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Bioinformatic and Phylogenetic Investigation of WRKY Genes Involved in Drought Stress in *Camelina sativa* Plant

Seyede Maryam Seyed Hassan Pour[1], Leila NejadSadeghi[*1], Danial Kahrizi[2], Zahra Sadat Shobbar[3]

[1]*Department of Genetic and Plant Breeding, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
[2]*Department of Agricultural Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran*
[3]*Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran*

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ABSTRACT

Camelina (*Camelina Sativa*) is a hexaploid dicotyledonous plant from the Brassicaceae family, which is very similar to the Arabidopsis. The number of camelina chromosomes is 40=2n. WRKY transcription factors are one of the most important gene families in plants that play an important role in regulating growth and development and response to various stresses. In this research, 224 WRKY genes were identified in the camelina plant by searching the database, and the chromosomal position, gene length, and conserved motifs were identified in the camelina plant based on the Arabidopsis. Also, in this study, in order to validate the research, 2 genes WRKY8 and WRKY57 under drought stress were investigated by the qPCR method. The results indicated that both the above-mentioned genes were strongly expressed under drought stress conditions in tolerant varieties compared to normal conditions, but the trend was opposite in sensitive varieties. This study has provided acceptable and valuable information for studying the evolution and function of the WRKY gene family in camelina.

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1. Introduction

Camelina with the scientific name *Camelina Sativa* is an allohexaploid annual plant, a member of the Brassicaceae family (Neupane *et al*., 2022), which, as an oilseed product, forms the second largest food reserve in the world after cereals (Vafaei *et al*., 2010). Camelina has a relatively short life cycle of 85-100 days, depending on the genotype and growth conditions (Moser, 2010). Compared with other commercialized oil crops, *C. sativa* has several prominent agronomic traits, such as strong tolerance to abiotic stress (salt, drought, cold), and high resistance to common pests and diseases infected in many cruciferous crops (Brock *et al*., 2018; Abdullah *et al*., 2016; Heydarian *et al*., 2018; Yuan *et al*., 2017). Camelina seeds accumulate high levels of oil (36%-047%) and protein (30%) as well as a variety of natural active ingredients.

Abiotic stresses seriously affect agricultural production, leading to a reduction in crop yield and quality (Heydarian *et al*., 2018). To adapt to diverse stresses, plants have evolved favorable strategies such as metabolic reconstruction, cell-tissue remodeling, and gene expression reprogramming. Transcriptional factors (TFs) can bind on cis-elements or interact with other regulatory factors to regulate expressions of the downstream defense-related genes (Ng *et al*., 2018). Increasing reports show that a number of different TFs play a significant regulatory role in plant stress responses, including bHLH (Li *et al*., 2015), MYB (Guo *et al*., 2017), bZIP (Ji *et al*., 2018), NAC (Yang *et al*., 2018), WRKY (Tang *et al*., 2014). Among them, WRKY proteins (WRKYs) are one of the largest TF families, with complex biological functions, and specific to many plant species from single-celled algae to higher plants. For instance, in *Arabidopsis*

* Corresponding author.
E-mail address: l.nejadsadeghi@scu.ac.ir

thaliana, overexpression of AtWRKY50 promoted the production of sinapic derivatives (Hussain *et al*., 2018). AtWRKY46, 54, and 70 have an important effect on activating the expression of brassinosteroid-mediated genes and restraining the drought gene response (Chen *et al*., 2017).

OsWRKY47 positively regulates both the yield and drought tolerance of rice (Raineri *et al*., 2015). TaWRKY33 significantly increased wheat drought tolerance (He *et al*., 2016a). PtrWRKY18 and PtrWRKY35 enhanced the resistance to Melampsora in Populus (Jiang *et al*., 2017).

All known WRKYs contain one or two unique DNA binding domains consisting of approximately 60 amino acids (aa) characterized by a highly conserved WRKYGQK sequence (designated as WRKY domain) at the N-terminus, followed by a C2H2 zinc-finger-like motif (C-X4-5C-X22-23-H-X1-H or C-X7C- X23-H-X1-C) at the C-terminus. The WRKY domain specifically binds to the consensus W-box (a cis-acting element with the core sequence TGAC) in promoters of the target genes (Jing and Liu, 2018; Rasouli and Fazeli-Nasab, 2014). According to the number of WRKY domains and the type of zinc-finger-like structure, WRKY proteins are generally classified into three main groups (I-III). Group I WRKY proteins contain two WRKY domains and the zinc finger motif of C-X4-C-X22-23-H-X1-H, whereas Group II and III have only one WRKY domain, with group II proteins sharing the same zinc finger motif as group I and group III proteins bearing the unique zinc finger motif of C-X4-5-C-X23-24-H-X1-H, respectively (Rushton *et al*., 2010). Group II WRKYs can be further divided into several distinct subgroups (IIa-e) based on their phylogenetic relationship. In addition, some special resistance protein (R-protein) WRKYs were found in several plant species, with three R-protein WRKYs in Arabidopsis (AtWRKY16, AtWRKY19 and AtWRKY52), and one R-protein WRKY in soybean (GmWRKY1) (Rinerson *et al*., 2015) and pineapple (AcWRKY23) (Xie *et al*., 2018) respectively. Such R-protein WRKY may further enhance signal diversity and even shorten the speed of signal transmission with other components of signaling pathways.

The purpose of this research is bioinformatic and phylogenetic investigation of camelina WRKY genes involved in drought stress.

2. Materials and methods

2.1. Bioinformatics part

This research was conducted in order to investigate the WRKY gene family in camelina plants under drought stress. The complete genome, proteome and CDS sequence files of *C. sativa* were downloaded from the webpage of NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF_000633955.1_Cs). The WRKY domain HMM (Hidden Markov Model) profile numbered PF03106 was extracted from the Pfam protein family database (http://pfam.xfam.org/) (Wu *et al*., 2017). The candidate WRKY protein sequences were discovered by the comprehensive research of utilization of HMMER (E-value cut-off < 1E-5) and BLAST analyses (75 AtWRKYs as queries) in *C. sativa* whole genome protein database (Wei *et al*., 2016). These CsWRKYs sequences were identified by checking the complete WRKY conserved domain with SMART (http://smart.embl-heidelberg.de/) and InterPro (http://www.ebi.ac.uk/interpro/), the redundant sequences were manually removed. The confirmed CsWRKYs used the ExPasy online tool website (http://web.expasy.org/protparam/) to calculate physicochemical properties including SL, MW and PI. Multiple sequence alignment of the CsWRKY domain was performed using ClustalW with Bioedit software. Based on the alignment WRKY domains of CsWRKYs and AtWRKYs, the phylogenetic tree was constructed with MEGA 7.0 using Neighbor-Joining (NJ) method and the parameters (Poisson model, pairwise deletion, and 1000 bootstrap replications). All identiied CsWRKYs were divided into different groups according to the classification of AtWRKYs sequences. The AtWRKYs sequences were obtained from NCBI. The MEME (http://meme.nbcr.net/meme/intro.html) was used to analyze CsWRKYs and searched for 10 conserved motifs. The interrelated parameters were as follows: the repetitive time was any, the maximum motif number was 20 and the motif width was between 5 and 50 residues. The MEME results were displayed with the TBtool software (Song and Peng, 2019).

