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Araştırma Makalesi / *Research Paper*

Genome-Wide Characterization and *In-silico* Transcriptional Expression Analysis of *PEBP* Family in *Solanum lycopersicum* L.

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ABSTRACT

Phosphatidylethanolamine-binding proteins (*PEBPs*) are an important gene family with highly conserved protein sequences represented in three taxonomic divisions. In plants, *PEBP* genes are an important actors in the regulation of flowering time, plant architecture and seed dormancy. Despite this, PEBP genes have not been genome-wide identified and systematically analyzed in tomato. In this study, the *PEBP* gene family in tomato, one of the economically important Solanum species, was comprehensively identified genome-wide and characterized by bioinformatics tools. Here, 12 *PEBP* genes were identified, which were classified into four clades based on their phylogenetic relationships and the presence of the structurally conserved domain/motif. In addition, the gene structure, conserved protein structure, promoter regions, and digital expression levels of these *PEBP* genes were determined. Digital expression profiling of *SlPEBP* transcripts revealed their expression in most developmental and anatomical tissues. These results will provide the further functional and evolutionary characterization of *PEBP* genes in tomato.

Keywords: Expression profiling, genome-wide, *PEBP*, phylogenetic analysis, tomato

Solanum lycopersicum L.'de *PEBP* Ailesinin Genom Çapında Karakterizasyonu ve *In-silico* Transkripsiyonel İfade Analizi

ÖZ

Fosfatidiletanolamin bağlayıcı proteinler (*PEBP*'ler), üç taksonomik bölümde temsil edilen yüksek oranda korunmuş protein dizilerine sahip önemli bir gen ailesidir. Bitkilerde *PEBP* genleri, çiçeklenme zamanı, bitki mimarisi ve tohum dormansisinin düzenlenmesinin önemli bir aktörüdür. Buna rağmen, *PEBP* genleri bugüne kadar domateste genom çapında tanımlanmamış ve sistematik olarak analiz edilmemiştir. Bu çalışmada, ekonomik açıdan önemli *Solanum* türlerinden domatesteki *PEBP* gen ailesi, genom çapında kapsamlı bir şekilde tanımlanmış ve biyoinformatik yöntemler ile karakterize edilmiştir. Bu çalışmada, filogenetik ilişkilerine ve yapısal olarak korunan domain/motif varlığına göre dört klad halinde sınıflandırılan 12 *PEBP* geni tanımlanmıştır. Ayrıca bu *PEBP* genlerinin gen yapısı, korunmuş protein yapısı, promotör bölgeleri ve dijital ekspresyon seviyeleri belirlenmiştir. *SlPEBP* transkriptlerinin dijital ekspresyon profili, çoğu gelişim aşamasında ve anatomik dokularda ekspresyonlarını ortaya çıkarmıştır. Bu sonuçlar, domateste *PEBP* genlerinin daha ileri fonksiyonel ve evrimsel karakterizasyonunun anlaşılmasını sağlayacaktır.

Anahtar Kelimeler: Ekspresyon profili, genom çapında tanımlama, *PEBP*, filogenetik analiz, domates

INTRODUCTION

Phosphatidylethanolamine-binding proteins (*PEBP*s) are a superfamily of genes that have containing the evolutionarily highly conserved PEBP domain and are represented in all three major phylogenetic divisions (Chautard et al., 2004; Zheng et al., 2016). Mammalian PEBPs are globular proteins consisting of acetate, phosphate groups, and a functional binding site for phosphatidyl ethanolamine, while the C-terminal sequences of plant *PEBP* homologs are very low conserved (Serre et al., 1998; Vallée et al. 2003). It has been reported in different studies that PEBP proteins act as serine proteases or RAF kinase inhibitors controlling cell growth and differentiation in animals (Hengst et al., 2001; Odabaei et al., 2004).

In *Arabidopsis thaliana*, the model plant of plant molecular biology, the *PEBP* gene family is mainly divided into three subfamilies: *FLOWERING LOCUS T* (*FT*)-like, *TERMINAL FLOWER 1* (*TFL1*)-like, and *FT MOTHER AND TFL1* (*MFT*)-like (Kardailsky et al.; 1999). *MFT-like* subfamily contains only one gene, *MOTHER OF FT AND TFL1* (*MFT*); the *FT-like* subfamily includes two genes, *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) and the *TFL-like* subfamily contains three genes, *TERMINAL FLOWER1* (*TFL1*), *ARABIDOPSIS THALIANA CENTRORADIALIS* (*ATC*) and *BROTHER OF FT AND TFL1* (*BFT*) (Yoo et al., 2010; Dong et al. 2020). It has been reported that *MFT-like* genes are present in both basal land plants and seed plants, but *FT-like* and *TFL1-like* genes are found only in gymnosperms and angiosperms, indicating that *MFT-like* is an ancestor of *FT-like* and *TFL1-like* subfamilies (Li et al., 2014; Wickland and Hanzawa, 2015). MFT-like proteins have critical roles in seed development and germination of gametophytes, sporophytes, and bryophytes, while FT-like and TFL1-like proteins are important components of the vegetative-to-reproductive transition in gymnosperms and angiosperms (Karlgren et al., 2011; Tao et al., 2014). In Arabidopsis, the expression of *MFT* is controlled by the ABA-INSENSITIVE3 (ABI3) and ABA-INSENSITIVE5 (ABI5) and regulates abscisic acid (ABA) and gibberellic acid (GA) signalling pathways (Xi et al., 2010).

BFT and *CEN* are two floral suppressors that play an important role in meristem growth in Arabidopsis and their overexpression results in a late flowering phenotype similar to *TFL1* (Mimida et al., 2001; Huang et al., 2012). The *FT* and *TFL1* genes have 60% homology at the amino acid level in Arabidopsis, but operate in opposite functions, moderating meristem identity, controlling flower transition and flowering architecture (Lee et al., 2019). *TSF*, the closest homolog of *FT* in Arabidopsis, acts as a floral inducer under non-inductive SD conditions and mutation of the *TSF* gene delays flowering.

