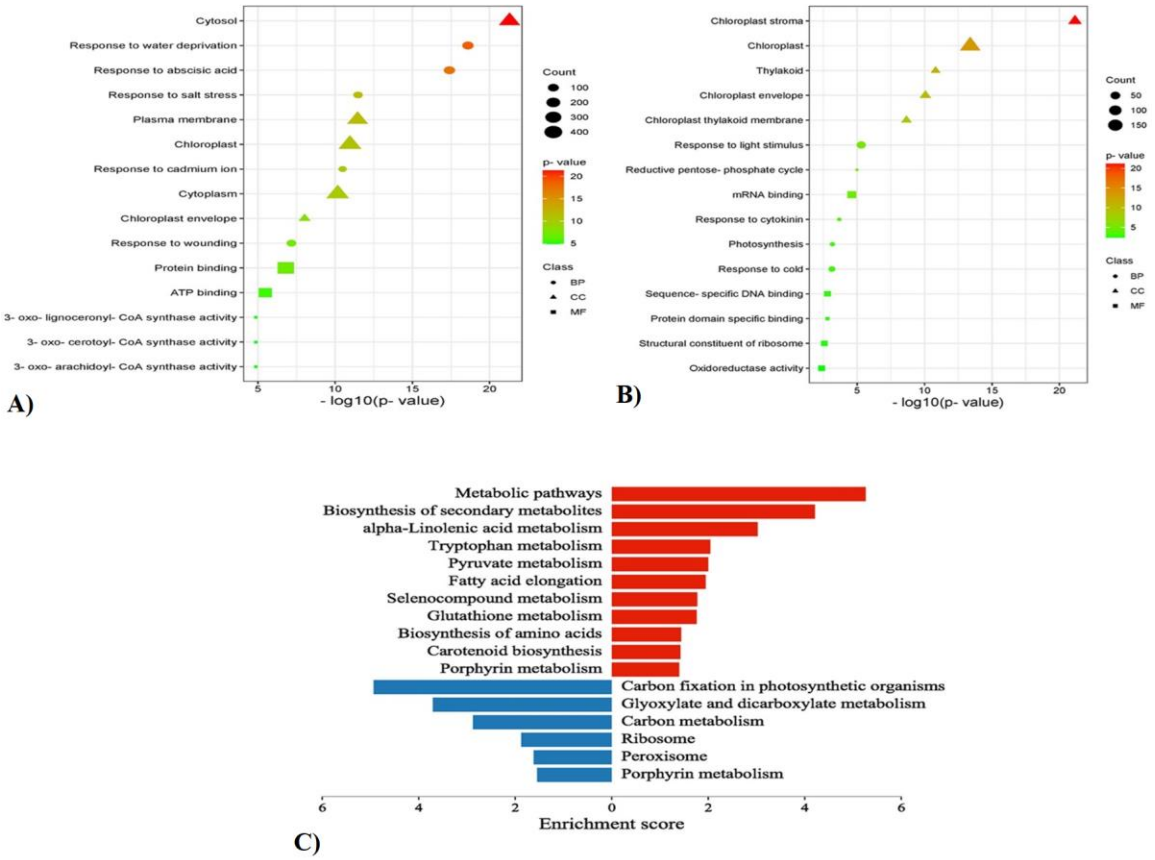


**Fig. 2.** Bubble plot for GO function enrichment analysis of DEGs: A and B) GO enrichment of up-regulated and down-regulated genes, respectivly; C) Bidirectional bar chart of KEGG pathway enrichment analysis of up-regulated genes (red) and down-regulated genes (blue) under drought stress conditions in Canola.

## Identification of hub genes in drought stress

The result illustrated that the seven genes, including *ACP4* (Acyl carrier protein 4), *PRK* (Phosphoribulokinase), *AT1G32470* (glycine decarboxylase complex H, *GDC* or *GDH3*), *AT5G58330* (NADP-dependent malate dehydrogenase, *NADP-MDH*), *FNR1* (Chloroplastic Ferredoxin-NADP reductase 1, *LNFR1*), *RCA* (Rubisco activase), and *HCEF1* (Cyclic electron flow around photosystem I, *CFBP1*) were common in 4 algorithms of Cytohubba plugin (Fig. 3A). They have closely protein-protein interaction with each other (Fig. 3B). These genes could be crucial in drought-stress conditions and are very effective on other genes. Therefore, these genes were selected as hub genes under drought stress. The pathway enrichment analysis demonstrated that the hub genes are involved in carbon metabolism, carbon fixation in photosynthetic organisms, and metabolic pathways (Fig. 3C).

## Potential regulatory miRNAs of hub genes

The result illustrated that the identified hub genes were regulated by 43 unique miRNAs. The result showed that the *FNR1* gene was regulated by bna-miR396a. The *RCA* gene was targeted by bna-miR390a, bna-miR390b, bna-miR390c, and bna-miR393 in Canola (Fig. 4, Supplement 3).

