Genome-wide identification, subcellular localization, and expression analysis of the *phosphatidyl ethanolamine-binding protein* family reveals the candidates involved in flowering and yield regulation of Tartary buckwheat (*Fagopyrum tataricum*)

Mengping Nie[1], Li Li[1], Cailin He[1], Jing Lu[1], Huihui Guo[1], Xiao'an Li[1], Mi Jiang[3], Ruiling Zhan[3], Wenjun Sun[1], Junjie Yin[2], Qi Wu[1*]

1 Key Laboratory of Coarse Cereal Processing, Ministry of Agriculture and Rural Affairs, Sichuan Engineering & Technology Research Center of Coarse Cereal Industralization, College of Food and Biological Engineering, Chengdu University, Chengdu 610106, Sichuan, China

2 State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Sichuan Agricultural University, Chengdu 611130, Sichuan, China

3 Key Laboratory of Wheat Crop Research in Ganzi Academy of Agricultural Sciences, Ganzi 626000, Sichuan, China

[*]Corresponding author: Qi Wu

Address: Chengluo road 2025, Longquanyi District, Chengdu 610106, Sichuan, China

E-mail: jerviswuqi@126.com

Abstract

Background. *PEBP* (*phosphatidyl ethanolamine-binding protein*) is widely found in eukaryotes, including plants, animals, and microorganisms. In plants, the PEBP family plays vital roles in regulating flowering time and morphogenesis and is highly associated with agronomic traits and yields of crops, which has been identified and characterized in many plant species but not well studied in Tartary buckwheat (*Fagopyrum tataricum* Gaertn.), an important coarse food grain with medicinal value.

Methods. Genome-wide analysis of *FtPEBP* gene family members in Tartary buckwheat was performed using bioinformatic tools. Subcellular localization analysis was performed by confocal microscopy. The expression levels of these genes in leaf and inflorescence samples were analyzed using qRT-PCR.

Results. ~~A total of fourteen~~Fourteen *Fagopyrum tataricum PEBP* (*FtPEBP*) genes were identified and divided into three sub-clades according to their phylogenetic relationships. Subcellular localization analysis of the FtPEBP proteins in tobacco leaves indicated that FT- and TFL-GFP fusion proteins were localized in both the nucleus and cytoplasm. Gene structure analysis showed that most *FtPEBP* genes contain four exons and three introns. *FtPEBP* genes are unevenly distributed in Tartary buckwheat chromosomes. Three tandem repeats were found among *FtFT5*/*FtFT6*, *FtMFT1*/*FtMFT2*, and *FtTFL4*/*FtTFL5*. ~~A total of five~~Five orthologous gene pairs were detected between *F. tataricum* and *F. esculentum*. A large number of light-responsive and hormone-related elements were detected in *FtPEBPs* promoters. We used real-time PCR to investigate the expression levels of *FtPEBP*s among two ~~flowering type~~flowering-type cultivars at floral transition time. We found *FtFT1*/*FtFT3* were highly expressed in leaf and young inflorescence of early-flowering type~~ whereas ,~~ whereas they were expressed at very low levels in ~~that of~~ late-flowering type cultivars. Thus, we deduced that *FtFT1*/*FtFT3* may be positive regulators for flowering and yield of Tartary buckwheat. These results lay an important foundation for further studies on the functions of *FtPEBP* genes, which may be utilized for yield improvement.

Keywords: *FT*/*TFL*, flowering, yield, Tartary buckwheat

Commented [TT1]: State how many elements

Introduction

Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is an important traditional coarse cereal with a long cultivation history in southwest China (Wen et al. 2021). It was the main food for the minorities in the marginal areas of Sichuan, Guizhou, and Yunnan provinces (Wu et al. 2017). Because Tartary buckwheat seeds are abundant in flavonoids, active peptides, and minerals (Ma et al. 2019; Huang et al. 2016), ~~it is~~they are usually used as food and medicine (Liu et al. 2021). Recently, with the increasing recognition ~~on the nutritional and medicinal value of Tartary buckwheat~~of Tartary buckwheat's nutritional and medicinal value, the global demand is growing rapidly. ~~Yet~~However, compared with the staple food crops, the yield of Tartary buckwheat still has a large room for improvement. Thus, expanding the planting area and improving the yield of Tartary buckwheat is necessary. Flowering regulation has been reported to ~~not only influence the inflorescence morphology and regional adaptation of plants, but also~~influence the inflorescence morphology and regional adaptation of plants and is closely related to crop yield (Song et al. 2015). Some ~~Tartary buckwheats with long growth periods usually could not survive the extreme~~Tartary buckwheats with long growth periods usually could not survive the extremely hot or cold weather to generate seeds. Rice Tartary buckwheat is ~~specific cultivar originated around Himalaya, which is favored by many people because of its easy-to-dehull property~~a specific cultivar that originated around the Himalayas and is favored by many people because of the easy-to-dehull properties of the seeds (Li et al. 2020). As its floral transition process is ~~hindered usually by temperature or light conditions in low-altitude planting area, rice Tartary buckwheat has a very long vegetative phase, in which inflorescence could not elongate~~usually hindered by temperature or light conditions in the low-altitude planting areas, rice Tartary buckwheat has a long vegetative phase in which inflorescence cannot elongate, resulting in very low florets production. Thus, ~~planting of rice Tartary buckwheat is narrowed to higher altitude area with lower temperature~~the planting of rice Tartary buckwheat is narrowed to higher altitude areas with lower temperatures. Therefore, exploration and utilization of flowering and inflorescence-related genes ~~is essential to expand the planting region and increase the yield of long-period Tartary~~are

