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Identification and expression profiles of tubby-like proteins coding genes in *Brassica rapa* (*B. rapa*) in response to hormone and drought stress

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Abstract

Background Tubby-like proteins (TLPs) are a widespread multigene family found in single-celled to multicellular eukaryotes. Despite their significance, no reports of TLPs in *B. rapa* have been made up to this point.

Results Herein, we identified 14 TLPs in the *B. rapa* genome and renamed them *BrTUB1-BrTUB14* based on their chromosomal location. The bulk of BrTUB proteins contain two characteristic domains: the F-box and Tubby domains. Subcellular localization prediction confirmed that BrTUBs are localized in the nucleus. Expression profiling showed that many BrTUB reacts to a variety of stressors, including drought stress and hormonal treatments (ABA and ethylene). In particular, the *BrTUB1* displayed elevated expression to ABA and the drought stress treatment.

Conclusion This study is the first thorough identification of the BrTUB family, providing critical insights into its function and regulation, and laying the groundwork for future functional analyses, particularly concerning drought tolerance of *B. rapa*.

Keywords Genome-wide analysis, *Brassica rapa*, Tubby-like proteins, Drought, Hormones

Introduction

Tubby-like proteins (TLPs) got their name when they were first identified in obese Tubby mice in the 1990s [1]. TLP genes, a class of Tubby-like proteins with two separate domains—the Tubby domain at the C-terminal and the F-box close to the N-terminal—encode these proteins [2]. As functional SCF-type E3 ligases, the highly conserved F-box domains play an essential role in interactions with other proteins, including specialized Skp1-like (SK) [2]. Through the action of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), the Tubby domain makes it easier for the protein to attach to the plasma membrane (PM) [3].

The TLP gene family is found in many plant species and plays important functions in organ growth and development [3]. Four TLP genes in tomato (*Solanum lycopersicum*) showed variable expression during fruit development, suggesting they are involved in ripening and softening [4]. The early stages of seedling growth and seed germination are also thought to be regulated by *OsTLP14* from rice (*Oryza sativa*) and *AtTLP9* from Arabidopsis (*A. thaliana*) [5]. Pollen grain shape is regulated

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by *AtTLP7*, *AtTLP2*, and *AtTLP6* [3]. In addition, it has been found that *AtTLP2* controls stem height [6] and regulates the accumulation of Rhamnogalacturonan I (RG-I) and homogalacturonan (HG) in seed coat mucilage [7]. The anti-gravity mechanism regulates root development angle by altering root tissue hardness via the TLP coding gene *EGT1* from barley (*Hordeum vulgare*) [8].

Angiosperms' complex physiology and developmental processes depend heavily on phytohormones [9, 10]. TLPs have become important mediators of several hormone responses and signaling cascades. Furthermore, treatment with cytokinin activates *AtTLP2* expression, while treatment with indole-3-acetic acid suppresses it [11]. The *AtTLP2* was transcriptionally activated by ABA, imparting ABA sensitivity. Conversely, *AtTLP7* could function antagonistically to *AtTLP2* within the ABA pathway [6]. Moreover, *AtTLP3* and *AtTLP9* participate in the ABA signaling pathway during seed germination and the initial stages of seedling development [5]. In tomatoes, the majority of *SlTLP* genes are activated by MeJA [12]. On top of that, TLPs are essential in the response to and resistance against both biotic and abiotic stressors. For example, four TLPs from *Arabidopsis* enhance plant immune responses [13]. Furthermore, *AtTLP2* is transcriptionally activated by saline conditions and desiccation [6]. The expression of all *OsTLPs* in rice is stimulated by bacterial infection, and eight *OsTLPs* also respond to injury [14]. Moreover, *SlTLFP8* modulated stomatal dimensions and diminished water loss, thereby enhancing water-use efficiency [12, 15].

The crops of the Brassica genus are extensively utilized for vegetables, oilseeds, sauces, and animal feed. China's yields presently constitute about 50% of global yields and 61% of yields in Asia (http://faostat.fao.org). *Brassica* vegetables mostly consist of *Brassica rapa* (*B.rapa*) and *Brassica oleracea*. *B. rapa* comprises multiple subspecies, including Chinese cabbage (*B. rapa ssp. pekinensis*), non-heading Chinese cabbage (*B. rapa ssp. chinensis*), and turnip (*B. rapa ssp. rapifera*) [16]. Chinese cabbage is a significant vegetable in China and is also widely farmed in Korea, Japan, Southeast Asia, the USA, and Europe. The genome of Chinese cabbage (Chiifu-401-42), due to its considerable economic importance and its close relation to Arabidopsis, was recently sequenced and assembled [16].

Herein, we investigated the response of *B.rapa* to drought and hormones (ABA and ethylene). Further, we identified the Tubby gene family in the *B. rapa* genome database and conducted numerous bioinformatic analysis to understand their putative functions. Our study will provide new insights to the role of Tubby genes in *B. rapa* subjected to drought stress and hormonal treatment.

Materials and methods

Identification and isolation of *BrTUB* genes from the *B. rapa* genome

The genomes of *B. rapa* were queried against the TLP proteins of *Arabidopsis thaliana* using the BLAST algorithm in Ensembl Plants in order to find every sequence associated with the TLP family. The CDD search and the Pfam database were used to analyze the non-redundant sequences of TLP in order to confirm the presence of the conserved domains [17]. Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/#) was utilized in this investigation to ascertain the subcellular location of every BrTUB protein.

Physical location and synteny of *BrTUB* genes

Through the use of TBtools (Toolbox for biologists) (v0.6655) and the extraction of gff3-files from the *B. rapa* genome database, the chromosomal distribution of the *BrTUB* genes was ascertained [18]. Gene duplication was defined by the alignment length, the aligned region's identity, and the fact that closely related genes experienced just one duplication event.

Phylogenetic analysis of BrTUB proteins

Using the amino acid sequences of *Brassica oleracea* (*B. oleracea*), *Solanum lycopersicum* (Tomato), and *B. rapa* BrTUB, a phylogenetic tree was created. First, all sequences were aligned using the multiple alignment tool Clustal-Omega [19, 20]. The Clustal-Omega data were then forwarded to the IQ-TREE website, which used a total of 1,000 bootstrap repeats and the Maximum Likelihood (ML) technique to assess the evolutionary relationships of TUB. In conclusion, the phylogenetic tree of TUB proteins was ultimately constructed with the assistance of the iTOL version 5 tool [21].

Conserved motif analysis

The BrTUB gene family's accession number, chromosomal location, ORF length, and exon-intron structure were searched in the *B. rapa* sequencing database. The BrTUB protein motif was examined using the MEME tool (http://meme-suite.org/index.html) with the followi ng parameters. There can be only one motif instance per site in each sequence. These motifs were visualized using TBtools (Toolbox for Biologists) (v0.6655) [18, 22].

Interactive protein partners

BrTUB sequences were uploaded to the STRING v11.5 database https://cn.string-db.org/ in order to construct the network of protein-protein interactions between BrTUB. After setting the maximum number of interactors to 5 for the first shell, the second shell had a maximum of 10. Finally, Cytoscape v3.8.2 was utilized to illustrate the networks of interactions.