2.2. Laboratory part

In this research, two double haploid lines, tolerant (DH 91) and sensitive (DH 101), were prepared in order to investigate the WRKY gene family in camelina plants under drought stress. The prepared seeds were cultivated in a medium pot (soil(50 percent), sand(40 percent), and

vermicompost(10 percent)) with 3 replications for each line and RNA extraction was done according to the method of Christou et al. (2014). For RNA extraction, leaves and roots of plants in different growth stages are used.. To perform the extraction steps, first, the sample (leaf or root) is ground well and powdered in a Chinese mortar with the help of liquid nitrogen and then the cold extraction buffer including sodium dodecyl sulfate (SDS) 1% by weight - volume and Tris-Hcl 0.5 M was added to it, and after that, phenol, chloroform, isoamyl alcohol (PCI) with a ratio of (1:24: 25) was added to the mixture and centrifuged at 14000 rpm for 7 minutes at 4 degrees Celsius in the machine. After the formation of 3 separate phases, the clear supernatant phase, containing RNA, was transferred to a new cold microtube and the same volume of PCI was added and centrifuged as in the previous step. 600 microliters of the sample were separated with a sampler in a cold environment and transferred to a new 2 ml microtube, and 600 microliters of PCI were added to it and centrifuged for 7 minutes at a temperature of 4 degrees Celsius with a speed of 14000 rpm. The bright and transparent phase of the supernatant was separated by a sampler and transferred to a new microtube, and 0.1 volume of 3 M sodium acetate with pH = 5.6 and 1 volume of cold absolute ethanol were kept for 20 minutes in a freezer at -80°C. After removing the samples from the freezer at -80 for 8 minutes at 4 degrees Celsius, they were centrifuged at a speed of 16,000. At this stage, carefully remove the ethanol and sodium acetate with the sampler so that only the precipitated plate (colorless or greenish-white spot) remains on the bottom of the microtube. At this stage, by adding cold 75% ethanol for washing, centrifugation was repeated for 3 minutes at 16,000 rpm. After completely removing the contents of the tube with ethanol at a dry ambient temperature and at the end, about 30 to 50 microliters of double distilled water were added to the sample depending on the amount of sediment formed and RNA was dissolved inside the microtube by pipetting.

2.3. Quantification of RNA

A nanodrop device was used to check the quantity and purity of the extracted RNA. The ratio of optical absorption (A260-A280 nm) indicates protein contamination, A230-A260 to check the amount of contamination of suspended substances and sugars in the extracted RNA solution, which was calculated by the device. Sterile double-distilled water was used to zero the device and 2 microliters of extracted RNA samples were placed on the sensor of the device for measurement.

2.4. Determination of RNA quality by agarose gel

In this research, electrophoresis with 1% agarose gel was used to check the quality of extracted RNA. In this way, RNA, after being dissolved with loading buffer (loading dye), was loaded individually in a well on 1% agarose gel and electrophoresis was performed with 100 voltage for 40 minutes. Then, the prepared gel was examined under ultraviolet light in a gel dock device (UV-transilluminator). In this method, if the band on the agarose gel is completely single and without any kind of background, it indicates the quality and absence of rupture and damage of the extracted RNA.

2.5. cDNA synthesis

RNA extracted in the previous step was used for cDNA synthesis according to the recipe of the NEB (New England Biolabs) kit. According to the mentioned kit, first DNase was used and the resulting mixture was placed in a thermocycler (SimpliAmp-Thermal Cycler). After finishing the steps of making cDNA, the samples were kept in a refrigerator at minus 20 degrees Celsius for the next steps.

2.6. Primer design of the desired gene

In order to investigate the change in the expression level of two candidate WRKY genes in two tolerant and sensitive lines under biological drought stress conditions, by real-time PCR (qRT-PCR), the number of 2 pairs of specific primers and 2 pairs of general primers PP2A and Beta-actin Suitable reverse primers were designed by Primer Premier 6 software and their synthesis was done by Metabion international AG in Germany. Each of the primers was diluted according to the respective formula and kept at minus 20 degrees Celsius. Table 1 is the list of primers and their sequence.

Table 1. Reverse primer sequence used in PCR and qRT-PCR reaction

Gene Name	Sequence (5´-3´)	Length
Csa11g065620.1-F	*TTACGAGAGTCAGCACAACCA*	*21*
Csa11g065620.1-R	*GCACACGGAAGAGATCATCATT*	*22*
Csa07g035970.1-F	*CTTCTTGTTCCTCATCCGTAGC*	*22*
Csa07g035970.1-R	*TTCTCCTTCACTGGTGTCTCC*	*22*
PP2A-F	*GTCAACAATCCGCACTACCTACA*	*23*
PP2A-R	*CAACCACGACGGGAAGAAAC*	*20*
Beta actin-F	TGGAATGGTCAAGGCTGGAT	*20*

3. Results and discussion

Environmental stress is one of the most important factors limiting growth and production in plants (Heydarian *et al*., 2018). Plants need a regulatory mechanism in response to biological stresses due to their inability to move and escape the stresses and changes created for survival, reproduction and production. Plants respond to stresses through the expression of genes, and in the meantime, transcription factors play a central and important role in regulating gene expression (Rushton *et al*., 2010).

As one of the largest TF families in higher plants, the WRKY family members participate in plant development and in response to various stresses. In this study, we used genome and RNA-seq data to identify 224 WRKY gene loci encoding 242 CsWRKY proteins in *C. sativa*. Compared to the quantity of WRKY family members detected in other higher plants like rice, soybean (Song *et al*., 2016a), pepper (Zheng *et al*., 2019), peanut (Song *et al*., 2016b), sesame (Li *et al*., 2017), CsWRKY family is the second large family after *Brassica napus* (He *et al*., 2016b) with 287 WRKY members. This indicates that a large-scale expansion of this family happened in the C. sativa genome, which might be the results of gene duplication As mentioned In this research, 224 WRKY genes were identified through databases. Also, to further investigate and identify the function of genes encoding WRKY transcription factors, 2 genes, WRKY8 and WRKY57, were randomly selected and analyzed to determine the level of expression in camelina plant leaf tissue under drought stress through qRT-PCR were placed. The results of increased expression of both genes under drought stress in the tolerant varieties indicated that these two genes clearly play an effective role in the occurrence of resistance and tolerance to biological stresses during the life of the plant.

3.1. Identification of WRKY family members in the camelina plant

As a result of the blast of the transcription factors downloaded from the database (PlantTFDB) with the transcription factors of the database (Camelina genome pororal), 7 new transcription factors were found. New transcription factors are listed in Table 2.

Due to the great similarity of the camelina plant to the Arabidopsis model plant, 106 possible new transcription factors were found in the camelina plant based on the Arabidopsis plant. New transcription factors are listed in Table 3.

Table 2. New transcription factors of camelina plant

Number	WRKY name	Chr	Lengh	Gene location
1	Csa11g068440.1|Camelina_sativa |WRKY|Csa11g068440.1	11	49703607	32171293 to 32172075
2	Csa18g008090.1|Camelina_sativa |WRKY|Csa18g008090.1	18	20869551	3065780 to 3066894
3	Csa18g010550.1|Camelina_sativa |WRKY|Csa18g010550.1	18	20869551	5538358 to 5538912
4	Csa02g004840.1|Camelina_sativa |WRKY|Csa02g004840.1	2	29139806	1119388 to 1120046
5	Csa13g009120.1|Camelina_sativa |WRKY|Csa13g009120.1	13	24101694	2808635 to 2808988
6	Csa03g062230.1|Camelina_sativa |WRKY|Csa03g062230.1	3	28500605	28036035 to 28036506
7	Csa11g011520.1|Camelina_sativa |WRKY|Csa11g011520.	11	49703607	4750420 to 4750822

