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GENOME-WIDE IDENTIFICATION AND CHARACTERIZATION OF TCP FAMILY GENES ASSOCIATED WITH FLOWER AND FRUIT DEVELOPMENT IN *FRAGARIA VESCA*

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Abstract

TEOSINTE-BRANCHED1/CYCLOIDEA/PCF (TCP) protein family is a large transcriptional regulator family in plant, which are important in various plant processes including leaf development, flower symmetry, shoot branching, and senescence. In this study, phylogenetic relationship, chromosome location, conservative domain structure and expression profiles in flower and fruit development of the TCP TFs family in woodland strawberry (*Fragaria vesca*) was performed. In total, 18 TCP members were identified in *F. vesca*. These 18 genes are evenly distributed on chromosome 4 to 7. All of them had highly conserved domain sequences through analyzing the functional domain of the 18 members. The TCP members were divided into TCP-C and TCP-P according to the difference of domain and phylogenetic analysis. Phylogenetic tree analysis showed that *F. vesca* TCP proteins were grouped into two subfamilies, class I and class II. Expression profile analysis of the FvTCP family, which is related to the flower and fruit development from the project site, indicated that 13 members were related to flower development and five members were related to fruit development. These results suggested the important role of FvTCP proteins in fruit and flower development.

Key words: TCP, *Fragaria vesca*, Phylogenetic analysis, Expression profile, Fruit and flower.

Introduction

TEOSINTE-BRANCHED1/CYCLOIDEA/PCF (TCP) domain proteins are members of a family of plant transcription factors (Cubas *et al*., 1999), which are known to play important regulatary effects in cell growth and proliferation. Transcription factors (TFs) control gene expression in plants (Ali *et al*., 2017).The TCP family genes all contain a conserved TCP domain. TCP transcription factor have a highly conserved 59-residue-long non-canonical basic helix-loop-helix (bHLH) structure at the N-terminus, involved in DNA binding, protein nuclear localization, and protein–protein interactions (Cubas *et al*., 1999). Part of the TCP protein also contains a hydrophilic R domain, which contains rich arginine, lysine, glutamic and other polar amino acids. TCP proteins could be grouped into two subfamilies, Class I and Class II: PCF is Class I, and Class II is represented by CYC and TB1 (Martin-Trillo *et al*., 2010).

TCP family is a kind of plant transcription factors which contains some genes regulating development of plant organs and some genes regulating the cell cycle. These findings indicated that most of the TCP family genes were related to the regulation of plant organ development, especially the floral organ development and developmental control of plant form (Lupas *et al*., 1991).

For example, the CYC gene *participates* in the control of floral symmetry, such as *Antirrhinum* (Luo *et al*., 1999), *Lotus japonicus* (Feng *et al*., 2006), and *Iberis amara* (Busch *et al*., 2007), inhibition of cell differentiation, the floral organ dorsal region, and restricting the formation of floral organs. AtTCP14 could regulate the growth of embryo in seed, and the length of internode is controlled by AtTCP15 (Kieffer *et al*., 2011). TCP transcription factors associate the Regulation of mitochondrial proteins with the circadian clock in *Arabidopsis thaliana* (Giraud *et al*., 2010). AtTCP16 is mainly expressed in the development of microspores, and it plays a crucial part in early pollen development in *Arabidopsis* (Takeda *et al*., 2006).

F. vesca is known as a model plant because of its small genome (240 Mb) (Kang *et al*., 2013).Distinguished from common plant of "the fruit develops from the ovary", strawberry fruit is derived from *the* inflated *receptacle, and* seeds (i.e. achenes) are covered on the surface of the receptacle.

The genome sequences of many plants, such as maize, *Arabidopsis thaliana*, rice, and soybean, have been published, so we can use bioinformatics methods to identify the TCP family in the whole genome-wide range. Recent availability of the completed genome sequence of *F.vesca* provided an excellent opportunity for whole-genome analysis of TCP transcription factors in the *F. vesca* genome.

This study provided comprehensive analysis on TCP family in *F. vesca*, including expression profiling during flower and fruit development, phylogenetic relationships, chromosomal location, alignment of the domain. The results showed vital functions of FvTCP proteins during plant growth and development.

Materials and Methods

Sequence identification of TCP proteins in *F. vesca*: The TCP gene family was obtained from various databases Phytozome (http://pfam.sanger.ac.uk/), Plant TFDB (http://planttfdb.cbi.pku.edu.cn/) and National Center for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov/) to collect data on all members of the *F. vesca* TCP gene family. The TCP domain

sequence of ZmTb1 were used as a query sequence, which were downloaded from NCBI database. By mining various databases, we initially identified 18 different *F. vesca* genes that encode putative TCP TFs. In order to confirm completion of the collection database searches, blastp was performed in the different databases. The combination of the database search results had redundancies which were removed. In order to ensure the accuracy of the results, InterPro (http:// www.ebi. ac.uk/ interpro/) (Quevillon *et al*., 2005) was used to identify whether the candidate genes have the TCP domain. Analysis of conserved domains of the FvTCP gene family multiple sequence alignment was performed to analyze the FvTCP family gene domain using ClustalX 2.0.12 (Larkin *et al*., 2007), and the alignment results were presented and manually modified using Gene Doc (Nicholas *et al*., 1997). The conservative structure of protein sequences was analyzed by this map.