Gene ontology analysis of *BrTUB* genes

Furthermore, the GO tool Blast2GO (Version 2.7.2) (http://www.blast2go.com) was used to examine BrTUB protein sequences (accessed on 3rd July 2024) [23]. The cellular component GO classification, molecular functions, and biological processes were reassembled into the three categories by using the procedures described in previous studies.

Promoter analysis of *BrTUB* genes

The PlantCARE database was used to screen each BrTUB gene's upstream region (1500 bp of ATG) in *B. rapa* for known *c*is-regulatory elements associated with growth, hormone response, and stress. The final phase involved categorizing the *c*is-regulatory elements according to their functions [18, 22].

Prediction of 3D protein structures

The BrTUB proteins' three-dimensional structure was obtained by use of the Phyre2 server following the procedures detailed in [24]. According to the procedure described in [25], the proteins were subjected to water molecule exclusion in Accelrys Discovery Studio v4.1 and then visualized with pyMOl.

Plant material and stress conditions

In this study Chinese cabbage (*B. rapa*) that was obtained from the Vegetable Research Institute, Guangdong Academy of Agricultural Sciences (GDAAS), was used for different stress treatments. The vigorous seeds that had a 100% germination rate were selected and sterilized with a 10% hypochlorous acid solution for 5 min. Cycling conditions of 25 °C and 16 h/8 h light/dark were used to grow seeds in the growth chamber. To determine the effect of the phytohormones, three-week-old seedlings were treated with 100 µM abscisic acid (ABA) and 100 µM ethylene by exogenously spraying [26]. Drought treatment was achieved by leaving the intact seedlings in the air without supplemented with water, followed by sampling at 0 (CK), 3, 6 and 12 h. Three biological replicates of each treatment were taken and rapidly frozen in liquid nitrogen and stored at – 80 °C.

Table 1 Physiochemical properties of *BrTUB* genes in *B. rapa* genome

Gene name	Locus ID	Chr. No	Domain	Subcellular
BrTUB1	BraA02g023830.3 C	A02	Tub	Nucleus
BrTUB2	BraA04g032500.3 C	A04	Tub	Nucleus
BrTUB3	BraA05g017150.3 C	A05	Tub	Nucleus
BrTUB4	BraA05g039050.3 C	A05	Tub	Nucleus
BrTUB5	BraA06g012090.3 C	A06	Tub	Nucleus
BrTUB6	BraA07g002450.3 C	A07	Tub	Nucleus
BrTUB7	BraA07g012700.3 C	A07	Tub	Nucleus
BrTUB8	BraA07g040380.3 C	A07	Tub	Nucleus
BrTUB9	BraA08g025350.3 C	A08	Tub	Nucleus
BrTUB10	BraA08g005240.3 C	A08	Tub	Nucleus
BrTUB11	BraA08g001590.3 C	A08	Tub	Nucleus
BrTUB12	BraA09g037650.3 C	A09	Tub	Nucleus
BrTUB13	BraA10g006660.3 C	A010	Tub	Nucleus
BrTUB14	BraA10g021470.3 C	A010	Tub	Nucleus

RNA extraction and qRT-PCR analysis

The qRT-PCR was performed for gene expression analysis at 0, 3, 6, and 12 h. Reactions will be conducted in a 20 µL volume using cDNA, primers, and qPCR Supermix. Cycling conditions followed the manufacturer's protocol. Ct values are determined automatically, normalized to GAPDH transcript, and fold differences are calculated using the $2^{-\Delta\Delta Ct}$ method. Primers designed using NCBI-Primer blast tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).

Statistical analysis

The statistical analysis was conducted using SPSS software. The data is presented as the average value plus or minus the standard deviation (SD). A one-way ANOVA was performed on the data, followed by Tukey's multiple comparison tests. A significant difference was indicated by different lowercase letters above the bars, with a *p-value* of less than 0.05. The graphical representation was created using GraphPad Prism version 10.3.1(509) from GraphPad Software, Inc. in La Jolla, CA, USA.

Results

Genome-wide characterization of BrTUB

We performed a sequence alignment search utilizing known AtTLP protein sequences from Arabidopsis as queries to identify BrTUB in the *B. rapa* genome. The obtained BrTUB sequences were subsequently validated with the HMMER software. Following manual verification and redundancy reduction, the 17 GA2ox members were obtained and given the names BrTUB1 to BrTUB14. The gene members were labelled BrTUB1 to BrTUB14 according to their distinct accession numbers (Table 1).

Chromosomal location of *BrTUB* genes.

A total of 14 *BrTUB* genes in *B. rapa* were unequally located on different chromosomes. Chromosome A07 and A08 anchored the highest number of *BrTUB* genes (*BrTUB6/7/8/9/10/11*), chromosomes A05 and A10 carried two members, and all others contained a single gene (Fig. 1).

Phylogenetic relationships of BrTUB with other TLPs

The phylogenetic tree was created to shed light on the evolutionary relationships between TLPs from *B. rapa*, *B. oleracea*, *B. juncea*, *S. lycopersicum*, and *Arabidopsis* (Fig. 2A). The TLP proteins were classified into six separate categories. Group I had three AtTLPs and

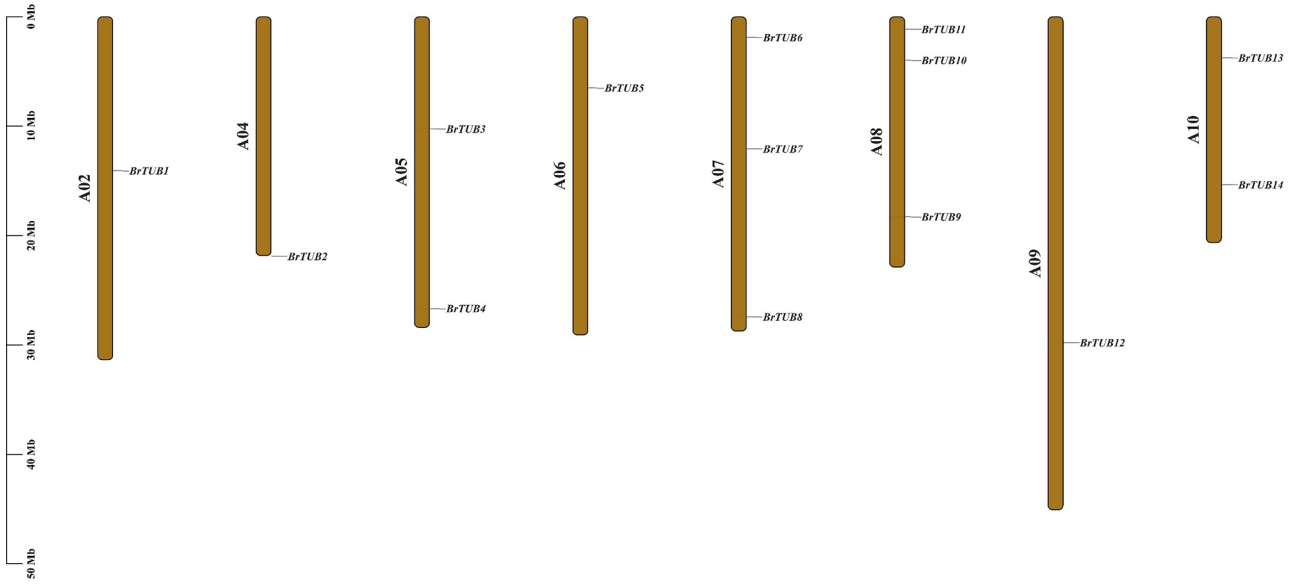

Fig. 1 Mapping of *BrTUB* genes on *B. rapa* chromosomes (A02-A10). The ruler represents the size of the chromosome. The relative positions of *BrTUB* genes are marked on the chromosomes, and the schematic representation was made using TBtools-II (Version 1.098765)

