Transcriptome and proteome analyses of the molecular mechanisms underlying changes in oil storage under drought stress in *Brassica napus* L.

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**Abstract**

Rapeseed (*Brassica napus* L.) is the second most important oilseed crop in edible vegetable oil and bioenergy; however, drought stress generally causes a decrease in rapeseed yield and oil content, especially during the reproductive stage. In our study, we measured the oil and protein contents and gibberellic acid (GA) and abscisic acid (ABA) levels in seeds that were acquired on the 30th, 40th, and 50th days after flowering under control and drought treatments. RNA and protein libraries were constructed from the stressed seeds to perform transcriptome and proteome analyses, respectively. Our results demonstrated that the oil content decreased due to four primary mechanisms: downregulation of fatty acid biosynthesis-associated genes and proteins; upregulation of fatty acid degradation-associated genes and proteins; enhancement of protein storage due to changes in the abundances of relevant genes and proteins; and upregulation of Gly-Asp-Ser-Leu (GDSL) gene expression, potentially as the result of upregulating the GA biosynthesis gene *GA20ox3* and downregulating the GA inactivating gene *GA2ox3* and thus an increase in GA content. During seed maturation, oil storage change may also relate to increasing ABA content as the upregulation of two members of *NCED6* (9-cis-epoxycarotenoid dioxygenase) gene family involved in ABA biosynthesis, and the upregulation of genes involved in ABA signal transduction. These results will help to establish a foundation for breeding excellent varieties of rapeseed with high oil content for areas with frequent droughts to promote the supply of edible vegetable oil and biofuel.

**Keywords**

*Brassica napus* L, Drought stress, proteome, seed oil content, transcriptome
1 INTRODUCTION

*Brassica napus* L. (AACC, 2n=38), known as rapeseed, an amphidiploid species formed by natural hybridization of two diploid progenitors, *Brassica rapa* (AA, 2n=20) and *Brassica oleracea* (CC, 2n=18), is the second most important oilseed crop and provides approximately 13%–16% of vegetable oil globally for edible and biofuel (Jian et al., 2016; Lohani et al., 2020; Song et al., 2020). Furthermore, rapeseed is the main crop converted to biofuel in the form of rapeseed methyl ester in the EU (Forleo et al., 2018; Thers et al., 2019). Improving rapeseed yield and oil content is necessary for sustainable biofuel production, which has become one of the main environmental challenges of the 21st century.

Drought stress, one of the primary abiotic stresses, causes significant losses in agricultural production and will increase in both intensity and frequency with increasing planet temperature (Elizamar et al., 2013; Xu et al., 2020). In semiarid areas, rapeseed production is often affected by drought stress (Poveda, 2020). Bouchereau et al. (1996) found that rapeseed yield and quality were greatly affected by growth conditions, especially water deficit at flowering and filling stages. The study conducted by RAD and Zandi (2012), Vahid et al. (2015), and Hatzig et al. (2018) also showed that water deficit caused reductions in parameters such as seed yield, oil content, and oil yield. Therefore, breeding drought-resistant varieties is a major approach for improving rapeseed and oil production and expanding rapeseed-growing areas.