Table 3. New plant transcription factors obtained from Arabidopsis model blast

Description	NEW TFs	Number
Csa16g002300.1|Camelina_sativa|WRKY|Csa16g002300.1	PREDICTED: probable WRKY transcription factor 68 [*C. Sativa*]	1
Csa20g009470.1|Camelina_sativa|WRKY|Csa20g009470.1	PREDICTED: probable WRKY transcription factor 26 isoform X2 [*C. Sativa*]	2
scaffold00047:248439-254447 Amino Acid Sequence (1,430 aa)	PREDICTED: LOW-QUALITY PROTEIN: disease resistance protein RRS1-like [*C. Sativa*]	3
Csa11g068440.1|Camelina_sativa|WRKY|Csa11g068440.1	PREDICTED: probable WRKY transcription factor 16 isoform X3 [*C. Sativa*]	4
XP_019097230.1 PREDICTED: probable WRKY transcription factor 16 isoform X3 [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 16 [*C. Sativa*]	5
XP_019097230.1 PREDICTED: probable WRKY transcription factor 16 isoform X3 [*C. Sativa*]	PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	6
XP_019097230.1 PREDICTED: probable WRKY transcription factor 16 isoform X3 [*C. Sativa*]	PREDICTED: protein DA1-related 4-like isoform X1 [*C. Sativa*]	7
XP_010492762.1 PREDICTED: protein DA1-related 4-like isoform X1 [*C. Sativa*]	PREDICTED: protein DA1-related 4 [*C. Sativa*]	8

Table 3. New plant transcription factors obtained from Arabidopsis model blast

Description	NEW TFs	Number
XP_010437375.1 PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	PREDICTED: protein SUPPRESSOR OF npr1-1, CONSTITUTIVE 1-like isoform X1 [*C. Sativa*]	9
XP_019097230.1 PREDICTED: probable WRKY transcription factor 16 isoform X3 [*C. Sativa*]	PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	10
XP_010437375.1 PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 68 [*C. Sativa*]	11
XP_010481629.1 PREDICTED: probable WRKY transcription factor 16 [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 26 isoform X2 [*C. Sativa*]	12
XP_010494626.1 PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	PREDICTED: LOW-QUALITY PROTEIN: disease resistance protein RRS1-like [C. Sativa]	13
Csa13g050240.1|Camelina_sativa|WRKY|Csa13g050240.1	PREDICTED: probable WRKY transcription factor 16 [*C. Sativa*]	14
XP_010439702.1 PREDICTED: probable WRKY transcription factor 19 [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 16 [*C. Sativa*]	15
XP_010434403.1 PREDICTED: probable WRKY transcription factor 19 isoform X2 [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 16 [*C. Sativa*]	16
XP_010439702.1 PREDICTED: probable WRKY transcription factor 19 [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 19 [*C. Sativa*]	17
XP_010481624.1 PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 19 isoform X2 [*C. Sativa*]	18
XP_019089546.1 PREDICTED: protein SUPPRESSOR OF npr1-1, CONSTITUTIVE 1-like isoform X1 [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 19 [*C. Sativa*]	19
XP_010492729.1 PREDICTED: disease resistance protein RML1A [*C. Sativa*]	PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	20
XP_010455633.1 PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	21
Csa13g050240.1|Camelina_sativa|WRKY|Csa13g050240.1	PREDICTED: disease resistance protein RML1A [*C. Sativa*]	22
XP_010439692.1 PREDICTED: disease resistance-like protein CSA1 isoform X1 [*C. Sativa*]	PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	23
XP_010455633.1 PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	PREDICTED: disease resistance-like protein CSA1 isoform X1 [*C. Sativa*]	24
XP_010449349.1 PREDICTED: disease resistance-like protein CSA1 [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 19 isoform X3 [*C. Sativa*]	25
XP_010436479.2 PREDICTED: probable WRKY transcription factor 19 [*C. Sativa*]	PREDICTED: disease resistance-like protein CSA1 [*C. Sativa*]	26
XP_010434410.1 PREDICTED: disease resistance-like protein CSA1 [*C. Sativa*]	PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	27
XP_010492729.1 PREDICTED: disease resistance protein RML1A [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 16 [*C. Sativa*]	28
XP_010492729.1 PREDICTED: disease resistance protein RML1A [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 16 [*C. Sativa*]	29
XP_010449922.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 16 [*C. Sativa*]	30
XP_019090355.1 PREDICTED: putative disease resistance protein At4g11170 [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 19 [*C. Sativa*]	31
XP_010455633.1 PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 19 isoform X2 [*C. Sativa*]	32
XP_019086568.1 PREDICTED: protein SUPPRESSOR OF npr1-1, CONSTITUTIVE 1-like isoform X2 [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 19 [*C. Sativa*]	33
XP_019090355.1 PREDICTED: putative disease resistance protein At4g11170 [*C. Sativa*]	PREDICTED: disease resistance-like protein CSA1 [*C. Sativa*]	34
XP_010441319.1 PREDICTED: disease resistance protein RML1A-like [*C. Sativa*]	PREDICTED: probable WRKY transcription factor 19 [*C. Sativa*]	35
XP_010449922.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: disease resistance protein LAZ5-like isoform X3 [*C. Sativa*]	36
XP_019084836.1 PREDICTED: LOW QUALITY PROTEIN: disease resistance protein RPS4 [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	37
XP_010449922.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	38
XP_019086568.1 PREDICTED: protein SUPPRESSOR OF npr1-1, CONSTITUTIVE 1-like isoform X2 [*C. Sativa*]	PREDICTED: putative disease resistance protein At4g11170 [*C. Sativa*]	39
XP_010441319.1 PREDICTED: disease resistance protein RML1A-like [*C. Sativa*]	PREDICTED: protein SUPPRESSOR OF npr1-1, CONSTITUTIVE 1-like isoform X2 [*C. Sativa*]	40

