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Identification and expression analysis of TALE superfamily genes explore their key roles in response to abiotic stress in *Brassica napus*

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Abstract

Background The three-amino-acid-loop-extension (*TALE*) superfamily genes are broadly present in plants and play important roles in plant growth, development, and abiotic stress responses. So far, the *TALE* family in *B.napus* have not been systematically studied, especially their potential roles in response to abiotic stress.

Results In this study, we identified 74 TALE family genes distributed on 19 chromosomes in the *B. napus* genome using bioinformatics methods. Phylogenetic analysis divided the BnTALE superfamily into two subfamilies, the BEL1-like (BLH/BELL homeodomain) and the KNOX (KNOTTED-like homeodomain) subfamilies. Moreover, the KNOX subfamily could be further categorized into three clades (KNOX Class I, KNOX Class II, and KNOX Class III). BnTALE members in the same subclass or branch of the phylogenetic tree generally showed similar gene structures and conserved domain compositions, which may indicate that they have similar biological functions. The *BnTALE* promoter regions contained many hormone-related elements and stress response elements. Duplication events identification analysis showed that WGD/segmental duplications were the main drivers of amplification during the evolution of *TALE* genes, and most of the duplicated *BnTALE genes* underwent purifying selection pressures during evolution. Potential protein interaction network analysis showed that a total of 12,615 proteins might interact with TALE proteins in *B. napus.* RNA-seq and qRT-PCR analyses showed that the expression of *BnTALE* was tissue-differentiated and can be induced by abiotic stresses such as dehydration, cold, and NaCl stress. In addition, weighted gene co-expression network analysis (WGCNA) identified four co-expression modules containing the most *BnTALE* genes, which would be notably related to dehydration and cold stresses.

Conclusions Our study paves the way for future gene functional research of *BnTALE* and facilitate their applications in the genetic improvement of *B. napus* in response to abiotic stresses.

Keywords *Brassica napus*, *TALE* gene family, Tissue expression, WGCNA, Abiotic stress

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Background

The homeobox genes encoding the transcriptional regulatory factors with highly conserved homeodomain play an important role in the growth and development of plants [1, 2]. In PlantTFDB, the homeobox genes were divided into five classes: homeodomain-leucine zipper (HD-ZIP), three-amino-acid-loop-extension (TALE), wuschel homeobox (WOX), homeobox-plant homeodomain (HB-PHD), and HB-other [3].

The TALE superclass, which consists of 63 amino acids forming two helices and three additional amino acid residues (P-Y-P) connecting the first and second helices, is a pivotal transcription factor that broadly exist in plants [1, 4–6]. The TALE family consists of the KNOX (KNOTTED-like homeodomain) and BELL (BEL1-Like homeodomain) subfamilies [7]. The KNOX proteins include four domains: KNOX1, KNOX2, ELK, and KN homeodomain and it can be divided into three classes according to the structure characteristics of homeodomain and expression patterns. Both class I and II KNOX proteins contain four members while Class III has only one member, KNATM, which lack the homeodomain and is only found in dicotyledons [4, 8–10]. BELL proteins contain SKY, BELL, and homeodomain [11]. The BELL and KNOX proteins have been shown to specifically recognize and bind to form the BELL-KNOX heterodimeric proteins [12], which are essential for nuclear localization, binding of target genes, and playing a regulatory role in biological processes of the two transcription factor proteins [13, 14].

The *TALE* gene family plays a regulatory role in plant growth, development [2], and different biological processes, such as meristem formation, organ morphogenesis, secondary cell wall development [15] and signal transduction [16]. Previous research has shown that the *KNOX1* gene plays an important role in the development and maintenance of meristem. The *KNOX2* gene plays a vital role in regulating the secondary growth of plant cell walls and the development of roots, stems, seed coats, and heartwood [17–20]. *BELL* gene plays essential regulatory roles in ovule development, frond development, and fruit development [21, 22]. The *LeT6/TKn2*, which belongs to KNOX class I was involved in morphological development in tomato fruit [23]. Kim et al. (2013) established that the AtBLH1 protein regulates seed germination and seedling development by cooperating with the AtKNAT3 protein [13]. GmSBH1 identified in *Glycine max*, could influences leaf phenotype [24]. ATH1 (*Arabidopsis thaliana* homeobox 1) interacts with STM (homeobox protein SHOOT MERISTEMLESS) and KNAT2 (*A. thaliana* KNOX 2) to participate in the development of meristems and inflorescence tissue [20, 25]. In *A. thaliana* KNOX Class I, AtKNAT2 showed expression in the internal vegetative shoot apical meristem (SAM) [26]. In *A. thaliana*, AtKNAT7 (class II KNOX protein) negatively regulates secondary cell wall deposition. It inhibits secondary cell wall lignin synthesis by forming a heterodimer with AtBLH6 [27, 28]. PoptrKNAT7, GhKNAT7-A03, and OsKNAT7 (Class II KNOX protein) were reported to be crucial for cell elongation and secondary cell wall (SCW) biosynthesis [7, 17, 29].

In addition, *TALE* are also involved in hormone regulatory pathways and response to a variety of abiotic stresses [30]. Ectopic expression of the maize KNOX-like gene *KN1* in leaves enhances auxin signaling in maize [31]. In *A. thaliana*, BLH1 activates the expression of abscisic acid (ABA) response gene abscisic acid insensitive 3 (*ABI3*) by forming a heterodimer with KNAT3, thereby promoting the plant response to ABA at seed germination and seedling stage [13]. Recent studies have identified 11 NaCl stress response genes from the poplar *TALE* transcription factor family. Among them, the expression of *ptTALE5*, a member of this family, was upregulated after NaCl stress treatment, which may play an important role in the response of poplar to NaCl stress [32]. Wang et al. (2021) reported that the expression level of *GmTALE* genes changes in response to NaCl stress and dehydration stress [2]. In poplars, the type I KNOX gene *PagKNAT2/6b* can enhance plant dehydration resistance by inhibiting gibberellin synthesis and adjusting plant phenotype [33]. Overexpression of the *Triticum aestivum* KNOX-like gene *TaKNOX11-A* in *A. thaliana* can enhance the NaCl tolerance and dehydration resistance of the plant [34].

Brassica napus, formed by spontaneous hybridization between *B. rapa* (AA genome) and *B. oleracea* (CC genome) [35], is the second largest oil crop in the world and plays a crucial role in the production of edible oil. During the growth and development of rapeseed, there are many abiotic stress problems, such as high or low temperature and soil salinity, which seriously affect the yield and quality of rapeseed. Previous studies on several plant species identified and analyzed *TALE* gene families at the genome-wide level in *Triticum aestivum* [34], cotton [7], soybean [2], tomato [16], and so on. However, no systematic study of the TALE family in *B. napus* and their expression pattern in tissues and under various stresses has been performed.

In this study, based on the published genome [35] and transcriptome datasets [36, 37], we identified members of the *TALE* gene family in the *B. napus* genome. The gene structure, position in the genome, phylogenetic relationships, cis-acting elements in the promoter region, physicochemical properties of coding proteins, tissue expression characteristics, and possible protein interaction network analysis were analyzed. Besides, the gene expression profiling in different tissues during rapeseed plant development, NaCl, and dehydration stresses were

also carried out by qRT-PCR. This study could lay a foundation to explore the role of *TALE* gene family in *B. napus* growth and development and identify some promising or key *TALE* genes that could be useful for genetic improvement of abiotic resistance in rapeseed.

Results

Identification of the TALE gene family members in *B. napus*

By using the Hidden Markov Model, we searched for ELK, KNOX1, KNOX2, POX, and Homeobox_KN structural domains in the rapeseed cultivar "*Darmor*-bzh" as a systematic screen for *TALE* genes at the genome-wide level. We identified a total of 74 *TALE* genes in the *B. napus* genome and named these *BnTALE* genes according to their location on the chromosomes. These *BnTALE* genes were unevenly distributed on the chromosomes (Table 1; Fig. 1). The A03 chromosome contained the largest number of *TALE* genes (7) while only one *TALE* gene was present on chromosomes A05, A07, and A10, respectively. Subcellular localization prediction showed that most of the BnTALE were located in the nucleus. According to domain differences, the TALE of *B. napus* can be divided into two subfamilies, BELL and KNOX. The sizes and physicochemical properties of the BnTALE vary greatly among the two sub-families. The number of exons contained in the BELL subfamily ranged from four to nine, while the number of exons for the KNOX subfamily members was between one to seven. The length of BELL subfamily proteins varied between 290 (BnTALE34 and BnTALE58) to 692 (BnTALE45) amino acids (aa), and the molecular weights varied from 32.98 to 75.64 kDa, with an average of 59.74 kDa. In the KNOX subfamily, BnTALE66 with 103 aa was the smallest TALE protein, while BnTALE59 was the largest protein with 445 AA, the molecular weights varied from 11.09 to 48.91 kDa, with an average of 32.69 kDa. Therefore, it can be concluded that the proteins of the BELL subfamily in *B. napus* are longer and bigger than the KNOX subfamily proteins. Isoelectric point analysis showed that the isoelectric point of BELL subfamily proteins ranged from 5.22 to 8.46, with 87.5% members (35/40) exhibiting acidic pI values. The isoelectric point of KNOX subfamily proteins ranged from 4.47 to 8.99, and with the exception of BnTALE25, all the proteins have isoelectric points less than seven.

Phylogenetic relationship analysis of the *TALE* gene family

To clarify the phylogeny and taxonomic relationships among TALEs in *B. napus*, a phylogenetic tree was constructed with the full-length sequence of 74 BnTALE proteins and 22 AtTALE proteins. Consistent with the classification of *(A) thaliana TALE* gene family, the *B. napus* TALE proteins were clearly divided into two main clades (The BELL subfamily and the KNOX subfamily) (Fig. 2). The BELL subfamily consisted of 40 BnTALE proteins and 13 AtTALE proteins. The KNOX subfamily had a total of 34 BnTALE proteins and nine AtTALE proteins, which can be further divided into class I, II, III, we can infer that functional differentiation may be existed in this *TALE* gene subfamily. Among them, Class II was the largest branch, consisting of 20 BnTALE proteins and four AtTALE proteins, Class I was comprised of 10 BnTALE proteins and four AtTALE proteins, and Class III consisted of four BnTALE proteins and one AtTALE protein. The evolutionary tree showed a one-to-many relationship between the *A. thaliana TALE* gene family and the *B. napus TALE* gene family, indicating that gene family replication events occurred after genome differentiation between *B. napus* and *A. thaliana*. From the phylogenetic tree, it can be inferred that the TALEs in *B. napus* and *A. thaliana* were evolutionary conserved.

Gene structure and domains analysis of the *TALE* gene family in *B. napus*

To obtain insights into structural feature of *BnTALE*, TBtools was used to display the gene structure. In the *TALE* gene family, 21 genes had 5′ and 3′ UTRs, 34 genes contained only one side of the UTR, and 19 genes did not have UTR (Fig. 3). Many genes in the same subfamily exhibit similar structures, especially in the KNOXII and KNOXIII subfamilies, indicating the high conservation. In other three subfamilies, although the length of gene sequences varied highly, the majority of the genes possessed similar exon numbers and arrangement order.

