Genome Wide Identification and Characters of TCP Family Genes in Brassica juncea var. tumida

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Quan Sun
Chongqing University of Posts and Telecommunications
sunquan@cqupt.edu.cn Corresponding Author
ORCiD: https://orcid.org/0000-0002-7228-9363

Jing He
Chongqing University of Posts and Telecommunications

Xiaohong He
Chongqing University of Posts and Telecommunications

Pingan Chang
Chongqing University of Posts and Telecommunications

Huaizhong Jiang
Chongqing University of Posts and Telecommunications

Daping Gong
Chinese Academy of Agricultural Sciences Institute of Tobacco Research

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Abstract

Background

Teosinte branched1/Cycloidea/Proliferating cell factor (TCP) proteins are plant-specific transcription factors, which widely involved in leaf development, flowering, shoot branching, and circadian rhythm. So far, TCP proteins function in tumorous stem mustard has not been reported. Here we identified and characterized the entire TCP protein family members in the tumorous stem mustard.

Results

We identified fifty-four TCP genes in Brassica juncea var. tumida, containing thirty-three Class I subfamily members and twenty-one Class II subfamily members. Fifty-three TCP genes are distributed on 15 chromosomes. Gene structure and conserved motif analysis showed that the same clade genes have similar gene intron/exon structure and conserved motifs. Cis-acting element results showed that the same clade genes also have similar cis-element, however subtle differences also imply the different regulated pathway. More than twice paralogs genes relation to diploid species in some members imply gene duplication events in evolution. The members of BjTCP18s are low expressed in DY strains and un-swelling stage of YA strains. After treatment with GA and SA, it was detected that the expression levels of multiple TCP genes were affected by these two hormones.

Conclusion

In this study, we perform the first genome-wide analysis of the tumorous stem mustard TCP gene family. The results provide valuable information for understanding the classification and functions of TCP genes in tumorous stem mustard.

Background

The Teosinte branched1/Cycloidea/Proliferating cell factor (TCP) family is a group of plant-specific transcription factors (TFs) that involved in embryonic growth, leaf development, branching, flowering, circadian rhythm, hormone signalling, stress responses and so on [1–9]. TCP family proteins contain a highly conserved TCP domain that formed with an N-terminal region enriched in basic amino acids followed by two amphipathic α-helices connected by a disordered loop[10, 11].

Base on their conserved domain, the TCP proteins are divided into two subfamilies: Class I and Class
Class I TCPs bind the DNA sequence GGNCCAC and Class II TCPs bind GTGGNCCC[12]. In Arabidopsis, TCP2–5, TCP10, TCP13, TCP17 and TCP24 were related to lateral organ organogenesis and controlled leaf development[13–16]. TCP1, TCP12 and TCP18 are similar homology genes in one subfamily, for the reason that Branched1(TCP18) and Branched2(TCP12) control branch outgrowth[17–20]. TCP18 interacts with the florigen proteins FLOWRING LOCUS T(FT) and modulates its activity in the axillary buds to repress the floral transition of axillary meristems[21]. TCP21 is involved in circadian clock through interacts with TIMING OF CAB EXPRESSION 1(TOC1) and CIRCADIAN AND CLOCK ASSOCIATED1 (CCA1) promoter[22]. The function of TCP proteins in plant growth and development is usually regulated by a series of phytohormone synthesis and metabolism, including brassinosteroids (BRs), jasmonic acid (JA), indole-3-acetic acid (IAA) and so on[7, 16, 23, 24].

In recently years, TCP proteins were demonstrated to be related with defence responses. TCP13, TCP14 and TCP19 were found to be directly targeted by effectors form both Pseudomonas syringae and Hyaloperonospora arabidopsidis[25]. Kim et al. found TCP8, TCP13, TCP15, TCP20, TCP22 and TCP23 can interact with the Arabidopsis immune adaptor SUPPRESSOR OF rps4-RLD1 (SRFR1) that is a negative regulator of effector-triggered immunity[26].

TCP gene family has been identified in many plant species, such as, 24 TCP genes in Arabidopsis [9], 28 TCP genes in Oryza sativa s, 30 TCP genes in Lycopersicon esculentum [27], 33 TCP genes in Populus euphratica [28], 27 TCP genes in Citrullus lanatuss [29], 66 TCP genes in Triticum aestivum [30], 75 TCP genes in Gossypium barbadense [31], 39 TCP genes in Brassica rapa L. ssp. Pekinensis [32] and 39 TCP genes in Brassica rapa ssp. rapa [33].

Tumorous stem mustard (Brassica juncea var. tumida Tsen et Lee) is an important crop with great economic benefits in China. Improve the yield of this crop is a key issue for the Chinese pickle industry. The growth of Tumorous stem mustard are divided into four stages: germination, seedling, stem swelling and flowering. Stem swelling is a key character of tumourous stem formation. The balance between stem swelling and flowering were directly related to the quality and yield of tumourous mustards. Tumourous stem mustard is an annual plant, and the stem does not swell
except in plants sown between mid-September and mid-October in Chongqing and the other valleys of the Yangtze River, China. Thus, the production period of edible stems is limited. As above reports, TCP proteins were extensively involved in branching, flowering and a series development processes, forming the shape of plant architecture[7, 17, 18, 21, 24, 34–46]. However, no report on tumorous stem mustard TCP family exists, and whether TCP family proteins control the stem swelling and flowering development of tumorous stem mustard are still unknown. 