Table 3. New plant transcription factors obtained from Arabidopsis model blast

Description	NEW TFs	Number
XP_010445862.2 PREDICTED: disease resistance protein RPS6-like [*C. Sativa*]	PREDICTED: LOW-QUALITY PROTEIN: disease resistance protein RPS4 [C. Sativa]	41
XP_010492729.1 PREDICTED: disease resistance protein RML1A [*C. Sativa*]	PREDICTED: disease resistance protein RPS6-like [*C. Sativa*]	42
XP_019088676.1 PREDICTED: disease resistance protein LAZ5-like isoform X3 [*C. Sativa*]	PREDICTED: disease resistance protein RML1A-like [*C. Sativa*]	43
XP_010445862.2 PREDICTED: disease resistance protein RPS6-like [*C. Sativa*]	PREDICTED: putative disease resistance protein At4g11170 [*C. Sativa*]	44
XP_010449922.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: probable disease resistance protein RPP1 [*C. Sativa*]	45
XP_010441603.1 PREDICTED: protein VARIATION IN COMPOUND TRIGGERED ROOT growth response-like isoform X2 [*C. Sativa*]	PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	46
XP_010449922.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: probable disease resistance protein RPP1 [*C. Sativa*]	47
XP_019090355.1 PREDICTED: putative disease resistance protein At4g11170 [*C. Sativa*]	PREDICTED: disease resistance protein RPP4-like isoform X1 [*C. Sativa*]	48
XP_010445862.2 PREDICTED: disease resistance protein RPS6-like [*C. Sativa*]	PREDICTED: disease resistance protein RML1B-like [*C. Sativa*]	49
XP_010418428.1 PREDICTED: disease resistance protein RML1B-like [*C. Sativa*]	PREDICTED: disease resistance protein RPS6-like [*C. Sativa*]	50
XP_010463849.1 PREDICTED: probable disease resistance protein RPP1 [*C. Sativa*]	PREDICTED: protein SUPPRESSOR OF npr1-1, CONSTITUTIVE 1-like isoform X1 [*C. Sativa*]	51
XP_010449922.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: inactive disease resistance protein RPS4-like [*C. Sativa*]	52
XP_010473654.1 PREDICTED: disease resistance protein RML1A-like isoform X1 [*C. Sativa*]	PREDICTED: protein VARIATION IN COMPOUND TRIGGERED ROOT growth response-like isoform X2 [*C. Sativa*]	53
XP_010422104.1 PREDICTED: probable disease resistance protein RPP1 isoform X1 [*C. Sativa*]	PREDICTED: probable disease resistance protein RPP1 [*C. Sativa*]	54
XP_010441319.1 PREDICTED: disease resistance protein RML1A-like [*C. Sativa*]	PREDICTED: protein VARIATION IN COMPOUND TRIGGERED ROOT growth response-like isoform X3 [*C. Sativa*]	55
XP_010418428.1 PREDICTED: disease resistance protein RML1B-like [*C. Sativa*]	PREDICTED: probable disease resistance protein RPP1 [*C. Sativa*]	56
XP_010472818.1 PREDICTED: protein SUPPRESSOR OF npr1-1, CONSTITUTIVE 1-like isoform X1 [*C. Sativa*]	PREDICTED: putative disease resistance protein At4g11170 [*C. Sativa*]	57
XP_010418428.1 PREDICTED: disease resistance protein RML1B-like [*C. Sativa*]	PREDICTED: disease resistance protein RPS6-like isoform X1 [*C. Sativa*]	58
XP_010449922.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: disease resistance protein RML1A-like isoform X1 [*C. Sativa*]	59
XP_010445862.2 PREDICTED: disease resistance protein RPS6-like [*C. Sativa*]	PREDICTED: probable disease resistance protein RPP1 isoform X1 [*C. Sativa*]	60
XP_010441319.1 PREDICTED: disease resistance protein RML1A-like [*C. Sativa*]	PREDICTED: disease resistance protein RML1A-like [*C. Sativa*]	61
XP_019084097.1 PREDICTED: disease resistance protein RPS6-like isoform X1 [*C. Sativa*]	PREDICTED: disease resistance protein RML1B isoform X2 [*C. Sativa*]	62
XP_010426315.1 PREDICTED: probable disease resistance protein RPP1 [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	63
XP_010419679.1 PREDICTED: probable disease resistance protein RPP1 [*C. Sativa*]	PREDICTED: disease resistance protein RML1B-like isoform X3 [*C. Sativa*]	64
XP_010442102.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	65
XP_010481472.2 PREDICTED: protein VARIATION IN COMPOUND TRIGGERED ROOT growth response-like isoform X3 [*C. Sativa*]	PREDICTED: disease resistance protein RPS6-like [*C. Sativa*]	66
XP_019084836.1 PREDICTED: LOW QUALITY PROTEIN: disease resistance protein RPS4 [*C. Sativa*]	PREDICTED: disease resistance protein RML1A-like isoform X1 [*C. Sativa*]	67
XP_010492729.1 PREDICTED: disease resistance protein RML1A [*C. Sativa*]	PREDICTED: disease resistance protein RPS6-like isoform X2 [*C. Sativa*]	68
XP_019084014.1 PREDICTED: mitogen-activated protein kinase kinase kinase 1-like [*C. Sativa*]	PREDICTED: probable disease resistance protein RPP1 isoform X2 [*C. Sativa*]	69
XP_019084014.1 PREDICTED: mitogen-activated protein kinase kinase kinase 1-like [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	70

Table 3. New plant transcription factors obtained from Arabidopsis model blast

Description	NEW TFs	Number
XP_019089940.1 PREDICTED: mitogen-activated protein kinase kinase kinase 9 [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	71
XP_010441603.1 PREDICTED: protein VARIATION IN COMPOUND TRIGGERED ROOT growth response-like isoform X2 [*C. Sativa*]	PREDICTED: protein VARIATION IN COMPOUND TRIGGERED ROOT growth response-like [*C. Sativa*]	72
XP_010429854.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: uncharacterized protein LOC104760394 isoform X3 [*C. Sativa*]	73
XP_010453999.1 PREDICTED: putative disease resistance protein At4g11170 [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	74
XP_010453999.1 PREDICTED: putative disease resistance protein At4g11170 [*C. Sativa*]	PREDICTED: mitogen-activated protein kinase kinase kinase 9 [*C. Sativa*]	75
XP_010492778.1 PREDICTED: putative disease resistance protein At4g11170 isoform X2 [*C. Sativa*]	PREDICTED: mitogen-activated protein kinase kinase kinase 1-like isoform X4 [*C. Sativa*]	76
XP_010473654.1 PREDICTED: disease resistance protein RML1A-like isoform X1 [*C. Sativa*]	PREDICTED: mitogen-activated protein kinase kinase kinase 9-like isoform X3 [*C. Sativa*]	77
XP_019095941.1 PREDICTED: uncharacterized protein LOC104760394 isoform X3 [*C. Sativa*]	PREDICTED: protein VARIATION IN COMPOUND TRIGGERED ROOT growth response-like isoform X1 [*C. Sativa*]	78
XP_010418428.1 PREDICTED: disease resistance protein RML1B-like [*C. Sativa*]	PREDICTED: protein SUPPRESSOR OF npr1-1, CONSTITUTIVE 1-like [*C. Sativa*]	79
XP_010450552.1 PREDICTED: mitogen-activated protein kinase kinase kinase 1-like isoform X4 [*C. Sativa*]	PREDICTED: putative disease resistance protein At4g11170 isoform X2 [*C. Sativa*]	80
XP_019095566.1 PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	PREDICTED: putative disease resistance protein At4g11170 isoform X1 [*C. Sativa*]	81
XP_010422104.1 PREDICTED: probable disease resistance protein RPP1 isoform X1 [*C. Sativa*]	PREDICTED: putative disease resistance protein At4g11170 isoform X2 [*C. Sativa*]	82
XP_019089677.1 PREDICTED: disease resistance protein RPS4-like isoform X2 [*C. Sativa*]	PREDICTED: disease resistance protein RML1A-like isoform X1 [*C. Sativa*]	83
XP_019095941.1 PREDICTED: uncharacterized protein LOC104760394 isoform X3 [*C. Sativa*]	PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	84
XP_019095566.1 PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	PREDICTED: disease resistance protein RML1B-like isoform X1 [*C. Sativa*]	85
XP_010418427.1 PREDICTED: disease resistance protein RML1B-like isoform X1 [*C. Sativa*]	PREDICTED: mitogen-activated protein kinase kinase kinase 1-like isoform X3 [*C. Sativa*]	86
XP_019083764.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: disease resistance protein RPS4-like isoform X2 [*C. Sativa*]	87
XP_019083764.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: probable disease resistance protein RPP1 isoform X2 [*C. Sativa*]	88
XP_010496944.1 PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	89
XP_019083764.1 PREDICTED: disease resistance protein TAO1-like [*C. Sativa*]	PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	90
XP_010418428.1 PREDICTED: disease resistance protein RML1B-like [*C. Sativa*]	PREDICTED: disease resistance protein LAZ5-like [*C. Sativa*]	91
XP_010434749.1 PREDICTED: disease resistance protein RPP4-like isoform X1 [*C. Sativa*]	PREDICTED: disease resistance protein RML1B-like [*C. Sativa*]	92
XP_019084014.1 PREDICTED: mitogen-activated protein kinase kinase kinase 1-like [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like isoform X2 [*C. Sativa*]	93
Csa13g050240.1|Camelina_sativa|WRKY|Csa13g050240.1	PREDICTED: disease resistance protein RML1A-like [*C. Sativa*]	94
Csa16g002300.1|Camelina_sativa|WRKY|Csa16g002300.1	PREDICTED: disease resistance protein TAO1-like isoform X1 [*C. Sativa*]	95
Csa20g009470.1|Camelina_sativa|WRKY|Csa20g009470.1	PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	96
scaffold00047:248439-254447 Amino Acid Sequence (1,430 aa)	PREDICTED: disease resistance protein TAO1-like isoform X3 [*C. Sativa*]	97
Csa11g068440.1|Camelina_sativa|WRKY|Csa11g068440.1	PREDICTED: disease resistance protein RML1B-like isoform X1 [*C. Sativa*]	98
XP_019097230.1 PREDICTED: probable WRKY transcription factor 16 isoform X3 [*C. Sativa*]	PREDICTED: protein SUPPRESSOR OF npr1-1, CONSTITUTIVE 1-like [*C. Sativa*]	99
XP_019097230.1 PREDICTED: probable WRKY transcription factor 16 isoform X3 [*C. Sativa*]	PREDICTED: mitogen-activated protein kinase kinase kinase 1 isoform X1 [*C. Sativa*]	100
XP_019097230.1 PREDICTED: probable WRKY transcription factor 16 isoform X3 [*C. Sativa*]	PREDICTED: mitogen-activated protein kinase kinase kinase 1-like [*C. Sativa*]	101