## Relative expression pattern of hub genes in tolerant and susceptible genotypes

The relative expression patterns of some hub genes including, *FNR1* and *PRK*, and *GDC* were investigated in tolerant and susceptible genotypes of Canola. The results showed that the relative expression of the investigated hub genes was significantly different in genotypes. The results showed that the expressions of *PRK* and *FNR1* were higher in the tolerant genotype and the expression of *GDC* was higher in the susceptible genotype (Fig. 5A-C).

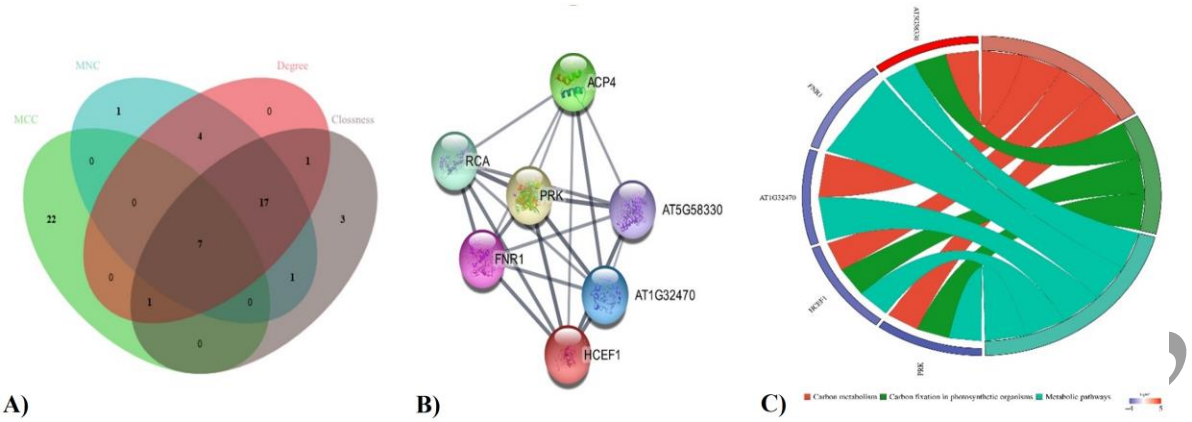


**Fig. 3.** Identified hub genes in this study: A) Venn diagram to identify significant hub genes from different algorithms; B) The PPI network of identified hub genes; C) Chord plot of hub genes based on pathway enrichment analysis by the DAVID at a p-value<0.05.