Furthermore, the number of domains contained in different TALE subfamilies varied from one to four (Fig. 3). Overall, the domain composition patterns of TALE proteins in the same subfamily were very similar, suggesting that the proteins were highly conserved. Almost all TALE proteins (87.8%) contained Homeobox_KN, indicating Homeobox_KN was very conservative and these *TALEs* may have common functions. The BELL subfamily of BnTALE contained two to three protein-conserved domains, while the domain of BnTALE in the KNOX subfamily varied between one to four. The domain composition pattern of the BELL subfamily of BnTALE was highly similar, and all contained POX and Homeobox_KN. The POX was specific to this subfamily and may be associated with subfamily-specific functions. In the KNOX subfamily, almost all the genes possessed KNOX1 or KNOX2, indicating that these two domains were strongly conserved and related to the function of the KNOX subfamily of the BnTALE. Furthermore, different subclasses of the KNOX subfamily contained different domains and patterns, for example, KNOX III contained the least number of domains (1 to 2), KNOX I contained two to four domains, while the number of domains contained

Table 1 The three-amino-acid-loop-extension (*TALE*) gene family members in *B. napus*

Gene	Name	Chr	Start	End	Amino acids	Exon number	pI	MW(kDa)	Subcellular Localization	Sub-family
BnaA01g00980D	*BnTALE1*	A01	535,291	540,515	670	4	6.59	73.51279	Nucleus	BELL
BnaA01g03890D	*BnTALE2*	A01	1,798,245	1,799,923	467	4	6.53	53.05888	Nucleus	BELL
BnaA02g17200D	*BnTALE6*	A02	10,314,345	10,316,831	479	4	6.56	54.26916	Nucleus	BELL
BnaA03g16450D	*BnTALE9*	A03	7,679,196	7,682,325	672	4	6.61	73.41444	Nucleus	BELL
BnaA03g39060D	*BnTALE11*	A03	19,441,616	19,443,428	450	4	5.82	50.26421	Nucleus	BELL
BnaA03g52290D	*BnTALE13*	A03	27,260,893	27,262,492	438	4	6.32	50.00129	Nucleus	BELL
BnaA03g52940D	*BnTALE14*	A03	27,658,343	27,665,800	686	6	7.31	64.21826	Nucleus	BELL
BnaA04g13860D	*BnTALE15*	A04	11,725,811	11,731,115	646	5	6.57	71.24468	Nucleus	BELL
BnaA04g15670D	*BnTALE16*	A04	12,928,777	12,931,158	449	4	7.26	50.4209	Nucleus	BELL
BnaA04g20970D	*BnTALE17*	A04	16,034,502	16,037,438	633	6	6.5	68.73016	Nucleus	BELL
BnaA05g08310D	*BnTALE18*	A05	4,587,942	4,591,572	629	7	6.33	69.40377	Nucleus	BELL
BnaA06g13850D	*BnTALE20*	A06	7,368,318	7,369,995	390	4	6.24	44.76677	Nucleus	BELL
BnaA07g21690D	*BnTALE22*	A07	16,762,939	16,765,267	474	4	6.29	53.73141	Nucleus	BELL
BnaA08g15450D	*BnTALE23*	A08	12,850,348	12,855,102	668	5	6.6	73.37995	Nucleus	BELL
BnaA08g21960D	*BnTALE26*	A08	16,112,826	16,115,895	507	6	5.65	57.18188	Nucleus	BELL
BnaA09g41850D	*BnTALE30*	A09	29,166,242	29,171,316	609	4	6.31	67.57958	Nucleus	BELL
BnaA10g27410D	*BnTALE33*	A10	17,257,903	17,261,512	579	5	7.12	62.49513	Nucleus	BELL
BnaAnng09210D	*BnTALE34*	Ann_random	9,702,570	9,704,594	290	5	5.8	32.99845	Nucleus	BELL
BnaAnng09220D	*BnTALE35*	Ann_random	9,706,433	9,709,540	517	5	6.33	58.95304	Nucleus	BELL
BnaAnng29380D	*BnTALE36*	Ann_random	33,665,323	33,668,807	575	5	6.8	61.93265	Nucleus	BELL
BnaC01g02010D	*BnTALE38*	C01	1,008,510	1,013,374	668	5	6.65	73.40157	Nucleus	BELL
BnaC01g05260D	*BnTALE39*	C01	2,691,346	2,692,991	462	4	6.32	52.67547	Nucleus	BELL
BnaC02g03640D	*BnTALE41*	C02	1,735,436	1,738,924	576	5	6.94	61.96476	Nucleus	BELL
BnaC03g19820D	*BnTALE45*	C03	10,358,826	10,362,353	692	6	6.74	75.64013	Nucleus	BELL
BnaC03g46250D	*BnTALE46*	C03	31,174,783	31,176,447	456	4	5.64	50.82279	Nucleus	BELL
BnaC03g61760D	*BnTALE47*	C03	50,983,453	50,988,199	671	5	6.68	74.04248	Nucleus	BELL
BnaC04g09330D	*BnTALE48*	C04	7,068,991	7,072,342	589	9	6.27	65.53572	Nucleus	BELL
BnaC04g38940D	*BnTALE50*	C04	40,015,293	40,018,054	450	4	8.46	50.69834	Nucleus	BELL
BnaC04g44970D	*BnTALE51*	C04	44,808,821	44,812,263	637	8	6.53	69.28185	Nucleus	BELL
BnaC04g56300D	*BnTALE52*	C04_random	3,995,247	4,001,065	649	4	6.56	71.45187	Nucleus	BELL
BnaC05g15280D	*BnTALE54*	C05	9,089,226	9,091,135	520	4	5.97	58.90236	Nucleus	BELL
BnaC06g22380D	*BnTALE56*	C06	24,450,176	24,452,898	504	5	6.08	56.71476	Nucleus	BELL
BnaC06g36180D	*BnTALE57*	C06	34,787,789	34,790,730	515	5	6.43	58.5538	Nucleus	BELL
BnaC06g36190D	*BnTALE58*	C06	34,794,361	34,796,367	290	5	5.94	32.98147	Nucleus	BELL
BnaC07g44050D	*BnTALE61*	C07	42,788,812	42,790,447	453	4	6.14	51.5971	Nucleus	BELL
BnaC08g19170D	*BnTALE63*	C08	22,140,847	22,142,559	419	5	5.22	47.23048	Nucleus	BELL
BnaC08g34350D	*BnTALE64*	C08	32,473,743	32,479,230	602	5	6.37	66.75979	Nucleus	BELL
BnaCnng03740D	*BnTALE68*	Cnn_random	2,892,511	2,896,094	576	5	7.14	62.15578	Nucleus	BELL
BnaCnng11290D	*BnTALE69*	Cnn_random	10,582,998	10,587,255	569	6	6.36	64.19103	Nucleus	BELL
BnaCnng21740D	*BnTALE71*	Cnn_random	20,394,052	20,396,527	472	4	6.51	53.4803	Nucleus	BELL
BnaA02g14950D	*BnTALE5*	A02	8,569,079	8,574,265	328	5	4.91	36.93633	Nucleus	KNOX I
BnaA03g23610D	*BnTALE10*	A03	11,303,177	11,306,833	403	6	6.1	46.35865	Nucleus	KNOX I
BnaA08g20500D	*BnTALE24*	A08	15,447,762	15,449,510	209	4	4.59	22.92107	Nucleus	KNOX I
BnaA09g13310D	*BnTALE28*	A09	7,413,897	7,416,888	384	4	6.12	43.10947	Nucleus	KNOX I
BnaA09g31100D	*BnTALE29*	A09	23,130,703	23,132,290	236	5	4.53	26.11447	Nucleus	KNOX I
BnaC02g19900D	*BnTALE42*	C02	16,260,036	16,265,948	328	5	4.9	36.89625	Nucleus	KNOX I
BnaC05g18670D	*BnTALE55*	C05	12,356,472	12,357,686	222	4	4.47	24.45232	Nucleus	KNOX I
BnaC08g06320D	*BnTALE62*	C08	8,766,857	8,772,834	315	5	5.22	35.23275	Nucleus	KNOX I
BnaC09g13580D	*BnTALE67*	C09	10,235,596	10,238,634	384	4	6.22	43.19965	Nucleus	KNOX I
BnaCnng59830D	*BnTALE73*	Cnn_random	59,614,805	59,616,584	350	5	6.17	40.19479	Nucleus	KNOX I
BnaA01g04870D	*BnTALE3*	A01	2,258,376	2,260,668	375	5	5.8	41.90902	Nucleus	KNOX II
BnaA02g00810D	*BnTALE4*	A02	304,150	306,956	394	6	5.91	44.28204	Nucleus	KNOX II

Table 1 (continued)

Gene	Name	Chr	Start	End	Amino acids	Exon number	pI	MW(kDa)	Subcellular Localization	Sub-family
BnaA02g32110D	*BnTALE7*	A02	23,108,587	23,111,116	421	6	5.62	46.28523	Nucleus	KNOX II
BnaA03g03190D	*BnTALE8*	A03	1,539,765	1,542,572	387	6	5.94	43.14774	Nucleus	KNOX II
BnaA03g51900D	*BnTALE12*	A03	27,019,152	27,020,903	383	6	5.72	43.17633	Nucleus	KNOX II
BnaA06g27560D	*BnTALE21*	A06	18,939,822	18,941,622	300	6	6.08	33.43057	Nucleus	KNOX II
BnaA08g20510D	*BnTALE25*	A08	15,451,084	15,453,936	122	4	8.99	14.02783	Nucleus	KNOX II
BnaA09g12980D	*BnTALE27*	A09	7,016,927	7,021,165	294	5	5.88	33.01723	Nucleus	KNOX II
BnaA09g52990D	*BnTALE32*	A09_random	786,104	789,831	295	6	6.14	33.10331	Nucleus	KNOX II
BnaAnng30720D	*BnTALE37*	Ann_random	35,042,409	35,043,398	205	5	6.23	23.25746	Nucleus	KNOX II
BnaC01g06410D	*BnTALE40*	C01	3,350,853	3,353,173	375	5	5.98	41.85796	Nucleus	KNOX II
BnaC02g40790D	*BnTALE43*	C02	43,802,090	43,804,797	405	6	5.68	44.84264	Nucleus	KNOX II
BnaC03g04580D	*BnTALE44*	C03	2,188,077	2,190,704	303	6	6.3	33.77428	Nucleus	KNOX II
BnaC04g20090D	*BnTALE49*	C04	21,173,379	21,173,759	105	2	5.03	11.45896	Cytoplasm	KNOX II
BnaC07g29530D	*BnTALE59*	C07	34,248,589	34,253,723	445	7	5.22	48.90775	Nucleus	KNOX II
BnaC07g43650D	*BnTALE60*	C07	42,610,676	42,612,632	401	6	5.59	45.346	Nucleus	KNOX II
BnaC09g12900D	*BnTALE66*	C09	9,424,016	9,424,324	103	1	5.35	11.09354	Cytoplasm	KNOX II
BnaCnng20070D	*BnTALE70*	Cnn_random	18,852,728	18,855,246	394	6	5.88	44.23395	Nucleus	KNOX II
BnaCnng51440D	*BnTALE72*	Cnn_random	50,877,922	50,881,532	295	5	6.06	33.08529	Nucleus	KNOX II
BnaCnng70390D	*BnTALE74*	Cnn_random	70,412,295	70,413,281	206	4	6.53	23.48277	Nucleus	KNOX II
BnaA06g09570D	*BnTALE19*	A06	5,123,287	5,124,136	142	3	4.66	16.25438	Nucleus	KNOX III
BnaA09g45470D	*BnTALE31*	A09	31,056,313	31,057,006	136	3	4.47	15.31036	Nucleus	KNOX III
BnaC05g10940D	*BnTALE53*	C05	6,337,554	6,338,427	138	3	5.07	15.70188	Nucleus	KNOX III
BnaC08g39310D	*BnTALE65*	C08	35,085,126	35,085,803	136	3	4.49	15.24631	Nucleus	KNOX III