In tomato, *SELF PRUNING* (*SP*) and *SINGLE FLOWER TRUSS* (*SFT*) which are homologs of *TFL1* and *FT*, regulate shoot architecture as well as flowering time (Pnueli et al., 1998). In spite of the extensive sequence similarity among *PEBP* members, their functions have diverged considerably over the evolutionary process (Wang et al., 2015). Therefore, the identification of *PEBP* family genes is becoming more important for molecular breeding studies in plant genomes at present. To date, *PEBP* family members have been identified in many different plant species such as Arabidopsis (Kobayashi et al., 1999, maize (Danilevskaya et al., 2008), grapevine (Carmona et al., 2007), legumes (Hecht et al., 2011), rice (Tamaki et al., 2007), and barley (Faure et al., 2007).

Due to its rich nutrient content, the tomato is not only a commercial but also a good model plant for the annotation of the whole genome sequence (Sato et al., 2012) and the elucidation of plant growth and development. However, to date, the *PEBP* gene family in tomato has not been identified genome-wide, and their potential biological functions remain unclear. In this study, *PEBP* genes in the tomato genome were identified and comparatively analyzed, including detailed protein trait analyses, exon-intron structure, phylogenetic tree, cis-elements of their promoters. Also, patterns of digital expression of PEBP genes at different developmental stages were analyzed. In summary, the results of this study may provide valuable information for understanding the distribution, structure and evolution of *PEBP* genes in tomato and may be the key to future functional characterization studies.

MATERIAL AND METHOD

Identification and Annotation of the *PEBP* Family in Tomato

To identification of *PEBP* genes in tomato, the Hidden Markov Model (HMM) profile of PEBP domain (PF01161) was downloaded from Pfam database (Mistry et al., 2021) and used as the query to search against the *Solanum lycopersicum* L. genomic database (Fernandez-Pozo et al., 2015. After HMM search analysis, candidate genes were uploaded to the Pfam and SMART database and the presence of the PEBP domain was confirmed. Finally, 12 PEBP family members were identified in the tomato genome.

Bioinformatics Analyses of the *PEBP* Family in Tomato

The basic biophysical properties, including the isoelectric point (*pI*), molecular weight (MW), instability index, and GRAVY of the PEBP proteins, were predicted by the ProtParam tool (Wilkins et al., 1999). The CELLO, online server (Yu et al., 2006) was used to predict the subcellular location of PEBP genes. The phylogenetic analysis was performed with 18 PEBP proteins (Figure 1) from *Solanum lycopersicum* L., and Arabidopsis. The phylogenetic tree was constructed using MEGA 11 software (Kumar et al. 2018) with the maximum likelihood estimation (MLE) method and was analyzed with 1000 bootstrap replications. Genomic and CDS sequences of *PEBP* genes were downloaded from Phytozome database (Goodstein et al., 2012) and used to develop an exon/intron map in the Gene Structure Display program (Hu et al., 2015). The conserved motifs of PEBP proteins were searched by the online software MEME Suite 5.4.1 (Bailey et al., 2015) with the default parameters except for two: any number of repetitions; maximum number of motifs: 5. The annotation of the motifs were performed in Pfam and NCBI Conserved Domain Search tools. Cis-regulatory elements for all the *SlPEBP* genes were identified by using the online Plant Promoter Analysis Navigator (Chow et al., 2019). To determine expression patterns of the *PEBP* genes in developmental stages of tomato, a comprehensive expression data was downloaded from Plant Omics Data Center database (Ohyanagi et al., 2015). Expression profiles were converted to log2 base and visualized with TBtools (Chen et al., 2020).

RESULTS AND DISCUSSION

In silico Identification and Characterization of *SlPEBP*s

In plants, the *PEBP* gene family controls many plant developmental processes, including flower transition, plant architecture, and seed germination (Liu et al., 2016; Jin et al., 2021). To date, 6 PEBP genes have been identified in Arabidopsis (Kardailsky et al., 1999), 20 in *Gossypium hirsutum* (Wang et al., 2019), 38 in *Triticum aestivum* (Dong et al., 2020), 15 in potato (Zhang et al., 2022), and 6 in *Dendrobium huoshanense* (Song et al., 2021).

After a systematic Hidden Markov Model (HMM) profile of the PEBP domain (PF01161) search against the tomato genome (Fernandez-Pozo et al., 2015), a total of 12 genes were obtained containing the typical PEBP domain. As shown in Table 1, basic information on *PEBP* genes in Arabidopsis and tomato was downloaded from the Phytozome database (Goodstein et al., 2012) and it was observed that the genes were randomly distributed on different chromosomes. The PEBP members of tomato were named according to the BLASTp (Altschul et al., 1990) similarity results. The polypeptide length of the SlPEBPs ranged from 222 aa (SlPEB7) to 73 aa (SlPEB12). The molecular weight (MW) of the SlPEBP proteins varied from the lowest 8.5 kDa (SlPEB12) to the highest of 24.8 kDa (SlPEB7). The predicted *pI* values ranged from 6.05 (SlPEB10) to 10.28 (SlPEB12), with an average of 8.1, suggesting that most SlPEBP proteins were weakly basic. According to subcellular analysis, SlPEBP proteins were predicted to be localized in the nucleus, cytoplasm, chloroplast, mitochondria, and extracellular space. The instability index of PEBP proteins ranged from 30.19 to 51.44, and all SlPEBP proteins except SlPEB10, SlPEB11, and SlPEB12 are in unstable form in the test tube. The calculated grand average of hydrophobicity (GRAVY) values ranged from -0.172 to -0.508, indicating all PEBP proteins were hydrophilic. In addition, the aliphatic index (AI) of PEBP proteins ranges from 76.4 to 92.36, and all PEBP proteins are thermostable. Based on the *Solanum lycopersicum* genome, the 12 *SlPEBP* genes were unevenly located on 6 chromosomes.