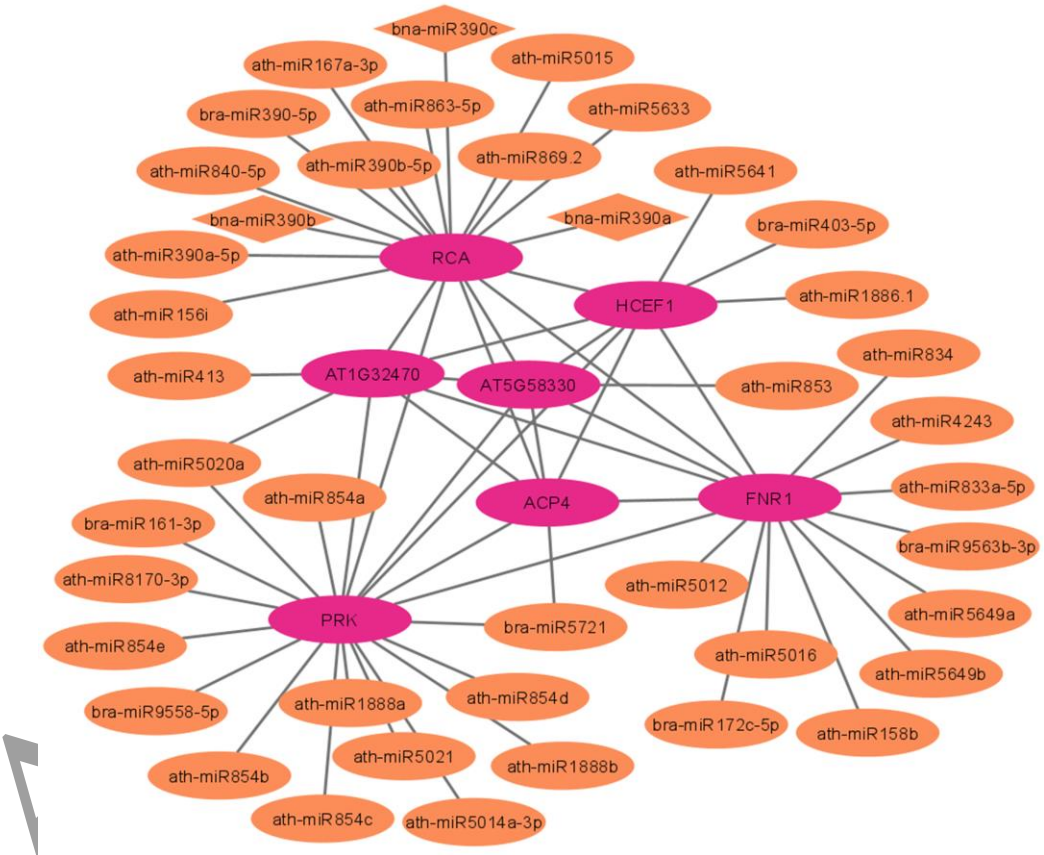


**Fig. 4.** The potential miRNA-hub genes network in Canola under drought stress: The purple color indicates hub genes and the orange color indicates their regulatory miRNAs in Canola (orange rhombus shape) and other close species, including *Arabidopsis thaliana*, *Brassica rapa*, and *Brassica oleracea* (orange ellipse shape).

## Discussion

The up-regulated genes involved in response to various stress conditions such as response to water deprivation, abscisic acid, salt stress, cold stress, and osmotic stress (Fig. 2A). The pathway analysis showed that the up-regulated genes were enriched in essential pathways such as metabolic pathways, biosynthesis of secondary metabolites, alpha-Linolenic acid metabolism, tryptophan metabolism, and pyruvate metabolism (Fig. 2C). These biological process

and pathways are very critical for the continued growth and development of Canola under drought stress conditions. It could be one of the main reasons for the upregulation of genes involved in these pathways. It shows the mutual role of up-regulated genes involved in drought stress in the vital biological pathways of the plant.

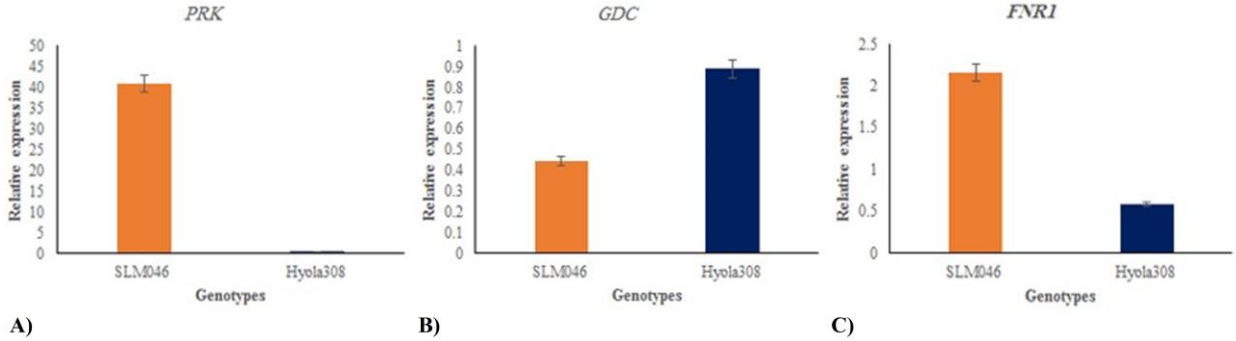


**Fig. 5.** The relative expression patterns of hub genes: A, B, and C) Comparing the relative expression patterns of *PRK*, *GDC*, and *FNR1* hub genes in tolerant and susceptible genotypes of Canola, respictivly.

The GO enrichment of down-regulated genes clearly showed that the photosynthesis and growth of Canola could decrease under drought stress (Fig. 2B). The results showed that the carbon metabolism and carbon fixation pathways were reduced under drought stress. It could lead to a decline in the growth and development of plant under drought-stress conditions. On the other hand, reducing the expression of genes involved in photosynthetic pathways could prevent the loss of water and energy in drought-stress conditions and cause the regulation of different drought-related functional pathways.

Acyl carrier proteins (ACPs) are a group of proteins that play a central role in fatty acid biosynthesis. The downregulation of *ACP4* might regulate the signaling mechanism under drought stress. It was found that the expression of *ACP4* was down-regulated under environmental stresses (Huang *et al*., 2017). The result showed that *ACP4* could be regulated by bra-miR5721 through cleavage of transcripts in Canola (Fig. 4, Supplement 3). It was found that the bra-miR5721 was influenced by heat stress in *Brassica rapa* (X. Yu *et al.*, 2012).