Table 3. New plant transcription factors obtained from Arabidopsis model blast

Description	NEW TFs	Number
XP_010492762.1 PREDICTED: protein DA1-related 4-like isoform X1 [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like isoform X1 [*C. Sativa*]	102
XP_010437375.1 PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	PREDICTED: disease resistance protein RPS4-like [*C. Sativa*]	103
XP_019097230.1 PREDICTED: probable WRKY transcription factor 16 isoform X3 [*C. Sativa*]	PREDICTED: disease resistance protein TAO1-like isoform X3 [*C. Sativa*]	104
XP_010437375.1 PREDICTED: disease resistance protein RRS1-like [*C. Sativa*]	PREDICTED: disease resistance protein RML1B-like isoform X1 [*C. Sativa*]	105
Csa16g002300.1|Camelina_sativa|WRKY|Csa16g002300.1	PREDICTED: probable WRKY transcription factor 68 [*C. Sativa*]	106

3.2. Identification of members of WRKY family transcription factors in camelina plant

Each type of protein has a unique sequence that plays an important role in the structure of the protein and ultimately its function. To investigate the WRKY family in the camelina plant, all the protein and gene sequences related to this plant through the databases downloaded. In this way, the number of 224 WRKY transcription factors were identified, which encode 224 proteins. The physical, chromosomal and length location of each gene and the distribution of each of them are shown in Table 4 and the factors involved in drought stress are highlighted in yellow (Fig. 1).

Table 4. Physical characteristics of WRKY gene family transcription factors

WRKY NAME	Chr	Length	Start	End
CsWRKY1	unknown	532aa	295	353
CsWRKY2	1	464aa	201	257
CsWRKY3	1	335aa	267	324
CsWRKY4	1	473aa	114	172
CsWRKY5	2	305aa	132	190
CsWRKY6	2	491aa	237	296
CsWRKY7	2	534aa	297	355
CsWRKY8	2	1275aa	228	285
CsWRKY9	2	321aa	136	196
CsWRKY10	2	150aa	70	128
CsWRKY11	2	411aa	231	289
CsWRKY12	2	408aa	221	279
CsWRKY13	2	180aa	97	155
CsWRKY14	2	555aa	194	251
CsWRKY15	2	796aa	370	427
CsWRKY16	2	704aa	278	335
CsWRKY17	2	194aa	108	166
CsWRKY18	3	525aa	241	297
CsWRKY19	3	543aa	239	298
CsWRKY20	3	543aa	239	298
CsWRKY21	3	300aa	153	210
CsWRKY22	3	289aa	142	199
CsWRKY23	3	435aa	216	274
CsWRKY24	3	435aa	216	274
CsWRKY25	4	400aa	226	283
CsWRKY26	4	516aa	254	310
CsWRKY27	4	218aa	129	184
CsWRKY28	4	400aa	226	283
CsWRKY29	4	278aa	70	128
CsWRKY30	4	392aa	181	239

Table 4. Physical characteristics of WRKY gene family transcription factors

WRKY NAME	Chr	Length	Start	End
CsWRKY31	4	429aa	163	219
CsWRKY32	4	379aa	179	236
CsWRKY33	4	291aa	161	221
CsWRKY34	4	333aa	148	208
CsWRKY35	4	222aa	147	204
CsWRKY36	4	301aa	110	170
CsWRKY37	5	343aa	175	231
CsWRKY38	5	351aa	182	238
CsWRKY39	5	302aa	113	173
CsWRKY40	5	223aa	148	205
CsWRKY41	5	333aa	148	208
CsWRKY42	5	292aa	163	223
CsWRKY43	5	513aa	177	234
CsWRKY44	5	432aa	166	222
CsWRKY45	5	412aa	182	240
CsWRKY46	5	383aa	316	374
CsWRKY47	5	470aa	242	299
CsWRKY48	5	240aa	103	163
CsWRKY49	5	355aa	214	273
CsWRKY50	5	282aa	141	198
CsWRKY51	5	381aa	201	260
CsWRKY52	5	311aa	123	184
CsWRKY53	5	232aa	70	128
CsWRKY54	6	678aa	251	307
CsWRKY55	6	263aa	128	188
CsWRKY56	6	302aa	125	185
CsWRKY57	6	276aa	70	128
CsWRKY58	6	556aa	75	122
CsWRKY59	6	431aa	165	221
CsWRKY60	6	517aa	181	238
CsWRKY61	6	290aa	161	221
CsWRKY62	6	332aa	147	207
CsWRKY63	6	167aa	145	166
CsWRKY64	6	297aa	107	167
CsWRKY65	6	357aa	189	245
CsWRKY66	7	288aa	121	188
CsWRKY67	7	227aa	89	127
CsWRKY68	7	381aa	35	9
CsWRKY69	7	180aa	14	53
CsWRKY70	7	308aa	120	181
CsWRKY71	7	387aa	320	378
CsWRKY72	7	393aa	165	222
CsWRKY73	7	202aa	80	122
CsWRKY74	7	352aa	211	270
CsWRKY75	7	282aa	141	198
CsWRKY76	7	385aa	204	263
CsWRKY77	7	200aa	106	164
CsWRKY78	7	365aa	288	345
CsWRKY79	7	362aa	282	339