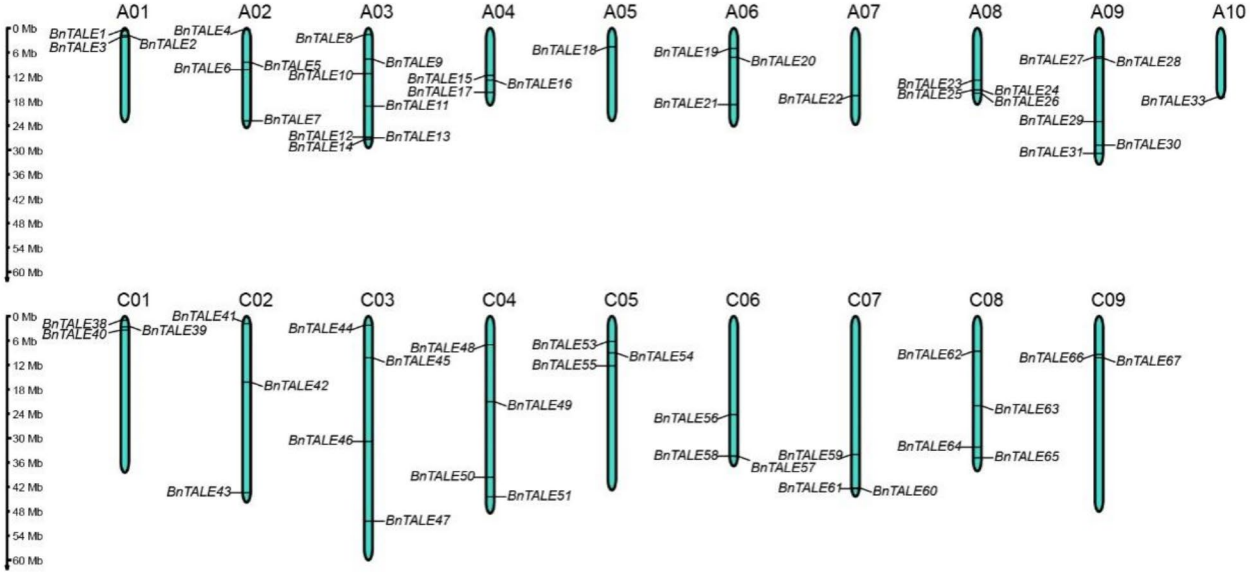

Fig. 1 Location of the *B. napus TALE* genes on chromosomes. The y-axes represented the chromosomes length

in the KNOX II subfamily proteins varied highly, ranging from 1 to 4.

Analysis of cis-acting elements within the promoters of *BnTALE*

Analysis of promoter characteristics of *B. napus TALE* gene family by PlantCARE software showed that the number of cis-acting elements contained in the *TALE* gene family varied widely (Fig. 4). *BnTALE25* contained the highest number of the cis-regulatory elements (25), followed by *BnTALE20* (24), *BnTALE3* (23), and *BnTALE29* (23), while *BnTALE16* contained only two elements (Table S1). As to the type of cis-acting elements, the highest number of cis-acting elements in the

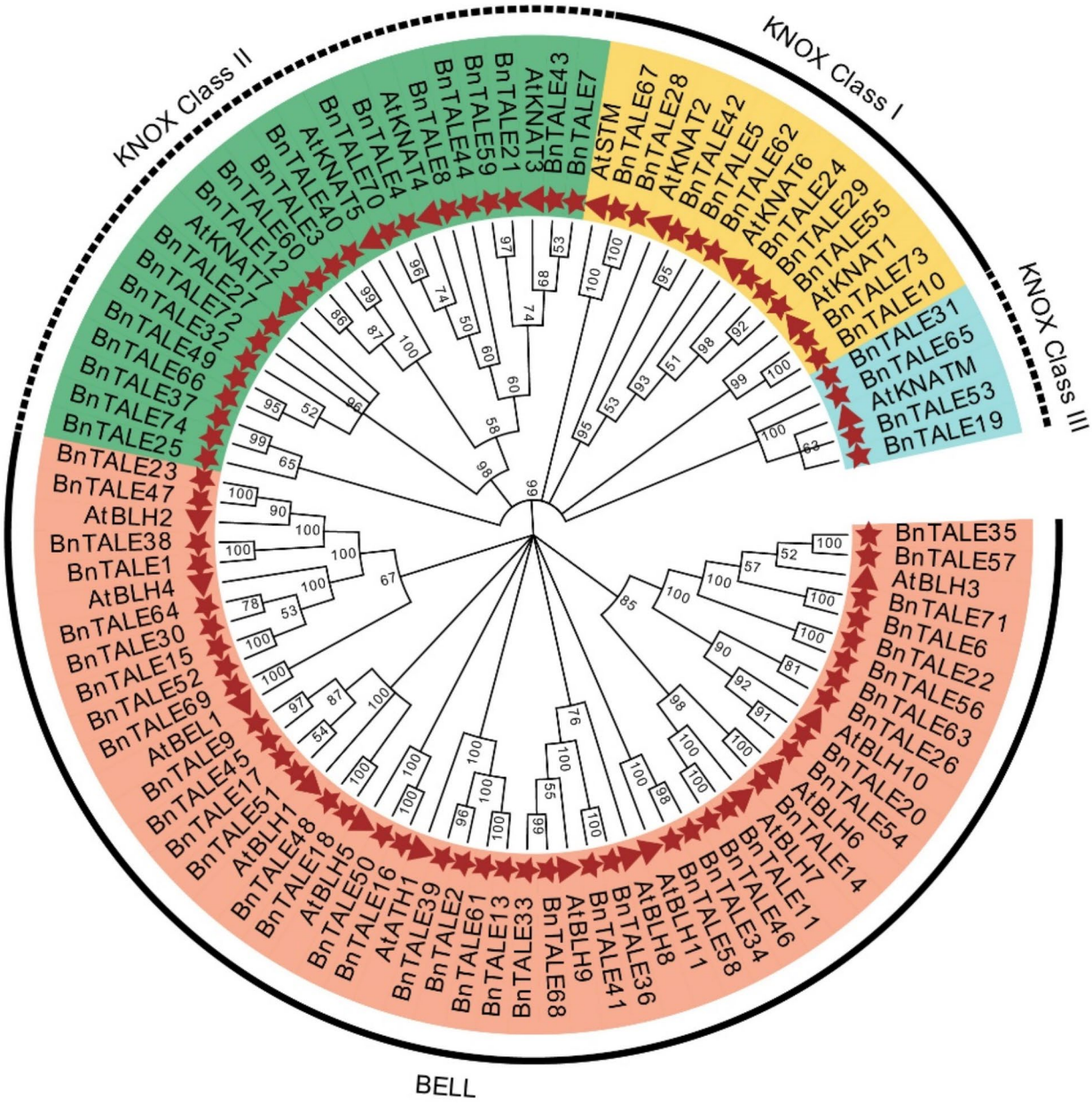

Fig. 2 Phylogenetic tree of the *B. napus* and *A. thaliana TALE* gene families. The neighbor-joining tree was generated using the amino acid sequences of the TALE proteins through the MEGA7 program and neighbor-joining (NJ) method, with 1000 bootstrap replicates. The two major phylogenetic clades (The BELL subfamily and the KNOX subfamily) are labeled and the TALEs from *B. napus* and *A. thaliana* are marked with asterisks and triangles, respectively

promoters of *BnTALE* genes were hormone-responsive elements (509), followed by environmental stress-responsive elements (347), and developmental-responsive elements (110). Among the hormone response elements, the most were ABRE elements (152), followed by ERE elements (148) and CGTCA-motif (87). Among the stress response elements, the most were ABRE elements (150), followed by WUN-motif elements (61). Specifically, the hormone response element ABRE, which is activated by ABA, could regulate the corresponding gene expression. *BnTALE25* contained the largest number of ABREs (9), followed by *BnTALE20* (6), *BnTALE4*, *BnTALE26*, *BnTALE3*, *BnTALE9*, and *BnTALE40* all contained five ABREs. Previous studies showed that there is a correlation between hormone response and plant resistance to abiotic stress [38]. Such as ABRE is associated with the plant response to drought stress [39], this implies that *BnTALE* may assist plants with adaptation to drought stress. *BnTALE64* contained the largest number of ERE components (6), *BnTALE71*, *BnTALE22*,