PRK is a vital enzyme of the Calvin Cycle in photosynthesis, and it catalyzes the ATP-dependent conversion of ribulose-5-phosphate to produce ribulose-1,5-bisphosphate (A. Yu *et al*., 2020). The repression of genes involved in the synthesis of Calvin Cycle enzymes under drought conditions infers a demand reduction for ATP and NADPH, resulting reduction in electron transport through photosystem II (Aranjuelo *et al*., 2011). The result showed that the relative expression pattern of these genes in the tolerant genotype was higher than susceptible genotype (Fig. 5A). Therefore, the electron transport through photosystem II could increase in the tolerant genotype under drought stress. The bra-miR5721 could regulate transcripts of *PRK* hub gene through cleavage inhibition (Fig. 4, Supplement 3). It was reported that bra-miR5721 was a heat-responsive miRNA in *Brassica rapa* (Yu *et al.*, 2012). Therefore, it is possible that the bra-miR5721 could be a drought-responsive miRNA in Canola. The result illustrated that bra-miR5721 regulates both *ACP4* and *PRK* transcripts in Canola under drought stress (Fig. 4, Supplement 3). The expression of *ACP4* is known to be correlated to the photosynthetic status (Jung *et al*., 2019). Therefore, the regulation of *PRK* and *ACP4* hub genes by bra-miR5721 may cause the coordinated regulation of fatty acid synthesis in relation to photosynthesis.

The result demonstrated that one of the identified regulatory miRNAs for *PRK* was bra-miR161-3p and bra-miR9558-5p through cleavage inhibition (Fig. 4, Supplement 3). It is reported that bra-miR161-3p is one of the salt stress-responsive miRNAs in *Brassica juncea* (Srivastava *et al.*, 2017). The miR9558 is a vital drought stress-responsive miRNA in oil crops (Schiessl *et al.*, 2020). The result illustrated that the *PRK* was targeted by ath-miR1888a and ath-miR1888b

with cleavage inhibition (Fig. 4, Supplement 3). A study reported that the ath-miR1888a expression in *Zanthoxylum bungeanum* was induced under drought stress (Fei *et al*., 2020). It is reported that the ath-miR1888a in Arabidopsis regulates the drought-responsive hub genes under drought stress (Pasandideh Arjmand *et al*., 2023a). The result illustrated that the ath-miR5021 regulated the *PRK* via cleavage of transcripts in Canola (Fig. 4, Supplement 3). The ath-miR5021 is a crucial stress-responsive miRNA in Arabidopsis, Rice, and Maize (Tahmasebi *et al*., 2021). The result showed that ath-miR854a/b/c/d/e regulate the *PRK* transcripts through translation inhibition (Fig. 4, Supplement 3). The ath-miR854 family has a vital role in the regulation of essential target genes under drought stress, metal stress, and pathogen infection of plants (Zhang *et al*., 2019). The result illustrated that the ath-miR5014a-3p and ath-miR5020a regulated the *PRK* through cleavage of transcripts in Canola (Fig. 4, Supplement 3). It is demonstrated that the ath-miR5014a-3p and ath-miR5020a regulate the important drought-responsive genes such as LEA (Late Embryogenesis Abundant) genes (Ceylan *et al*., 2019). Another identified *PRK*-related miRNA was ath-miR8170. It is reported that ath-miR8170 is one of the stress-related miRNAs in plants (Pandita, 2022).

The relative expression of *AT1G32470* (*GDC*) in the susceptible genotype was higher than the tolerant genotype (Fig. 5B). It was found that the expression level of *GDC* in Arabidopsis was down-regulated under aluminum stress. *GDC* is one of the vital photorespiratory enzymes capable of controlling photorespiratory flux and photosynthetic capacity (Goodwin and Sutter, 2009). Therefore, it could be a crucial drought-responsive gene under drought stress.

The result showed that ath-miR5020a and ath-miR413 could regulate *AT1G32470* through cleavage of the transcripts in Canola under drought stress (Fig. 4, Supplement 3). It was found that the miR5020 regulates the stress-responsive genes such as AP2/ERF and LEA under stress conditions (Ceylan *et al*., 2019). The miR413 could regulate drought stress-responsive genes in Arabidopsis (Pasandideh Arjmand *et al*., 2023a).

The result demonstrated that the *AT5G58330* (NADP-MDH) was one of the hub genes in Canola (Fig. 4), and it was up-regulated under drought stress. MDH catalyzes the conversion of oxaloacetate to malate using NAD(P)H. The activity of NADP-MDH and, as a result, the rate of malate export from the chloroplast increases, when the amount of $NADP^+$ is limited. Therefore, the NADP-MDH plays an essential role in the regulation of the stromal NADP(H) in stress response (Hebbelmann *et al*., 2012). The result showed that the *AT5G58330* was regulated by ath-miR853 (Fig. 4, Supplement 3). A study reported that the miR853 might have been significantly involved in the plant thermos-tolerance mechanism (Ijaz *et al*., 2020).