Table 1. The predicted characteristics of PEBP proteins in tomato and Arabidopsis

Gene name	AA length	Molecular weight	Theoretical pI	Instability index	Aliphatic index	GRAVY	Subcellular location	Chromosome	Start	Stop
SlPEB1	177	20	6.74	46.74	79.15	-0.388	Nuclear	3	29218238	29222055
SlPEB2	174	20	8.75	42.11	83.33	-0.37	Extracellular	11	2854836	2857237
SlPEB3	175	19.5	5.26	30.19	85.09	-0.199	Extracellular	5	63274186	63276271
SlPEB4	140	16	6.08	33.16	92.36	-0.172	Cytoplasmic	5	64601916	64603867
SlPEB5	175	19.5	9.23	50.02	75.71	-0.362	Nuclear	3	3442048	3443000
SlPEB6	172	19.5	8.89	50.2	76.4	-0.377	Cytoplasmic	9	3005801	3008374
SlPEB7	222	24.8	9.63	40.56	89.05	-0.218	Mitochondrial	1	3790533	3792319
SlPEB8	173	19.3	8.69	30.95	86.07	-0.221	Cytoplasmic	1	3775892	3776790
SlPEB9	175	19.9	8.72	50.26	75.03	-0.262	Cytoplasmic	6	43636030	43638303
SlPEB10	181	20.1	6.05	37.06	72.71	-0.458	Cytoplasmic	2	41887985	41889245
SlPEB11	173	19.1	8.6	39.32	82.14	-0.155	Cytoplasmic	3	62245871	62247505
SlPEB12	73	8.5	10.28	38.24	68.08	-0.508	Extracellular	11	2866944	2867166
AtFT	175	19.8	7.75	48.81	88.97	-0.259	Extracellular	1	24331427	24333934
AtBFT	177	20	9.16	48.12	74.35	-0.271	Chloroplast	5	24922809	24923709
AtCEN	175	19.8	7.01	33.33	78.91	-0.275	Cytoplasmic	2	11773250	11774681
AtTFL1	177	20.1	9.69	44.52	81.36	-0.224	Cytoplasmic	5	1024640	1025812
AtTSF	175	19.6	7.76	41.54	85.6	-0.283	Extracellular	4	11000770	11002996
AtMFT	173	19.1	7.93	51.44	81.04	-0.179	Cytoplasmic	1	6227216	6230188

Phylogenetic Analysis of *SlPEBP* Genes

In order to explore the evolutionary relationship of the tomato PEBP family, an unrooted phylogenetic tree was constructed using the maximum likelihood estimation (MLE) executed in MEGA XI with the Jones–Taylor–Thornton (JTT) model based on multiple sequence alignment of six PEBP members from Arabidopsis (Figure 1). The phylogenetic tree demonstrated that 18 PEBP proteins were clustered in four subfamilies which is compatible with the classification of the PEBP family from *Gossypium hirsutum* (Wang et al., 2019). Subgroup FT-clade was the largest with 7 genes, of which had 5 PEBP genes from tomato, and subgroup MFT-clade was the smallest with 3 genes, of which had 2 PEBP genes from tomato. In addition, the BFT-clade and TFL1-clade subgroups consisted of four genes each with 3 and 2 PEBP genes from tomato, respectively.

Gene Structure and Motif Identification of *SlPEBP* Genes

Structural differences in the exon-intron, which determine the expression and function of genes, also play an important role in understanding the evolution of plant gene families (Wang et al. 2014). In order to reveal the gene structure of *SlPEBP* genes, the UTR, CDS, domain, and intron regions were downloaded from Phytozome and submitted to the GSDS server for visualization (Figure 2A). The results showed that all *SlPEBP* genes were found to consist of four exons and three introns, except for the intronless *SlPEBP12* gene. In addition, consistent with previous studies, the sizes of the second and third exons of the *SlPEBP* genes were found to be 62 and 41 bp, respectively (Zhao et al., 2020).

Further, the conserved motifs of all SlPEBP genes were analyzed based on MEME software, and 5 conserved motifs were identified (Figure 2B-2C). The analysis showed that, in terms of width, motif 1 was the largest (50 each), with motif 5 (15 each) was the smallest. According to the analysis, motifs 1-5 are represented in all SlPEBP genes, except SlPEBP12 that contains only motif 2 and motif 5.

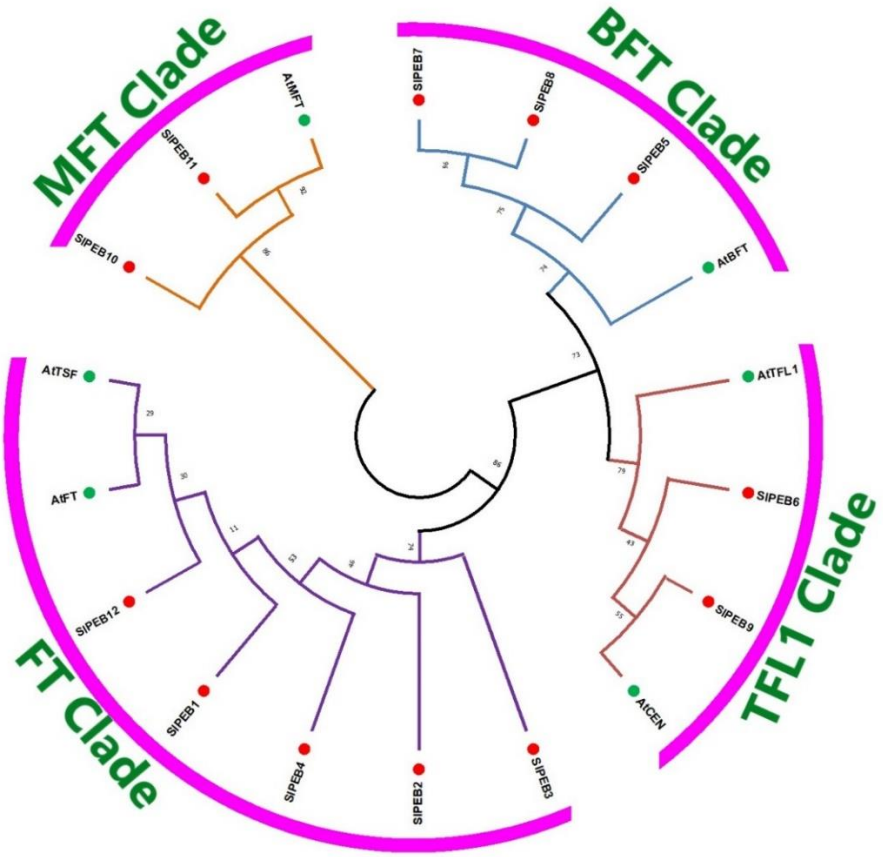

Figure 1. Phylogenetic analysis of PEBP proteins. All selected proteins, together with tomato and Arabidopsis were classified into four clade and marked with red and green, respectively. The values at the branch nodes showed the confidence levels from 1000-replication bootstrapping