When drought stress occurs, various genes are induced in drought signal transduction, which consists of complex networks of protein–protein reactions, transcription factors (TFs), and promoters (Liu et al., 2015). In the abscisic acid (ABA)-dependent pathway, pyrabactin resistance proteins/PYR-like proteins/components of ABA receptors (PYR/PYL/RCAR) receive ABA to inhibit type 2C protein phosphatases (PP2Cs), thereby increasing the levels of SNF1-related kinase 2 (SnRK2) to activate TFs such as ABA-responsive element-binding factors (AREBs), ABA binding factors (ABFs), and myeloblastosis-related proteins/myelocytomatosis-related proteins (MYBs/MYCs). In addition, APETALA2/ethylene-responsive element binding factors (AP/ERF), *Arabidopsis* transcription activation factor (ATAF), no apical meristem proteins (NAC), and zinc finger homeodomain (ZF-HD) family members have been determined to be induced under drought in an ABA-independent pathway (Liu et al., 2015; Yoshida et al., 2014). Gibberellic acid (GA) also plays an important role in drought stress and seed development. The DELLA proteins comprise a class of GA signaling repressors, such as REPRESSOR of gal-3 (RGA), GA-INSENSITIVE (GAI), RGA-LIKE1 (RGL1), RGA-LIKE2 (RGL2), and RGA-LIKE3 (RGL3; Chen et al., 2012). Prior studies have shown that DELLA accumulation is linked to growth restriction upon exposure to abiotic stress and inhibits the accumulation of reactive oxygen species (ROS), which accumulate under biotic and abiotic stress (Achard et al., 2008; Colebrook et al., 2014). Recently, the genes encoding several Gly-Asp-Ser-Leu (GDSL) proteins, which were named seed fatty acid reducer (SFAR) genes and observed to be downregulated by DELLA proteins, were reported to reduce fatty acid storage in *Arabidopsis* and *B. napus* (Chen et al., 2012; Huang et al., 2015; Karunarathna et al., 2020).

Transcriptome profiling enables researchers to investigate plant regulation and identify genes involved in stress tolerance mechanisms (Liu et al., 2015; Song, Huang, et al., 2016). Proteomics, which is the study of the structural and functional features of all the proteins in an organism, is an important means of understanding complex biological mechanisms, including plant responses to abiotic stress tolerance (Deshmukh et al., 2014). Using transcriptomic or proteomics sequencing, many genes associated with drought response were identified in a number of plants, such as *Arabidopsis* (Cho et al., 2013; Matsu et al., 2008; Meng et al., 2019), rice (Hamzelou et al., 2020; Li et al., 2019), wheat (Fotovat et al., 2017; Kang et al., 2012; Ma et al., 2017; Michaletti et al., 2018), maize (Zeng et al., 2019; Zheng et al., 2020), soybean (Song, Prince, et al., 2016; Wang & Komatsu, 2018), tobacco (Chen et al., 2019; Khan et al., 2019), and others (An et al., 2020; Ghatak et al., 2017; Jangpromma et al., 2010; Ngara & Ndimba, 2013; Raney et al., 2014; Rodziewicz et al., 2019; Singh et al., 2017; Utsumi et al., 2012; Wisniewski et al., 2009; Yang et al., 2017). In rapeseed, numerous candidate genes associated with drought response have also been identified by transcriptomic and proteomics analyses. Liu et al. (2015) characterized a comparative network related to phytohormone signal transduction and AREB/ABF, AP2/EREBP, NAC, WRKY, and MYC/MYB transcription factors (TFs), and provided a view of different stress tolerance mechanisms between roots and leaves. Wang et al. (2017) identified 169 highly differentially expressed genes in response to drought stress, including 37 drought-resistant cultivar-related genes, 35 drought-sensitive cultivar-related genes, and 97 cultivar nonspecific genes. The comparative proteomic analysis of the response to drought stress explored 417 proteins that showed obvious changes in abundance (Koh et al., 2015). Wang et al. (2016) discovered approximately 138 differentially abundant proteins and 1232 phosphoproteins containing 4469 phosphopeptides and demonstrated that phosphorylation of β-carbonic anhydrase 1 might be important for adaptation to drought stress in *B. napus*.

To date, there is limited information regarding the molecular mechanism governing the decrease in seed oil content under drought stress in *B. napus*. In our study, we
measured the oil and protein contents and GA and ABA levels in seeds that were acquired on the 30th, 40th, and 50th days (30 days, 40 days, and 50 days) after flowering under control and drought treatments. RNA and protein were extracted from the stressed seeds at the three above-mentioned times for sequencing and analysis. The results help elucidate the molecular mechanism for breeding drought-resistant varieties and improving the oil content for areas with frequent droughts to increase the supply of edible vegetable oil and biofuel.