The result showed that *FNR1* was down-regulated under drought stress and could be regulated by various miRNAs such as bra-miR172c-5p, bra-miR9563b-3p, ath-miR833a-5p, ath-miR834, ath-miR158b, ath-miR4243, ath-miR5012, ath-miR5016, ath-miR5649a/b in Canola (Fig. 4, Supplement 3). *FNR1* is one of the main NADPH sources in the chloroplasts of plants, which is finally used in the Calvin cycle (Corpas *et al*., 2021). The result showed that the relative expression pattern of *FNR1* in the tolerant genotype was higher than the susceptible genotype (Fig. 5C). Therefore, it could be an important drought-responsive hub gene in Canola.

The miR172c is a biotic and abiotic stress-responsive miRNA in plants (Pasandideh Arjmand *et al*., 2023a). The miR9563 was found to be associated with photosynthesis genes, like Q2 subunit of photosystem II (PSII) (Schiessl *et al*., 2020). It was demonstrated that the expression of miR833 has been induced under heavy metal conditions (He *et al*., 2014).

The ath-miR834 is involved in the antioxidant system under drought stress (Fei *et al*., 2020). It was demonstrated that the ath-miR158b regulates ERF family TF target genes in Arabidopsis (Rakhmetullina *et al*., 2021). It was reported that the ath-miR4243 and ath-miR5012 regulate the drought-responsive LEA genes (Ceylan *et al*., 2019). The miR5016 is involved in plant growth and development (Dou *et al*., 2022). The result illustrated that the *RCA* (Rubisco activase) was another hub gene in Canola under drought stress (Fig. 4). The

inactive form of Rubisco is converted to an active form by the RCA, utilizing ATP. Therefore, the activation of Rubisco by RCA is a critical factor in photosynthesis for plants under stress conditions (Suganami *et al*., 2021).

The result showed that the *RCA* could be regulated by bra-miR390-5p, bna-miR390a/b/c, ath-miR863-5p, ath-miR390a/b-5p, ath-miR156i, ath-miR840-5p, and ath-miR869.2 through cleavage inhibition. The ath-miR167a-3p, ath-miR5633, and ath-miR5015 regulate the *RCA* via translation inhibition (Fig. 4, Supplement 3). The bra-miR390 is a highly conserved miRNAs in Arabidopsis and Brassicaceae that responses to heat stress (Yu *et al*., 2012). The ath-miR863-5p regulates phosphofructokinase family protein (PFP), an important carbohydrate metabolism enzyme, in Arabidopsis (Shao *et al*., 2013).

It is reported that the ath-miR156i regulates transcripts of drought-responsive hub genes through cleavage inhibition in Arabidopsis (Pasandideh Arjmand *et al*., 2023a). It is demonstrated that the miR156 family regulates various developmental processes in plants (Srivastava *et al*., 2013). It is demonstrated that the ath-miR840 regulates the genes involved in the antioxidant system in *B. juncea* and *B. rapa* under drought and heat stresses (Verma and Singh, 2021). It is demonstrated that the ath-miR869.2 regulates drought-responsive genes such as MAPK and LEA genes in plants (Ceylan *et al*., 2019). The ath-miR167a-3p is a drought-responsive miRNA that regulates antioxidant system genes (Fei *et al*., 2020). It is demonstrated that the miR167 is directly involved in auxin signaling in plants, which could play an essential role under stress conditions (Fasani *et al*., 2021). The ath-miR5633 regulates the nitrate-responsive genes in Arabidopsis (Vidal *et al*., 2013). In addition, the ath-miR5633 is involved in lipid production in oilseed crops (Yin *et al*., 2022). The ath-miR5015 also regulates the LEA genes (Ceylan *et al*., 2019).

Cyclic electron transport around photosystem I (CEF) produces ATP without NADPH accumulation in chloroplast (Shikanai, 2014). CEF has been proposed to balance the chloroplast energy budget of photosynthesis. Hydrogen peroxide acts as a signaling agent for activating the CEF in plants (Strand *et al*., 2015). The result showed that the *HCEF1* could be regulated by bra-miR403-5p and ath-miR5641 through cleavage inhibition and ath-miR1886.1 via translation inhibition (Fig. 4, Supplement 3). The miR403 was found to be associated with drought stress in various plants. The ath-miR5641 regulates the drought-responsive hub genes in plants (Pasandideh Arjmand *et al*., 2023a). The ath-miR1886.1 regulates drought stress-responsive genes in plants (Ceylan *et al*., 2019).