Phylogenetic analysis of the FvTCP gene family: We imported the amino acid sequences of FvTCP proteins into MEGA6.0 (Tamura *et al*., 2013) and then generated an unrooted phylogenetic tree based on the neighbor-joining method after 1,000 bootstrap replications. The parameter settings were model adopt poisson process and the gap was the "pairwise deletion."

Expression profiling of FvTCP genes: The transcription of genes related to the development of strawberry flowers and fruit development were downloaded from the appendixes of the paper (Hollender *et al*., 2014; Kang *et al*., 2013), then determined the related FvTCP genes. The heat maps for the expression profile in early flowering and fruit development were generated based on the FvTCP family gene transcription values using TIGR Multi Experiment Viewer (MeV4,) software package (Saeed *et al*., 2006). The default setting was "single-color array." Thus, the function of FvTCP gene family in early flower and fruit development could be inferred.

Results

Identification of the TCP family transcription factors in *F. vesca* genome: By mining various databases, we initially identified 18 candidate TCP genes. Furthermore, to predict whether they contained the TCP domain, we analyzed all non-redundant candidate TCP sequences to examine the presence of the conserved TCP domain using the InterPro program. The results indicated that all of the 18 *F. vesca* TCP genes had the conserved TCP domain. The length of the 18 TCP TFs ranged from 175 to 1,147 amino acids. Meanwhile, we acquired basic information about the FvTCP gene family, such as the protein length, pI, molecular weight, accession number, score, E-value, and chromosome location in the database (Tables 1 and 2).

In this table, pI: isoelectric point. The accession numbles were from NCBI database (www.ncbi. nlm. nih.gov/). Other content of this table is available at PlantTFDB (http://planttfdb.cbi.pku.edu.cn/).

According to the position of the gene on the chromosome, the *F. vesca* TCP gene family was named from FvTCP1 to FvTCP18 (*F. vesca* abbreviated as Fv). An almost even distribution of genes in the genome is shown in Table 2. Chromosomes 3-7 contained the most number of FvTCP genes, while only one gene was assigned to chromosome 4. The distribution of the other chromosomes was relatively uniform.

All the information in the table comes from Phytozome database URL http://pfam.sanger.ac.uk/)

Multiple sequence alignment and sequence features of FvTCPs

We analyzed the conservative structure of protein sequences. The results revealed that the domains of these protein sequences were highly conserved (Fig. 1). Three hydrophilic R domains were found in the 18 TCP protein sequences, and multiple sequence alignment was carried out on the protein sequences. The results showed that the R domain of these protein sequences were highly conserved (Fig. 2).

Table 1. The information of TCP genes identified in *F. vesca*.

Name	Protein length (aa)	pI	Molecular weight (Da)	Accession No.	Score	E-value
FvTCP1	460	7.9322	49952.5	XP_004293547.1	73.6	1.5E-15
FvTCP2	231	5.1431	25832.5	XP_011462025.1	60.5	9.7E-12
FvTCP3	543	9.736	58452.9	XP_004294116.1	75.1	5.4E-16
FvTCP4	407	10.0898	45971.2	XP_004298309.1	94	6.0E-23
FvTCP5	1147	7.9715	123285	XP_004298742.1	38.9	6.5E-04
FvTCP6	423	7.2767	45402.7	XP_004299376.1	37	3.4E-03
FvTCP7	376	9.0287	41441.1	XP_004301109.1	63.5	3.1E-12
FvTCP8	451	6.9661	50831.2	XP_004301078.1	92	4.4E-22
FvTCP9	284	9.8083	29893.1	XP_004301414.1	40.4	1.9E-04
FvTCP10	263	9.024	28678.1	XP_011468122.1	36.6	3.5E-03
FvTCP11	366	6.8972	40307.1	XP_004303161.1	70.1	1.3E-14
FvTCP12	487	7.6091	54779.6	XP_011467828.1	89	8.6E-21
FvTCP13	366	6.8798	40186.1	XP_004304223.1	68.9	3.4E-14
FvTCP14	418	10.1077	45499.8	XP_011468196.1	37.7	1.6E-03
FvTCP15	398	9.3438	42943.5	XP_004308417.1	38.5	9.1E-04
FvTCP16	159	8.2335	17553.6	XP_011459878.1	85.9	9.9e-27
FvTCP17	175	4.3664	18747.6	XP_004299376.1	36.6	2.5E-03
FvTCP18	327	7.1666	34335.7	XP_004309238.2	36.6	4.3E-03

Table 2. Location information of the FvTCP gene family on chromosomes.