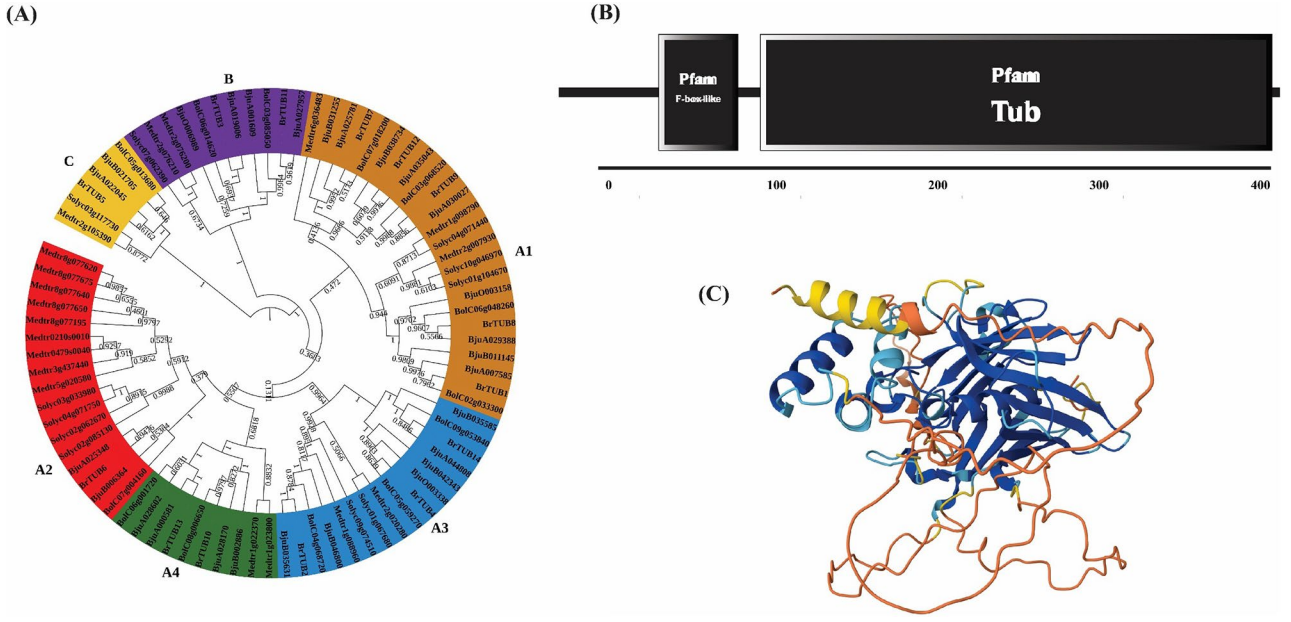

Fig. 2 (**A**) Neighbor-joining tree illustrating evolutionary relationships among TLP proteins from *B.rapa Brassica oleracea*, *B. juncea*, *S. lycopersicum*, and *Arabidopsis*. (**B**) Structural domain of BrTUB gene. (**C**) 3D protein structure of TUB domain

five BrTUBs (BrTUB1, BrTUB7, BrTUB8, BrTUB9, BrTUB12). A single BrTUB6 was clustered in Group II. BrTUB2 BrTUB4 and BrTUB14, which demonstrated a close evolutionary affinity to AtTLP7, were classified inside Group III. Group IV consisted of two BrTUBs (BrTUB10, BrTUB13) and two AtTLPs (AtTLP2, AtTLP6). Group V consists exclusively of BrTUB5. Finally, Group VI was found with a total of two BrTUB proteins, including BrTUB3 and BrTUB11. The domain structure analysis revealed a primary TUB domain is present in all the BrTUB proteins (Fig. 2B). The 3D homology of BrTUB protein consist of less residual noise along a highly conserved TUB domain region (Fig. 2C).

Synteny analysis of *BrTUB* genes

The synteny relationships between the genomes of *B. rapa* and *B. oleracea* were analyzed to ascertain the potential functions of the *BrTUB* genes. In the genomes of *B. oleracea* (~55%) and *B. rapa* (~70%), all *BrTUB* genes exhibited synteny linkages, as illustrated in (Fig.

3A). Correspondingly, significant syntenic connections were identified between *B. rapa* (~70%) and *S. lycopersicum* (~60%) (Fig. 3B). The profound evolutionary relationships and significant rearrangement events of *B. rapa* chromosomes are evidenced by extensive synteny interactions at the gene level during genome evolution.

Conserved motifs of BrTUB proteins

To understand the conserved nature of BrTUB proteins, we conducted motif analysis. A total of 10 conserved motifs were identified in the protein sequence of BrTUB genes (Fig. 4). Almost all the BrTUB proteins possessed 8 to 9 motifs. The motifs 7, 3, and 1 were identified in all the BrTUB proteins. BrTUB5 contained the least number of motifs among all other BrTUB proteins.

CRE in BrTUB promoter

The *BrTUB* promoters were shown to have a wide range of cis-regulatory element (CRE) patterns, including tissue-selective expression, adversity stress, phytohormone responses, and environmental stimuli (Fig. 5). Two to eight light response CREs were present in all *BrTUB* promoters, suggesting that light exposure may have an effect on gene expression in this family. The presence of meristem-specific expression CRE in the promoter region shows their function in controlling gene expression in various tissue types. Environmentally relevant CREs that were detrimental to drought, low temperature, and stress defense were present in all *BrTUB* promoters. Furthermore, four phytohormone response cis-regulatory elements associated with ABA, auxin, gibberellin, and MeJA were found in the promoters.

Gene ontology (GO) of BrTUB genes

The Gene Ontology (GO) analysis is essential for understanding gene activities. The Gene Ontology analysis of *BrTUB* genes demonstrated their unique roles in *B. rapa* development and stress response (Fig. 6). Major biological activities regulated by *BrTUB* genes include leaf growth, plant organ, pollen development, ABA signaling transduction pathway, ion carriers and response to fungus. All *BrTUB* genes are localized in the nucleus and exhibit transcription factor-related activity, as shown by cellular processes (CP). The molecular functions indicate that *BrTUB* genes demonstrate DNA binding activity, a hallmark of transcription factors.