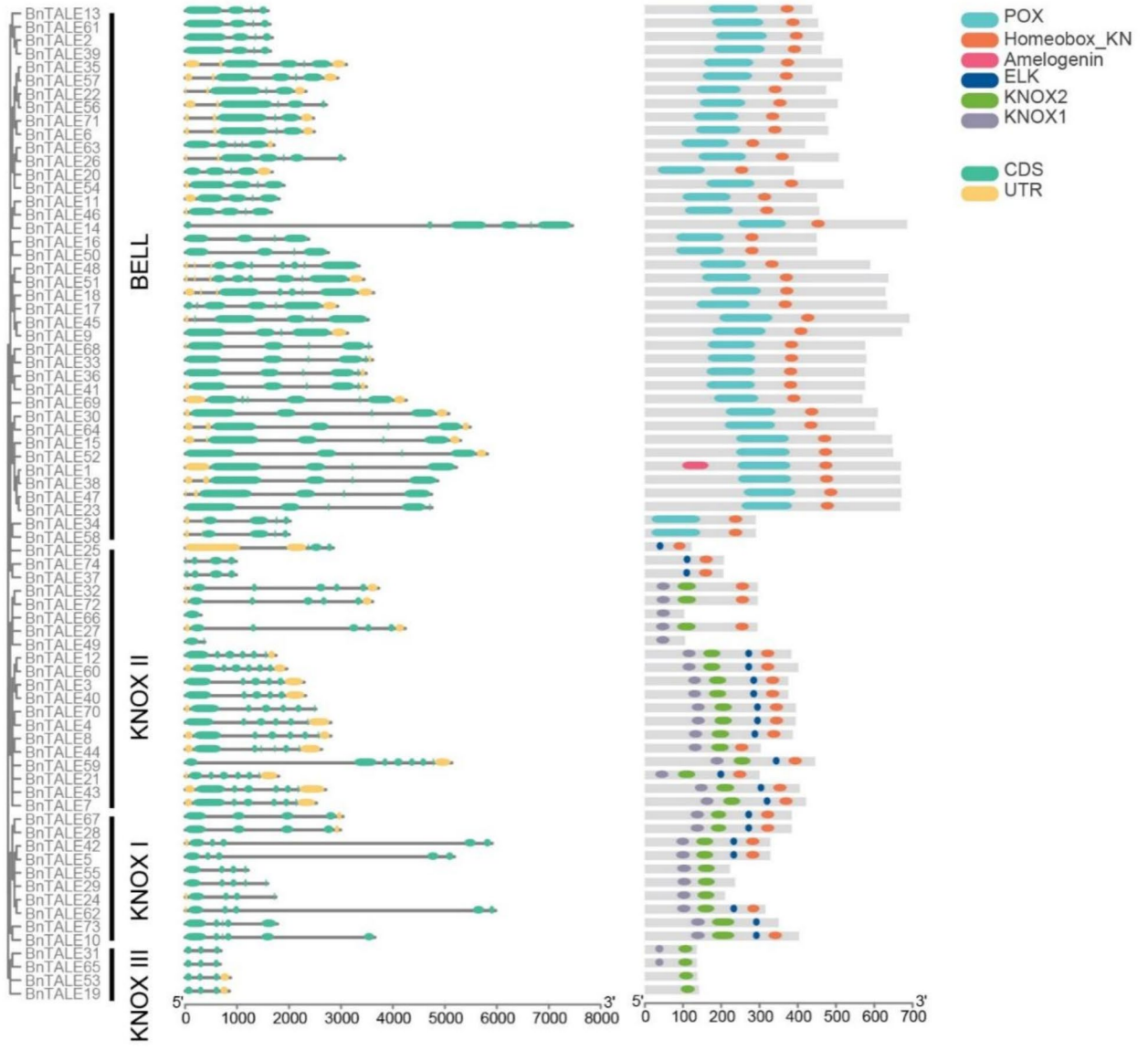

Fig. 3 *B. napus TALE* family gene structures and TALE proteins domains. The yellow box, the green box and the horizontal line represent UTRs, CDSs and introns, respectively. The length of the yellow box, the green box and the horizontal line represents the relative lengths of the corresponding UTRs, CDSs and introns

BnTALE70, *BnTALE74* also contained many ERE elements. *BnTALE20* contained more CGTCA elements (5). *BnTALE29* contained the largest number of ARE elements (8), followed by *BnTALE3* (6). The promoter of the *B. napus TALE* gene family contained a small number of TCTC box (3), MBSI (5), and GC motif (6).

The duplication events of *BnTALE* genes

To explore the expansion patterns of the *B. napus TALE* genes, gene duplication events were identified. A total of 105 *TALE* gene pairs were obtained by MCScanX program. Among them, 24 pairs were found in the A subgenome, 15 pairs occurred in the C subgenome, and the other 66 pairs derived from the A and C subgenomes respectively (Fig. 5). Most of the *TALE* genes (65/74) were defined as whole-genome duplication (WGD) or segmental duplication events, and only nine genes were caused by dispersed duplication. Therefore, the increase of *TALE* genes in the *B. napus* genome may be mainly attributed to WGD/segmental duplication. To assess the direction and strength of natural selection pressure of the *TALE* family during evolution, the non-synonymous/synonymous substitution ratio (Ka/Ks) for each duplicated gene pair was counted. The Ka/Ks ratio of the duplicated *TALEs* in *B. napus* varied from 0.0384 to 1.7325 with an average of 0.2291 (Table S2). Except *BnTALE27* and

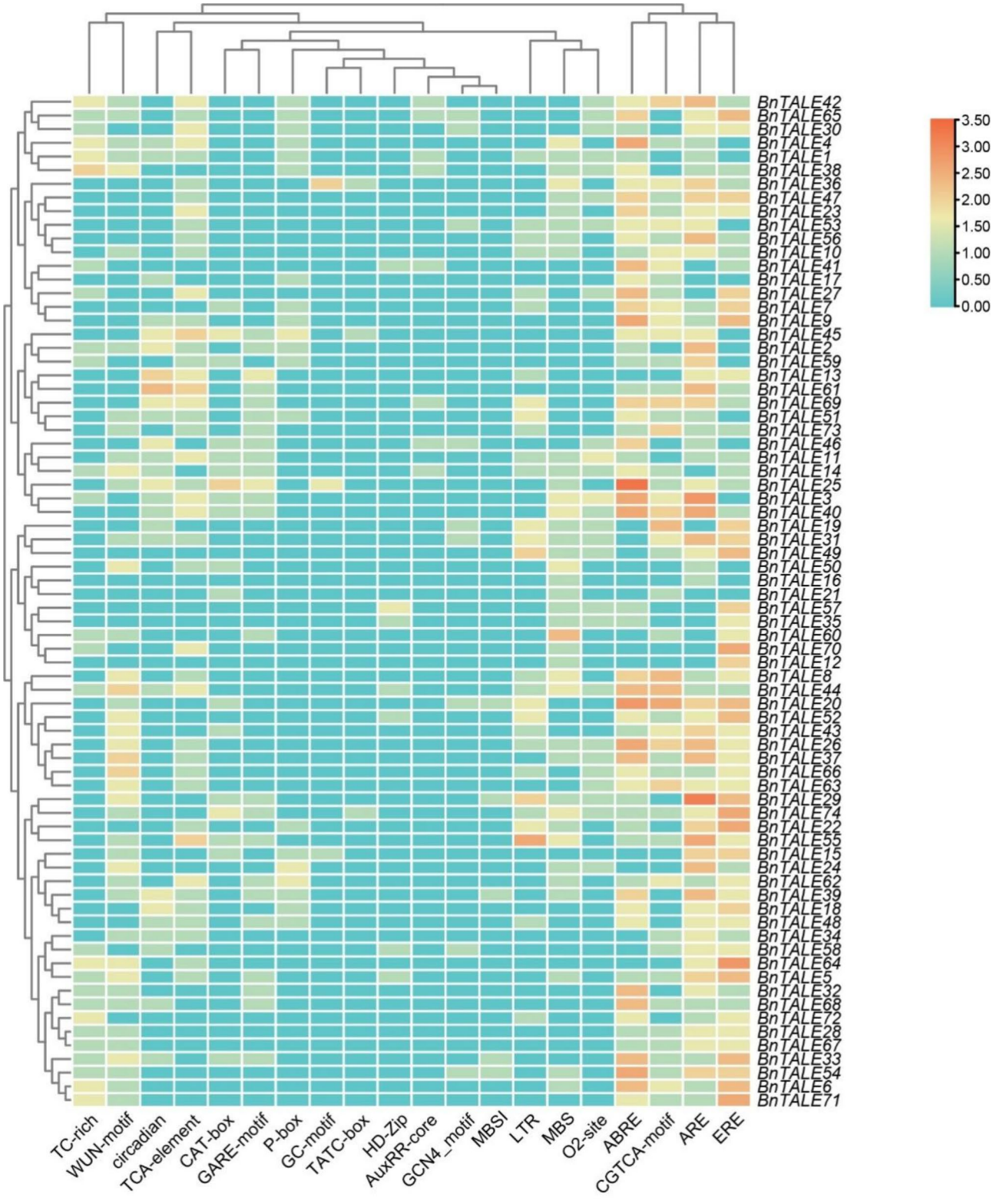

Fig. 4 Heatmap of the cis-regulatory elements for 74 *TALE* genes in *B. napus* normalized to log2 transformation. The color scale represents the number of elements from low (blue color) to high (red color)

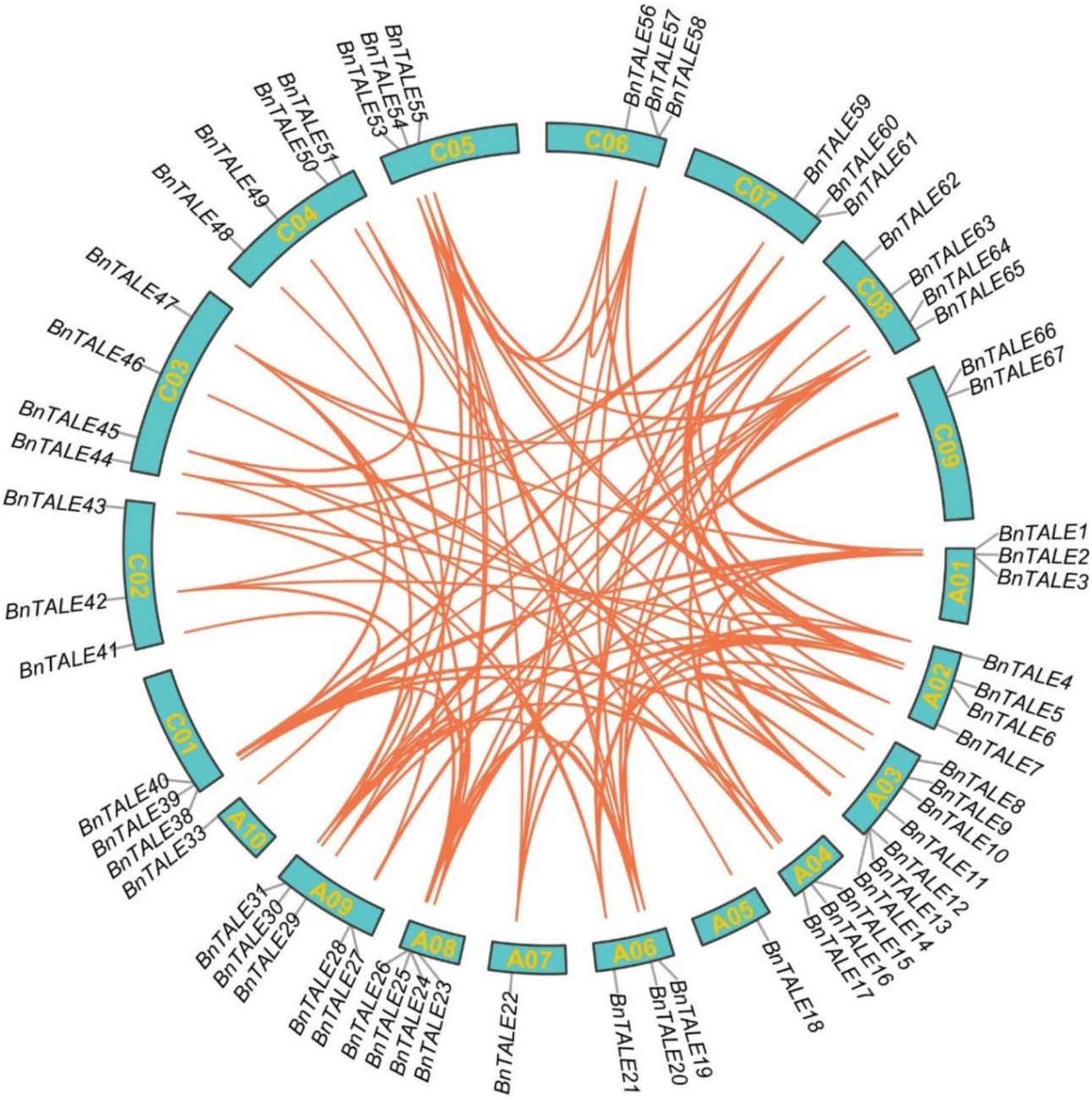

Fig. 5 The duplicate gene pair analysis of *TALE* genes in *B. napus*. Red lines indicate duplicated *TALE* gene pairs. Chromosome numbers are shown in the green box. The name and location of the *TALE* gene is marked on the respective chromosome

BnTALE66 (1.7325), the other duplicated *BnTALE* had experienced purifying selection pressure during their development, as shown by the fact that these Ka/Ks values of the duplicated *TALEs* gene pairs were less than 1.