2 MATERIALS AND METHODS

2.1 Plant materials

Six lines with different oil contents were identified by measuring the oil content of recombinant inbred lines (RILs) (Table S1). The RILs were derived from GH06 × P174, two parent lines with significantly different oil contents (Table S1), and were planted with normal management in the research field located at the Chongqing Engineering Research Center for Rapeseed, Southwest University in Beibei, Chongqing, China. In 2016, the six lines were planted under a waterproof shed with normal experimental management until the initial flower stage. Subsequently, drought stress with 7%–12% soil water content and control treatments were employed for each line. The soil water content was measured using a moisture meter (TZS-1K, Zhejiang Top Instrument Co., Ltd). At 30 days, 40 days, and 50 days after flowering, six lines of fresh seeds that had received the same treatment were mixed equally and stored at −80°C for multiomics analysis, electron microscopy observation, and oil content measurement. The measured seeds were harvested for oil and protein content measurements.

2.2 Measurement of oil and protein contents

The oil and protein contents of mature seeds were measured using a FOSS NIR Systems 5000 in triplicate. The oil contents of fresh seeds were measured using the Soxhlet extraction technique (Dongre, 2011), with three repeats. The weight of dry filter paper was recorded as \( W_0 \), and the weight of dry filter paper with samples was recorded as \( W_1 \). After extraction, the weight of the fully dried sample bags was recorded as \( W_2 \). The oil content was calculated using the following formula:

\[
\text{Oil content (\%) } = \frac{W_1 - W_2}{W_1 - W_0} \times 100.
\]

2.3 Observation of the oil body

The oil body was observed using transmission electron microscope analysis. Seed cotyledons were isolated from dry seeds, fixed immediately in 2.5% glutaraldehyde, and post-fixed with a 1% osmium tetroxide solution. After dehydration using an acetone series (30%, 50%, 75%, 90%, and 100%), the cotyledons were infiltrated and subsequently embedded in epoxy resin. Ultrathin sections were prepared and stained using uranyl acetate and lead citrate. The stained sections were examined on an H-7650 transmission electron microscope (Jeol; Hu et al., 2009, 2013).

2.4 GA and ABA content measured by HPLC

Approximately 0.1 g seed was frozen in liquid nitrogen and finely ground. The extraction and measurement were carried out according to Forghani et al. (2018) in triplicate using a Rigol L3000 high-performance liquid chromatograph (HPLC).

2.5 RNA extraction and transcriptome analysis

Total RNA of six mixed samples (two treatments × three development times) was extracted using a Trizol kit (Invitrogen). The purity concentration and integrity of RNA were determined using a Nanodrop 2000 and Agilent 2100 Bioanalyzer.

Six qualified RNA samples were used to construct libraries using oligo (dT) magnetic beads, fragmentation buffer, random hexamers, 3’ end adenylation and AMpure XP beads. Qubit 2.0 and Agilent 2100 instruments were employed to assess the quality of libraries. Next, the effective concentration of libraries was accurately quantified by qRT-PCR. Qualified libraries were subjected to cluster generation on cBot in Biomarker Technologies Co., Ltd., Beijing, China and sequenced on an Illumina Hiseq 2000 platform (Illumina) with paired-end 100-bp reads. The original data were uploaded to NCBI under GEO ID: GSE160342, and the URL is https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE160342.

Low-quality reads were filtered out using NGS QC toolkit v2.2.3 (https://omictools.com/ngs-qc-toolkit-tool), and clean reads were mapped in the reference genome of “Darmor-bzh” (http://www.genoscope.cns.fr/brassicaneu/). The obtained genes from the previous step were quantitatively analyzed using Cluffquant and Cluffnorm of Cufflinks 2.0.0 (http://cole-trapnell-lab.github.io/cufflinks/releases/v2.0.0/). FPKM (fragments per kilobase of transcript per million
fragments mapped) was employed to measure transcription. The screening conditions for differentially expressed genes (DEGs) were a fold change greater than 2 and a false discovery rate (FDR) less than 0.01.