As the whole genome of tumorous stem mustard is sequenced[47], a genome wide analysis of TCP genes is performed for the first time in the current study. Fifty-four BjTCP genes were identified in the tumorous stem mustard genome, and their phylogenetic relationship, gene structure, protein motifs, chromosome location, and expression profile in different tissue were analysed. The results provide information on the classification and detail of BjTCPs and lay the foundation on the stem swelling and flowering regulation mechanism of TCP proteins in tumorous stem mustard.

Results
Identification of members of the TCP family in Brassica juncea var. tumida
Fifty-four TCP family genes were identified in Brassica juncea var. tumida, based on the similarity with Arabidopsis thaliana homology genes, the tumorous stem mustard TCP genes were named with BjTCP1a-BjTCP27b (Table 1). The coding amino acids were 171–457, with the molecular weight (MW) of 18.6–50.08 kDa and the isoelectric point (pI) of 5.5–10.18. Except BjTCP27b anchored in contig6125, TCP genes were located on 15 of 20 chromosomes. There are one TCP gene on chromosomes A10, B01 and B06, two TCP genes on A06, as well as three to seven members on the other chromosomes (Figure 1). Except BjTCPa-c, other BjTCP proteins all are localized in nuclear (Table 1), that is a typical feature of transcription factors.

The phylogenetic tree of BjTCPs and AtTCPs
Multiple sequence alignment of TCP proteins showed the conserved region mainly focused on the TCP domain (Figure S1).

To assess the phylogenetic relationships of the TCP family, the predicted TCP proteinsequences in Brassica juncea var. tumida and Arabidopsis thaliana were used to construct a phylogenetic tree. The results indicated that all the TCP proteins were divided into two groups, Class I and Class II TCPs
In the Class II TCPs cluster, these proteins were further divided into three subgroups, CYC, TB1 and CIN. The CYC subgroup was mainly cluster with AtTCP1 and AtTCP12, containing four AtTCP1 homology proteins BjTCP1a-d and two AtTCP12 homology proteins BjTCP12a-b. The TB1 subgroup was composed of AtTCP18 and four homology TCP proteins BjTCP18a-d. In CIN cluster, no proteins were found to be homology with AtTCP2-4 and AtTCP10, the other TCP proteins were found two or three homology proteins like AtTCP24 (three homology proteins BjTCP24a-c), AtTCP13 (three homology proteins BjTCP13a-c), AtTCP27 (two homology proteins BjTCP27a-b) and AtTCP5 (three homology proteins BjTCP5a-c). In the Class I TCPs cluster, except AtTCP6, AtTCP11, AtTCP16, AtTCP23 cannot found homology proteins in Brassica juncea var. tumida, the other TCP proteins all have multiple homology proteins, such as AtTCP15 and AtTCP21 even have six homology proteins, respectively. The BjTCP proteins of mustard have a typical bHLH motif in all other identified TCP proteins, (Figure 2b). As TCP proteins of Arabidopsis thaliana, the main different of Class I and Class II TCP family proteins were the identity of the residue at position 10-15 of the domain. Most Class I BjTCP proteins lose four amino acids between position 9-13 and have a Gly at position 15, whereas Class II BjTCP proteins have an Asp at this position in the TCP domain (Figure 2b).

The gene structures and conserved motifs analysis of BjTCPs

The intron-exon gene structure results showed that most BjTCP genes only have one exon except BjTCP18s, BjTCP12s, BjTCP20b, BjTCP13b containing two and more exons. The genetic structure and evolutionary relationships of all TCP family members of stem mustard are also closely related. Genes within the same subfamily often have similar gene structures. BjTCP12a and BjTCP12b genes were composed of two exons, and BjTCP18a-d were more than three exons (Figure 3A and B). The conserved motifs in these BjTCPs also showed the similar characters in the same subgroup, such as three similar motifs in all BjTCP15 homology proteins and five similar motifs in all BjTCP21 homology proteins (Figure 3C).

The gene duplication of BjTCP members

The gene duplication events were further analysed in the tumorous stem mustard TCP gene family. As shown in Table 2, 67 paralogous and/or orthologous pairs were identified. The Ka/Ks ratio is widely
applied to measure genetic evolution and selection pressure. The ratios of forty-eight homologous pairs were greater than 1, in contrast other nineteen homologous pairs were smaller than 1, indicating positive selection and negative selection during evolution, respectively (Table 2). Meanwhile, the divergence times of the TCPs were also calculated. The results indicated that these *BjTCPs* underwent duplication events from ~2.65 to 37.39 million years ago (MYA).

**The promoter cis-acting element analysis of *BjTCPs***

The cis-acting element in the promoter of a gene usually regulated the expression and function. Multiple cis-acting elements were found in these TCP gene promoters, such as plant hormone responses element, light responses element, stress responses element, meristem expression, circadian control, low-temperature and wound responses element and so on (Figure 4).