No.	Name	Phytozome identifier	Chromosome	Start position (bp)	End position (bp)
1.	FvTCP1	mrna30370	Chr.3	2019762	2021144
2.	FvTCP2	mrna29800	Chr.3	4801104	4801799
3.	FvTCP3	mrna27171	Chr.3	23742492	23744166
4.	FvTCP4	mrna03882	Chr.4	25735315	25736538
5.	FvTCP5	mrna32370	Chr.5	938645	947383
6.	FvTCP6	mrna25985	Chr.5	7001299	7002570
7.	FvTCP7	mrna26101	Chr.5	7791074	7792204
8.	FvTCP8	mrna26211	Chr.5	8363266	8364751
9.	FvTCP9	mrna12294	Chr.5	25912686	25913540
10.	FvTCP10	mrna09614	Chr.6	9038760	9039551
11.	FvTCP11	mrna17805	Chr.6	21077341	21078441
12.	FvTCP12	mrna10333	Chr.6	32172515	32180202
13.	FvTCP13	mrna04255	Chr.6	32858223	32859323
14.	FvTCP14	mrna01346	Chr.6	37767489	37774114
15.	FvTCP15	mrna18961	Chr.7	4288013	4289423
16.	FvTCP16	mrna21453	Chr.7	13267257	13267736
17.	FvTCP17	mrna21454	Chr.7	13285100	13285627
18.	FvTCP18	mrna13351	Chr.7	22107988	22108971

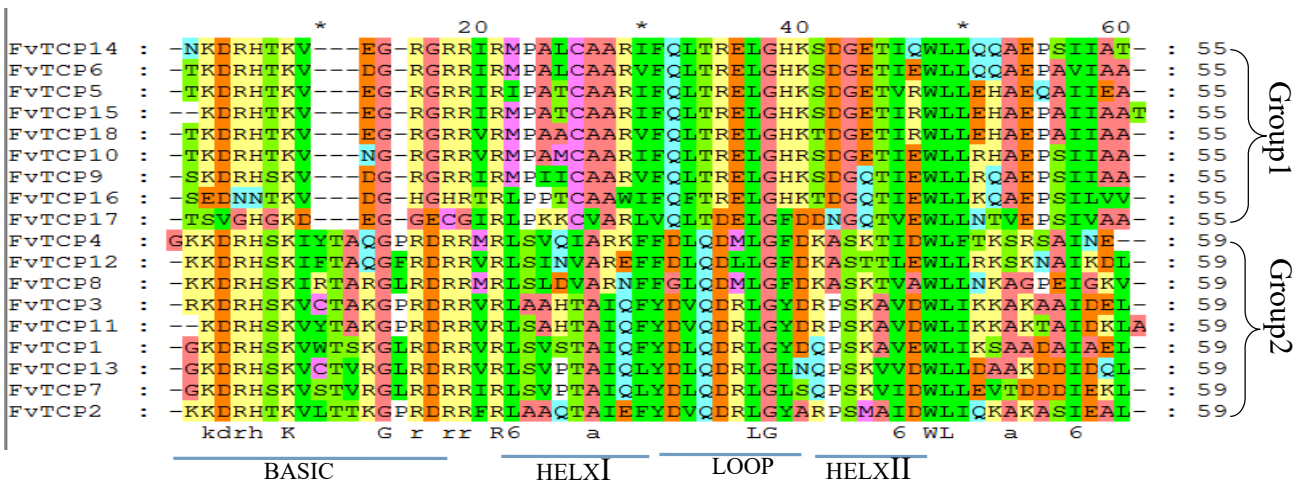

Fig. 1. Alignment of the TCP domain for the predicted *F. vesca* TCP proteins. The basic, helix I, loop, and helix II regions are indicated.

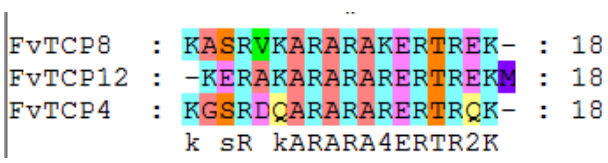

Fig. 2. Alignment of the R-domain of FvTCPs. Sequences were aligned with ClustalX 2.0.12 and visualized with GeneDoc.

To investigate the characteristics of the TCP domain in the FvTCP family, we analyzed their predicted secondary structures. The results showed that all the proteins contained a bHLH domain. The basic region of the bHLH domain had 15 or 19 residues. The first helix contained 11 residues, and the second helix had nine residues. Within the bHLH domain, the basic region was highly conserved, but the helices were not very conservative and the loop region changed greatly. This conservation indicated that the TCP domain played an important part in the function of the TCP protein.

Based on the number of conserved residues, we divided the TCP gene family into two groups, Group 1 and Group 2. Group 1 contained 55 conserved amino acids, and Group 2 contained 59 conserved amino acids. In the basic region, Group 1 had 15 amino acids and Group 2 contained 19 amino acids, suggesting that the members in Group 1 and Group 2 had unique DNA-binding characteristics. The basic region had lots of positively charged residues, Lys (K) and Arg (R), and possibly contributed to a bipartite nuclear localization signal (Yao *et al*., 2007). Three hydrophobic residues, Ala (A), Leu (L), and Trp (W), were abundant in the helical regions, and the loops linking two helices contained a conserved Gly (G).