Potential protein interaction network analysis of *B. napus* TALE family members

In order to uncover the potential interaction of BnTALE and the participating pathway at the molecular level, possible protein-protein interaction (PPI) network was predicted and enrichment analysis of GO and KEGG were conducted.

The PPI network analysis showed that there were 12,615 proteins possibly interacted with BnTALE proteins (Fig. 6A), and some BnTALE proteins could also interact with each other, especially between the BELL subfamilies and the KNOX subfamilies. GO enrichment of proteins interacting with BnTALE showed that the first three enriched GO terms were plastid organization, chlorophyl II biosynthetic process, response to far red light

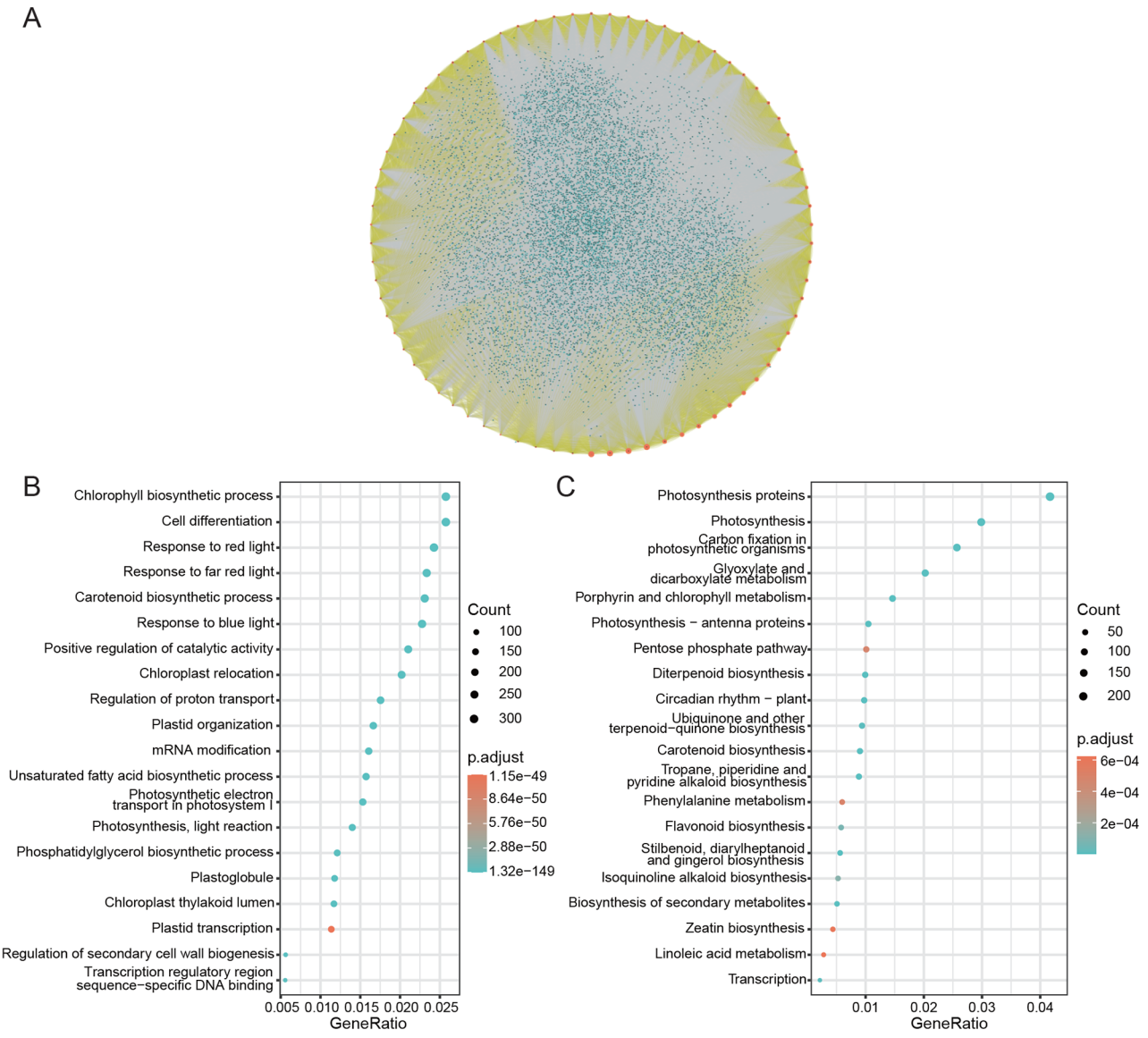

Fig. 6 Potential protein interaction network of *TALE* family members and enrichment analysis in *B. napus*. **A.** Potential protein interaction network of *TALE* family members in *B. napus* displayed by Cytoscape. Red dots indicate TALEs in *B. napus*, indigo-blue dots indicate other proteins, yellow lines indicate the relationship between different TALEs and gray lines indicate the relationship between TALEs and other proteins. **B.** GO enrichment of proteins interacting with *B. napus* TALEs, the x-axes represented the gene ratio and the y-axes represented the GO categories. **C.** KEGG pathway enrichment of proteins interacting with *B. napus* TALEs, the x-axes represented the gene ratio and the y-axes represented the KEGG categories. The circle size represented the gene number, and the circle color represented the adjusted-p value

(Fig. 6B, Table S3). According to the KEGG annotations of the interacting proteins, the top enriched pathways were photosynthesis proteins, photosynthesis, carbon fixation in photosynthetic organisms (Fig. 6C, Table S4).

Expression of *TALEs* in different tissues of *B. napus*

The analysis of the expression characteristics of *TALE* gene family members based on transcriptome data showed that the expression of *BnTALE* was quite different in different tissues (root, leaf, bud, silique, stamen, pistil, blossomy petal, wilting petal, stem, sepal, ovule, and pericarp) of *B. napus* [40]. Based on the expression patterns, the *BnTALE* could be divided into three clusters (I–III) (Fig. 7). Cluster I was composed of 31 genes that showed very low or no expression in these 12 tissues. There are a few exceptions, like the expression of *BnTALE53* in the bud was relatively high, suggesting that the encoded proteins may be required for the development of the bud, and the expressions of *BnTALE73* and *BnTALE10* were relatively high in the root and stem. Cluster II consisted of 11 genes which were highly expressed in multiple tissues. These genes were

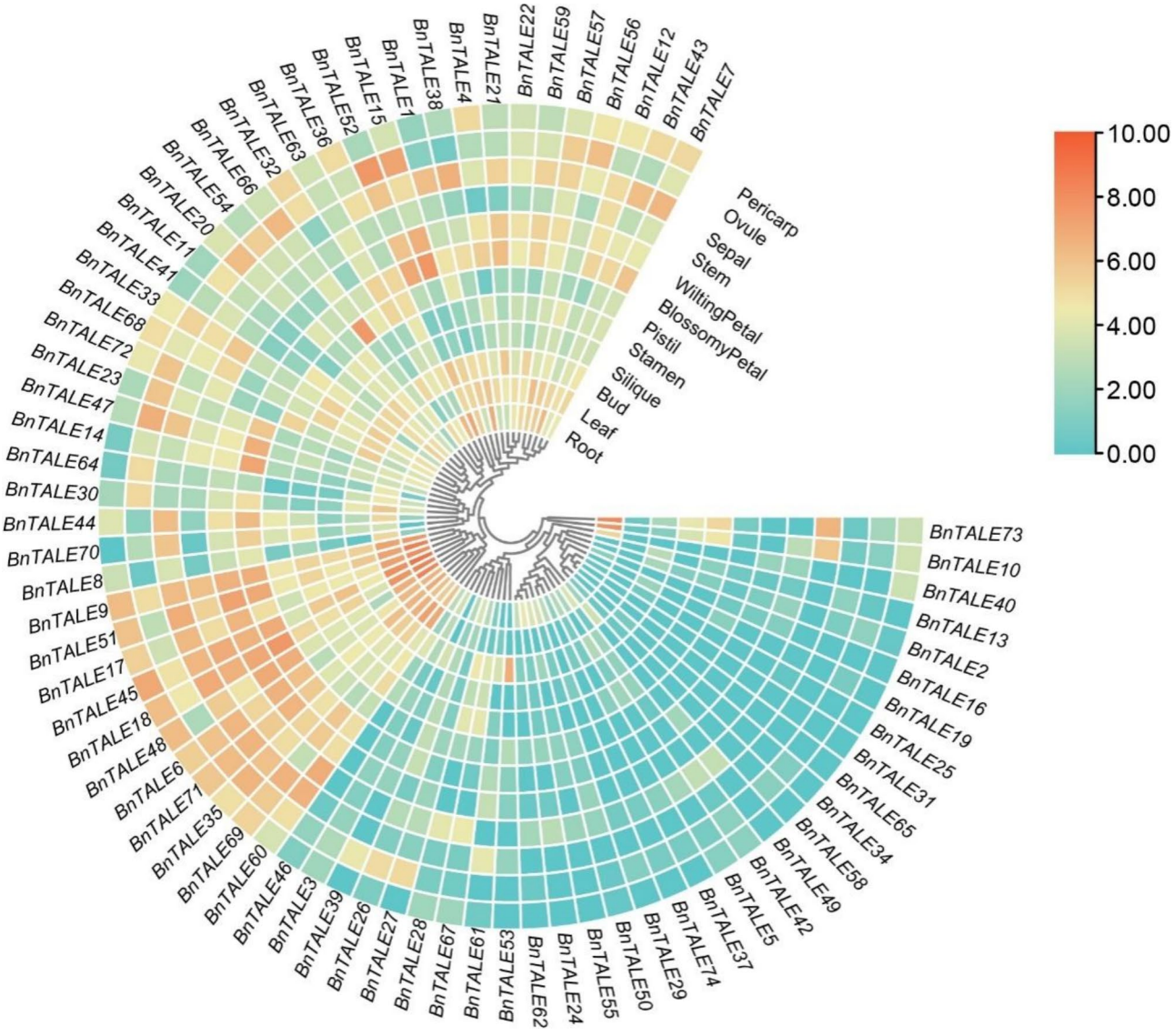

Fig. 7 Heatmap representation and hierarchical clustering of *TALE* genes in different tissues. Expression data were processed with log2 normalization. The color scale represents relative expression levels from low (green color) to high (red color)

particularly higher expressed in the sepal, wilting petal, leaf, and root. For example, *BnTALE45* and *BnTALE18*, with similar expression patterns, were highly expressed in various tissues, especially in the root and leaf. The expressions of *BnTALE9*, *BnTALE51*, *BnTALE6*, and *BnTALE71* were also higher in various tissues. Cluster III was comprised of 32 moderately expressed *BnTALE genes*. The *BnTALE15* and *BnTALE52* genes were highly expressed in the ovule, wilting petal, and blossomy petal, displaying similar expression patterns, while the *BnTALE66* was highly expressed in the pistil. The expression profile of *BnTALE* in different tissues indicated that the *BnTALE* may have certain tissue-specific properties and fulfill different functions in different tissues.