Stomatal closure in response to drought stress prevents transpiration, but it produces additional light energy. It could cause the decrease of Calvin cycle-related genes such as *PRK* and *RCA* hub genes. In addition, the downregulation of the *FNR1* hub gene may cause a decrease in NADPH generation and its transfer to the Calvin cycle under drought stress (Corpas *et al*., 2021). The downregulation of *RCA* expression can reduce the Calvin cycle activity. The down-regulation in *GDC* expression may cause the regulation of photosynthetic process under drought stress. It seems that the *HCEF1* could balance the difference between the ATP/NADPH under drought-stress conditions. Excess light energy can lead to ROS production under drought stress. The ROS may cause the regulation of *HCEF1* under drought stress. In addition, the *ACP4* is required to perceive the stress signal in plants. Therefore, it could regulate the expression of other genes responding to drought stress. The upregulation of *NADP-MDH* could affect the regulation of the stromal NADP(H) redox state and the maintenance of redox homeostasis in response to drought stress (Hebbelmann *et al*., 2012). It could be involved in the regulation of antioxidant activity and the accumulation of the $H_2O_2$ signal under drought stress. The result illustrated that the relative expression pattern of investigated hub genes was different in tolerant and susceptible genotypes. Therefore, these hub genes could be considered for the detection of tolerant genotypes in the genetic engineering programs of Canola.

## Conclusion

The regulation of gene expression involved in the carbon metabolism pathway could prevent the loss of water and energy under drought stress

and cause the regulation of different biological pathways in tolerance to drought stress. The identified hub genes appear to play an essential role in the regulation of carbon metabolism, activation of stress signaling, the regulation of the stromal NADP(H), and the maintenance of redox homeostasis in drought stress response.

**Conflicts of interest**

Authors declare that there is no conflict of interests regarding the publication of this article.

**References**


Aranjuelo, I., Molero, G., Erice, G., Avice, J. C., & Nogues, S. (2011). Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.). *Journal of Experimental Botany*, 62(1), 111-123. https://doi.org/10.1093/jxb/erq249

Ceylan, K. B., Ceylan, Y., Ustaoğlu, B., Baloğlu, M. C., & Altunoğlu, Y. Ç. (2019). Molecular identification and characterization of LEA proteins in jujube genome. *Kastamonu University Journal of Engineering and Sciences*, 5(2), 101-146. https://dergipark.org.tr/en/pub/kastamonujes

Corpas, F. J., González-Gordo, S., Palma, J. M. (2021). Nitric oxide and hydrogen sulfide modulate the NADPH-generating enzymatic system in higher plants. *Journal of Experimental Botany*, 72, 830-847. https://doi.org/10.1093/jxb/eraa440

Dou, J., Duan, S., Umer, M. J., Xie, K., Wang, Y., Kang, Q., ... & Zhao, F. (2022). Genome-wide analysis of IQD proteins and ectopic expression of watermelon ClIQD24 in tomato suggests its important role in regulating fruit shape. *Frontiers in Genetics*, 13, 993218. https://doi.org/10.3389/fgene

Fasani, E., DalCorso, G., Zorzi, G., Vitulo, N., & Furini, A. (2021). Comparative analysis identifies micro-RNA associated with nutrient homeostasis, development and stress response in *Arabidopsis thaliana* upon high Zn and metal hyperaccumulator *Arabidopsis halleri*. *Physiologia Plantarum*, 173(3), 920-934. https://doi.org/10.1111/ppl.13488

Fei, X., Li, J., Kong, L., Hu, H., Tian, J., Liu, Y., & Wei, A. (2020). miRNAs and their target genes regulate the antioxidant system of *Zanthoxylum bungeanum* under drought stress. *Plant Physiology and Biochemistry*, 150, 196-203. https://doi.org/10.1016/j.plaphy.2020.01.040

Goodwin, S. B., & Sutter, T. R. (2009). Microarray analysis of *Arabidopsis* genome response to aluminum stress. *Biologia Plantarum*, 53, 85-99. https://doi.org/10.1007/s10535-009-0012-4

He, Q., Zhu, S., & Zhang, B. (2014). MicroRNA–target gene responses to lead-induced stress in cotton (*Gossypium hirsutum* L.). *Functional and Integrative Genomics*, 14, 507-515. https://doi.org/10.1007/s10142-014-0378-z

Hebbelmann, I., Selinski, J., Wehmeyer, C., Goss, T., Voss, I., Mulo, P., ... & Scheibe, R. (2012). Multiple strategies to prevent oxidative stress in *Arabidopsis* plants lacking the malate valve enzyme NADP-malate dehydrogenase. *Journal of Experimental Botany*, 63(3), 1445-1459. https://doi.org/10.1093/jxb/err386

Huang, J., Xue, C., Wang, H., Wang, L., Schmidt, W., Shen, R., & Lan, P. (2017). Genes of ACYL CARRIER PROTEIN family show different expression profiles and overexpression of ACYL CARRIER PROTEIN 5 modulates fatty acid composition and enhances salt stress tolerance in Arabidopsis. *Frontiers in Plant Science*, 8, 987. https://doi.org/10.3389/fpls.2017.00987