Alignment of the TCP domain of 18 TCP proteins showed distribution of the conserved amino acids. A total of 16 conserved amino acids were same in at least 80% of the 18 bHLH domains: 10 amino acids in the basic region (K, D, R, H, K, G, R, R, R, R), eight of which are positively charged and alkaline residues; the remaining two are not charged hydrophilic amino acids. Two hydrophobic residues in the two helices respectively, Ala (A) and Leu (L), constituted the basic structure of the hydrophobic region. The loops linking two helices contained conserved Gly (G) and Trp (W).

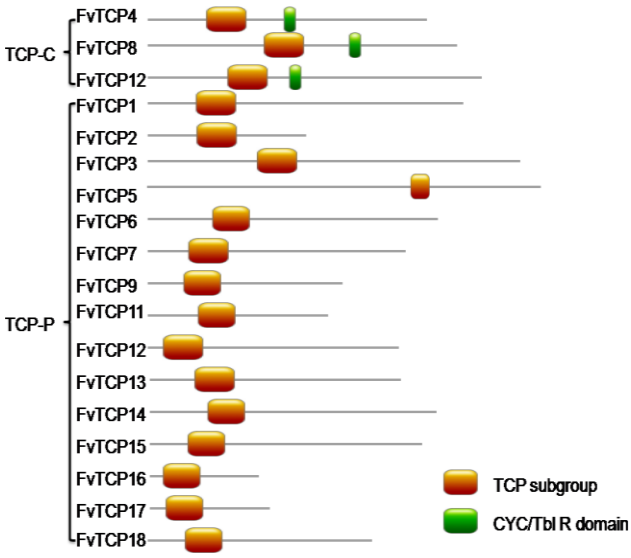

Fig. 3. Schematic organization of FvTCPs. The TCP subgroup domain and CYC/Tbl R domain are shown in brown and green, respectively.

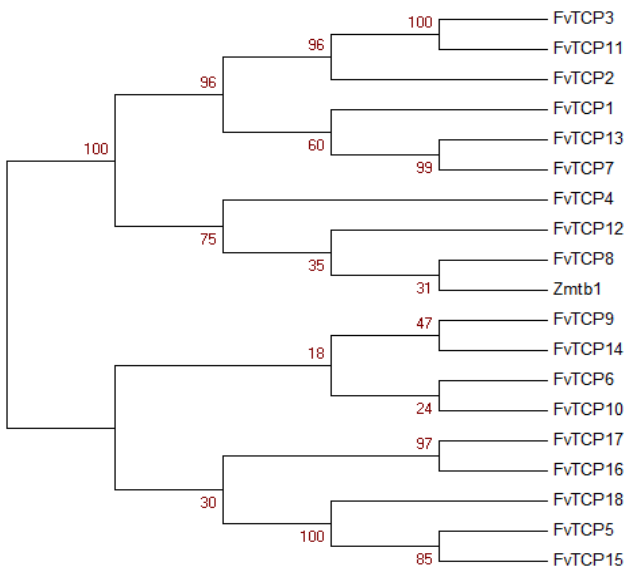

Fig. 5. The phylogenetic tree of FvTCPs and ZmTb1. The unrooted phylogenetic tree was construct- ed using the neighbor-joining method after 1,000 bootstrap replications.

Based on sequence comparison of the TCP domain, FvTCP genes could be divided into two subfamilies (Fig. 3). one with close orthologs to CYC and TB1 (the TCP-C) and the other closer to the PCF (the TCP-P). Most of the FvTCP members belong to the TCP-P subfamily. They all contained the TCP domain, composed of 59 or 55 amino acids, which could form the bHLH structure. Besides the bHLH structure, several TCP-C members also shared an R-domain outside the conserved TCP. Only three of FvTCPs had the R-domain, which has lots of polar residues and is expected to form a hydrophilic α-helix. Because CYC and Tb1 both have a conserved R-domain, they can form a coiled structure resembling a leucine zipper, which did not exist in PCF1 and PCF2.

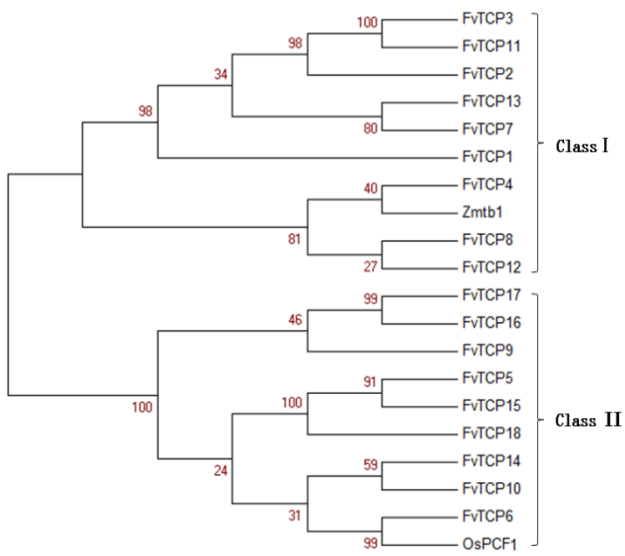

Fig. 4. The phylogenetic tree of FvTCP, ZmTb1, and OsPCF1.The unrooted phylogenetic tree was constructed using the neighbor-joining method after 1,000 bootstrap replications.