2.6 Protein extraction and SDS-PAGE

Six mixed samples were fully ground into a fine powder in liquid nitrogen for protein extraction using the TCA/acetone precipitation method (Wang et al., 2010). The concentration of the protein solution was measured using bicinchoninic acid (BCA; Smith et al., 1985). Protein samples (15 μg) were separated using 12% SDS-PAGE. Then, the gel was stained using Coomassie blue stain (Candiano et al., 2004) and scanned using ImageScanner. The protein extraction experiment was considered to be successful if the scanned SDS-PAGE figure that we scanned was consistent with that obtained by consulting a paper (http://www.docin.com/p-1622267830.html).

2.7 Proteome analysis

Protein digestion and TMT labeling were performed according to Yang et al. (2019). The samples were fractionated using a C18 chromatographic column (250 mm × 4.6 mm). The dry peptides were dissolved and separated using 0.1% formic acid and a reversed-phase analytical column (Hajduch et al., 2006; Yang et al., 2019). The peptide fragment data gathered by elution liquid chromatography were collected using LC-MS/MS and analyzed by Proteome Discoverer 2.2 (http://www.protein.iastate.edu/docs/ProteomeDiscovererInstructions.pdf) according to the oilseed proteomics server (http://oilseedproteomics.missouri.edu).

2.8 Analysis of genes with different expression or protein levels

For analysis of the functions of genes with different expressions or protein levels, we employed the R package in the OmicShare cloud platform (https://www.omicshare.com) to analyze the gene ontology (GO) and KEGG enrichment and draw heatmaps.

2.9 QRT-PCR

The primers were designed using Primer Premier 5 and synthesized by Sangon Biotech Co., Ltd. (Table S2). qRT-PCR was performed using a Bio-Rad CFX96 Real-time System with SYBR® Green PCR Supermix in triplicate. BnActin7 was employed as a control. Each reaction included 10 μl SYBR® Green PCR Supermix, 0.4 μl primer F, 0.4 μl primer R, 7.2 μl ddH2O, and 2 μl cDNA model.

3 RESULTS

3.1 Effects of drought stress on seed oil, protein, and phytohormone contents

Compared with the control, inflorescence length, silique number per branch, silique length, and silique seed number decreased under drought stress (Figure S1). The oil body observation of mature seeds in RIL194 indicated that the size of individual oil bodies was smaller, the sectional area of the oil bodies in a single cell was reduced, the number of oil bodies in the intercellular space was fewer, and the arrangement of the oil bodies was looser under drought stress (Figure 1a–h). Moreover, drought stress reduced the oil content and accumulation rates (Figure 1i,k). Furthermore, we found that drought stress increased the quantity of protein bodies and protein storage (Figure 1a–h,j). To further characterize the changes in oil content, we measured the GA and ABA contents of seeds at 30 days, 40 days, and 50 days using HPLC and found that GA levels raised more than sevenfold at 30 days and 40 days and twofold at 50 days (Figure 1l), and ABA content increased significantly only at 50 days under drought.