Table 4. Physical characteristics of WRKY gene family transcription factors

WRKY NAME	Chr	Length	Start	End
CsWRKY80	7	237aa	110	169
CsWRKY81	7	254aa	96	155
CsWRKY82	7	570aa	328	386
CsWRKY83	7	194aa	111	169
CsWRKY84	8	151aa	71	129
CsWRKY85	8	100aa	20	77
CsWRKY86	8	542aa	225	284
CsWRKY87	8	292aa	113	172
CsWRKY88	8	303aa	115	172
CsWRKY89	8	174aa	112	170
CsWRKY90	8	266aa	204	260
CsWRKY91	8	314aa	136	196
CsWRKY92	8	1231aa	587	644
CsWRKY93	8	1153aa	228	285
CsWRKY94	8	557aa	304	363
CsWRKY95	8	304aa	132	190
CsWRKY96	8	304aa	132	190
CsWRKY97	8	215aa	130	148
CsWRKY98	8	273aa	112	172
CsWRKY99	9	518aa	256	312
CsWRKY100	9	230aa	94	154
CsWRKY101	9	305aa	127	187
CsWRKY102	9	278aa	71	129
CsWRKY103	9	200aa	106	164
CsWRKY104	9	309aa	232	289
CsWRKY105	9	369aa	288	345
CsWRKY106	9	270aa	143	202
CsWRKY107	9	304aa	146	205
CsWRKY108	9	572aa	328	386
CsWRKY109	9	194aa	111	169
CsWRKY110	10	343aa	261	319
CsWRKY111	10	308aa	173	231
CsWRKY112	10	332aa	252	309
CsWRKY113	10	477aa	180	236
CsWRKY114	10	564aa	214	271
CsWRKY115	10	514aa	138	195
CsWRKY116	10	361aa	287	345
CsWRKY117	10	319aa	148	208
CsWRKY118	10	309aa	140	198
CsWRKY119	10	360aa	117	175
CsWRKY120	10	325aa	175	232
CsWRKY121	10	181aa	98	156
CsWRKY122	11	343aa	261	319
CsWRKY123	11	632aa	188	246
CsWRKY124	11	333aa	253	310
CsWRKY125	11	476aa	175	231
CsWRKY126	11	649aa	242	299
CsWRKY127	11	565aa	214	271
CsWRKY128	11	701aa	141	181
CsWRKY129	11	360aa	286	344
CsWRKY130	11	317aa	146	206
CsWRKY131	11	309aa	140	198
CsWRKY132	11	541aa	298	356
CsWRKY133	11	324aa	174	231
CsWRKY134	11	179aa	96	154
CsWRKY135	11	329aa	184	241
CsWRKY136	11	1454aa	1334	1372
CsWRKY137	11	319aa	113	169
CsWRKY138	11	137aa	57	115
CsWRKY139	11	415aa	235	293
CsWRKY140	11	362aa	172	230
CsWRKY141	11	522aa	193	250
CsWRKY142	11	720aa	294	351

Table 4. Physical characteristics of WRKY gene family transcription factors

WRKY NAME	Chr	Length	Start	End
CsWRKY143	11	700aa	274	331
CsWRKY144	11	102aa	16	74
CsWRKY145	12	303aa	221	279
CsWRKY146	12	323aa	243	300
CsWRKY147	12	307aa	172	230
CsWRKY148	12	509aa	180	236
CsWRKY149	12	564aa	214	271
CsWRKY150	12	514aa	141	198
CsWRKY151	12	361aa	286	344
CsWRKY152	12	314aa	148	208
CsWRKY153	12	309aa	140	198
CsWRKY154	12	550aa	307	365
CsWRKY155	12	322aa	172	229
CsWRKY156	13	273aa	111	171
CsWRKY157	13	314aa	130	188
CsWRKY158	13	152aa	72	130
CsWRKY159	13	554aa	232	291
CsWRKY160	13	547aa	225	284
CsWRKY161	13	292aa	113	172
CsWRKY162	13	275aa	116	146
CsWRKY163	13	174aa	112	170
CsWRKY164	13	217aa	149	206
CsWRKY165	13	314aa	136	196
CsWRKY166	13	1700aa	231	288
CsWRKY167	13	532aa	295	353
CsWRKY168	13	491aa	238	297
CsWRKY169	14	304aa	132	190
CsWRKY170	14	519aa	233	289
CsWRKY171	14	541aa	240	299
CsWRKY172	14	265aa	75	133
CsWRKY173	14	288aa	141	198
CsWRKY174	14	434aa	216	274
CsWRKY175	15	395aa	222	279
CsWRKY176	15	471aa	206	262
CsWRKY177	15	831aa	271	324
CsWRKY178	15	468aa	116	174
CsWRKY179	16	286aa	128	186
CsWRKY180	16	312aa	123	184
CsWRKY181	16	384aa	317	375
CsWRKY182	16	393aa	165	222
CsWRKY183	16	110aa	86	107
CsWRKY184	16	244aa	103	163
CsWRKY185	16	353aa	212	271
CsWRKY186	16	142aa	1	60
CsWRKY187	16	272aa	131	188
CsWRKY188	16	385aa	204	263
CsWRKY189	16	200aa	106	164
CsWRKY190	16	310aa	233	290
CsWRKY191	16	330aa	250	307
CsWRKY192	16	270aa	143	202
CsWRKY193	16	254aa	96	155
CsWRKY194	16	571aa	327	385
CsWRKY195	16	194aa	111	169
CsWRKY196	17	526aa	241	297
CsWRKY197	17	540aa	239	298
CsWRKY198	17	384aa	75	133
CsWRKY199	17	288aa	141	198
CsWRKY200	17	435aa	216	274
CsWRKY201	17	624aa	329	386
CsWRKY202	18	328aa	183	240
CsWRKY203	18	1313aa	1204	1264
CsWRKY204	18	316aa	113	169
CsWRKY205	18	150aa	70	128

Table 4. Physical characteristics of WRKY gene family transcription factors

WRKY NAME	Chr	Length	Start	End
CsWRKY206	18	380aa	205	258
CsWRKY207	18	430aa	240	298
CsWRKY208	18	568aa	206	263
CsWRKY209	18	701aa	274	331
CsWRKY210	18	193aa	107	165
CsWRKY211	19	465aa	200	256
CsWRKY212	19	336aa	268	325
CsWRKY213	19	473aa	114	172
CsWRKY214	20	266aa	111	171
CsWRKY215	20	314aa	130	188
CsWRKY216	20	150aa	70	128
CsWRKY217	20	542aa	225	284
CsWRKY218	20	293aa	113	172
CsWRKY219	20	307aa	115	176
CsWRKY220	20	111aa	49	107
CsWRKY221	20	113aa	68	102
CsWRKY222	20	319aa	107	163
CsWRKY223	20	328aa	183	240
CsWRKY224	17	88aa	59	87

3.3. Identifying the function of unknown camelina motifs based on phylogenetic analysis

The conserved motifs in WRKY proteins were identified and analyzed using the MEME database. By placing the unknown motifs of the camelina plant in front of the known motifs of the Arabidopsis plant and drawing a phylogenetic tree, motifs with the same sequence and functional function in Arabidopsis plant were placed in the same branch, through which the function and The function of each camelina motif was also identified (Fig. 2).

3.4. Alignment of protein sequences and phylogenetic analysis

The transcription factor motif of camelina was blasted with the Arabidopsis model plant and the position of each motif on the gene was marked with different colors (Fig. 3).