For hormone-related cis-acting elements, the abscisic acid (ABA) responsive elements were identified at least two or more ABRE cis-acting elements in the tumorous stem mustard TCP gene promoters expect *BjTCP5s, BjTCP18s, BjTCP19* and *BjTCP24s*. Auxin responsive elements include AuxRP and TGA elements. AuxRP has a relative small number of components, mainly in the *BjTCP5, BjTCP19* and *BjTCP20* homologous gene promoters. The distribution of TGA-element is relatively more extensive. The MeJA responsive related elements CGTCA and TGACG are found on most gene promoters, except for *BjTCP12, BjTCP19* and *BjTCP20* homologous genes. A number of other hormones related cis-element, such as ethylene (ET) responsive element ERE, gibberellin (GA) responsive elements GARE, P-box and TATC-box, and salicylic acid (SA) responsive element TCA-element are also present in promoters of some *BjTCP* genes.

In addition, a large number of cis-acting elements related to light responsive have been found on these TCP gene promoters, including 3-AF1 binding site, ACE, AE-box, TCT-motif, ATC-motif, Box 4, GATA-motif, G-Box, GT1-motif and I-box. Others include WUN-motif (related to wound), meristem-element (related to meristem), circadian element (related to circadian control), LTR element (related to low-temperature induction), defence and stress responsiveness elements (include MBS, Myb, Myc, STRE, TC-rich, W box and ARE elements). In particular, MYB and MYC-motif elements are identified on almost all TCP gene promoters.
Tissue-specific expression profiles of *BjTCPs*

In *A. thaliana*, TCP proteins were mainly involved in development and defence. Tumorous stem mustard is mainly used for a vegetable crop. The expression patterns of all TCP genes during the different development periods and tissues were analysis base on the previous RNA-seq data[48]. The results showed the expression of *BjTCP1a* and *BjTCP5c* cannot be detected in all samples. Twenty-six *TCP* genes were highly expressed in at least two tissues (log2(FPKM) > 3, the subgroup half bottom in Figure 5). *BjTCP13b* and *BjTCP5a* were weakly expressed in DA sample. The expression of *BjTCP1b*, *BjTCP1c*, *BjTCP1d*, *BjTCP5c*, *BjTCP7c*, *BjTCP13a*, *BjTCP13c*, *BjTCP18d* and *BjTCP19* were not detected in DY stem tissue. *BjTCP12a*, *BjTCP12b* and *BjTCP18c* have weakly expression in DY, YA1 and/or YA2 (Figure 5).

**Expression Analysis of *BjTCP* Genes in response to Exogenous hormones**

To predict possible functions of *TCP* genes in environmental adaptation, we investigated the transcriptional profile of *TCP* genes under SA and GA treatment.

For SA treatment, almost all of the detected *BjTCPs* are upregulated after treatment for 2-8 hours expected *BjTCP1*, *BjTCP12* and *BjTCP17*, whereas decreased to be at a low level at 24h. The *BjTCP9*, *BjTCP13*, *BjTCP15*, *BjTCP18*, *BjTCP19*, *BjTCP22* and *BjTCP24* are induced at the early stages and maintained at a relatively high level until 8h. *BjTCP17* are induced slowly and the high expression level appear at 24h after treatment (Figure 6).

Under GA treatment, the levels of *BjTCP12*, *BjTCP17* and *BjTCP20* cannot be detected. In contrast, the expression of other genes were induced at the early stages but then decreased to a low level mainly at 8h (Figure 6).

**Discussion**

As plant-specific TFs, the TCP TFs play a various function role in plant growth and development processes. In tumorous stem mustard, 54 family members were found. As other plants, the general organization of these TCP family are also conserved and significantly more members in the class I subfamily than those in the class II[28–33, 49–52]. As a tetraploid plant, the number of TCP proteins in tumorous stem mustard is significantly more than twice as abundant as Arabidopsis (24 TCP proteins), which suggest that some genes are doubled in the process of evolution.
In addition, the exon/intron structure, conserved motif distribution patterns and domain of BjTCP homology genes often showed high similarity, such as BjTCP21a-f, BjTCP12a/b and so on. These similarities cluster homology genes members might play similar function during tumorous stem mustard growth and development.

Interesting, a series of genes such as BjTCP15b, c and BjTCP1b and BjTCP22b are located on the same chromosome A07. But the homology gene of these genes can be found (BjTCP15b and BjTCP15f, BjTCP15e and BjTCP15c, BjTCP1b and BjTCP1c, BjTCP22b and BjTCP22d) in series and showed the same order on the corresponding B03 chromosome (Figure 1 and 2). The allopolyploid tumorous stem mustard (B.juncea, AABB) may form by hybridization between the diploid ancestors of B.rapa (AA) and B.nigra (BB), followed by spontaneous chromosome doubling. These results indicated that the formation of fragments between the four genes of two chromosome A07 and B03 may be obtained from B.rapa (AA) and B.nigra (BB), respectively. The most similarity of the eight genes were aqueried in B.rapa (AA) and B.nigra (BB) using the blastP program, and four gene were selected out in B.rapa (AA) and B.nigra (BB), respectively. The evolutionary relationships of the sixteen genes coding proteins showed the four genes located on the same chromosome A07 were cluster with the homology genes of A subgenome ancestor B.rapa (AA) in one branch, respectively (Figure S2). And the four genes on chromosome B03 are also corresponding to B subgenome ancestor B.nigra (BB), respectively (Figure S2). These results also indicted that the division of BjTCP15b and BjTCP15c may be earlier than the formation of tetraploids.