3.2 Analysis of DEGs under drought stress

After removing low-quality sequences, more than 6.06 GB of clean data were obtained for each sample. The Q30 was more than 90.82%, the GC content was between 46.55% and 49.02%, and more than 70.49% of reads were mapped (Table S3). The results of correlation analysis and principal component analysis (PCA) showed that the difference between the control (CK) and drought stress (DS) was largest at 40 days and 50 days, and the difference between CK at 30 days and DS at 30 days was lowest (Figure 2a,b). By screening DEGs, the greatest number of downregulated genes was observed at 50 days, and the lowest was observed at 30 days (Figure 2e). The GO terms associated with fatty acid metabolism were enriched in the upregulated gene cluster at 40 days and 50 days (Figure 2a,b) and the downregulated gene cluster at 50 days (Figure 2e). The protein storage-related terms were enriched in DEGs at 30 days (Figure 2a,b) and in upregulated gene clusters at 40 days (Figure 2b). Hormones including auxin, JA, SA, GA, ABA, CTK, drought response-, and reactive oxygen species-related terms were enriched in each DEG cluster, especially upregulated gene pools (Figure 2a,b). The KEGG enrichment results showed that the fatty-acid-related pathways were mainly enriched, except for the downregulated gene clusters at 30 days and 40 days (Figure 2c,d). For example, fatty acid metabolism
and biosynthesis of fatty acid and unsaturated fatty acid were enriched in upregulated gene clusters at 30 days and 40 days (Figure 3c,d); cutin, suberine, and wax biosynthesis pathway was enriched in upregulated gene cluster at 40 days and in downregulated gene cluster at 50 days (Figure 3d,e); fatty acid degradation was enriched in upregulated gene clusters at 50 days (Figure 3e). Otherwise, glycolysis/gluconeogenesis and starch and sucrose metabolism pathways were enriched in the DEG clusters at 30 days (Figure 3c), and the MAPK signaling pathway was only enriched in the upregulated gene cluster at 50 days (Figure 3e).

### 3.3 Analysis of genes with different protein expression levels

The data produced during protein extraction and SDS-PAGE are provided in the supplementary material (Tables S4 and S5; Figures S2 and S3). A total of 2098 proteins were identified by quantitative and qualitative proteomics analyses (Tables S6–S8). The correlation and PCA showed that there were powerful relations between 40 days and 50 days and slight relations between 30 days and other times (Figure 2c,d). By filtering according to $|\log_{2}FC|$(fold change) ≥ 1 and...
Figure 2  Correlation analysis and principal component analysis and different expression (protein level) gene statistics in transcriptome and proteome data. (a), Correlation analysis of the transcriptome; (b), principal component analysis of the transcriptome; (c), correlation analysis of the proteome; (d), principal component analysis of the proteome; (e), statistics of the differentially expressed genes; (f), statistics of the differentially expressed genes at the protein level.


**Figure 3**  GO and KEGG enrichment of differentially expressed genes in the transcriptome. (a) The GO terms related to fatty acid, protein storage, and drought response in downregulated gene pools with \( q < 0.01 \). (b) The GO terms related to fatty acid, protein storage, and drought response in upregulated gene pools with \( q < 0.01 \). (c–e) The KEGG enrichment of different expression genes at 30, 40, and 50 days, respectively, the inside was about downregulated genes and the outside was about upregulated genes.

\( p < 0.05, 112, 151, \) and 138, proteins were found at different levels between the two water conditions at 30 days, 40 days, and 50 days, respectively (Figure 2f). GO and KEGG enrichment analyses of different protein-level genes (DPLGs) were performed. Most DPLGs were enriched in the cytosol and plastid, exhibited binding and activity processes, and participated in the modification of RNA and the progression of glycometabolism and stimulus response (Figure 4a–c). In the downregulated DPLGs at 30 days and 40 days, the GO terms associated with lipids were enriched, especially fatty acid biosynthesis and metabolism. Fatty acid degradation, unsaturated fatty acid biosynthesis, and metabolism pathways were enriched in upregulated DPLGs at 40 days (Figure 4f,g). For the upregulated DPLGs observed at 30 days, 40 days, and 50 days, KEGG pathways were enriched and associated with the TCA cycle, carbon metabolism, glycometabolism, and peroxisomes (Figure 4e,g,i).