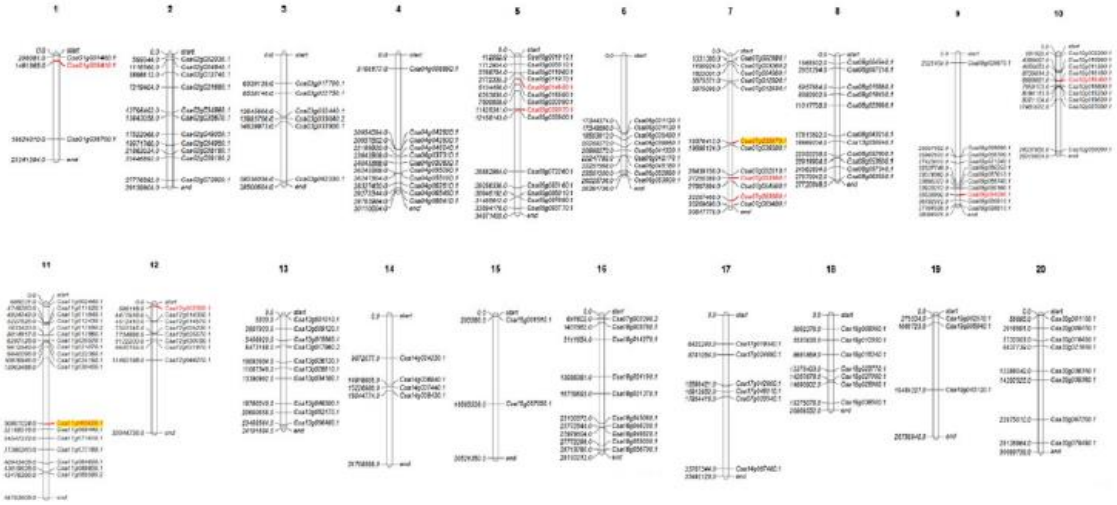

Figure 1. Distribution of 224 camelina transcription factors on 20 camelina chromosomes. Factors involved in drought stress are highlighted in yellow

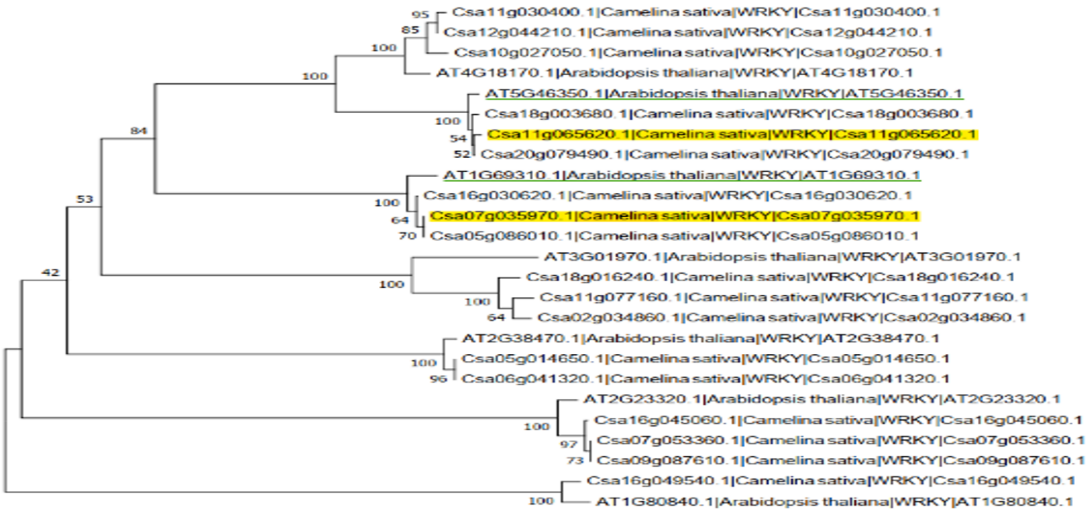

Figure 2. Identifying the function of unknown motifs of camelina based on the known motifs of Arabidopsis (motifs involved in drought stress are highlighted in yellow)

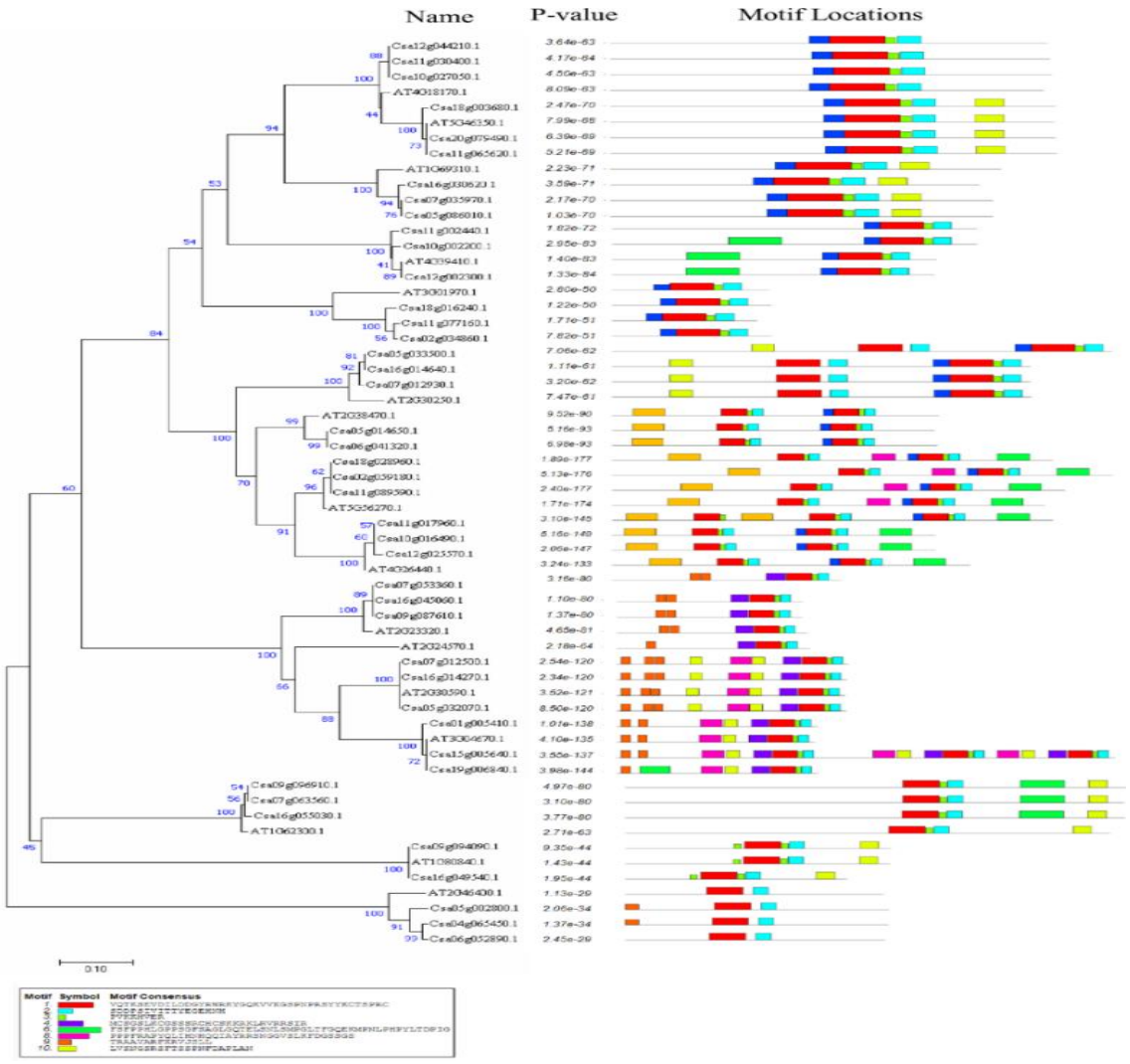

Figure 3. Phylogenetic tree of motifs involved in WRKY transcription factors based on Arabidopsis model plant transcription factors

3.5. Investigating the expression of two candidate genes in the WRKY gene family

In order to investigate and identify more WRKY genes of the WRKY gene family, 2 genes were selected and analyzed to determine the expression levels in the leaf tissue under drought stress in the camelina plant through qRT-PCR analysis. First, for each sample, the Ct level was determined using the reference gene by determining the baseline. Ct analysis was performed using the relative quantification method - ΔΔCt2. The expression pattern of both selected genes under stress indicates the importance of the effect of that gene. As result of the experiment, it showed that both candidate genes under drought stress had the highest expression in the tolerant varieties, and in the sensitive varieties, their expression was lower than in the control condition, which shows the importance of these two genes in increasing the resistance of the variety. The tolerant species are under drought stress. In order to investigate and identify more WRKY genes of the WRKY gene family, 2 genes were selected and analyzed to determine the expression levels in the leaf tissue under drought stress in the camelina plant through qRT-PCR analysis. First, for each sample, the Ct level was determined using the reference gene by determining the baseline. Ct analysis was performed using the relative quantification method - ΔΔCt2. The expression pattern of both selected genes under stress indicates the importance of the effect of that gene. As result of the experiment, it showed that both candidate genes under drought stress had the highest expression in the tolerant lines, and in the sensitive lines, their expression was lower than in the control condition, which shows the importance of these two genes in increasing the resistance of the variety. The tolerant species are under drought stress.