Protein interactive analysis

The investigation of protein-protein interactions using the STRING online database is an effective method for elucidating the characteristics of specific proteins or protein groups involved in developmental biology or stress response. Here, our analysis suggested that BrTUB interacts with an array of other proteins (Fig. 7A). For instance, our reference protein BrTUB1 interacts with PER6, PROT3, and TET13. Other proteins of high interest namely TULP group and NHL25 interact strongly with BrTUB3 and BrTUB3 (Fig. 7A). The interactive map retrieved from the STRING database also displayed numerous proteins that are engaged with BrTUB in certain ways (Fig. 7B). The highest interacted proteins

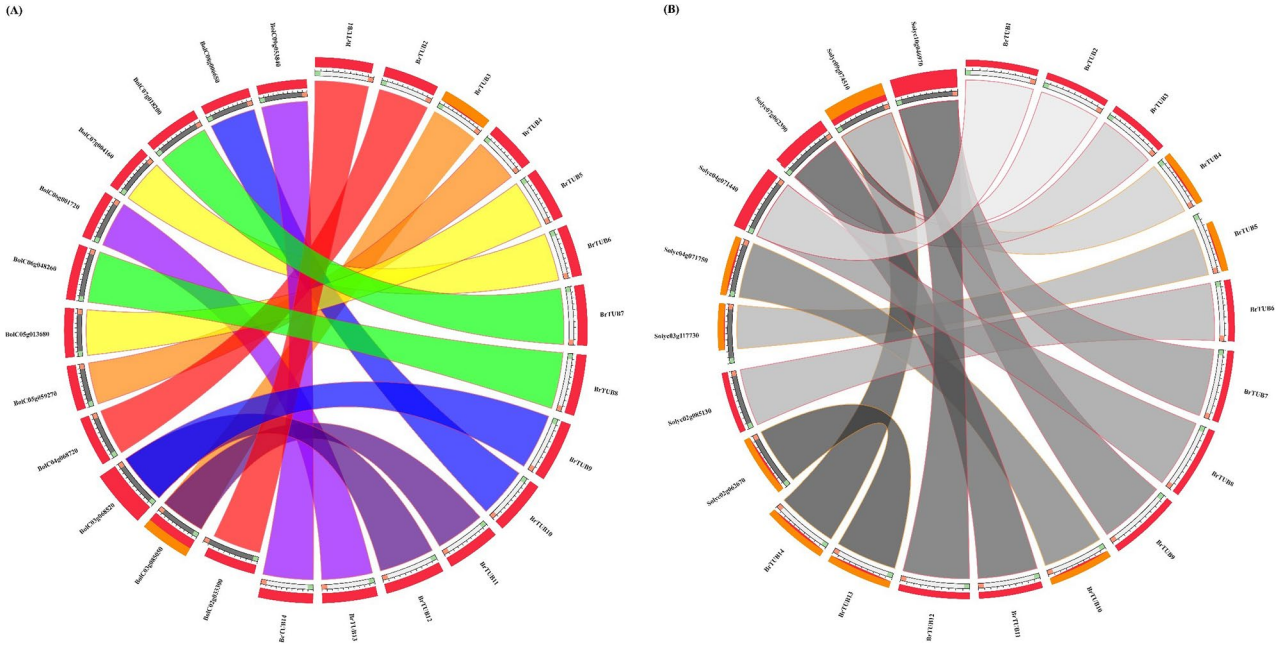

Fig. 3 Syntenic analysis of BrTUB genes in *B. rapa* genomes. (**A**) Syntenic links were drawn between the *B. rapa* and *B. oleracea* TLP genes. (**B**) Syntenic relationship between *B. rapa* and *S. lycopersicum* TLP genes

Fig. 4 Conserved motif analysis of BrTUB proteins. Different colors represent different motifs and the designated number is assigned

belong to Tubby genes group that are crucial in growth and stress response. The GPA1, a key flower and stomatal development gene interacted strongly with our BrTUB1 reference proteins (Fig. 7B).

Expression of *BrTUB* genes under ABA treatment

ABA is the master regulator of plant stress response. The presence of ABA-responsive *cis*-elements in the promoter region of *BrTUB* genes allowed us to investigate their expression under ABA treatment. The expression of *BrTUB1* following ABA treatment increased significantly at 3, 6, and 12 h and reached its maximum of 10 folds at 12 h compared to 0 h (Fig. 8). The *BrTUB3* induced at 3 and 6 h and declined at 12 h. The *BrTUB6* gene reduced sharply at 3 h: however, no significant difference was observed between 0, 6, and 12 h. In addition, the *BrTUB9* gene displayed a downregulated expression pattern at 6 and 12 h whereas no significant difference was recorded between 0 and 3 h. The *BrTUB12* gene showed an obvious increase in expression and reached a maximum of 35 folds at 12 h. The *BrTUB14* at 6 h peaked at a maximum of 3 folds compared to 1 fold at 0 h. A significant difference between 0 h and 3 and 12 h was also recorded for the *BrTUB14* gene.

Expression of *BrTUB* genes under Ethylene treatment

Ethylene, a gaseous hormone is crucial for plant growth and stress response [27]. Herein, we performed an expression analysis of *BrTUB* genes under ethylene treatment. The expression of *BrTUB1* following ethylene treatment declined at 3, 6, and 12 h. Similarly, the *BrTUb9*, *BrTUB12*, and *BrTUB14* sharply declined in the ethylene-treated samples. On the other hand, the *BrTUB3* and *BrTUB6* induced at all time points under ethylene treatment (Fig. 9). The highest expression for *BrTUB6* was recorded at 6 and 12 h where it reached a maximum of 3 folds compared to that 1 fold at 0 h.

Expression of *BrTUB* genes under drought stress

Drought stress is a serious environmental stress, damaging the crop and overall yield at a significant proportion [9, 28, 29]. Here the expression of *BrTUB* genes was analyzed in the brassica rapa plants subjected to drought stress. For instance, the expression of *BrTUB1* inclined sharply at 3, 6, and 12 h and reached a maximum of 15