By comparison of the protein sequence of the R-domain in FvTCPs, we found that the R-domain all had 18 amino acids. Alignment of the protein sequence indicated that the R-domain was highly conserved (Fig. 2). New researches have revealed that the R-domain of the TCP protein was speculated to modify the function of the TCP gene (Cubas *et a1*., 1999).

Phylogenetic analysis of the FvTCP family genes: In order to clarify the phylogenetic relationships of the TCP TF family in *F. vesca*, OsPCF1, and ZmTb1, we constructed a phylogenetic tree with MEGA6.0 by the neighbor-joining method. According to the phylogenetic tree, we divided the 18 TCPs into two subfamilies, Class I and Class II (Fig. 4). The phylogenetic tree showed that Class I formed a clade with ZmTb1 gene, and Class II with the OsPCF1 gene formed a clade, implying that Class I with close homologs to Tb1 and Class II was more closely related to PCF1. The results indicated that members of Class I belonged to Tb1-like, while the members of Class II belonged to PCF-like, which was consistent with previous studies of the classification of the TCP family (Doebley *et al*., 1997; Luo *et al*., 1996).

The Tb1 gene regulates the fate of rice and maize axillary meristems, which can prevent bud growth at lower nodes and promote the formation of female flowers at higher nodes. A phylogenetic tree was constructed including the typical Tb1 gene of maize (ZmTb1) and 18 FvTCP protein sequences. The results showed that the FvTCP8 gene and ZmTb1 clustered into the same clade (Fig. 5). Multiple sequence alignment was performed with FvTCP8, ZmTb1, AtBRC1, and Ostb1, from which results showed that the protein sequences were highly conserved and similar (Fig. 6), implying that FvTCP8 may be the homologous counterpart of Tb1 gene, which may control the tillering of *F. vesca*.

According to the results of the protein sequence analysis of ZmTb1, OsTb1, FvTCP8, and AtBRC1, these protein sequences all shared the R-domain. Their R-domain protein sequences are very conserva- tive (Fig. 7), and the similarity is very high.

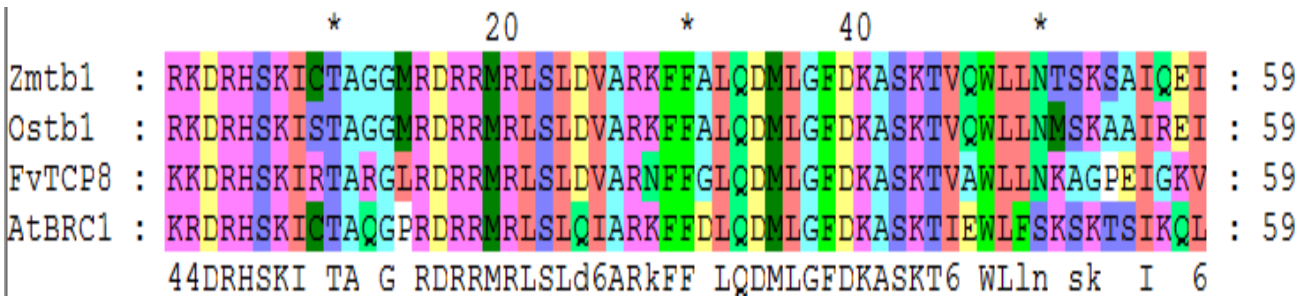

Fig. 6. Alignment of conserved sequence from ZmTb1, OsTb1, FvTCP8, and AtBRC1.

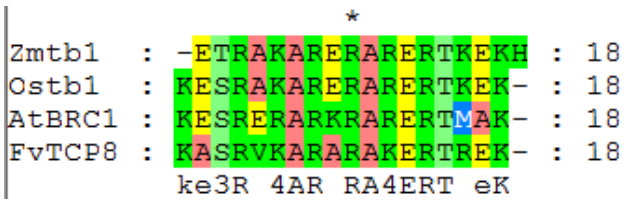

Fig. 7. Alignment of the R-domain from ZmTb1, OsTb1, FvTCP8, AtBRC1.