There are 24 TCP genes in Arabidopsis, whereas some corresponding homologous TCP genes cannot find in tumorous stem mustard, such as TCP2–4, 6, 11 and 16, which may due to occurred losing event during the evolution process. Tumorous stem mustard is a tetraploid plant that belongs to the cruciferous near-source species of Arabidopsis. In theory, each of the Arabidopsis TCP genes has two orthologous genes in the stem mustard. However, some TCP genes can find out more than two paralogous genes, such as BjTCP1 (four homology genes), BjTCP18 (four homology genes), BjTCP21 (six homology genes), BjTCP15 (six homology genes) and so on. These genes may be caused by multiple gene doubling events, the function of these paralogous genes have similarity and gradual
differentiation in the process of evolution. First of all, from the characteristics of the cis-elements of these genes, most of the paralogous genes have similar cis-acting elements, but there are also a few differences, such as the four paralogs of BjTCP18 are no ABA and auxin cis-acting elements, but BjTCP18b is the only member of the four genes with circadian regulatory elements, suggesting that the BjTCP18b gene may be involved in circadian rhythm (figure 4). Correspondingly, the expression patterns of these paralogous genes are also different (Figure 5).

Previous studies demonstrated that *B.juncea* genome underwent genome duplication events about 0.039–0.055 million years ago [47]. In this study, all the duplication pair gene was occurred before 2.65 MYA, suggesting that the gene duplication events occurred before the *B.juncea* forming. This results also proved the guess above that the *BjTCP* genes clustered together on the chromosome A07 and B03 were formed before the formation of tetraploids.

The Arabidopsis *BRANCHED1*(BRC1),, the rice *TB1* and maize *TB1* gene were demonstrated for its role as a negative regulator in the growth of axillary buds and branching[17, 36, 53, 54]. *BRC1* is ortholog with *TB1* and rice *TB1* that may play a similar roles and molecular mechanism in development of the primary shoot architecture and negatively regulating lateral branching[17–20, 36, 53, 55]. Moreover, OsTB1 can be regulated by IPA1 to suppress tillering in rice and TB1 can interact with FT1 to regulat inflorescence architecture in bread wheat[36, 54, 56]. For tumorous stem mustard, a close phylogenetic relationship with Arabidopsis, BjTCP18s may also play the similar function in branching. But four BjTCP18s homology genes in tumorous stem mustard, which may form by gene duplication. Functional differentiation may occur between the four TCP18 genes. From the perspective of their expression patterns, the four genes are not consistent during tissue development. In this study, the expression profiles of tumorous stem mustard seedlings and tumour stems were used, and the expression levels of these four genes were low in these tissues. The expression levels of the four genes gradually decreased with the swelling of the tumorous stems (Figure S3). The flowering stage of the tumorous stem mustard is mainly characterized by the swelling of the tumorous stem. At this time, there will be a bolting and flowering phenomenon similar to *Arabidopsis*. Since BRC1 has the function of inhibiting branching and flowering, the gradual down-regulation of its mRNA levels may
reflect the gradual decrease ability in inhibition of branching and flowering. The results also indicated that the tumorous mustard is about to enter the period of reproductive growth.

According to reports, there are 16 varieties of mustard species identified, which the mainly difference of these vegetables tissues are used for food and shape, including root, stem, leaf and branch[57]. BRC1 gene controls plant branching and flowering, and four identified BjBRC1 may imply the further function differentiation of branch development and flowering during the development of mustard. Among these BjTCP genes, multiple BjTCP15s and BjTCP21s in DY and/or the early stage of seedling and tumour stem per-swelling stage were higher expression than swelling stage. DY is a no swelling mutant line tissues sample, and YA1 and YA2 are also not begin to swelling. This result may indicate that these genes are involved in the process of stem swelling in tumorous stem mustard. Increasing evidence verified that TCP proteins are involved in responses to plant hormone[37, 58, 59]. In this study, most of tumorous stem mustard TCP genes appear to be regulated by SA and GA (Figure 6). In Arabidopsis thaliana, several TCPs interact with the SA biosynthetic enzyme ISOCHORISMATE SYNTHASE 1 gene and enhanced the gene’s expression through binding to the TCP-binding motif in its promoter region [60]. In our results, there are many SA-related cis-elements in the promoter regions of BjTCPs (Figure 4), and the expression levels of several BjTCP genes significantly increased after the SA treatment (Figure 6), indicating that BjTCPs may be involved in the signal transduction of SA. In addition, most BjTCP genes have polytype gibberellin (GA) responsive elements GARE, P-box and TATC-box in their promoters (Figure 4), which may lead to more complex regulation of their expression and more diverse expression patterns (Figure 6).

This study was the first to identify 54 BjTCP gene family members in tumorous stem mustard and to investigate their roles in stem development. Our results provide a foundation to further determine the molecular mechanisms of TCP genes in the development of tumorous stem.

Conclusions

We performed a genome-wide analysis and identify 54 TCP genes in Brassica juncea var. tumida. These genes are divided into two subfamilies, 33 Class I and 21 Class II. Chromosomal mapping showed that 53 BjTCP genes were heterogeneously distributed on 15 chromosomes. Structural
analyses of BjTCP genes showed that 44 genes had no introns, most of the BjTCP genes in the same cluster had similar patterns of exon length, intron number, and conserved motifs. We identified several genes that are highly expressed in development of tumorous stem mustard and branching relation genes were low expression in swelling stage of vegetative growth.