Identification of *Cis-Acting* Regulatory Elements in the Promoters of *SlPEBP* Genes

Cis-acting elements are regulators that control gene expression by combining with specific transcription factors during plant development and stress response, and their distribution is closely related to gene function (Le et al., 2012; Biłas et al., 2016). To better understand the cis-acting regulatory elements in the promoter of *SlPEBP*s, 2 kb upstream sequences of the transcription initiation site were used to search in PlantPAN 3.0 database. Except for the conventional promoter elements (such as TATA-box and CAAT-box), a total of ten cis-acting elements were found in the *SlPEBP* promoters (Table 2).

As a result of the analysis, regulatory elements that play a role in many different stages from germination to fruit ripening were determined (Table 2). To summarize, *SlPEBPs* have various cis-elements involved in flower development (AT-Hook), responses to abiotic stress (NAC), cell elongation (bZIP), and fruit development (MADS box), light signaling (bHLH).These results indicate that cis-acting regions of *SlPEBP* genes were important for plant growth and development.

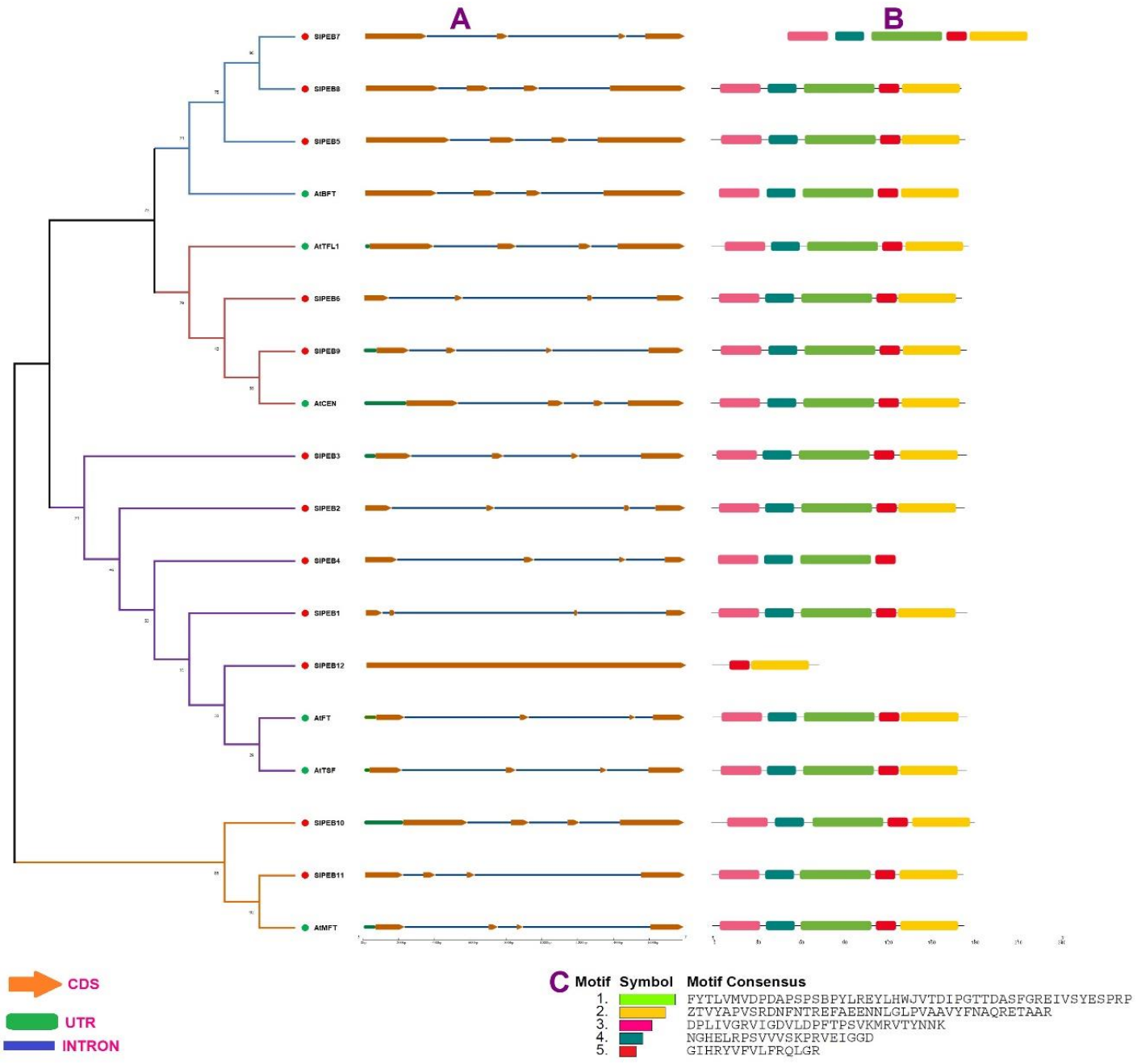

Figure 2. Gene structure and conserved motifs distribution of PEBP proteins in tomato and Arabidopsis. (**A**) Exon-intron structure of *PEBP* genes. (**B-C**) Distribution of conserved motifs identified from PEBP proteins