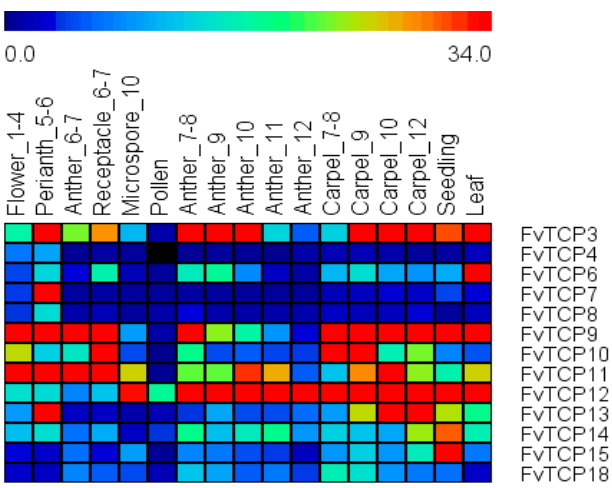

Fig. 8. Heatmap representation for expression patterns of FvTCP genes in flower development. The color scale for the fold-change values is shown at the top.

Expression profiles of TCP genes in flower development in *F. vesca*: The transcript profiles of genes related to the development of strawberry flowers were downloaded (Hollender *et al*., 2014), then the related FvTCP gene was determined. A heat map for gene expression patterns in early flower development was generated based on expression values of the FvTCP genes with the software Multi Experiment Viewer (MeV4). The results showed that 13 FvTCPs (FvTCP3, FvTCP4, FvTCP6, FvTCP7, FvTCP8, FvTCP9, FvTCP10, FvTCP11, FvTCP12, FvTCP13, FvTCP14, FvTCP15, and FvTCP18) were expressed in the early flower development stage. The expression profile data of the FvTCP genes were obtained across the development of all seven parts of the plant, including flower, perianth, receptacle, anther, microspore, pollen, seeding, leaf, and carpel (Fig. 8).

As indicated in Fig. 9, (1) the expressions files of FvTCP4, FvTCP7, FvTCP13, and FvTCP16 were greatly increased and FvTCP2 decreased in the development of embryos, showing that these five genes may take effect in the development of the embryo. (2) The expressions of FvTCP13 and FvTCP16, especially FvTCP7 were at high level in ovule development, implying their specific roles in the regulation of ovule development. (3) FvTCP4 and FvTCP16 showed gradual upregulation. The expression of FvTCP7 decreased firstly and then increased, implying that the three genes may play regulatory parts at the ghost development stage. (4) The expression levels of FvTCP2 and FvTCP13 were increased in the development of ovary wall tissues, indicating that they may play an important role in the formation of the wall. (5) In the development of the seeds and cortex, the expression of these five genes were all low, and there was no obvious change, implying that these five FvTCP genes have no significant effect on these two stages. (6) In addition, FvTCP4 was first expressed at high level and then showed gradual upregulation at the pith development stage with obvious changes, implying that FvTCP4 may be active in the development of the pith. In general, these five genes coregulate the development of the fruit.

Discussion

In our research, 18 TCP members were identified in *F. vesca*, and they all have a conserved TCP domain, which contains a non-canonical bHLH structure. According to the different domains of the protein, the FvTCP transcription factors can be divided into two subfamilies, namely TCP-C and TCP-P.

Analysis of the phylogenetic tree and the alignment of the TCP domains indicated that *F. vesca* TCP proteins could be divided into class I and class II. Class I has high degree of homology with Tb1, which belongs to the Tb1-like gene class, whereas Class II has a high degree of homology with PCF, which belongs to the PCF-like gene class.

Previous studies indicated that the Tb1 gene of maize and rice regulated the tillering number. Analysis of the phylogenetic tree and the alignment of the TCP domains indicated that FvTCP8 had the highest degree of orthology with OsTb1, ZmTb1, and AtBRC1. Thus, it is speculated that FvTCP8 may regulate tillering in strawberry. *F. vesca* has a very high nutritional and medicinal value. FvTCP8 might control the tillering of strawberry. Therefore, it can be used as a candidate gene to improve the tillering of strawberry and increase the yield of strawberry.

The recent research of TCP family is mainly about the environmental stress responses (cold and salt stresses) (Wang *et al*., 2014, Emre *et al*., 2018), hormone signal responses (Lei *et al*., 2017), stem and ear growth (Chai *et al*., 2017), regulating plant height (Shi *et al*., 2016). This study was focuced on the role of TCP family in the regulation of flower and fruit development, which world be valuable for uncovering the potential functions of TCP genes.

A relative contrast of expression profiles of all 18 TCPs showed a relatively higher expression of FvTCP3, FvTCP4, FvTCP6, FvTCP7, FvTCP8, FvTCP9, FvTCP10, FvTCP11, FvTCP12, FvTCP13, FvTCP14, FvTCP15, and FvTCP 18 in flower development and FvTCP2, FvTCP4, FvTCP7, FvTCP13, and FvTCP16 in fruit development. These genes were differentially expressed in flower and fruit development, suggesting that they might regulate the development of the two tissues. Strawberry fruit is referred to as the "fruit queen," and is rich in vitamin C. The fleshy fruit of *F. vesca* is in fact the stem tip and the receptacle, while the achene is the true fruit. Our results indicated that FvTCP8, FvTCP9, FvTCP10 clearly regulate receptacle development, which will be conducive to the transformation of the fruits of the forest strawberry, suggesting that they might affect the size of the development of the fruit and improve the economic value. The genes related to fruit development were not clear in the regulation of seed development, implying that they did not play a significant role at the fruit ripening stage. Thirteen FvTCP genes were related to flower development, and only five genes were related to fruit development, which again verified the main role of TCP TFs in the regulation of floral organ development (Carpenter *et a1*., 1990; Coen *et a1*., 1991; Davies *et a1*., 1994).