Ijaz, U., Ali, M. A., Nadeem, H., Tan, L., & Azeem, F. (2020). RNA world and heat stress tolerance in plants. *Physiological, Molecular and Genetic Perspectives*, 167-187. https://doi.org/10.1002/9781119432401.ch8

Jung, K. W., Cho, C., Lee, S., Kim, J. H., Bai, H. W., Park, Y. D., ... & Lee, D. G. (2019). Identification of γ-radiation-responsive proteins in *Arabidopsis thaliana*. *Journal of Radiation Industry*, 13(2), 83-91. https://www.kns.org

Kõressaar, T., Lepamets, M., Kaplinski, L., Raime, K., Andreson, R., & Remm, M. (2018). Primer3_masker: integrating masking of template sequence with primer design software. *Bioinformatics*, 34(11), 1937-1938. https://doi.org/10.1093/bioinformatics/bty036

Mahdavi Mashaki, K., Garg, V., Nasrollahnezhad Ghomi, A. A., Kudapa, H., Chitikineni, A., Zaynali Nezhad, K., ... &

Thudi, M. (2018). RNA-Seq analysis revealed genes associated with drought stress response in kabuli chickpea (*Cicer arietinum* L.). *PLoS One*, 13(6), e0199774. https://doi.org/10.1371/journal.pone.0199774

Mohsenzadeh Golfazani, M., Taghvaei, M. M., Samizadeh Lahiji, H., Ashery, S., & Raza, A. (2022). Investigation of proteins' interaction network and the expression pattern of genes involved in the ABA biogenesis and antioxidant system under methanol spray in drought-stressed rapeseed. *3 Biotech*, 12(9), 217. https://doi.org/10.1007/s13205-022-03290-4

Moradyar, M., Zamani, M., Motallebi, M., Jourabchi, E. (2023). Analysis of the effect of chimeric chitinase expressed by synthetic promoter in T2 generation of transgenic canola. *Journal of Genetic Resources*, 9(1), 48-58. https://doi.org/10.22080/jgr.2023.24506.1336

Morsi, N. A., Hashem, O. S., El-Hady, M. A. A., Abd-Elkrem, Y. M., El-temsah, M. E., Galal, E. G., ... & Abdelkader, M. A. (2023). Assessing drought tolerance of newly developed tissue-cultured canola genotypes under varying irrigation regimes. *Agronomy*, 13(3), 836. https://doi.org/10.3390/agronomy13030836

Nadarajah, K., & Kumar, I. S. (2019). Drought response in rice: The miRNA story. *International Journal of Molecular Sciences*, 20(15), 3766. https://doi.org/10.3390/ijms20153766

Pandita, D. (2022). Role of miRNA technology and miRNAs in abiotic and biotic stress resilience. *In Plant Perspectives to Global Climate Changes*. Academic Press. https://doi.org/10.1016/B978-0-323-85665-2.00015-7

Pasandideh Arjmand, M., Samizadeh Lahiji, H., Mohsenzadeh Golfazani, M., & Biglouei, M. H. (2023a). New insights on the regulatory network of drought-responsive key genes in *Arabidopsis thaliana*. *Genetica*, 151(1), 29-45. https://doi.org/10.1007/s10709-022-00177-3

Pasandideh Arjmand, M., Samizadeh Lahiji, H., Mohsenzadeh Golfazani, M., & Biglouei, M. H. (2023b). Evaluation of protein's interaction and the regulatory network of some drought-responsive genes in Canola under drought and re-watering conditions. *Physiology and Molecular Biology of Plants*, 29(8), 1085-1102. https://doi.org/10.1007/s12298-023-01345-1

Rakhmetullina, A., Zielenkiewicz, P., Pyrkova, A., Uteulin, K., & Ivashchenko, A. (2021). Prediction of characteristics of interactions of miRNA with mRNA of GRAS, ERF, C2H2 genes of *A. thaliana*, *O. sativa* and *Z. mays*. *Current Plant Biology*, 28, 100224. https://doi.org/10.1016/j.cpb.2021.100224

Schiessl, S. V., Quezada-Martinez, D., Orantes-Bonilla, M., & Snowdon, R. J. (2020). Transcriptomics reveal high regulatory diversity of drought tolerance strategies in a biennial oil crop. *Plant Science*, 297, 110515. https://doi.org/10.1016/j.plantsci.2020.110515

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature protocols*, 3(6), 1101-1108. https://doi.org/10.1038/nprot.2008.73

Shahriari, A. G., Soltani, Z., Tahmasebi, A., & Poczai, P. (2022). Integrative system biology analysis of transcriptomic responses to drought stress in soybean (*Glycine max* L.). *Genes*, 13(10), 1732. https://doi.org/10.3390/genes13101732