Table 2. *Cis*-acting element analysis of the *SlPEBP* gene family

Cis element	Function	References
AT-Hook	plant reproductive development, flower development.	(Gallavotti et al., 2011)
NAC	developmental programs, defense, responses to abiotic stress.	(Xie et al., 1999**;** Hegedus et al., 2003; Tranbarger et al., 2017)
bZIP	cell elongation, seed storage protein gene regulation	(Fukazawa et al., 2000; Lara et al., 2003)
MADS box	regulatory role in the development of flower organs, regulating the development of seeds and fruits.	(Michaels et al., 2003; Dong et al., 2013).
AP2	leaf epidermal cell determinacy, spikelet meristem differentiation and floral organ patterning	(Aukerman and Sakai, 2004)
bHLH	light signaling, flowering and fruit development.	(Castelain et al., 2012, Li et al., 2019)
WRKY	trichome morphogenesis, dormancy and germination	(Johnson et al., 2002**;** Zhang et al., 2004)
EIN3/EIL	regulate the growth, development and senescence	(Davies, 1993)
GATA	carbon and nitrogen metabolism, seed germination, cotyledon development	(Bi et al., 2005; Liu et al., 2005**;** Luo et al., 2010)
HD-ZIP	apical meristem differentiation, microtubule formation	(Ariel et al., 2007)

Digital Expression Profiling of *PEBP* Genes Various Tissues of Tomato

Revealing the expression profile of gene families is key to understanding the system and form of plants throughout growth and development. RNA-seq, based on the assumption that the depth of coverage of a sequence is proportional to the expression of the gene of interest, provides a better alternative to gene expression analysis compared to hybridization-based methods (Aceituno et al. 2008). Therefore, to understand the roles of *PEBP* genes in tomato growth and development, we downloaded publicly available transcriptomic data from the PODC (Ohyanagi et al., 2015) and analyzed and visualized it with TBtools (Chen et al., 2020). In particular, it was observed that SlPEBP7 was strongly expressed in the root, and *SlPEBP3* and *SlPEB10* in the leaf. In addition, *SlPEBP12* was found not to be expressed in any of the tissues tested. Among all *SlPEBP* genes, *SlPEBP10* was highly expressed in all major tissues, making it a strong candidate for future functional characterization studies.

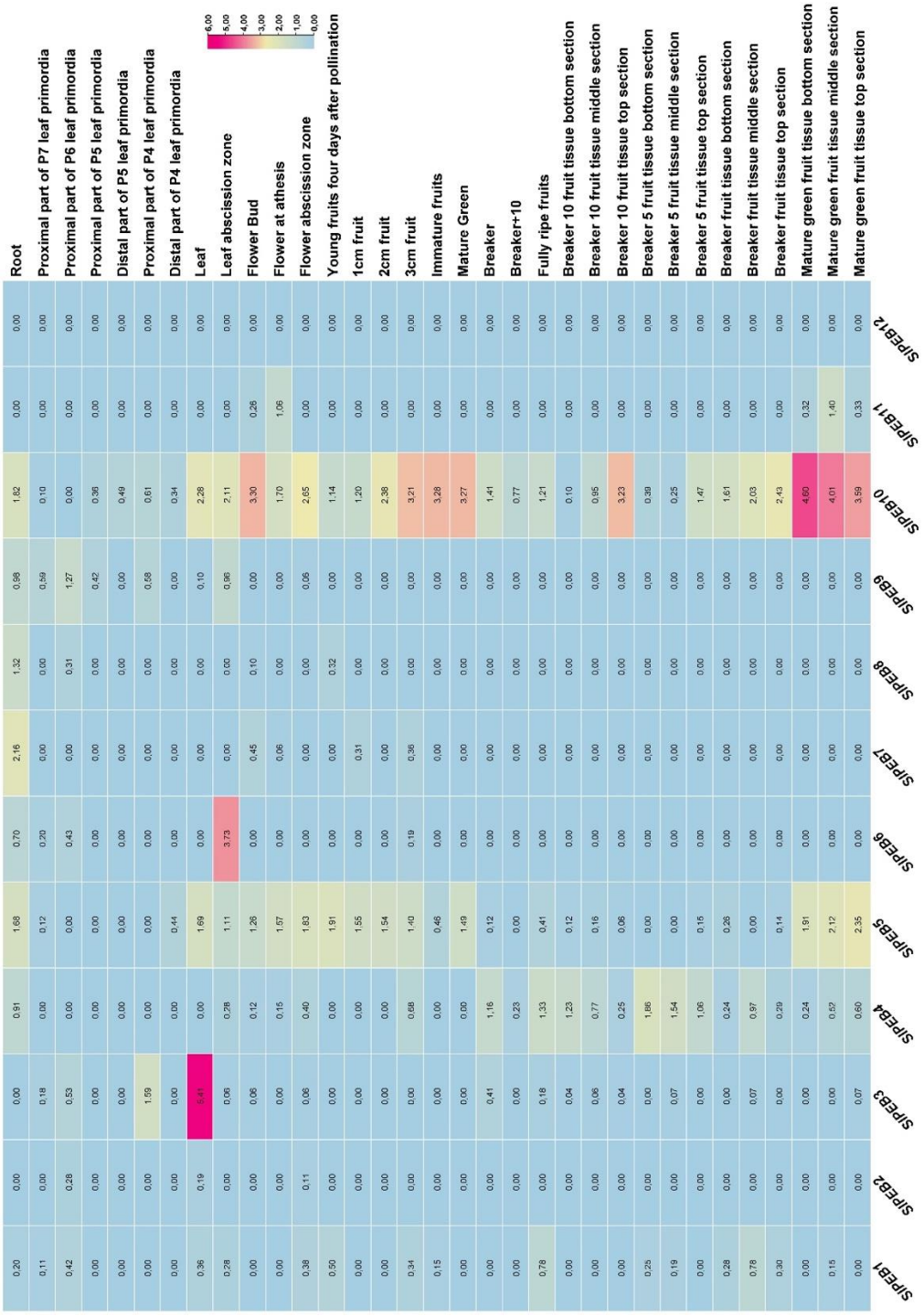

Figure 3. Heatmap of digital expression profiles for SlPEBP-genes in different developmental stages. Blue and red indicated the expression values decreased and increased, respectively