Materials And Methods

Plant materials growth conditions and treatments.

In this study, tumorous stem mustard cultivar Yong An Xiao Ye (YA) was used for gene expression pattern analysis. The seeds were sowed into 2:1 vermiculite: turfy soil, cultured under constant light at 22 °C with a 16/8 h light/dark regime in culture room. Three-week-old seedlings were used for exogenous hormone treatments. For SA and GA3 treatment, the seedlings were sprayed with 100 µM SA and 100 µM GA, respectively. The second true leaf on each plant was sampled at 0 (control), 2, 4, 6, 8 h and 24h after spraying. All treatments were repeated three times and each treatment contained at least 20 seedlings. All materials were frozen at -70 °C until RNA isolation.

Identification of TCP protein in tumorous stem mustard

The genome sequences of Brassica juncea var. tumida, Brassica nigra and Brassica rapa were downloaded from the Brassica database (BRAD, http://brassicadb.org/brad/index.php)[47, 61]. The TCP domain in the Pfam database with accession number PF03634 were download[62], and searched for the domain in the Brassica juncea var. tumidadatabases using HMMER 3.0 with an E-value<1e-6[63]. To confirm the results obtained using the HMMER algorithm, the protein domain were further verify in Pfam and Smart databases[62, 64, 65]. The TCP family protein sequence of Arabidopsis thaliana were download the Arabidopsis information resource website (https://www.arabidopsis.org).

Sequence and phylogenetic analyses

Multiple alignments of TCP protein sequences from Brassica juncea var. tumida and Arabidopsis thaliana were performed using the ClustalW programme[66]. Phylogenetic tree were constructed with the MEGA 7.0 software using the neighbour joining method and a bootstrap test replicated 1000 times[67]. The gene structure diagram was drawn using the online software of the GSDS2.0 server (http://gsds.cbi.pku.edu.cn/)[68]. The physical location data of BjTCP genes were retrieved from the Brassica juncea var. tumida genomes. The mapping of these TCP genes was subsequently performed
using MapInspect software. Default parameters were used for the Multiple Em for Motif Elicitation (MIME, http://meme-suite.org/) programme for the identification of conserved protein motifs and a maximum number of 12 motifs. Subcellular localizations of BjTCPs were predicted using ProComp9.0. The identified protein motifs were further annotated using Weblogo (http://weblogo.berkeley.edu/).
The 2000 bp of the 5′ sequence as the promoter domain of each TCP genes were used to analyse the cis-acting elements using the online software PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [69].

Chromosomal location and gene duplication
The physical location data of BjTCP genes were retrieved from the Brassica juncea var. tumida genomes. The mapping of these TCP genes was subsequently performed using MapInspect software. Gene duplication was defined according to the criteria described in previous studies: the aligned region of two sequences covers over 80% of the longer sequence, and the similarity of the aligned region is over 80%. In addition, the KaKs Calculator software was employed to calculate Ka (nonsynony-mous substitution rate) and Ks (synonymous substitution rate). The divergence time was calculated with the formula $T = \frac{Ks}{2r}$, the $r$ was taken to be $1.5 \times 10^{-8}$ synonymous substitutions per site per year for dicotyledonous plants.

The expression profile of TCP genes
The RNA-seq data were we previously reported and can downloaded from NCBI SRA database (http:www.ncbi.nlm.nih.gov/sra/) with the accession number were SRX108496(DY), SRX108498(YA1), SRX108499(YA2), SRX108500(YA3), SRX108501(YA4) and SRX108502(YAr), respectively[48]. Then the clean reads that were filtered from the raw reads were mapped onto B. juncea genome using Tophat2 [70, 71]. Gene expression levels of individual genes were quantified using RPKM values (fragments per kilobase of exon per million fragments mapped) by the Cufflinks 2.2.1 [70].

RNA extraction and real-time quantitative PCR analysis
The total RNA was extracted from different plant materials using RNA plant plus reagent (Tiangen Biotech Co., Ltd., Beijing, China). The total RNA samples were treated with DNase I (Takara, Qingdao, China) to remove contaminating genomic DNA. First-strand cDNA was synthesized from the total RNA using a Hiscript II 1st strand cDNA synthesis Kit (Vazyme, Nanjing, China). Real-time qPCR (qRT-PCR)
was performed using TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, Qingdao, China). BjActin was used as the internal reference gene for qRT-PCR, and the gene-specific primers are listed in Supplementary Table S1. The relative gene expression level was calculated using the $2^{-\Delta\Delta Ct}$ method.