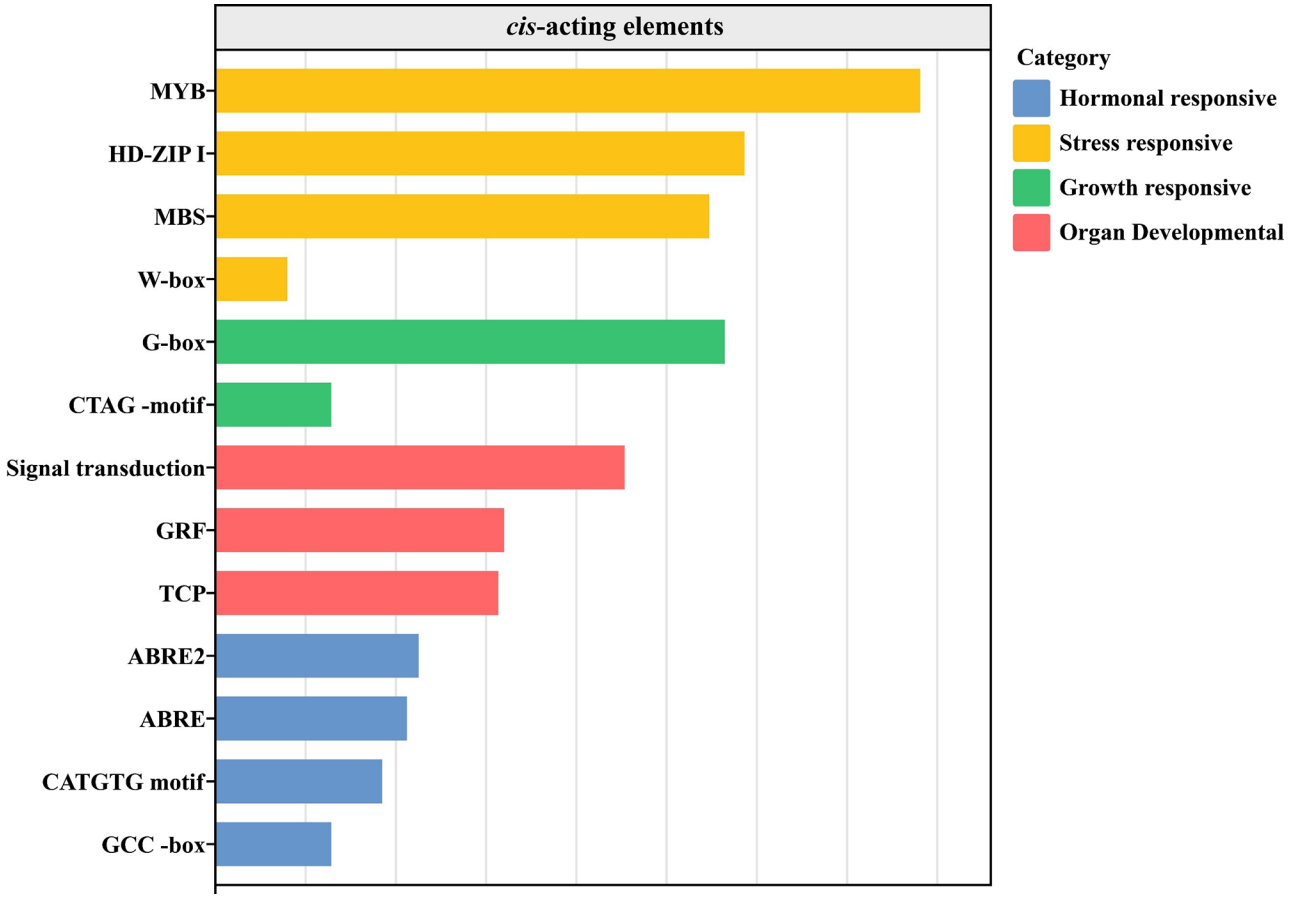

Fig. 5 Predicted *cis*-elements in the *BrTUB* genes promoters. Different colors of bars represent different categories of *cis*-elements

folds at 12 h. On the other hand, no significant difference was recorded for *BrTUB3* at all the time points (Fig. 10). The transcriptional level of *BrTUB6*, *BrTUB9*, and *BrTUB14* increased many folds compared to that of 0 h. Compared to 0 h, only the expression of *BrTUB12 was* downregulated under drought treatment at all the time points.

Discussion

Addressing the escalating global demand for *B. rapa* is increasingly difficult due to climate change, as drought conditions jeopardize oilseed production in numerous regions worldwide. Transcription factors (TFs) play a key role in regulating the response of *B. rapa* to drought stress. Despite the identification of TLP protein-coding genes in other species, including Arabidopsis [5], Soybean (*Glycine max*) [30], and rice [31], literature pertaining specifically to walnut is limited. Our investigation has effectively discovered 14 members of the *BrTUB* family in the *B. rapa* genome.

The BrTUB proteins can be categorized into six classes according to the evolutionary tree (Fig. 2A). All BrTUB proteins, except for BrTUB, have two characteristic domains: the F-Box and Tubby (Fig. 2B). This observation is notably compelling, as plants are characteristically differentiated from other eukaryotic TLPs by the existence of an N-terminal F-box domain [11]. Consequently, BrTUB3 may signify a crucial juncture in the evolutionary trajectory of TLP genes, connecting plants and eukaryotic organisms. Additionally, the F-box domain contains the conserved Skp1 binding site, which is essential for the regulation of protein ubiquitination and degradation. Since the BrTUB3 is devoid of this domain, it may circumvent ubiquitination and undergo degradation by this mechanism [32]. Processes such as segmental duplication, co-evolution, random translocation, expansion, and insertion may give rise to the F-box and Tubby domains of TLPs [33]. Segmental duplication is a significant contributor to the proliferation and maintenance of gene family clusters [34]. Our analysis of the link between *BrTUBs* revealed that six pairs of *BrTUBs* are associated with duplicated genomic regions (Fig. 3). Several newly found proteins are incorporated into the interaction network calculated using BrTUB as queries (Fig. 7). The Two pore calcium channel (TPC) protein serves as a pivotal nexus for protein interactions, significantly regulating basal jasmonate signaling and defensive responses. The peroxidase proteins are key in regulating the plant