3.6. Expression pattern of CSWRKY genes under drought stress using qRT-PCR method

With the increase in the duration of drought and dehydration in camelina plants, the selected genes (WRKY 57 & WRKY 8) are expressed in a very low amount in the leaf tissue under normal conditions but strongly expressed in drought treatments in the tolerant varieties. They had a very significant increase in expression, but in sensitive varieties, they had an opposite trend. This issue shows the importance of these two genes in increasing resistance in tolerant lines under drought stress (Fig. 4 and 5).

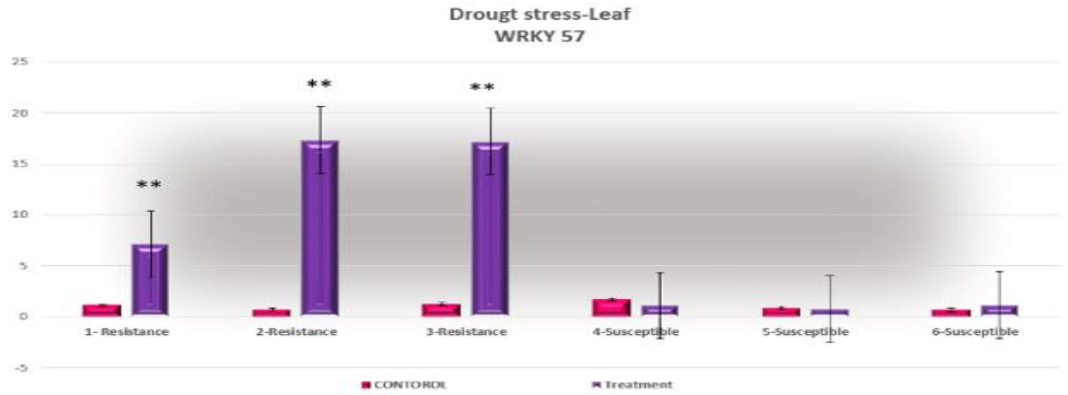

Figure 4. WRKY 57 gene expression pattern under drought stress in two tolerant and sensitive lines

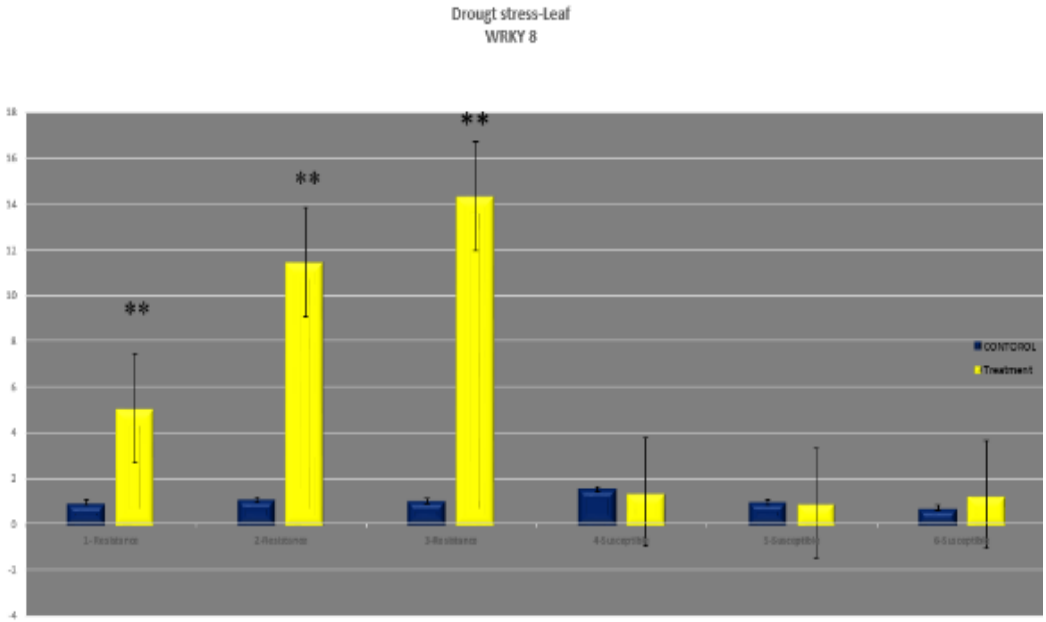

Figure 5. WRKY 8 gene expression pattern under drought stress in two tolerant and sensitive varieties

In this research, the expression of WRKY8 and WRKY 57 genes in camelina was investigated by RNA-seq technique and the expression of the mentioned genes under drought stress was very significant in two sensitive and resistant varieties, as Shi et al. (2018), by researching the soybean plant genome (*Glycine max*), they found that a WRKY gene named GmWRKY12 is involved in stress conditions and resistance to salinity and drought based on RNA-seq and qRT-PCR. GmWRKY12 is expressed in a very low amount in different tissues under normal conditions, but it is strongly expressed in salt and drought treatments. Also, in Wang et al. (2019), by researching wheat plants (*Triticum aestivum*), found that TaWRKY10 and TaWRKY45 genes had increased expression under drought stress in both tolerant and sensitive wheat varieties. In this research, Wang et al. (2019) concluded that TaWRKY45 increased expression in the tolerant variety under drought stress conditions, and the genes TaWRKY41, TaWRKY8, TaWRKY19, TaWRKY22, TaWRKY15 increased in expression in two sensitive and tolerant varieties. They

were completely the opposite. Wang et al. (2019) also investigated the cotton (*Gossypium hirstum*) genome and found that there is a special WRKY protein in the leaves of the cotton plant called GhWRKY33. By transferring GhWRKY33 to the Arabidopsis model plant, the transgenic Arabidopsis plant is overexpressed under drought stress and causes the transgenic plant to wilt faster than its wild type, and the stomata of Arabidopsis transgenic with the GhWRKY33 gene are more open than those of the wild type. In addition, they found that GhWRKY33 is also involved in the abscisic acid signaling pathway and reduces the transgenic plant's sensitivity to abscisic acid. In general, during this research, they found that GhWRKY33 has both negative and positive regulatory roles. Environmental stress is one of the most important factors limiting growth and production in plants. Plants need a regulatory mechanism in response to biological stresses due to their inability to move and escape the stresses and changes created for survival, reproduction and production. Plants respond to stresses through the expression of responsive genes, among which transcription factors play a central and important role in regulating gene expression. In this research, 209 WRKY genes were identified through the database, and to further investigate and identify the function of genes encoding WRKY transcription factors, 2 genes, WRKY8 and WRKY57, were randomly selected to determine the level of expression in camelina plant leaf tissue under drought stress were selected and analyzed through qRT-PCR. The results of increased expression of both genes under drought stress in the tolerant lines indicate that these two genes clearly play a role in the occurrence of resistance and tolerance to biological stresses during the life of the plant.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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