Declarations

Authors’ contributions

QS conceived and designed the experiments, carried out the main bioinformatics analyses, and drafted the manuscript. JH and XHH performed the experiments. PAC helped to improve the manuscript. DPG collected the public dataset and assisted with data analysis. HZJ and XHH participated in its design and helped to revise the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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**Tables**

Table 1. The TCP protein family members in *Brassica juncea var. tumida*

| ID   | pfam | name   | chr | star  | end  | sence | Subcellular localization | homolog   | P | MWkD | protine(aa) |
|------|------|--------|-----|-------|------|-------|--------------------------|------------|---|------|-------------|
| BjuA00 | 7230 | BjTCP 1a | A02 | 1061  | 4941 | -     | Nuclear                  | AtTCP1     | 5 | 39.28 | 346         |
| BjuA02 | 7377 | BjTCP 1b | A07 | 2910  | 6097 | -     | Nuclear                  | AtTCP1     | 6 | 38.96 | 343         |
| BjuB03 | 0534 | BjTCP 1c | B03 | 2993  | 3758 | -     | Nuclear                  | AtTCP1     | 5 | 39.1  | 344         |
| BjuB04 | 3984 | BjTCP 1d | B03 | 2773  | 4025 | -     | Nuclear                  | AtTCP1     | 5 | 39.57 | 348         |
| BjuA04 | 1558 | BjTCP 12a | A02 | 1171  | 6146 | -     | Nuclear                  | AtTCP12    | 8 | 37.88 | 322         |
| BjuB01 | 0789 | BjTCP 12b | B05 | 5502  | 4870 | -     | Nuclear                  | AtTCP12    | 8 | 39.61 | 352         |
| BjuA01 | 1472 | BjTCP 13a | A03 | 1817  | 1755 | +     | None                     | AtTCP13    | 6 | 35.69 | 320         |
| BjuA02 | 1096 | BjTCP 13b | A05 | 3126  | 6872 | -     | None                     | AtTCP13    | 6 | 35.72 | 321         |
| Accession | Locus | ID | Type | Description | Length | Identity | Parent Accession |
|-----------|-------|----|------|-------------|--------|----------|-----------------|
| BjuB02    | 6804  | 58 | BJTCP| 13c | None | AtTCP13 | 7 34.47 309   |
| BjuA00    | 7311  | 58 | BJTCP| 15a | Nucle | AtTCP15 | 6 33.84 321   |
| BjuA01    | 6487  | 55 | BJTCP| 15b | Nucle | AtTCP15 | 6 34.06 321   |
| BjuA02    | 7170  | 56 | BJTCP| 15c | Nucle | AtTCP15 | 7 33.96 322   |
| BjuB00    | 0709  | 50 | BJTCP| 15d | Nucle | AtTCP15 | 8 32.02 300   |
| BjuB00    | 3932  | 57 | BJTCP| 15e | Nucle | AtTCP15 | 7 33.74 319   |
| BjuB03    | 0482  | 55 | BJTCP| 15f | Nucle | AtTCP15 | 7 33.49 321   |
| BjuA02    | 2523  | 1  | BJTCP| 14a | Nucle | AtTCP14 | 7 36.45 344   |
| BjuB01    | 9669  | 10 | BJTCP| 14b | Nucle | AtTCP14 | 6 49.33 457   |
| BjuA01    | 2606  | 15 | BJTCP| 18a | Nucle | AtTCP18 | 8 48.45 425   |
| BjuB00    | 7175  | 15 | BJTCP| 18b | Nucle | AtTCP18 | 7 40.12 350   |
| BjuB00    | 7177  | 15 | BJTCP| 18c | Nucle | AtTCP18 | 6 50.08 437   |
| BjuB02    | 5473  | 15 | BJTCP| 18d | Nucle | AtTCP18 | 8 46.6 404    |
| BjuA00    | 7026  | 52 | BJTCP| 19  | Nucle | AtTCP19 | 5 30.18 281   |
| BjuA02 4339 | 67 30 | BJTCP 20a | A06 | 2559 6111 | 2559 7025 | + Nuclear AtTCP20 | 7 32.22 305 |
| BjuA03 1722 | 53 23 | BJTCP 20b | A09 | 3333 673 | 3334 532 | - Nuclear AtTCP20 | 5 25.25 241 |
| BjuB03 7176 | 65 30 | BJTCP 20c | B02 | 5607 9378 | 5608 0310 | - Nuclear AtTCP20 | 7 32.61 311 |
| BjuA00 9108 | 31 20 | BJTCP 21a | A03 | 1689 417 | 1690 118 | + Nuclear AtTCP21 | 1 24.26 234 |
| BjuA04 1017 | 32 20 | BJTCP 21b | A02 | 1466 529 | 1467 236 | + Nuclear AtTCP21 | 9 24.54 236 |
| BjuA04 7338 | 31 20 | BJTCP 21c | A10 | 1749 1637 | 1749 2344 | + Nuclear AtTCP21 | 7 24.73 236 |
| BjuB01 2430 | 31 20 | BJTCP 21d | B05 | 1700 1527 | 1700 2243 | - Nuclear AtTCP21 | 9 25.05 239 |
| BjuB04 0955 | 33 20 | BJTCP 21e | B08 | 2452 006 | 2452 713 | + Nuclear AtTCP21 | 1 24.46 236 |
| BjuB04 8495 | 28 20 | BJTCP 21f | B02 | 5055 0592 | 5055 1305 | - Nuclear AtTCP21 | 7 24.94 238 |
| BjuA00 7449 | 57 19 | BJTCP 22a | A02 | 1336 5484 | 1336 6605 | + Nuclear AtTCP22,AtTCP23 | 8 39.06 374 |
| BjuA04 3373 | 40 20 | BJTCP 22b | A07 | 3137 8278 | 3137 9348 | + Nuclear AtTCP22,AtTCP23 | 6 37.34 357 |
| BjuB01 0697 | 56 18 | BJTCP 22c | B05 | 5979 0473 | 5979 1573 | - Nuclear AtTCP22,AtTCP23 | 8 38.41 367 |
| BjuB04 4035 | 45 16 | BJTCP 22d | B03 | 3847 3563 | 3847 4609 | + Nuclear AtTCP22,AtTCP23 | 6 36.59 349 |
| BjuA03 46 13 | BJTCP A09 | 3131 3131 | + Nuclear AtTCP24 | 7 35.29 318 |
| Accession | Genotype | Chromosome | Region | Start | End | Name | Description | Length | Parental nucleosome size |
|-----------|----------|------------|--------|-------|-----|------|-------------|--------|-------------------------|
| BjUB02    |          | B04        |        | 1472  | 2425| ar   | AtTCP24     |        | 5.7                     |
| BjUB03    |          | B03        |        | 1292  | 2492| 24c  | AtTCP24     |        | 7.6                     |
| BjUA00    |          | A03        |        | 1624  | 1625| 17a  | AtTCP17     |        | 0.1                     |
| BjUB02    |          | B04        |        | 1846  | 1863| 17b  | AtTCP5      |        | 0.5                     |
| BjUB03    |          | B02        |        | 5934  | 5934| 5a   | AtTCP5      |        | 0.2                     |
| BjUB03    |          | B07        |        | 1284  | 1284| 5c   | AtTCP5      |        | 0.4                     |
| BjUA02    |          | A06        |        | 2016  | 2016| 7a   | AtTCP7      |        | 0.3                     |
| BjUA03    |          | A09        |        | 5290  | 5290| 7b   | AtTCP7      |        | 0.1                     |
| BjUA04    |          | A02        |        | 3458  | 3458| 7c   | AtTCP7      |        | 0.2                     |
| BjUB03    |          | B07        |        | 1270  | 1270| 7d   | AtTCP7      |        | 0.3                     |
| BjUB04    |          | B02        |        | 6085  | 6085| 7e   | AtTCP7      |        | 0.5                     |
| BjUA03    |          | A09        |        | 1519  | 1519| 8a   | AtTCP8      |        | 0.0                     |
Table 2  Ks, Ka calculation and divergent time of the duplicated *BjTCP* genes