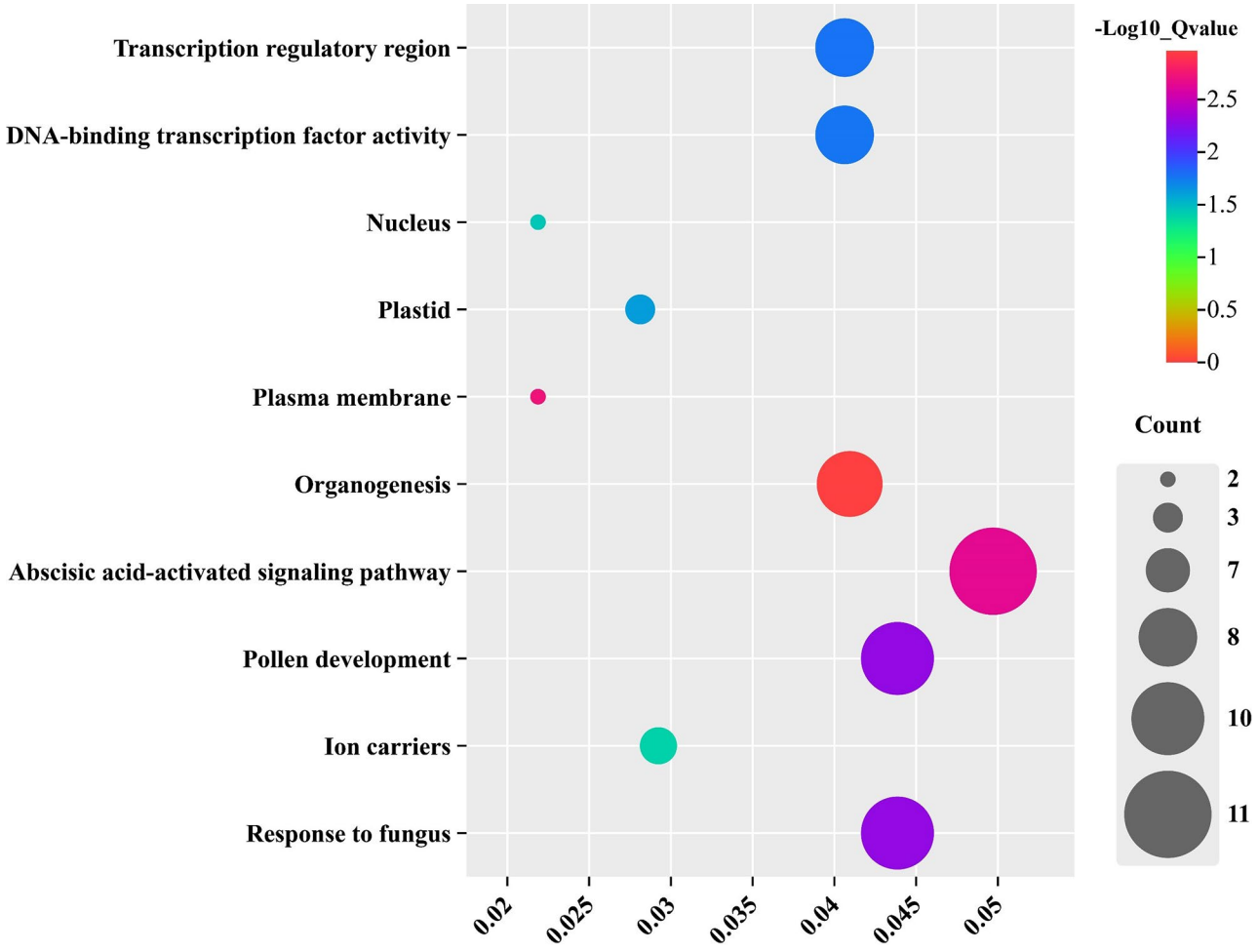

Fig. 6 The gene ontology of *BrTUB* genes was split into 3 categories (Biological processes: BP, Cellular processes: CP, Molecular function: MF), and the final presentation was made using the ChiPlot online server

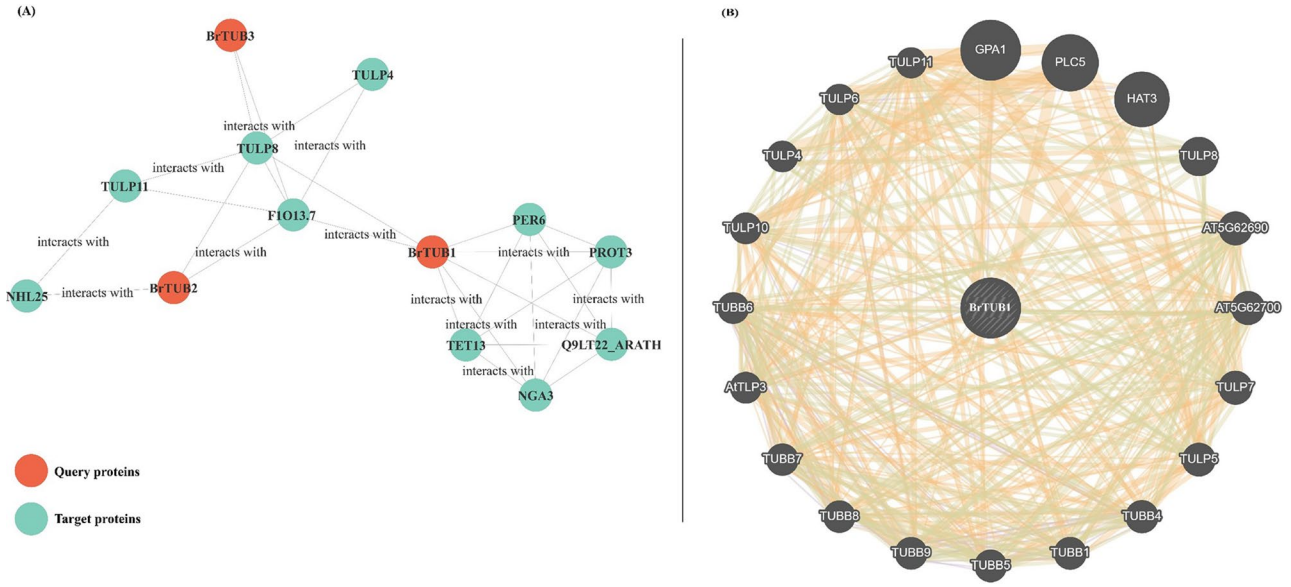

Fig. 7 Protein interaction and co-expression network analysis of BrTUB proteins. (**A**) The Protein-Protein interaction (PPI) was drawn using the BrTUB proteins as a reference to identify their predicted functional partner. (**B**) The co-expression analysis of *BrTUB* genes with their potential interactive partners

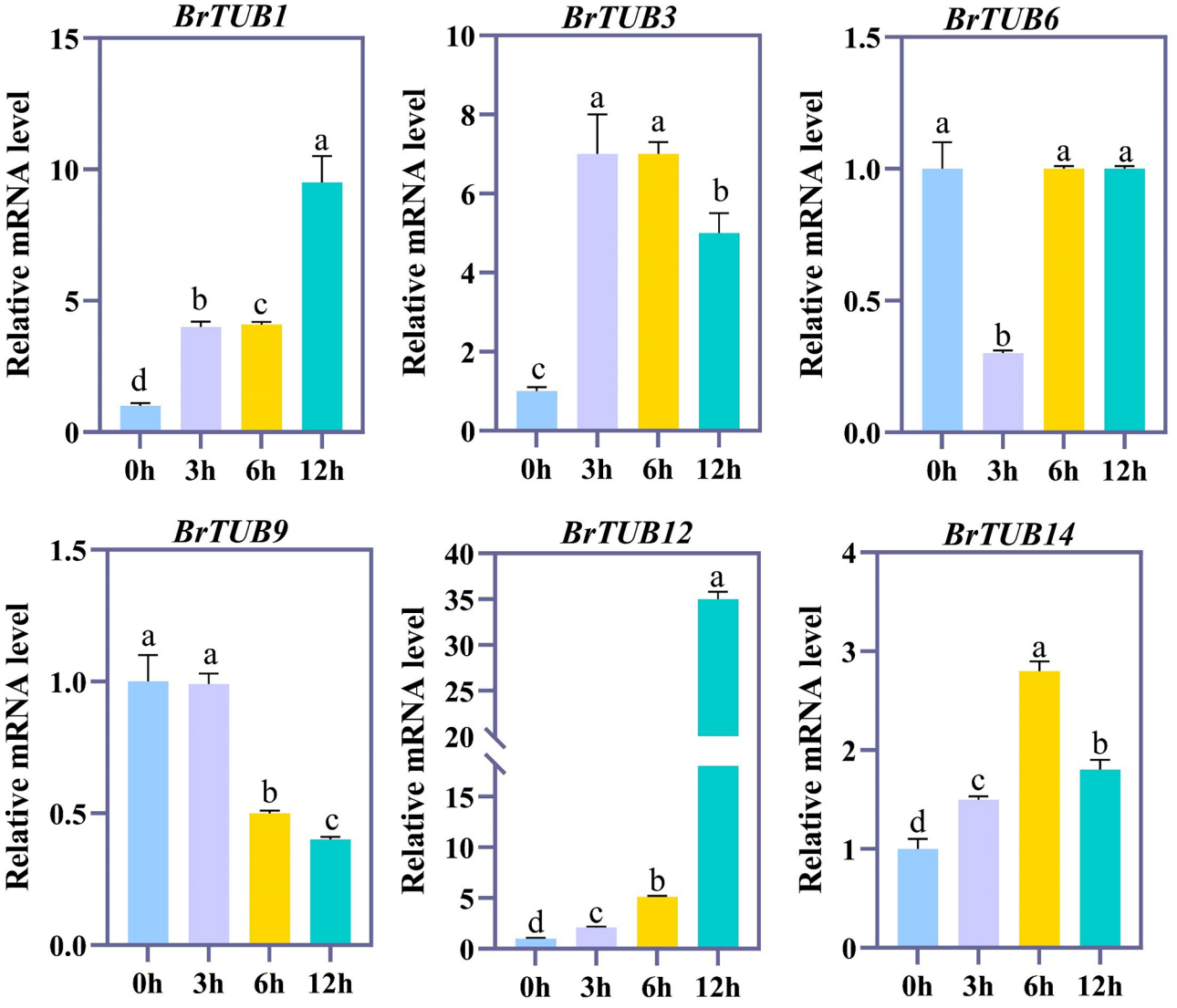

Fig. 8 ABA treatment-induced changes in *BrTUB* transcript levels. The graph displayed data from four different time points: 0, 3, 6, and 12 h. Three replicates' mean values ± standard deviation (SD) is shown in bars. Significant differences at $P < 0.05$ are indicated by different small letters