Expression profiles of the *BnTALE* genes under abiotic stresses

To explore the potential function of *BnTALE* in response to abiotic stresses, expression patterns were detected using the published RNA-seq data [41]. It displayed that the *BnTALE* genes were differentially expressed under dehydration, cold, ABA, and NaCl stresses (Fig. 8). Under dehydration stress, the genes with high expression were *BnTALE17*, *BnTALE59*, *BnTALE21*, *BnTALE51*, *BnTALE45*, and *BnTALE48*. The expressions of *BnTALE7* and *BnTALE43* were also high at 8 h of dehydration. Under 24 h cold stress, *BnTALE17*, *BnTALE59*, *BnTALE21*, and *BnTALE51* were highly expressed. The expression levels of *BnTALE*18, *BnTALE*45, and *BnTALE*48 were relatively high. After ABA treatment for

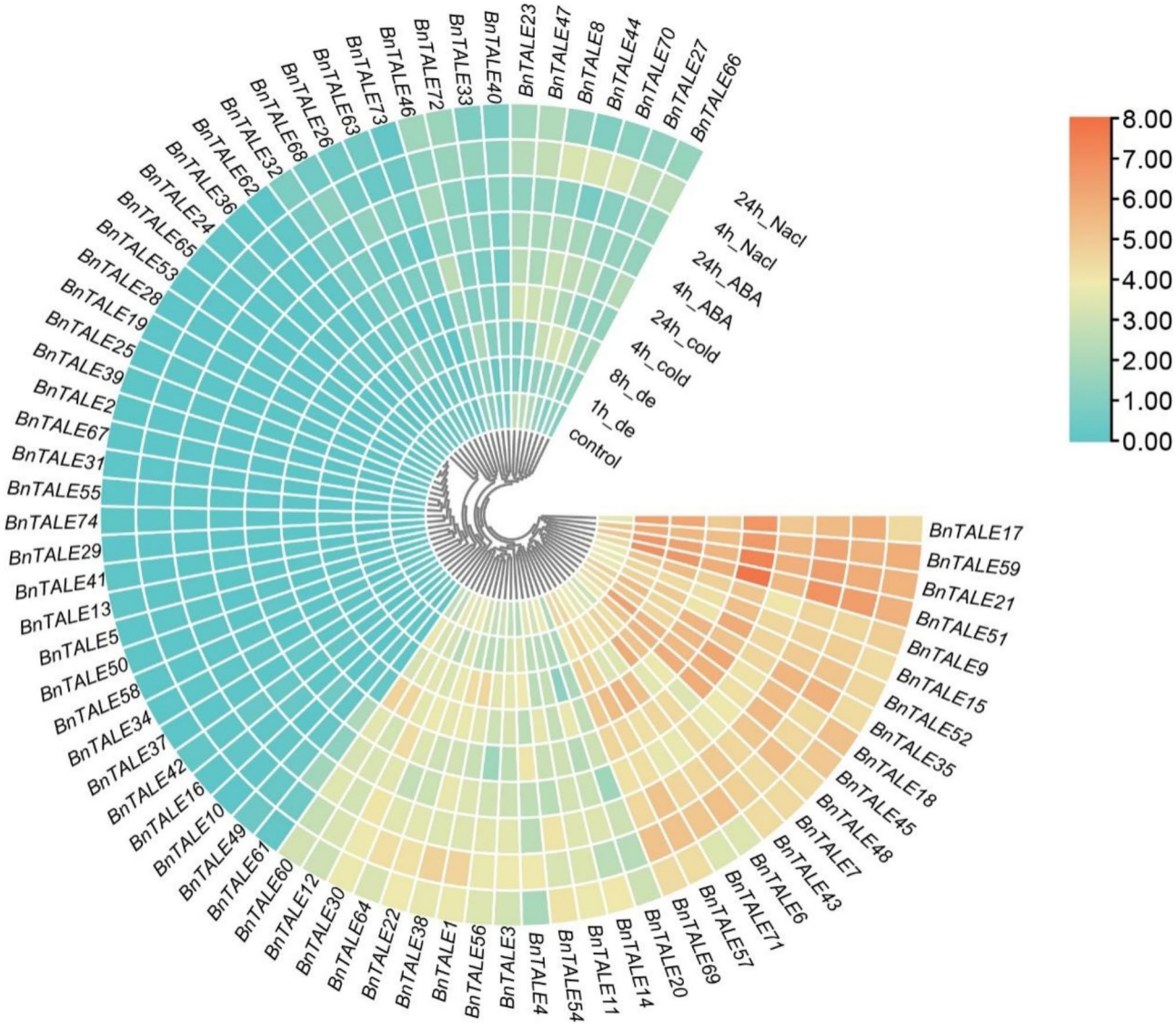

Fig. 8 Heatmap of the expression of 74 *TALE* genes under dehydration, cold, ABA and NaCl treatment at 4 h and 24 h. Expression data were processed with log2 normalization. The color scale represents relative expression levels from low (green color) to high (red color)

24 h and NaCl treatment for 4 h, the expression levels of *BnTALE*17, *BnTALE*59, *BnTALE*21, and *BnTALE*51 were higher. According to the gene expression level under stress treatment, *BnTALE* genes can be divided into three clusters. Cluster I contained 17 highly expressed genes under different stresses. Cluster II consisted of 43 genes with low expression. Cluster III was comprised of 14 genes with expression levels between Cluster I and Cluster II. In Cluster I, *BnTALE59*, *BnTALE21*, and *BnTALE51* had high expression levels under different stresses, especially at cold stress for 24 h, followed by *BnTALE17*, which also had relatively high expression levels under different stresses, indicating that they may be key genes in response to different stresses. *BnTALE45* and *BnTALE48* genes had relatively high expression levels under dehydration and cold stress, *BnTALE7* and *BnTALE43* genes were highly expressed after 8 h of dehydration and had similar expression patterns. The expression of *BnTALE35* and *BnTALE18* genes were higher under cold stress. Besides, the expression of *BnTALE35* was higher under ABA stress for 24 h and NaCl stress for 4 h, and the expression of *BnTALE18* was higher under ABA stress for 24 h and NaCl stress for 24 h. The expression patterns of *BnTALE6* and *BnTALE71* were similar under different stresses, and their expression levels were higher at 4 h of cold stress and 4 h of NaCl treatment. The expression patterns of *BnTALE57* and *BnTALE69* were also similar, and their expression were higher under 4 h of NaCl stress and 4 h of cold stress. In Cluster III, the overall expression level of the *BnTALE* genes were relatively low, the expressions of *BnTALE1* and *BnTALE38* were relatively high at 4 h of NaCl stress

and the expressions of *BnTALE12* and *BnTALE60* were slightly higher at 4 h of cold stress. The *TALE* genes in Cluster II were not expressed or had very low expression levels under stresses, indicating that these genes were not involved in the stress response of *B. napus*. Further, qRT-PCR expression patterns of nine representative *TALE* genes under cold stress, dehydration stress, ABA stress, and NaCl stress in *B.napus* cultivar ZS11 showed that the expression profiles of the selected *TALE* genes were generally consistent with the results of the analysis of the previously published RNA-seq data (Fig. 9).

Generally, genes perform their biological functions by cooperating with a series of genes with similar expression patterns, therefore, investigating the co-expression modules associated with *BnTALE* could obtain a better understanding of their functions. Herein, WGCNA was performed to study the associated co-expression modules of *BnTALE* under abiotic stresses. A total of 25 co-expression modules were detected, and 37 *BnTALE* genes were clustered in these modules. Among them, 9, 7, 7, and 7 *BnTALE* genes were found in the brown, green, blue, and turquoise modules, respectively (Table S5). According to the relationships between modules and stresses, these four modules were significantly related to 8 h_dehydration, 4 h_cold, 24 h_cold, 8 h_dehydration, and 24 h_cold, respectively (Fig. 10). These thirty genes clustered in the four modules would be potential key genes for genetic improvement of dehydration and cold resistance in rapeseed. In the brown module, the most enriched KEGG pathways were autophagy, peroxisome, fatty acid degradation, valine, leucine, and isoleucine degradation, and biosynthesis of unsaturated fatty acids (Fig. S1A). In the green module, the most enriched KEGG pathways were circadian rhythm, flavonoid biosynthesis, stilbenoid, diarylheptanoid, gingerol biosynthesis, and vitamin B6 metabolism (Fig. S1B). In the blue module, the most enriched KEGG pathways were ribosome biogenesis in eukaryotes, citrate cycle, protein export, phagosome, and secretion system (Fig. S1C). In the turquoise module, the most enriched KEGG pathways were proteasome, GTP-binding proteins, autophagy, and SNARE interactions in vesicular transport (Fig. S1D). According to the