| Duplicated Gene Pairs | Ka    | Ks     | Ka/Ks | P-Value(Fisher) | Divergence-Time(Ma) |
|-----------------------|-------|--------|-------|-----------------|---------------------|
| BjTCP20a&BjTCP9a      | 0.999293 | 1.00215 | 0.997151 | 1                | 33.41               |
| BjTCP20a&BjTCP9d      | 1.01737  | 0.946396 | 1.075 | 0.179341        | 31.55               |
| BjTCP20a&BjTCP22d     | 0.978041 | 1.06173 | 0.921176 | 0.0709694       | 35.39               |
| BjTCP20a&BjTCP8a      | 1.01664  | 0.954319 | 1.06531 | 0.207383        | 31.81               |
| BjTCP20a&BjTCP15d     | 1.0129  | 0.962903 | 1.05192 | 0.30836         | 32.10               |
| BjTCP20a&BjTCP13a     | 1.01885  | 0.942488 | 1.08102 | 0.122359        | 34.38               |
| BjTCP20c&BjTCP21a     | 0.989309 | 1.0313  | 0.959288 | 0.482644        | 32.01               |
| BjTCP20c&BjTCP21c     | 1.01341  | 0.960297 | 1.05531 | 0.326765        | 29.82               |
| BjTCP20c&BjTCP9b      | 1.03638  | 0.894697 | 1.15836 | 0.0455288       | 29.28               |
| BjTCP20c&BjTCP11b     | 0.999191 | 1.00276 | 0.996436 | 1                | 33.43               |
| BjTCP20c&BjTCP1d      | 1.02992  | 0.898138 | 1.14672 | 0.0072974       | 29.94               |
| BjTCP20c&BjTCP1a      | 1.01515  | 0.949568 | 1.06907 | 0.181948        | 31.65               |
| BjTCP20c&BjTCP5c      | 1.05632  | 0.7937  | 1.33088 | 9.74E-06        | 26.46               |
| BjTCP20c&BjTCP5a      | 1.04315  | 0.84631 | 1.23258 | 0.000574845     | 28.21               |
| BjTCP20c&BjTCP24a     | 1.02067  | 0.937008 | 1.08929 | 0.109641        | 31.23               |
| BjTCP20c&BjTCP24b     | 1.03281  | 0.892867 | 1.15673 | 0.00840374      | 29.76               |
| BjTCP21a&BjTCP21c     | 0.194528 | 0.145215 | 1.33958 | 0.150138        | 4.84                |
| BjTCP21a&BjTCP9b      | 0.98743  | 1.03637 | 0.952782 | 0.394321        | 34.55               |
| BjTCP21a&BjTCP11b     | 1.08642  | 0.715504 | 1.5184  | 6.06E-08        | 23.85               |
| BjTCP21a&BjTCP1d      | 1.01932  | 0.929203 | 1.09698 | 0.176264        | 30.97               |
| BjTCP21a&BjTCP1a      | 1.09159  | 0.714456 | 1.52787 | 3.78E-08        | 23.82               |
| Comparison            | Value1  | Value2  | Value3  | Value4  | Value5  |
|-----------------------|---------|---------|---------|---------|---------|
| BjTCP21a&BjTCP5c      | 1.02937 | 0.908039 | 1.13362 | 0.10476 | 30.27   |
| BjTCP21a&BjTCP5a      | 1.0257  | 0.91267 | 1.11578 | 0.126757| 30.64   |
| BjTCP21a&BjTCP24a     | 1.07085 | 0.797343 | 1.34302 | 9.27E-05| 26.58   |
| BjTCP21a&BjTCP24b     | 1.09267 | 0.738083 | 1.48042 | 2.30E-08| 24.60   |
| BjTCP21c&BjTCP9b      | 1.036   | 0.914091| 1.13074 | 0.0274717|30.47    |
| BjTCP21c&BjTCP9a      | 1.0225  | 0.92785  | 1.11578 | 0.126757| 30.64   |
| BjTCP21c&BjTCP24a     | 1.07085 | 0.797343 | 1.34302 | 9.27E-05| 26.58   |
| BjTCP21c&BjTCP24b     | 1.09267 | 0.738083 | 1.48042 | 2.30E-08| 24.60   |
| BjTCP21c&BjTCP9b      | 1.036   | 0.914091| 1.13074 | 0.0274717|30.47    |
| BjTCP21c&BjTCP9a      | 1.0225  | 0.92785  | 1.11578 | 0.126757| 30.64   |
| BjTCP21c&BjTCP24a     | 1.07085 | 0.797343 | 1.34302 | 9.27E-05| 26.58   |
| BjTCP21c&BjTCP24b     | 1.09267 | 0.738083 | 1.48042 | 2.30E-08| 24.60   |
| BjTCP21c&BjTCP9b      | 1.036   | 0.914091| 1.13074 | 0.0274717|30.47    |
| BjTCP21c&BjTCP9a      | 1.0225  | 0.92785  | 1.11578 | 0.126757| 30.64   |
| BjTCP21c&BjTCP24a     | 1.07085 | 0.797343 | 1.34302 | 9.27E-05| 26.58   |
| BjTCP21c&BjTCP24b     | 1.09267 | 0.738083 | 1.48042 | 2.30E-08| 24.60   |
| BjTCP21c&BjTCP9b      | 1.036   | 0.914091| 1.13074 | 0.0274717|30.47    |
| BjTCP21c&BjTCP9a      | 1.0225  | 0.92785  | 1.11578 | 0.126757| 30.64   |
| Pair                        | $t_{1}$ | $t_{2}$ | $t_{3}$ | $t_{4}$ | $t_{5}$ |
|-----------------------------|---------|---------|---------|---------|---------|
| BjTCP1a&BjTCP5a             | 1.05563 | 0.823698| 1.28158 | 0.00486352| 27.46   |
| BjTCP1a&BjTCP24a            | 1.02273 | 0.929274| 1.10057 | 0.255428 | 30.98   |
| BjTCP1a&BjTCP24b            | 1.00908 | 0.972336| 1.03779 | 0.646827 | 32.41   |
| BjTCP5c&BjTCP5a             | 0.150359| 0.287462| 0.523056| 0.00041802| 9.58    |
| BjTCP5c&BjTCP24a            | 0.86782 | 0.888744| 0.976457| 0.774868 | 29.62   |
| BjTCP5c&BjTCP24b            | 0.940256| 1.01511 | 0.926263| 0.402502 | 33.84   |
| BjTCP5a&BjTCP24a            | 0.803529| 0.715009| 1.1238  | 0.250546 | 23.83   |
| BjTCP5a&BjTCP24b            | 0.892262| 1.00693 | 0.886122| 0.193768 | 33.56   |
| BjTCP24a&BjTCP24b           | 0.108959| 0.0796321| 1.36828 | 0.286969 | 2.65    |