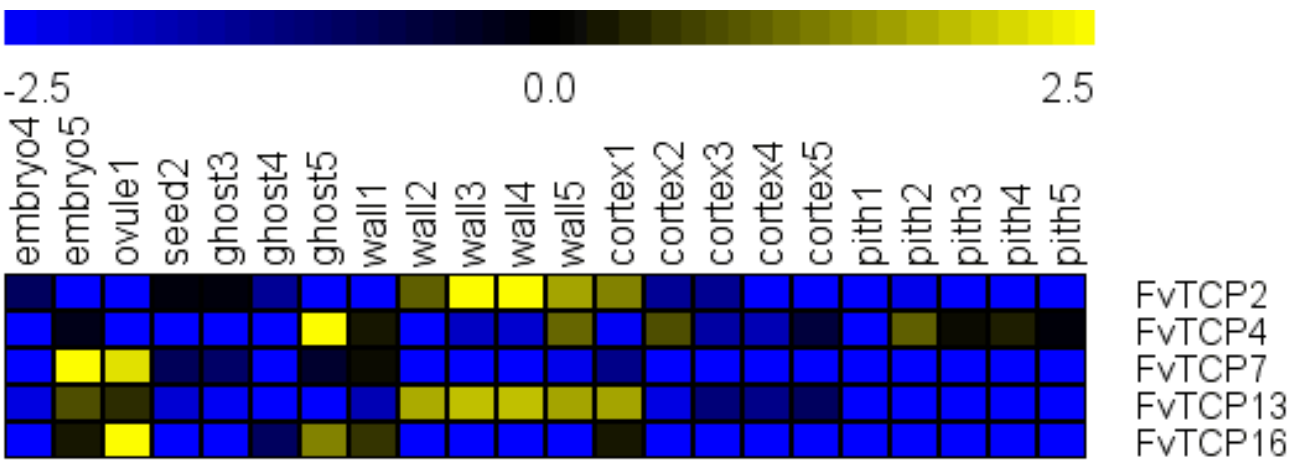

Fig. 9. Heatmap representation for expression patterns of the FvTCP gene in early fruit development. The color scale for fold-change values is shown at the top.

Conclusions

In conclusion, 18 TCP members in *F. vesca* were identified and the basic information of the 18 genes was also obtained. Phylogenetic analysis, multiple sequence alignment, expression profiling in flower and fruit development were all performed in our study .Based on phylogenetic tree and multiple sequence alignment, the FvTCP famliy can be divided into TCP-C and TCP-P, or class I and class II . We also hypothesized that FvTCP8 might be used as a candidate gene to improve the tillering of strawberry and increase the yield of strawberry, 13 and 5 TCP members were related to flower development and fruit development were identified. Therefore, this study revealed that TCP gene played an important role in the in the development of strawberry flower and fruit and enriched our knowledge of TCP transcription factor in plants.

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References

Ali, M.A., K.B. Alia, R.M. Atie, I. Rasul, H.U. Nadeem, A. Shahid and F. Azeem. 2017. Genome-wide identification and comparative analysis of SQUAMOSA-Promoter Binding Proteins (SBP) transcription factor family in *Gossypium raimondii* and *Arabidopsis thaliana*. *Pak. J. Bot.*, 49(3): 1113-1126.

Busch, A. and S. Zachgo. 2007. Control of corolla monosymmetry in the Brassicaceae Poeris. Amara. *Proc. Natl. Acad. Sci. USA*., 104(42): 16714-16719.

Carpenter, R. and E.S. Coen. 1990. Floral homeotic mutations produced by transposon- mutagenesis in *Antirrhinum majus*. *Genes Dev*., 4: 1483-1493.

Chai ,W., P. Jiang, G. Huang , H. Jiang and X. Li. 2017. Identification and expression profiling analysis of TCP family genes involved in growth and development in maize. *Phy. & Mol. Biol. Plants*, 23(4): 779-791.

Coen, E.S. and E.M. Meyerowitz. 1991. The war of the whorls: genetic interactions controlling flower development. *Nature*, 353: 31-37.

Cubas, P., N. Lauter, J. Doebley and E. Coen. 1999. The TCP domain: a motif found in proteins regulating plant growth and development. *Plant J.*, 18(2): 215-220.

Davies, B. and Z. Schwarz-Sommer. 1994. Control of floral organ identity by homeotic MADS-box transcription factors. In: *Results and Problems in Cell Differentiation* (Ed.): L. Nover. Berlin: Springer-Verlag, pp. 235-258.

Doebley, J, A. Stec and L. Hubbard. 1997. The evolution of apical dominance in maize. *Nature*, 386: 485-488.