CONCLUSION

Tomato is the largest widely cultivated vegetable crop favored by consumers worldwide. Due to its high genetic diversity, it is a model plant for the study of plant growth, development, and responses to stress. PEBPs are evolutionarily conserved proteins that are significantly associated with the growth and development of plants and their seasonal adaptability (Pnueli et al., 1998). In this study, 12 PEBP genes were identified in the tomato genome and their sequence, cis-acting elements, structure and expression during development were analyzed to reveal their potential biological functions in the plant kingdom and their possible roles in regulatory pathways. The number of *PEBP* genes identified in tomato is higher than in 6 genes in Arabidopsis; but fewer than in maize (24), soybean (23), rice (19), sorghum (19), and *Setaria italica* (20). These results show that there is no direct relationship between the number of PEBP family members and the genome size of plants. Moreover, although the number of PEBP gene family members differed between monocotyledons and dicotyledons, the sequences were highly conserved, suggesting that PEBP-like genes played an important role in the evolutionary process. Phylogenetic tree analysis demonstrated that 12 SlPEBP genes, unlike other plant species, can be divided into four main groups: FT-like, TFL1-like, BFT-like and MFT-like genes (Kardailsky et al., 1999). As with the PEBP genes in Arabidopsis, apple (*Malus domestica*) and pear (*Pyrus communis*), all tomato PEBP genes, except SlPEBP12, consist of four exons and three introns, while exon second contains 62 bases and exon third contains 41 bases. Expression profiles of *SlPEBP* genes were analyzed from publicly available RNA-seq data, revealing that most of the transcripts are expressed at different levels in developmental stages and anatomical tissues.

To summarize, the comprehensive analysis of the PEBP family in this study provides new evidence to better understand the structure, evolution and function of PEBP family genes among different plant groups. Furthermore, the results of this study revealed the potential role of SlPEBP genes in tomato growth and development.

REFERENCES

Aceituno, F.F., Moseyko, N., Rhee, S. Y., Gutiérrez, R.A. (2008). The rules of gene expression in plants: organ identity and gene body methylation are key factors for regulation of gene expression in Arabidopsis thaliana. *BMC Genomics*, 9(1): 1-14.

Altschul, S.F., Gish, W., Miller, W., Myers, E. W., Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3): 403-410.

Ariel, F.D., Manavella, P.A., Dezar, C.A., Chan, R.L. (2007). The true story of the HD-Zip family. *Trends In Plant Science*, 12(9): 419-426.

Aukerman, M.J., Sakai, H. (2003). Regulation of flowering time and floral organ identity by a microRNA and its APETALA2-like target genes. *The Plant Cell*, 15(11): 2730-2741.

Bailey, T.L., Johnson, J., Grant, C.E., Noble, W.S. (2015). The MEME suite. *Nucleic Acids Research*, 43(W1): W39-W49.

Bi, Y.M., Zhang, Y., Signorelli, T., Zhao, R., Zhu, T., Rothstein, S. (2005). Genetic analysis of Arabidopsis GATA transcription factor gene family reveals a nitrate-inducible member important for chlorophyll synthesis and glucose sensitivity. *The Plant Journal*, 44(4): 680-692.

Biłas, R., Szafran, K., Hnatuszko-Konka, K., Kononowicz, A.K. (2016). Cis-regulatory elements used to control gene expression in plants. *Plant Cell, Tissue and Organ Culture*, 127(2): 269-287.

Carmona, M.J., Calonje, M., Martínez-Zapater, J.M. (2007). The FT/TFL1 gene family in grapevine. *Plant Molecular Biology*, 63(5): 637-650.

Castelain, M., Le Hir, R., Bellini, C. (2012). The non-DNA-binding bHLH transcription factor PRE3/bHLH135/ATBS1/TMO7 is involved in the regulation of light signaling pathway in Arabidopsis. *Physiologia Plantarum*, 145(3): 450-460.

Chautard, H., Jacquet, M., Schoentgen, F., Bureaud, N., Bénédetti, H. (2004). Tfs1p, a member of the PEBP family, inhibits the Ira2p but not the Ira1p Ras GTPase-activating protein in Saccharomyces cerevisiae. *Eukaryotic Cell*, 3(2): 459-470.

Chen, C., Chen, H., Zhang, Y., Thomas, H.R., Frank, M.H., He, Y., Xia, R. (2020). TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Molecular Plant*, 13(8): 1194-1202.

Chow, C.N., Lee, T.Y., Hung, Y.C., Li, G.Z., Tseng, K.C., Liu, Y.H., Chang, W.C. (2019). PlantPAN3. 0: a new and updated resource for reconstructing transcriptional regulatory networks from ChIP-seq experiments in plants. *Nucleic Acids Research*, 47(1): 1155-1163.

Danilevskaya, O.N., Meng, X., Hou, Z., Ananiev, E.V., Simmons, C.R. (2008). A genomic and expression compendium of the expanded PEBP gene family from maize. *Plant Physiology*, 146(1): 250-264.

Abeles, F.B., Morgan, P.W., Saltveit Jr, M.E. (2012). *Ethylene in plant biology*. Academic Press; DOI:10.1016/C2009-0-03226-7

Dong, L., Lu, Y., Liu, S. (2020). Genome-wide member identification, phylogeny and expression analysis of PEBP gene family in wheat and its progenitors. *PeerJ*, 8: 10483; DOI: 10.7717/peerj.10483

Dong, T., Hu, Z., Deng, L., Wang, Y., Zhu, M., Zhang, J., Chen, G. (2013). A tomato MADS-box transcription factor, SlMADS1, acts as a negative regulator of fruit ripening. *Plant Physiology*, 163(2): 1026-1036.

Faure, S., Higgins, J., Turner, A., Laurie, D.A. (2007). The FLOWERING LOCUS T-like gene family in barley (Hordeum vulgare). *Genetics*, 176(1): 599-609.

Fernandez-Pozo, N., Menda, N., Edwards, J.D., Saha, S., Tecle, I.Y., Strickler, S.R., Mueller, L.A. (2015). The Sol Genomics Network (SGN)—from genotype to phenotype to breeding. *Nucleic Acids Research*, 43: 1036-1041.

Fukazawa, J., Sakai, T., Ishida, S., Yamaguchi, I., Kamiya, Y., Takahashi, Y. (2000). Repression of shoot growth, a bZIP transcriptional activator, regulates cell elongation by controlling the level of gibberellins. *The Plant Cell*, 12(6): 901-915.