*Mya, million years ago

**Figures**
Figure 1

The gene locations of BjTCP genes family. The chromosome name is at the top of each bar.

The scale of the chromosome is in millions of bases (Mb).
Evolutionary relationships of taxa. A. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid
substitutions per site. The analysis involved 78 amino acid sequences. All ambiguous positions were removed for each sequence pair. There were a total of 636 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. B. A conserved motif in the Class I subfamily of the BjTCP gene family. C. A conserved motif in the Class II subfamily of the BjTCP gene family. The consensus sequences were displayed using Weblogo (http://weblogo.berkeley.edu).
Genomic structure and motif composition of BjTCPs. A. The phylogenetic tree of BjTCP proteins. B. Genomic structure of BjTCPs family members in tumorous stem mustard. Exons and introns are represented with blank boxes and blank lines. C. The conserved motifs in tumorous stem mustard TCP proteins were identified using MEME. Each motif is represented with a specific color and the characters sequence were showed below.
Figure 4

Cis-acting elements on promoters of BjTCP genes. The colour bar showed the number of cis-acting elements.
Expression patterns of TCP genes in different tissues and development stages of Brassica juncea var. tumida. DY, Dayejie stems were collected 22 weeks after seeding (daye3bianzhong); YA1-4, The stems of Yong’an were collected 18, 20, 22, and 25 weeks after seeding; YAr, The mix roots samples of 18 and 22 weeks after seeding. The expression levels are represented by the color bar (log2-transformed).
Expression levels of BjTCPs under SA and GA treatment by qRT-PCR. The number represented the treatment times (hours). The colour scales represented relative expression data.

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