response to drought stress in ABA dependent or independent manner [35]. Our reference protein BrTUB1 strongly interacts with the PER6 (a peroxidase genes) (Fig. 7A) and therefore maybe involved in fine-tuning the *B. rapa* response to drought stress. The promoter region of PER6 contains ABRE *cis*-elements similar to that of *BrTUB1* (Fig. 5). It can be suggested that ABA activates the expression of *PER6* and *BrTUB1* that further stimulates the tolerance level of *B. rapa* to drought stress.

Phytohormones are recognized for their essential function in orchestrating plant responses to environmental stressors [23, 36–38], with ABA identified as the principal regulator of abiotic stress tolerance [39, 40]. The endogenous synthesis of ABA in the plant is induced by many stress signals including drought, likely including the activation of genes that encode enzymes converting β-carotene into ABA [41]. The putative ABA-responsive (ABRE), low-temperature responsive (LTR), and dehydration-responsive (DRE) components were found on all fifteen *Zea mays* TLP promoters [42]. Numerous transcriptomic investigations indicate that 50% of ABA-regulated genes are influenced by drought stress. Of these, 245 genes have been identified in Arabidopsis [43]. Similarly, 43 out of 73 stress-responsive genes in rice have been documented as regulated by ABA and drought stress [43]. Similarly in our study, the expression of *BrTUB* genes elevated significantly following ABA treatment. Our study suggests that *BrTUB* genes in particular *BrTUB12* (Fig. 8) could be involved in modulating *B. rapa* response to drought stress.

Ethylene, a gaseous plant hormone, regulates drought stress-mediated signal transduction on plant adaptation

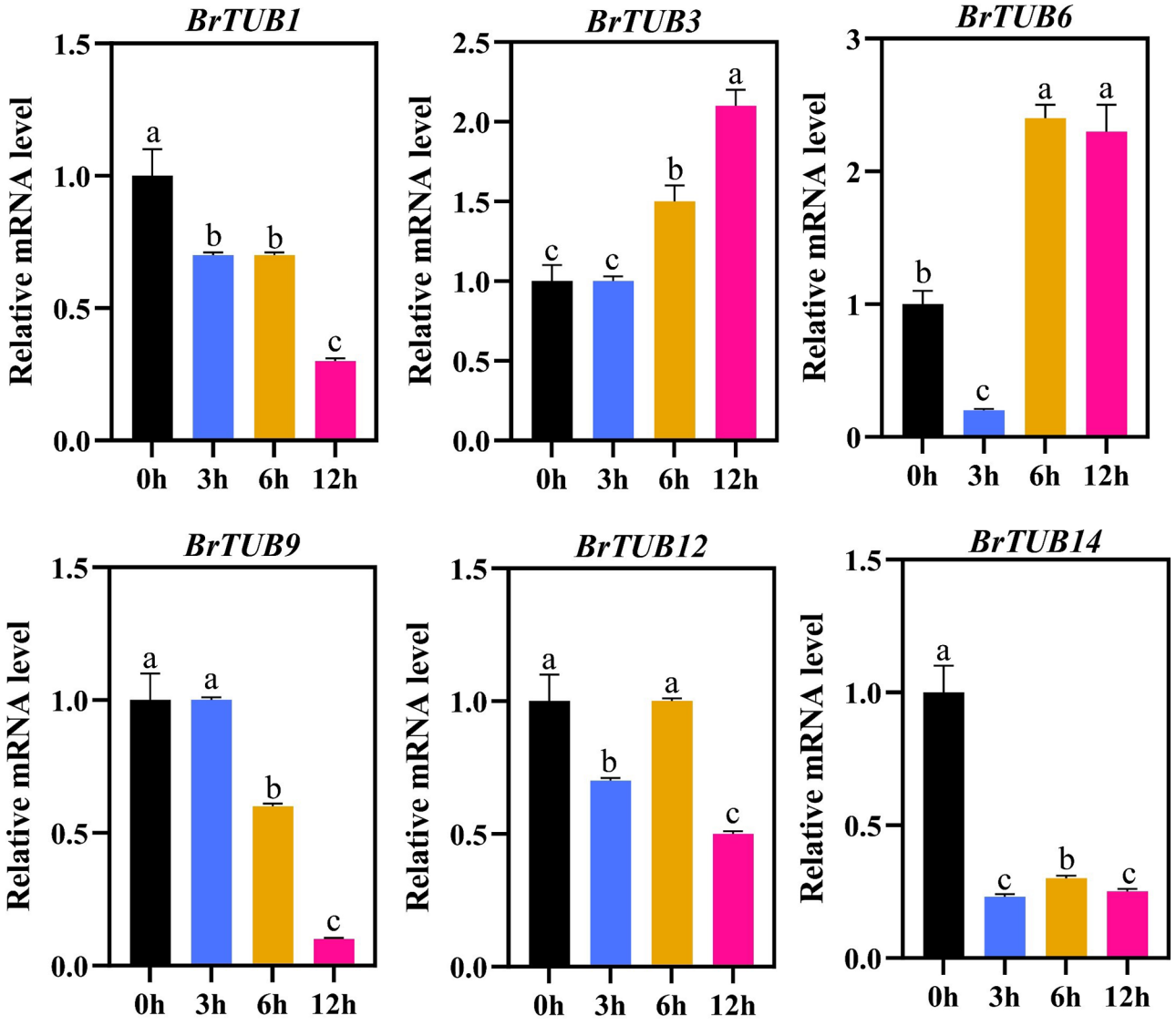

Fig. 9 Ethylene treatment-induced changes in *BrTUB* transcript levels. The graph displayed data from four different time points: 0, 3, 6, and 12 h. The bars show three replicates' mean values ± standard deviation (SD)

[44–46]. At drought stress, ethylene works as a pro-oxidant (ROS accumulator) and antioxidant (ROS scrapper). Osmotic adjustments and ROS regulation in Arabidopsis are linked to ethylene's upregulation of soluble sugars, proline acquisition, and enzymatic antioxidant activities [44]. On the other hand, ethylene modulates the expression of key TFs that are instrumental in drought stress response. For instance, Arabidopsis modified with a constitutive 35 S promoter: ERF1 exhibits drought tolerance, and the ERF protein directly binds to the DRE element of the RD29B promoter [47]. A/GCCGAC is a fundamental sequence of the DRE element, which constitutes a *cis*-acting promoter element involved in gene expression and regulation under drought conditions, characterized by high survival rates and poor yields [47]. Here in our study, the expression of majority of *BrTUB* genes displayed downregulated expression trend except the *BrTUB3* and *BrTUB6* (Fig. 9). ABA and ethylene crosstalk have also been discussed concerning drought stress. The exogenous ethylene application hinders the ABA-mediated stomatal closure and causes drought sensitivity in the process [48]. In our study, *BrTUB12* under ABA showed an induced expression pattern but was suppressed by ethylene (Figs. 8 and 9). It can be suggested that *BrTUB12* augments the response of *B. rapa* to drought stress only in an ABA-dependent manner.