Gallavotti, A., Malcomber, S., Gaines, C., Stanfield, S., Whipple, C., Kellogg, E., Schmidt, R.J. (2011). BARREN STALK FASTIGIATE1 is an AT-hook protein required for the formation of maize ears. *The Plant Cell*, 23(5): 1756-1771.

Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R.D., Fazo, J., Rokhsar, D.S. (2012). Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Research*, 40: 1178-1186.

Hecht, V., Laurie, R.E., Vander Schoor, J.K., Ridge, S., Knowles, C.L., Liew, L.C., Weller, J.L. (2011). The pea GIGAS gene is a FLOWERING LOCUS T homolog necessary for graft-transmissible specification of flowering but not for responsiveness to photoperiod. *The Plant Cell*, 23:147-161.

Hegedus, D., Yu, M., Baldwin, D., Gruber, M., Sharpe, A., Parkin, I., Lydiate, D. (2003). Molecular characterization of Brassica napus NAC domain transcriptional activators induced in response to biotic and abiotic stress. *Plant Molecular Biology*, 53(3): 383-397.

Hengst, U., Albrecht, H., Hess, D., Monard, D. (2001). The phosphatidylethanolamine-binding protein is the prototype of a novel family of serine protease inhibitors. *Journal of Biological Chemistry*, 276(1): 535-540.

Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J., Gao, G. (2015). GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics*, 31(8): 1296-1297.

Huang, N.C., Jane, W.N., Chen, J., Yu, T.S. (2012). Arabidopsis thaliana CENTRORADIALIS homologue (ATC) acts systemically to inhibit floral initiation in Arabidopsis. *The Plant Journal*, 72(2): 175-184.

Jin, S., Nasim, Z., Susila, H., Ahn, J.H. (2021). Evolution and functional diversification of FLOWERING LOCUS T/TERMINAL FLOWER 1 family genes in plants. *In Seminars in Cell & Developmental Biology*, 109: 20-30.

Johnson, C.S., Kolevski, B., Smyth, D.R. (2002). TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. *The Plant Cell*, 14(6): 1359-1375.

Kardailsky, I., Shukla, V.K., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T., Weigel, D. (1999). Activation tagging of the floral inducer FT. *Science*, 286: 1962-1965.

Karlgren, A., Gyllenstrand, N., Källman, T., Sundström, J.F., Moore, D., Lascoux, M., Lagercrantz, U. (2011). Evolution of the PEBP gene family in plants: functional diversification in seed plant evolution. *Plant Physiology*, 156(4): 1967-1977.

Kobayashi, Y., Kaya, H., Goto, K., Iwabuchi, M., Araki, T. (1999). A pair of related genes with antagonistic roles in mediating flowering signals. *Science*, 286(5446): 1960-1962.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6): 1547; DOI: 10.1093/molbev/msy096

Lara, P., Oñate-Sánchez, L., Abraham, Z., Ferrándiz, C., Díaz, I., Carbonero, P., Vicente-Carbajosa, J. (2003). Synergistic activation of seed storage protein gene expression in Arabidopsis by ABI3 and two bZIPs related to OPAQUE2. *Journal of Biological Chemistry*, 278(23): 21003-21011.

Le, D.T., Nishiyama, R., Watanabe, Y., Vankova, R., Tanaka, M., Seki, M., Tran, L.S.P. (2012). Identification and expression analysis of cytokinin metabolic genes in soybean under normal and drought conditions in relation to cytokinin levels. *Plos One*, 7(8): 42411; DOI: 10.1371/journal.pone.0042411

Lee, C., Kim, S.J., Jin, S., Susila, H., Youn, G., Nasim, Z., Ahn, J.H. (2019). Genetic interactions reveal the antagonistic roles of FT/TSF and TFL1 in the determination of inflorescence meristem identity in Arabidopsis. *The Plant Journal*, 99(3): 452-464.

Li, H., Gao, W., Xue, C., Zhang, Y., Liu, Z., Zhang, Y., Zhao, J. (2019). Genome-wide analysis of the bHLH gene family in Chinese jujube (Ziziphus jujuba Mill.) and wild jujube. *BMC Genomics*, 20(1): 1-13; DOI:10.1186/s12864-019-5936-2

Li, Q., Fan, C., Zhang, X., Wang, X., Wu, F., Hu, R., Fu, Y. (2014). Identification of a soybean MOTHER OF FT AND TFL1 homolog involved in regulation of seed germination. *PLoS One*, 9(6): 99642; DOI: 10.1371/journal.pone.0099642

Liu, P.P., Koizuka, N., Martin, R.C., Nonogaki, H. (2005). The BME3 (Blue Micropylar End 3) GATA zinc finger transcription factor is a positive regulator of Arabidopsis seed germination. *The Plant Journal*, 44(6): 960-971.

Liu, Y.Y., Yang, K.Z., Wei, X.X., Wang, X.Q. (2016). Revisiting the phosphatidylethanolamine-binding protein (PEBP) gene family reveals cryptic FLOWERING LOCUS T gene homologs in gymnosperms and sheds new light on functional evolution. *New Phytologist*, 212(3): 730-744.

Luo, X. M., Lin, W. H., Zhu, S., Zhu, J.Y., Sun, Y., Fan, X.Y., Wang, Z.Y. (2010). Integration of light-and brassinosteroid-signaling pathways by a GATA transcription factor in Arabidopsis. *Developmental Cell*, 19(6): 872-883.

Michaels, S.D., Ditta, G., Gustafson-Brown, C., Pelaz, S., Yanofsky, M., Amasino, R.M. (2003). AGL24 acts as a promoter of flowering in Arabidopsis and is positively regulated by vernalization. *The Plant Journal*, 33(5): 867-874.

Mimida, N., Goto, K., Kobayashi, Y., Araki, T., Ahn, J.H., Weigel, D., Sakamoto, W. (2001). Functional divergence of the TFL1-like gene family in Arabidopsis revealed by characterization of a novel homologue. *Genes to Cells*, 6(4): 327-336.

Mistry, J., Chuguransky, S., Williams, L., Qureshi, M., Salazar, G.A., Sonnhammer, E.L., Bateman, A. (2021). Pfam: The protein families database in 2021. *Nucleic Acids Research*, 49: 412-419.