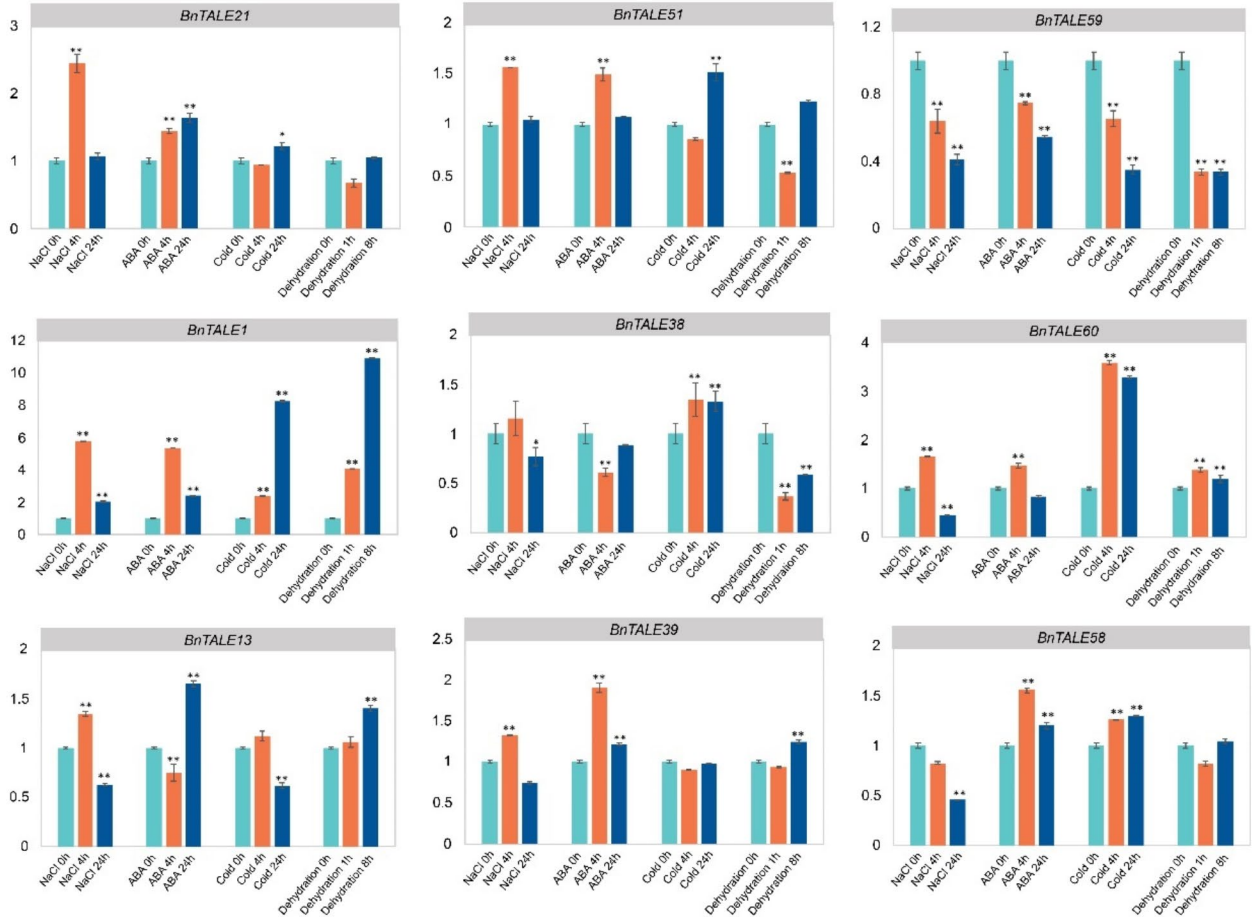

Fig. 9 qRT-PCR analysis of *BnTALE* genes expression under cold and osmotic stresses (dehydration, ABA and NaCl). The error bars represent standard deviations. The y-axis represents relative expression levels and x-axis represents different stresses of ZS11

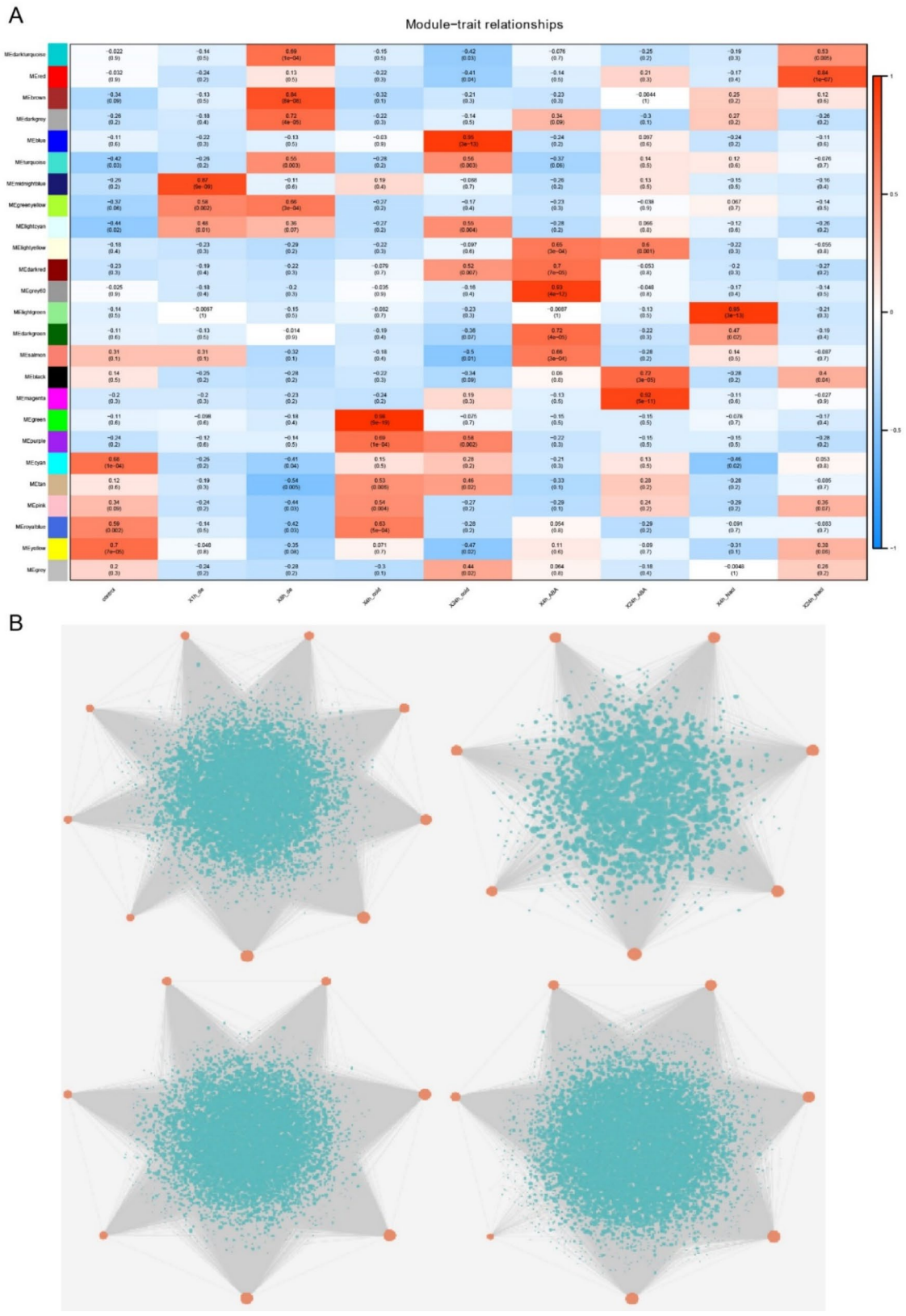

Fig. 10 Co-expression modules under abiotic stresses identified by weight gene co-expression networks analysis (WGCNA). **A.** Relationship between co-expression modules and abiotic stresses. **B.** *BnTALE* genes involved in the brown, green, blue, turquoise modules. Red dots indicate *TALEs* and green dots indicate other co-expressed genes

expression heatmap (Fig. 8), cluster I was identified as highly expressed genes under different stresses, among them, *BnTALE59*, *BnTALE21*, *BnTALE51*, and *BnTALE17* were the highest. Now with the help of WGCNA results, *BnTALE59* and *BnTALE21* were found in the blue module related to 24 h_cold, while *BnTALE51* and *BnTALE17* were in the turquoise module related to 24 h_cold and 8 h_dehydration, which would function through different pathways. These results indicated that *BnTALE*, as the important transcription factor, could cooperate with other genes to respond to various abiotic stresses by different pathways.

Discussion

The *TALE* superfamily genes ubiquitously exist in plant genomes and play an important role in regulating plant growth, development, cell differentiation, and stress responses [2, 7, 32]. Till now, *TALE* genes have been studied in a variety of plants, however, the genome-wide identification and characterization of *B. napus TALE* superfamily members have not been studied.

In this study, we identified a total of 74 *TALE* family genes in *B. napus*, the number of *TALE* genes in the *B. napus* genome is approximately the same as in *Triticum aestivum* (70), three times that of *A. thaliana* (22) and rice (22) [42], and twice that of poplar [32]. We speculated that the number of gene family members correlates with the size of the genome and the degree of polyploidy, similar to the findings in soybeans [2]. Phylogenetic tree analysis showed that, the members of the *B. napus* TALE family can be classified into two groups, namely, the KNOX subfamily and the BELL subfamily. The KNOX subfamily can be further divided into three classes. The classification of the *B. napus* TALE family was consistent with the result of *A. thaliana*, cotton, and poplar, indicating that the amino acid sequence of the TALE family in plants is highly conserved. The TALE members in the same phylogenetic cluster of different species generally indicate their analogous biological functions, which is consistent with previous studies in soybean [2]. BnTALE39, BnTALE2, BnTALE61, and BnTALE13 clustered with AtATH1. AtATH1 has been shown to affect the growth of nutrient or reproductive organs and inhibit stem development [43]. Thus, we hypothesized that the above four *TALE* genes in *B. napus* have growth-related functions similar to AtATH1.

In the present study, BnTALE members in the same subclass or branch of the phylogenetic tree generally have similar gene structures and protein-conserved domains, which may indicate that they have similar biological functions in general and further validate our classification of the *B. napus* TALE family. From the physicochemical characteristics of the members of the *B. napus* TALE family, it can be seen that there are significant differences between the KNOX subfamily and the BELL subfamily members. The amino acid number and molecular weight of TALE members in the BELL subfamily of *B. napus* are much larger than those of the KNOX family, which is consistent with the characteristics of TALE members in soybean [2], cotton [7], and poplar [32].

Gene structure analysis showed that out of 74 *BnTALE* genes, 39 genes had 5′ UTR, and 37 genes had 3′ UTR. Given that the 5′ UTR plays a role in regulating mRNA stability and the 3′ UTR may function as a miRNA binding site, we suggest that the *B. napus TALE* genes exert complex regulatory properties on downstream genes [44–47]. Specific domains or motifs were reported to play important roles in DNA binding and protein interactions [48]. The Homebox KN domain is located at the C-terminal end of the protein. It is involved in DNA-binding functions and transcriptional regulation [47, 48]. The domain analysis of *B. napus* TALE proteins showed that 87.8% of members contained Homebox_KN domain. Specifically, all BELL subfamily members of the *B. napus* TALE family contain the Homebox_KN domain. With the exception of nine proteins, members of the KNOX subfamily all contain Homebox_KN domains, suggesting that *B. napus* TALE proteins possess DNA-binding, and potential protein-interaction functions. Previous research has shown that the ELK domain of the *TALE* gene family can act as a nuclear localization signal and is involved in transcriptional regulation, which is associated with transcriptional repression [49, 50]. Most members of the KNOX subfamily of the BnTALE family contain the ELK domain, whereas members of the BELL subfamily do not. TALE proteins typically function as dimers. Zhao found that in poplar, different TALE proteins can form heterodimers [32]. Yang presumed that the KNOX and BELL subfamilies of the *Prunus mume* TALE proteins can form heterodimers that affect early stem development [51]. In this study, most members of the KNOX subfamily of the BnTALE family contained the KNOX2 structural domain, which was considered essential for homodimerization and was critical for protein function [52]. All BELL subfamily members contain the POX domain. Studies in *A. thaliana* have shown that BEL1-like proteins containing the POX structural domain interact with KNAT2 and KNAT5 proteins to influence plant development [53, 54]. The potential protein interaction of the two components of the heterodimer TALE proteins can occur between TALE and non-TALE members or between members of different TALE families [55]. In this study, the PPI network analysis also indicated that some BnTALE proteins could also interact with each other, especially between the BELL subfamilies and the KNOX subfamilies.