The TLP genes have been reported for their role in tailoring plant response to abiotic stresses including drought. For instance, In *Cicer arietinum*, the *CaTLP1* overexpressing transgenic plants showed increased resistance to oxidative stress, salt, drought, and ABA [49]. The ectopic expression of cotton *GhTULP30* in

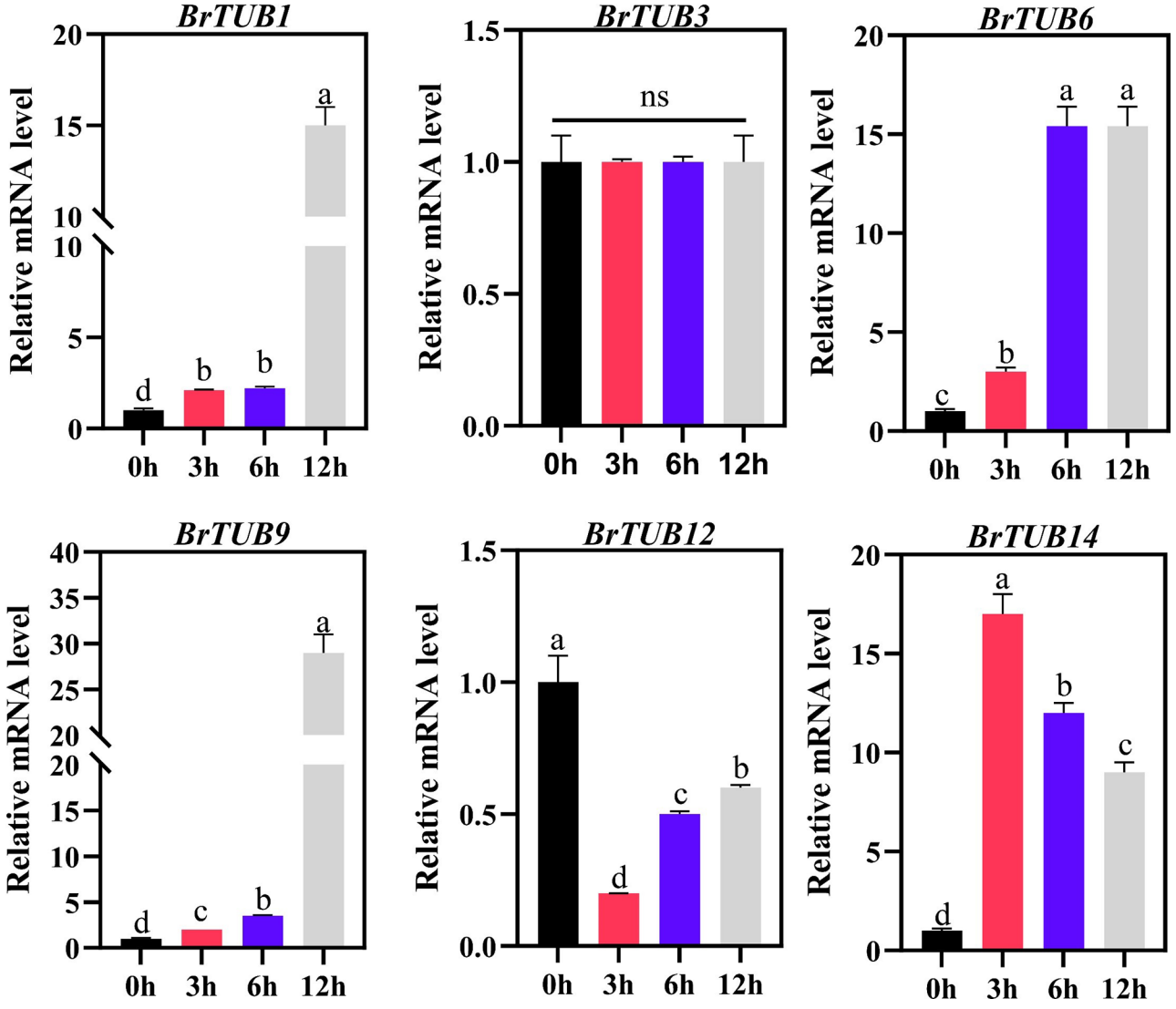

Fig. 10 Drought treatment-induced changes in *BrTUB* transcript levels. The graph displayed data from four different time points: 0, 3, 6, and 12 h. The bars show the three replicates' mean values ± standard deviation (SD)

yeast significantly enhanced the tolerance of yeast cells to salt and drought conditions [50]. The overexpression of *GhTULP30* enhanced the drought and salt stress resistance of Arabidopsis seeds during germination and accelerated the stomatal closure rate of the plant under drought-stress conditions. The silencing of *GhTULP30* in cotton via virus-induced gene silencing (VIGS) technology reduced the stomatal closure rate during drought stress and diminished stomata's length and width [50]. It was shown that *GmTLP8* overexpressed soybean plants were more resilient to salt and drought stressors than *GmTLP8*-RNAi lines [30]. In our study, the expression of *BrTUB* genes displayed varied expression trends in the *B. rapa* plants subjected to drought stress (Fig. 10). Drought stress enhanced *BrTUB1*, *BrTUB6*, and *BrTUB9* while suppressing *BrTUB12* and *BrTUB14* (Fig. 10). The downregulation of *BrTUB12* during drought stress, along with its sensitivity to ABA, further supports its function in ABA-mediated drought tolerance in *B. rapa*.

Conclusion

Here in, we extracted a total of 14 *BrTUB* genes from the Brassica genome database. Based on domain and structural features, the *BrTUB* family was classified into six subfamilies. The expansion and evolution of the *BrTUB* gene family are primarily driven by segmental duplications, contributing significantly to both conservation and variability. Notably, the promoter region of *BrTUB* contains CREs that are essential for regulating responses to plant hormones and potentially drought stress. The differential expression of *BrTUB1* and *BrTUB12* in response to ABA and ethylene highlights their potential

in regulating drought stress response of *B. rapa*. This initial discovery and comprehensive study provide significant knowledge that will guide future investigations into the functions of *BrTUB* genes in *B. rapa* drought stress biology.

Supplementary Information

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Supplementary Material 1

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Author contributions
Author Contributions: I.K planned and designed the research. I.K and J.G. collected the plant materials, performed the experiment, and analyzed the data. I.K., J.G., U.K., and G.L. wrote the manuscript. J.G., M.F. contributed to sample data collection. All authors have read and agreed to the published version of the manuscript.

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Data availability
The published article and its supplementary materials include all the data generated in this study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

New clinical tools and procedures
Not applicable.

Competing interests
The authors declare no competing interests.

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