Odabaei G, Chatterjee D, Jazirehi A.R. (2004). Raf-1 kinase inhibitor protein: structure, function, regulation of cell signaling, and pivotal role in apoptosis. *Advances in Cancer Research*, 91: 169; DOI: 10.1016/S0065-230X(04)91005-6

Ohyanagi, H., Takano, T., Terashima, S., Kobayashi, M., Kanno, M., Morimoto, K., Yano, K. (2015). Plant Omics Data Center: an integrated web repository for interspecies gene expression networks with NLP-based curation. *Plant and Cell Physiology*, 56(1): 9; DOI: 10.1093/pcp/pcu188

Pnueli, L., Carmel-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Lifschitz, E. (1998). The SELF-PRUNING gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. *Development*, 125(11): 1979-1989.

Sato, S., Tabata, S., Hirakawa, H. (2012). The tomato genome sequence provides insights into fleshy fruit evolution. *Nature*, 485(7400): 635–641.

Serre, L., Vallée, B., Bureaud, N., Schoentgen, F., Zelwer, C. (1998). Crystal structure of the phosphatidylethanolamine-binding protein from bovine brain: a novel structural class of phospholipid-binding proteins. *Structure*, 6(10): 1255-1265.

Song, C., Li, G., Dai, J., Deng, H. (2021). Genome-Wide Analysis of PEBP Genes in Dendrobium huoshanense: Unveiling the Antagonistic Functions of FT/TFL1 in Flowering Time. *Frontiers in Genetics*, 12: 687689; DOI: 10.3389/fgene.2021.687689

Tamaki, S., Matsuo, S., Wong, H.L., Yokoi, S., Shimamoto, K. (2007). Hd3a protein is a mobile flowering signal in rice. *Science*, 316(5827): 1033-1036.

Tao, Y. B., Luo, L., He, L.L., Ni, J., Xu, Z.F. (2014). A promoter analysis of MOTHER OF FT AND TFL1 1 (JcMFT1), a seed-preferential gene from the biofuel plant Jatropha curcas. *Journal of Plant Research*, 127(4): 513-524.

Tranbarger, T.J., Fooyontphanich, K., Roongsattham, P., Pizot, M., Collin, M., Jantasuriyarat, C., Morcillo, F. (2017). Transcriptome analysis of cell wall and NAC domain transcription factor genes during Elaeis guineensis fruit ripening: evidence for widespread conservation within monocot and eudicot lineages. *Frontiers in Plant Science*, 8: 603; DOI: 10.3389/fpls.2017.00603

Vallee, B.S., Coadou, G., Labbe, H., Sy, D., Vovelle, F., Schoentgen, F. (2003). Peptides corresponding to the N- and C-terminal parts of PEBP are well-structured in solution: new insights into their possible interaction with partners in vivo. *The Journal of Peptide Research*, 61(2): 47-57.

Wang, L., Zhu, W., Fang, L., Sun, X., Su, L., Liang, Z., Xin, H. (2014). Genome-wide identification of WRKY family genes and their response to cold stress in Vitis vinifera. *BMC Plant Biology*, 14(1): 1-14; DOI: 10.1186/1471-2229-14-103

Wang, M., Tan, Y., Cai, C., Zhang, B. (2019). Identification and expression analysis of phosphatidy ethanolamine-binding protein (PEBP) gene family in cotton. *Genomics*, 111(6): 1373-1380.

Wang, Z., Zhou, Z., Liu, Y., Liu, T., Li, Q., Ji, Y., Tian, Z. (2015). Functional evolution of phosphatidylethanolamine binding proteins in soybean and Arabidopsis. *The Plant Cell*, 27(2): 323-336.

Wickland, D.P., & Hanzawa, Y. (2015). The FLOWERING LOCUS T/TERMINAL FLOWER 1 gene family: functional evolution and molecular mechanisms. *Molecular Plant*, 8(7): 983-997.

Wilkins, M.R., Gasteiger, E., Bairoch, A. (1999). Protein identification and analysis tools in the ExPASy server. *Methods in Molecular Biology*, 112: 531–552.

Xi, W., Liu, C., Hou, X., Yu, H. (2010). MOTHER OF FT AND TFL1 regulates seed germination through a negative feedback loop modulating ABA signaling in Arabidopsis. *The Plant Cell*, 22(6): 1733-1748.

Xie, Q., Sanz-Burgos, A.P., Guo, H., García, J. A., Gutiérrez, C. (1999). GRAB proteins, novel members of the NAC domain family, isolated by their interaction with a geminivirus protein. *Plant Molecular Biology*, 39(4): 647-656.

Yoo, S.J., Chung, K.S., Jung, S.H., Yoo, S.Y., Lee, J.S., Ahn, J.H. (2010). BROTHER OF FT AND TFL1 (BFT) has TFL1-like activity and functions redundantly with TFL1 in inflorescence meristem development in Arabidopsis. *The Plant Journal*, 63(2): 241-253.

Yu, C.S., Chen, Y.C., Lu, C.H., Hwang, J.K. (2006). Prediction of protein subcellular localization. *Proteins: Structure, Function, and Bioinformatics*, 64(3): 643-651.

Zhang, Z.L., Xie, Z., Zou, X., Casaretto, J., Ho, T.H.D., Shen, Q.J. (2004). A rice WRKY gene encodes a transcriptional repressor of the gibberellin signaling pathway in aleurone cells. *Plant Physiology*, 134(4): 1500-1513.

Zhao, S., Wei, Y., Pang, H., Xu, J., Li, Y., Zhang, H., Zhang, Y. (2020). Genome-wide identification of the PEBP genes in pears and the putative role of PbFT in flower bud differentiation. *PeerJ*, 8: 8928; DOI: 10.7717/peerj.8928

Zheng, X.M., Wu, F. Q., Zhang, X., Lin, Q.B., Wang, J., Guo, X.P., Wan, J.M. (2016). Evolution of the PEBP gene family and selective signature on FT-like clade. *Journal of Systematics and Evolution*, 54(5): 502-510.