### 3.4 The influence of drought stress on fatty acid metabolism and protein storage

In fatty acid biosynthesis, 45 DEGs were identified, including the genes encoding ACCase, FabH, FabF, FabI, FabZ, FatA, LACS, FAD3 GPAT, and PAP, and most of them were upregulated at 30 days and downregulated at 40 days.
and 50 days under drought stress (Figure 5a). Furthermore, drought stress downregulated the members of LEAFY COTYLEDON 1 (LEC1) and WRINKLED 1 (WRI1; Figure 5a). There were 35 DEGs participating in fatty acid degradation, engaging in activities such as degrading fatty acids to alcohol, hexadecanoyl-CoA, and acetyl-CoA, and most of these genes were upregulated under drought stress (Figure 5a). The protein level of FabI, involved in the process from Enoyl-ACP to Butyryl-ACP, was upregulated at 30 days and downregulated at 40 days. Four ADH proteins, two ALDH proteins, and three KAT proteins, which played roles in fatty acid degradation, were upregulated under drought stress (Figure 5b).

In all, 14 DEGs associated with protein storage were upregulated at 40 days and did not exhibit significant differences at 30 days and 50 days, including one cruciferin
3 (CRU3), five CRUCIFERINA (CRA1), one RmlC-like cupin superfamily protein gene, two AT2S3, and five AT2S4 (Figure 5c). A total of seven proteins exhibited different levels between the two treatments. At 30 days, CRU3 and CRA1 encoded by BnaC01g09900D were upregulated. Drought stress upregulated the CRA levels at 40 days and 50 days and the AT2S4 levels at 50 days (Figure 5d). The change trends of DEGs and DPLGs concerning fatty acid metabolism and protein storage were consistent where the analyses were repeated (Figure 5).

### 3.5 Influence of drought on GA biosynthesis and transduction

The DEGs and DPLGs involved in GA biosynthesis and transduction were further investigated. The results showed that under drought stress, GA20ox3 (BnaA03g55500D and BnaC09g4830D) was upregulated, GA2ox3 (BnaC04g10660D) was downregulated (Figure 6a), and four genes encoding DELLA and 14 GDSL genes were upregulated (Figure 6b). Four SFAR homologous genes had
expression changes, including two members of SFAR4 and two members of SFAR5, which were upregulated at 50 days (Figure 6c).

### 3.6 Gene expression changes in the ABA pathway under drought stress

At 50 days after drought treatment, BnaA07g06050D and BnaC07g07580D (9-cis-epoxycarotenoid dioxygenase, NCED6), which play vital roles in the first step in ABA biosynthesis, exhibited higher expression under drought stress (Figure 6d). The major genes involved in ABA signal transduction were highly expressed under drought stress, including PYL/PYR, PP2C, SnRK2, and ABF, such as BnaA06g40220D, BnaA03g13020D, BnaA01g23120D, and BnaA05g08020D (Figure 6d).

### 3.7 The gene relative expression analysis

The expressions of key DEGs at 50 days were validated using qRT-PCR. Under drought stress, the genes involved in fatty acid biosynthesis, including BnFAB1, BnFAD3, and BnKASIII, were downregulated. In fatty acid degradation, BnLACS7 and BnACX2 were upregulated; BnHAL3 and BnABI5, the genes in ABA signaling pathways, were upregulated. The expression tendencies of genes participating in the stress response and transcription factors, including BnCCH, BnFSD1, BnCSY3, BnLTI65, BnHSL2, BnICL, BnNAC032,
and BnMYB122, were consistent with the RNA-seq data (Figure 7).

4 | DISCUSSION

Abiotic stresses such as drought, salinity, heat stress, and water logging are the main climatic factors for decreasing the growth, development, yield, and quality of crops (Arvin et al., 2012; Bagheri, 2013; Cramer et al., 2011; Mosavendi et al., 2019; Rezaeizadeh et al., 2019; Sabagh, Hossain, Barutçular, & Saneoka, 2019). Drought is the most important stress that negatively affects the yield and quality of rapeseed, plant growth, physiology, and metabolism (Alqudah et al., 2011; Islam et al., 2011), and restricts the sustainable production of bioenergy. A number of previous studies showed that the reduction in oil content was associated with a decrease in oleic acid and an increase in erucic acid and glucosinolate (Jense et al., 1996; Rezaeizadeh et al., 2019; Sabagh, Hossain, Barutçular, Islam, et al., 2019). Mohammadi et al. (2018) determined that the reduction in oil content is due to a decrease in carbohydrates for oil synthesis and an increase in oil oxidation under drought stress. The result of this study, that is, a decrease in oil content because of drought stress, was verified by oil body observations and oil content measurements.