Emre, İ., İ. Büyük and B. İnal. 2018. Transcriptome-Scale characterization of salt responsive bean TCP transcription factors. *Gene*, 642: 64-73.

Feng, X, Z. Zhao, Z. Tian, S. Xu, Y. Luo, Z. Cai, Y. Wang, J. Yang, Z. Wang, L. Weng, J. Chen, L. Zheng, X. Guo, J. Luo, S. Sato, S. Tabata, W. Ma, X. Cao, X. Hu, C. Sun and D. Luo. 2006. Control of petal shape and floral zygomorphy in *Lotus japonicas*. *Proc. Natl. Acad. Sci. USA*., 103: 4970-4975.

Giraud, E., S. Ng, C. Carrie, O. Duncan, J. Low, C.P. Lee, O. Van Aken, A.H. Millar, M. Murcha and J. Whelan. 2010. TCP transcription factors link the regulation of genes encoding mitochondrial proteins with the circadian clock in *Arabidopsis thaliana*. *Plant Cell*, 22: 3921-3934.

Hollender, C.A., C. Kang, O. Darwish, A. Geretz, B.F. Matthews, J. Slovin, N. Alkharouf and Z. Liu. 2014. Transcriptomes in Woodland Strawberry Uncover Developing Receptacle and Anther Gene Network. *Plant Physiol*, 165(7): 1062-1075.

Kang, C., O. Darwish, A. Geretz, R. Shahan, N. Alkharouf and Z. Liu. 2013. Genome-scale transcriptomic insights into early-stage fruit development in woodland strawberry *Fragaria vesca*. *The Plant Cell*, 25(6): 1960-1978.

Kieffer, M., V. Master, R. Waites and B. Davies. 2011. TCP14 and TCP15 affect internode length and leaf shape in *Arabidopsis*. *Plant J*., 68: 147-158.

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, Wilm A, R. Lopez, J.D. Thompson, T.J. Gibson and D.G. Higgins. 2007. W. Clustal and X. Clustal version 2.0. *Bioinformatics*, 23(21): 2947-2948.

Lei, N., X. Yu, S.X. Li, C.Y. Zeng, L.P. Zou, W.B. Liao and M. Peng. 2017. Phylogeny and expression pattern analysis of TCP transcription factors in cassava seedlings exposed to cold and/or drought stress. *Scientific Reports*, 30: 1-13.

Luo, D., R. Carpenter, C. Vincent, L. Copsey and E. Coen. 1996. Origin of floral asymmetry in *Antirrhinum*. *Nature*, 383: 794-799.

Luo, D., R. Carpenter, L. Copsey, C. Vincent, J. Clark and E. Coen. 1999. Control of organ asymmetry in flowers of *Antirrhinum Cell*, 12: 367-376.

Lupas, A., M. Van Dyke and J. Stock. 1991. Predicting coiled coils from protein sequences. *Sci.*, 252: 1162-1164.

Martin-Trillo, M. and P. Cubas. 2010. TCP genes: a family snapshot ten yearslater. *Trends Plant Sci*., 15: 31-39.

Nicholas, K.B., H.B.J. Nicholas and D.W.I. Deerfield. 1997. Gene Doc: Analysis and visualization of genetic variation. *EMBNEW News*, 4: 14.

Quevillon, E., V. Silventoinen, S. Pillai, N. Harte, N. Mulder, R. Apweiler and R. Lopez. 2005. InterProScan: Protein domains identifier. *Nucl. Acids Res*., 33: 116-120.

Saeed, A.I., N.K. Hagabati, J.C. Braisted, W. Liang, V. Sharov, E.A. Howe, J.Li, M. Thiagarajan, J.A. White and J. Quackenbush. 2006. TM4 microarray software suite. *Methods Enzymol*., 411: 134-193.

Shi, P., K.M. Guy, W. Wu, B. Fang, J. Yang, M. Zhang and Z. Hu. 2016. Genome-wide identification andexpression analysis of the ClTCP transcription factors in *Citrullus lanatus*. *BMC Plant Biol.*, 16: 85-97.

Takeda, T. K., Amano, M. Ohto, K. Nakamura, S. Sato, T. Kato, S. Tabata and C. Uequchi. 2006. RNA interference of the *Arabidopsis* putative transcription factor TCP 16 gene results in abortion of early pollen development. *Plant Mol. Biol*., 61: 165-177.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol*., 30: 2725-2729.

Wang, S.T., X.L. Sun, Y. Hoshino, Y. Yu, B. Jia, Z.W. Sun, M.Z. Sun, X.B. Duan and Y.M. Zhu. 2014. *MicroRNA319* positively regulates cold tolerance by targeting *OsPCF6* and *OsTCP21* in rice (*Oryza sativa* L.). *Plos One*, 9(3): e91357.

Yao, X., H. Ma, J. Wang and D.B. Zhang. 2007. Genome-wide comparative analysis and expression pattern of TCP gene families in *Arabidopsis thaliana* and *Oryza sativa*. *J. Int. Plant. Biol*., 49(6): 885-897.

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