Commented [TT2]: Give examples of the staple food crops

essential to expanding the planting region and increasing the yield of long-period Tartary buckwheat.

Flowering is an ~~important~~ essential process ~~standing~~ for the transition from vegetative to reproductive growth (Song et al., 2015) and is influenced by internal and environmental factors (Hemming et al., 2008). The *phosphatidyl ethanolamine-binding protein* (*PEBP*) gene family is widespread in many species, ~~spanning from bacteria, animals~~including bacteria, animals, and plants (Karlgren et al., 2011). The *PEBP* family members are tightly associated with plant growth and development. Many *PEBP* family members have been identified in various plants, such as Arabidopsis (*Arabidopsis thaliana*) (Wigge et al. 2005; Fryxell, 1996), rice (*Oryza sativa*) (Tamaki et al. 2007; Zhao et al. 2022), soybean (*Glycine max*) (Wang et al. 2015; Chengming Fan et al. 2014), maize (*Zea mays*) (Meng et al. 2011; Danilevskaya et al. 2008) and potato (*Solanum tuberosum*) (Navarro et al. 2011; Zhang et al. 2022). Three sub-clades were classified according to the structure and function in plants: *FT*-like, *TFL1*-like, and *MFT*-like subgroups (Karlgren et al. 2011). *FLOWERING LOCUS T* (*FT*) encodes for florigen protein that moves through the phloem from leaves to the shoot apical meristem (SAM) to activate flowering~~, whereas *TERMINAL*~~. In contrast, TERMINAL *FLOWER 1* (*TFL1*) ~~acts as~~is a flowering repressor (Wickland and Hanzawa 2015). *MOTHER OF FT AND TFL1* (*MFT*) is homologous to both *FT* and *TFL1*, and constitutive expression of *MFT* resulted in slightly earlier flowering under long days (Chen et al. 2018). Besides, *MFT* plays ~~an important~~a vital role in seed germination and development, which promotes embryo growth through a negative feedback loop in the ABA signaling pathway (Xi et al. 2010).

To date, many studies have proved the roles of the *PEBP* genes in agronomic ~~traits~~ trait regulation. When *Hd3a* was suppressed, the transgenic plants showed a later flowering time and a reduction in the number of branches compared to the ~~wild type~~wild-type (WT) plants (Tsuji et al. 2015). Overexpression of ~~*RCN1*or~~ *RCN1 or RCN2*, rice *TFL1*/*CEN* homologs, caused a delayed transition to the reproductive phase and displayed a more branched, denser panicle morphology (Nakagawa et al. 2002). The wheat *TaTFL1-5* mutation reduced the tiller numbers per plant during ~~vegetative~~

~~period and decreased the number of effective tillers and spikelets at maturity stage (Sun et al. 2023a)~~the vegetative period and decreased the number of effective tillers and spikelets at the maturity stage (Sun et al.,. Overexpression of *HbMFT1* resulted in delayed seed germination, seeding growth, and flowering in transgenic Arabidopsis (Bi et al., 2016). The maize plants ectopic expressing *ZCN8* had earlier flowering ~~time~~ times (Meng et al. 2011; Danilevskaya et al. 2008). Yet, some *PEBP* genes within the same subfamily may have differing roles. In soybean, *GmFT1a* ~~has been shown to be~~is a flowering inhibitor (Liu et al. 2018; Jiang et al. 2019). *GmFT*4, another homolog of *FT*, also acts as a flowering repressor (Zhai et al. 2014). Those two genes have ~~contrast~~ contrasting roles to the other flowering promoters *GmFT2a*/*5a* (Nan et al. 2014). In addition to flowering controlling, *FT*/*TFL1* is also involved in the development of plant organs. In transgenic onions (*Allium cepa* L.), *AcFT1* promotes bulb formation, whereas *AcFT4* prevents the up-regulation of *AcFT1* and inhibits bulb formation (Lee et al. 2013; Rashid et al. 2019; Manoharan et al. 2016). Overexpression of *StSP6A* induces rapid tuberization and increases tuber yield, while up-regulation of *StSP6A* could inhibit bud development (Park et al. 2022; Navarro et al. 2011).