The GO and KEGG enrichment analyses showed that the GO terms or pathways associated with fatty acid metabolism were enriched in most DEG or DPLG clusters in the transcriptome and proteome. In fatty acid biosynthesis, the downregulated genes included LECI and WRI1, the key regulators that coordinate the expression of fatty acid biosynthesis genes (Mu et al., 2008; Yang et al., 2015), and Fabl, a component of the fatty acid synthase complex that participates in critical process of fatty acid elongation (Dayan et al., 2008; Wu et al., 2015). In fatty acid degradation, acyl-CoA oxidase (ACX) is the first and most important step controlling enzymes involved in fatty acid β-oxidation (Xin et al., 2019). MFP2 is a multifunctional peroxisomal isozyme that adds H₂O over the 2-trans-enoyl-CoA double bond and oxidizes L-3-hydroxyacyl-CoA to 3-keto-acyl-CoA with NAD⁺ in β-oxidation (Arent et al., 2010). The last step of the β-oxidation cycle requires a KAT enzyme catalyzing fatty ketoacyl-CoA to produce one acetyl-CoA molecule (Jiang et al., 2011). Alcohol dehydrogenase is the first enzyme and a rate-limiting enzyme in the oxidation of fatty acids in peroxide and is a key enzyme involved in the conversion of ethanol into acetaldehyde during the process of fatty acid degradation (Manriquez et al., 2006; Shi et al., 2017). In this study, the members of the ACX and MFP2 gene families involved in the progression of β-oxidation exhibited higher expression, and the expression of the ADH, ALDH, and KAT genes and proteins was upregulated under drought stress, although the protein levels of some genes could not be identified at some time points.

The increased expression of the ADH and ALDH genes and proteins might enhance the degradation of fatty acids and thus decrease the oil content. These results revealed that drought decreased oil content by downregulating fatty-acid-associated genes and proteins and upregulating fatty-acid-degradation-associated genes and proteins in rapeseed.

Gami et al. (1977) and Goffman et al. (2005) reported a negative correlation between fatty acid and protein content. The main storage proteins in B. napus are napin (2S storage of protein) and cruciferin (12S globulin protein), which constitute 20% and 60% of the total protein, respectively (Murphy et al., 1989; Perera et al., 2016). In Arabidopsis, there are five 2S albumin genes, including AT2S1, AT2S2, AT2S3, AT2S4, and AT2S5 (van der Klei et al., 1993; Krebbers et al., 1988), and four 12S globulin genes, including CRA1 (AT5G444120), CRU2 (AT1G03880), CRU3 (AT4G28520), and Rmlc-like cupin superfamily protein genes (ATIG03890; Li et al., 2007; Pang et al., 1988). Some studies have shown that napins are encoded by a large gene family of 12–19 genes in B. napus (Guerci et al., 1990; Josfesson et al., 1987; Scofield & Crouch, 1987). Our results revealed that drought stress increased protein storage. Moreover, seven napin genes were upregulated at 40 days after drought treatment, including two AT2S3 homologs and five AT2S4 homologs. The expression of five homologs of CRA1 and one homolog of CRU3 was upregulated at 40 days. The protein levels of one AT2S4, two CRU3, and three CRA1 homologs were also increased in response to drought stress. These were regarded as reasons for the decrease in oil content under drought stress.