Shao, C., Wu, Q., Qiu, J., Jin, S., Zhang, B., Qian, J., ... & Meng, Y. (2013). Identification of novel microRNA-like-coding sites on the long-stem microRNA precursors in *Arabidopsis*. *Gene*, 527(2), 477-483. https://doi.org/10.1016/j.gene.2013.06.070

Shikanai, T. (2014). Central role of cyclic electron transport around photosystem I in the regulation of photosynthesis. *Current Opinion in Biotechnology*, 26, 25-30. https://doi.org/10.1016/j.copbio.2013.08.012

Singroha, G., Sharma, P., & Sunkur, R. (2021). Current status of microRNA-mediated regulation of drought stress responses in cereals. *Physiologia Plantarum*, 172(3), 1808-1821. https://doi.org/10.1111/ppl.13451

Srivastava, A. K., Sablok, G., Hackenberg, M., Deshpande, U., & Suprasanna, P. (2017). Thiourea priming enhances salt tolerance through co-ordinated regulation of microRNAs and hormones in *Brassica juncea*. *Scientific Reports*, 7(1), 45490. http://doi.org/10.1038/srep45490

Srivastava, S., Srivastava, A. K., Suprasanna, P., & D'souza, S. F. (2013). Identification and

profiling of arsenic stress-induced microRNAs in *Brassica juncea*. *Journal of Experimental Botany*, 64(1), 303-315. https://doi.org/10.1093/jxb/ers333

Strand, D. D., Livingston, A. K., Satoh-Cruz, M., Froehlich, J. E., Maurino, V. G., & Kramer, D. M. (2015). Activation of cyclic electron flow by hydrogen peroxide *in vivo*. *Proceedings of the National Academy of Sciences*, 112(17), 5539-5544. https://doi.org/10.1073/pnas.1418223112

Suganami, M., Suzuki, Y., Tazoe, Y., Yamori, W., & Makino, A. (2021). Co-overproducing Rubisco and Rubisco activase enhances photosynthesis in the optimal temperature range in Rice. *Plant Physiology*, 185(1), 108-119. https://doi.org/10.1093/plphys/kiaa026

Tahmasebi, A., Khahani, B., Tavakol, E., Afsharifar, A., & Shahid, M. S. (2021). Microarray analysis of *Arabidopsis thaliana* exposed to single and mixed infections with *Cucumber mosaic virus* and turnip viruses. *Physiology and Molecular Biology of Plants*, 27, 11-27. https://doi.org/10.1007/s12298-021-00925-3

Tang, C. N., Wan Abdullah, W. M. A. N., Wee, C. Y., Balia Yusof, Z. N., Yap, W. S., Cheng, W. H., ... & Lai, K. S. (2023). Promoter Cis-element analyses reveal the function of αVPE in drought stress response of *Arabidopsis*. *Biology*, 12(3), 430. https://doi.org/10.3390/biology12030430

Verma, D., & Singh, K. (2021). Understanding role of glutathione reductase gene family in drought and heat stresses in *Brassica juncea* and *B. rapa*. *Environmental and Experimental Botany*, 190, 104595. https://doi.org/10.1016/j.envexpbot.2021.104595

Vidal, E. A., Moyano, T. C., Krouk, G., Katari, M. S., Tanurdzic, M., McCombie, W. R., ... & Gutiérrez, R. A. (2013). Integrated RNA-seq and sRNA-seq analysis identifies novel nitrate-responsive genes in *Arabidopsis thaliana* roots. *BMC Genomics*, 14(1), 1-15. https://doi.org/10.1186/1471-2164-14-701

Yin, X., Guo, X., Hu, L., Li, S., Chen, Y., Wang, J., ... & Hu, Z. (2022). Genome-wide characterization of DGATs and their expression diversity analysis in response to abiotic stresses in *Brassica napus*. *Plants*, 11(9), 1156. https://doi.org/10.3390/plants11091156

Yu, A., Xie, Y., Pan, X., Zhang, H., Cao, P., Su, X., ... & Li, M. (2020). Photosynthetic phosphoribulokinase structures: Enzymatic mechanisms and the redox regulation of the Calvin-Benson-Bassham cycle. *The Plant Cell*, 32(5), 1556-1573. https://doi.org/10.1105/tpc.19.00642

Yu, X., Wang, H., Lu, Y., de Ruiter, M., Cariaso, M., Prins, M., ... & He, Y. (2012). Identification of conserved and novel microRNAs that are responsive to heat stress in *Brassica rapa*. *Journal of Experimental Botany*, 63(2), 1025-1038. https://doi.org/10.1093/jxb/err337

Zhang, X., Fan, B., Yu, Z., Nie, L., Zhao, Y., Yu, X., ... & Ma, Y. (2019). Functional analysis of three miRNAs in *agropyron mongolicum* keng under drought stress. *Agronomy*, 9(10), 661. https://doi.org/10.3390/agronomy9100661