Gene replication patterns, including tandem, fragment, and genome replication, are important factors

affecting biological evolution and the amplification of different gene families in eukaryotic genomes [56]. In this study, most of the *TALE* genes were derived from whole-genome duplication (WGD) or segmental duplication events, suggesting that WGD/segmental duplication was the main driving force for the expansion of *TALE* genes in the *B. napus* genome. With the exception of *BnTALE27* and *BnTALE66* (1.7325), all *BnTALE* gene pairs had Ka/Ks ratios less than 1, indicating that these genes have evolved under the influence of purifying selection, which is consistent with research in sweet orange [43]. Since purification selection limited gene differentiation, it can be inferred that the duplicated *TALE* genes in *B. napus* were relatively conserved in evolution and may have similar functions [57].

Cis-element analysis of the promoter region revealed that the promoter sequence of the *BnTALE* genes contains several cis-elements related to hormone response, abiotic stress, and development. This was consistent with the TALE family in soybean, wheat, pomegranate [1], and cotton, indicating that the *B. napus TALE* genes may be associated with abiotic stress and plant development regulation. The main cis-acting elements of *B. napus TALE* genes are hormone response elements (509), such as ABA-responsive element (ABRE) and estrogen-responsive element (ERE), followed by environmental stress response elements. The effects of transcription factors on growth, development, and plant stress resistance are usually closely related to hormonal pathways, for example, analysis of ethylene-related gene expression models suggests that ethylene may indirectly be involved in the induction of dormancy, thereby improving cold/freeze tolerance in *P. mume* [58]. Previous studies have shown that the function of the *TALE g*enes were related to the hormone pathway of plants [59]. Here, we infer that *B. napus TALE* genes may participate in the response to abiotic stress through the hormone pathway.

The expression profile of *BnTALE* genes showed that the expression of *BnTALE* genes were higher in roots and leaves, which may be related to the fact that the root system is an important sensory organ that responds to various abiotic stresses. Previous studies showed that sweet orange *TALE* genes were highly expressed in stems [60]. The majority of the *PgTALE* genes were expressed in pomegranate; nevertheless, distinct *PgTALE* genes were expressed in various tissues, indicating expression differentiation [1]. The differences in the expression positions of the *TALE* gene in plants may be due to species differences. The expression profiles of *BnTALE* genes under various abiotic stresses including NaCl, ABA, cold, and dehydration stresses revealed that *BnTALE59*, *BnTALE21*, and *BnTALE51* had high expression levels under different stresses, especially at cold stress for 24 h. In this study, four co-expression modules brown, green, blue, and turquoise colors detected by WGCAN were significantly associated with dehydration and cold stress. *BnTALE59* and *BnTALE21* were found in the blue module, while *BnTALE51* were present in the turquoise module. Thus, we infer that these genes, together with other genes in the module, are involved in certain metabolic pathways through co-expression in response to abiotic stresses. We supposed that signal-regulated pathways of plants in response to different abiotic stresses may be interrelated and the above three genes were possibly key responsive genes in different stress-specific regulatory networks. In addition, further studies on the functions of these genes should be investigated so as to provide important genetic resources for breeding for stress tolerance in *B. napus*.

Conclusions

In the present study, we identified 74 *TALE* superfamily members in the genome of *B. napus*. The BnTALE members were further divided into the BEL1-like subfamily and the KNOX subfamily. BnTALE members in the same subfamily or clade displayed universal similarities, indicating their analogous biological functions. Whole-genome duplication (WGD) or segmental duplications played a major role in the expansion of *BnTALE* superfamily. The Ka/Ks ratios indicate that the *BnTALE* genes have evolved under the influence of purifying selection, and it is inferred that the duplicated *TALE* genes in *B. napus* were relatively conserved in evolution and may have similar functions. Potential protein interaction analysis showed that TALE proteins were involved in response to drought, temperature, and regulation of defense response. By analyzing cis-element in gene promoter regions, combined with transcriptome data and quantitative RT-PCR investigations, several *BnTALE* genes such as *BnTALE59*, *BnTALE21*, and *BnTALE51* have been proposed to play potential roles during *B. napus* development and abiotic stress responses. In addition, WGCNA analysis detected four modules what would be notably related to dehydration and cold stresses. To conclude, our work laid a foundation for the biological functions study of *BnTALE* genes in the future, which may provide genetic resources for the genetic improvement of *B. napus* and the breeding of new varieties resistant to various abiotic stresses.

Materials and methods

Genome wide identification of *TALE* gene family members in *B. napus*

The conserved TALE ELK (PF03789), KNOX1 (PF03790), KNOX2 (PF037901), POX (PF07526), Homeobox_KN (PF05920) protein domains from the Pfam website were used to build the Hidden Markov Model profiles (http://hmmer.janelia.org/) to search against the whole-genome

protein database of *B. napus* cultivar '*Darmor*-bzh' (the Brassicaceae Database: *B. napus* v4.1) [35]. The *TALE* gene sequences of *A. thaliana* were also used to search against the *B. napus* genome to obtain the homologous sequences in *B. napus*. The proteins obtained by both methods were further verified through submissions to Pfam (http://pfam.xfam.org/), NCBI-CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and Smart (http://smart.embl-heidelberg.de/) for domain predictio n. After manual screening, the *TALE* genes for *B. napus* were obtained. MG2C v2 (http://mg2c.iask.in/) was used to display the location of the *TALE* gene on the chromosome [60]. ExPASY (https://web.expasy.org/compute_pi/) was used to analyze the isoelectric point and molecular weight of the TALE protein, and CELLO (http://cello.life.nctu.edu.tw/) was used to predict the subcellular localization of the TALE protein [61].

Construction of the *B. napus* TALE protein family phylogenetic tree

The TALE protein sequences of *B. napus* and *A. thaliana* were aligned together using ClustalW (http://www.clustal.org/clustal2/) [62] and a phylogenetic tree was constructed using MEGA10 (https://www.megasoftware.net/) with the neighbor-joining method and 1000 replicate iterations [63]. Evolutionary trees were decorated using Evolview (http://www.evolgenius.info/evolview/#/) [64].

TALE gene structure, domain, and promoter element analysis

BnTALE sequence information was extracted from the *B. napus* reference genome (the Brassicaceae Database: *B. napus* v4.1). Pfam (http://pfam.xfam.org/), NCBI-CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and Smart (http://smart.embl-heidelberg.de/) were used to predict the domains in the protein sequences. The locations of UTR, CDS and domains were displayed by TBtools (https://tbtools.updatestar.com/en) [65]. The 2 kb sequence upstream of *TALE* CDS was extracted and analyzed for cis-acting elements using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [66]. The pheatmap package of R was used to draw the heatmap representing the number of cis-elements.

Gene duplication events identification analysis

Duplicated genes in the *TALE* family of *B. napus* were analyzed using BLASTP and MCScanX (https://github.com/wyp1125/MCScanX? tab=readme-ov-file) [67]. The position and relationship of the duplicated genes were showed by Circos software (https://circos.ca/) [68]. The protein sequences of the duplicated gene pair were aligned by Muscle (https://link.zhihu.com/?target=https%3 A//www.drive5.com/muscle/) [69]. To evaluate the selection pressure, the Ka/Ks values were calculated with the KaKs_Calculator (https://ngdc.cncb.ac.cn/biocode/tools/BT000001) [70].

Potential protein interaction analysis

The protein interaction information for TALEs in *A. thaliana* was obtained by STRING (https://www.string-db.org/) and used to search the corresponding potential interaction network of *B. napus* TALE proteins based on the homology between *A. thaliana* and *B. napus* TALEs. The potential protein interaction relationship was displayed by Cytoscape (http://www.cytoscape.org/) [71]. KEGG and GO enrichment analysis were conducted on the genes encoding proteins that interacted with TALE proteins using the R package clusterProfiler [72].

Gene expression analysis

To investigate the expression patterns of *BnTALE* genes, we downloaded RNA-seq data of different tissues (root, leaf, bud, silique, stamen, pistil, blossomy petal, wilting petal, stem, sepal, ovule, and pericarp) and various abiotic stresses (dehydrate, cold, ABA, and NaCl) [36, 37]. RNA-seq reads were mapped to the *B. napus* genome using Hisat2 and the expression levels were calculated using Stringtie. The expression data of *TALEs* were extracted and displayed with the R package pheatmap.

To further uncover the critical *BnTALE* genes in response to abiotic stresses, weight gene co-expression network analysis (WGCNA) was performed using RNA-seq data from those four stresses. Power value was set as 8 to get the original adjacency matrix, minModuleSize and cutHeight were set as 50 and 0.25, respectively. Cytoscape (http://www.cytoscape.org/) [71] was used to visua lize the interested module. Package ClusterProfile [72] in R was selected for GO and KEGG enrichment analysis.

Quantitative reverse transcription polymerase chain reaction

B. napus ZS11 seedlings were grown in a growth room at 24 °C with a 16/8 h light/dark photoperiod. The leaves, stems and roots were collected from 20-day-old seedlings, buds were collected from 70-day-old seedlings, and siliques were harvested 90 days after germination. For cold and NaCl stresses treatment research, leaf samples from three weeks old plants of *B. napus* ZS11 were collected at 1 h and 8 h after dehydration while 4 h and 24 h of ABA (25 μM), NaCl (200 mM), and cold (4 °C) treatment, as described in [37]. Samples were stored in liquid nitrogen immediately after collection. Total RNA was extracted using the TRIzol reagent (Invitrogen, 15596026, USA) according to the product manual. Reverse transcription was performed using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Japan). The relative expression of *BnTALE* genes was quantified using quantitative real time-PCR (qRT-PCR) on the

CFX96 Real-time PCR System using gene-specific primers (Table S6). The SYBR Green Real-time PCR Master Mix was used for the qRT-PCR (Bio-Rad, USA). The internal standard was the *B. napus* histone gene. The PCR program was as follows: 95 °C for 30 s followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. All assays were carried out for three biological repeats, each with three technical repeats. The quantification methods used for the expression of *BnTALE* genes in different tissues and under different stresses were $2^{-\triangle CT}$ and $2^{-\triangle\triangle CT}$ [73], respectively.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05953-1.

Supplementary Material 1: Additional file 1: Figure S1 KEGG pathway enrichment of module brown (A), green (B), blue (C), and turquoise (D).

Supplementary Material 2: Additional file 2: Table S1 Numbers of cis-acting elements in the promoter of *B. napus TALEs*.

Supplementary Material 3: Additional file 3: Table S2 Ka/Ks analysis for paralogous gene pairs of *B. napus TALEs*.

Supplementary Material 4: Additional file 4: Table S3 GO enrichment of proteins interacting with *B. napus TALEs*.

Supplementary Material 5: Additional file 5: Table S4 KEGG pathway enrichment of proteins interacting with *B. napus TALEs*.

Supplementary Material 6: Additional file 6: Table S5 Gene names and IDs of four modules.

Supplementary Material 7: Additional file 7: Table S6 qRT-PCR primer sequence for the three-amino-acid-loop-extension (*TALE*) genes in *B. napus*.

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Not applicable.

Author contributions
Meili Xie analyzed the data and provided manuscript preparation and editing. Xiaojuan Zhang performed the experiments and manuscript preparation. Kexin Liu performed part of the experiments. Zhixian Qiao provided data analysis assistance. Xiaohui Cheng designed the research and modified this manuscript.

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Data availability
The datasets supporting the conclusions of this study are available within the article and its supplementary materials.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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