Transcriptomic and metabolomic analyses of emmer wheat by Krugman et al. (2011) suggest that drought stress downregulates GA 2-beta-dioxygenase (GA2ox1), which may thereby cause an increase in bioactive GAs. GA20ox and GA3ox also catalyze the production of bioactive GAs (GA1, GA₃, GA₄, and GA₇) from GA12 aldehyde (Huang et al., 2012). In our results, GA20ox3 was upregulated, whereas GA2ox3 was downregulated. This result is consistent with the higher content of GA under drought stress. DELLA proteins influence Arabidopsis growth and development with both redundant and partially specialized functions and facilitate fatty acid storage by suppressing the expression of SFAR genes (Chen et al., 2012; Huang et al., 2015; Karunarathna et al., 2020; Wang et al., 2009). GA-induced degradation of DELLA proteins has been reported via the ubiquitin-26S proteasome system (Dill et al., 2001; Feng et al., 2008; Itoh et al., 2002; Silverstone et al., 2001). Karunarathna et al. (2020) found that seed oil content was significantly increased after knocking out members of the BnSFAR4 and BnSFAR5 gene families. Our study showed that drought stress induced the expression of 4 DELLA and 14 GDSL genes, including two members of the SFAR4 family and two members of the SFAR5 family. Ding et al. (2019) found that the constitutive overexpression of AtGDSL1 and BnGDSL1 promoted
lipid catabolism and decreased the seed oil content. Our results further suggest that the upregulation of BnSFAR4 and BnSFAR5 promoted lipid degradation and decreased seed oil content under drought stress, and other GDSL genes associated with lipid catabolism may also affect seed oil storage.

ABA regulates many important aspects of plant development, including the synthesis of seed storage proteins and lipids, seed desiccation tolerance, maturation, and dormancy (Finkelstein et al., 2002). Under drought stress, ABA accumulates during seed maturation and controls seed development and germination in addition to promoting the closure of stomata in guard cells to maintain water in plants (Nakashima & Yamaguchi-Shinozaki, 2013). Lefebvre et al. (2006) showed that NCED6 and NCED9 are required for ABA biosynthesis during seed development. Drought did not change the ABA content or the expression of ABA biosynthesis-associated genes at 30 days and 40 days. At 50 days, we found that ABA content increased with the upregulated expression of two members of the NCED6 gene family, and the major genes involved in ABA signal transduction were highly expressed. Finkelstein and Someville (1989) thought that exogenous ABA and osmotic conditions influenced the distribution of fatty acids between the pathways leading to desaturation or elongation in Brassica napus L. Qi et al. (1998) also indicated that ABA affects the accumulation of very-long-chain monounsaturated fatty acids in rapeseeds. Under drought stress for 50 days, ABA accumulation might result in seed dormancy and changes in the distribution of desaturated or elongated fatty acids.

5 | CONCLUSIONS

Our study suggests that drought stress causes reduced oil content and increased protein content during seed development. Transcriptome and proteome analyses revealed that gene and protein expression decreased in fatty acid biosynthesis and increased in fatty acid degradation and protein storage. Furthermore, the increased GA content in response to drought stress could promote GDSL gene expression, leading to a decrease in fatty acid content under drought stress. The increased ABA synthesis and signal transduction after long-term drought stress may be related to oil storage changes in developing seeds. The results revealed the molecular mechanisms underlying the decrease in seed oil content under drought stress, which may help breed new cultivars of Brassica napus L. with improved drought resistance for the production of edible oil and biofuel.

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AUTHORS’ CONTRIBUTIONS

Yangyang Li and Linxue Zhang prepared the plant materials, analyzed the data, and wrote the paper; Jinfeng Zhang, Sheng Hu, Lin Wang, and Xiaoke Ping measured the traits. Jia Wang, Jiana Li, Kun Lu, Zhanglin Tang, and Liezhao Liu designed the experiments.

DATA AVAILABILITY STATEMENT

RNA-seq data are available on NCBI under GEO ID: GSE160342, and the URL is https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE160342.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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