These studies provide a deep understanding of the functions of plant *PEBP* members, but the function of the Tartary buckwheat *PEBP* gene family is still unknown. In this study, based on the published genome sequence of Tartary buckwheat, we identified fourteen *PEBP* family genes in the genome~~, and then~~. Then, we analyzed their phylogenetic relationships, gene structures, conserved motifs, chromosome location, and duplication events. We further analyzed the expression levels of *PEBP* genes in two flowering-type cultivars and identified the candidate *FT* genes for buckwheat flowering. This~~e results of this study will help understand the functions of *PEBP* members and provide~~study helps understand the functions of PEBP members and provides potential candidates for Tartary buckwheat breeding.

MATERIALS AND METHODS

Identification of PEBP family genes in Tartary buckwheat

The genome sequences of Tartary buckwheat (*Fagopyrum tataricum*) and common buckwheat (*Fagopyrum esculentum*) were obtained from the Tartary buckwheat

Genome Project (TBGP; https://www.mbkbase.org/Pinku1/) and the Chinese National Genomics Data Center database (https://bigd.big.ac.cn/) under the BioProject accession numbers PRJCA009237 (He et al. 2023), respectively. The protein sequences of Arabidopsis (*A. thaliana*) and rice (*Oryza sativa*) were downloaded from Phytozome V13 (https://phytozome-next.jgi.doe.gov). Two programs were used to identify *PEBP* family genes in the Tartary buckwheat genome. First, the sequences of six Arabidopsis PEBP proteins were used as queries to identify the candidate PEBP proteins by using the BLASTP program with E-value<1.0e-10. Second, the Hidden Markov Model (HMM) profiles of the PEBP consensus conserved seed file (PF01161) were downloaded from the Pfam database (Jaina et al. 2020) and used as a query to screen the candidate PEBP proteins by the Simple HMM search tool on TBtools (E-value<1.0e-10) (Wu et al. 2022; Chen et al., 2020). Then, all PEBP candidate proteins from the two parts were merged, ~~the NCBI-CDD (Bauer et al. 2014) and InterPro databases (Matthias et al. 2020) were used to verify the PEBP proteins that~~ and the NCBI-CDD (Bauer et al. 2014) and InterPro databases (Matthias et al. 2020) were used to verify the PEBP proteins obtained previously. All the PEBP protein sequences can be found in **Dataset 1**. The theoretical isoelectric point (pI) and molecular weight (Mw) of PEBP proteins were predicted by the ProtParam program (https://web.expasy.org/protparam/). ProtComp 9.0 in Softberry tool (http://linux1.softberry.com/berry.phtml) was used for PEBP subcellular location analysis.~~.~~

Phylogenetic analysis

Based on multiple sequence alignment results of Tartary buckwheat, common buckwheat, Arabidopsis~~ and rice PEBP amino acid sequences obtained by using CLUSTALW (Thompson et al. 2002)~~, and rice PEBP amino acid sequences obtained by using CLUSTALW [illegible], a~~,~~ phylogenetic tree was constructed using MEGA 11.0 (Tamura et al. 2021) based on the Neighbor-Joining method (Liu et al. 2019)with a bootstrap value of 1000. Evolview (http://evolgenius.info/) was used to add colorful visualization plots.

Gene structure and conserved motif, chromosomal locations analysis

~~On the basis of~~Based on the genome sequences and general feature format (GFF) files, intron and exon structures and the physical location of *PEBP* genes on chromosomes were determined and visualized ~~by~~ using the two programs ~~of~~ Gene Structure View, and Gene Location Visualize~~,~~ in TBtools (Chen et al. 2020). Multiple Em for Motif Elicitation (MEME) program (https://meme-suite.org/meme/tools/meme) was used to identify the conserved motifs in PEBP proteins by setting the maximum motif count at eight, the minimum and maximum motif lengths at four and fifty amino acids, respectively (Bailey et al. 2009). The motif analysis results were displayed using the Gene Structure View program in TBtools (Chen et al. 2020).

Duplication and synteny analysis of *PEBP*s between Tartary buckwheat and other species

Multiple Collinearity Scan toolkit (MCScanX) with the default parameters was used to analyze the gene duplication events (Wang et al. 2012). To investigate the homologous gene pairs of the *PEBP* gene family between Tartary buckwheat and the other species, we also used TBtools to analyze the inter-genomic collinearities (Chen et al. 2020).

***Cis*-acting element analysis**

The upstream 2,000 bp sequences of the transcription start site of *FtPEBP* genes were extracted from the Tartary buckwheat genome sequences by TBtools (Chen et al. 2020). The *cis*-acting elements were screened and predicted using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/), and TBtools was used to visualize these promoter elements (Chen et al. 2020).

Gene expression analysis of *FtPEBP* genes during floral transition

To investigate the relationships between the expression levels of *PEBP* genes and the flowering time, two cultivars (MQ-Miqiao 1# and KQ-KQ178) with different flowering ~~time~~ times were used. MQ, a rice Tartary buckwheat, has a long vegetative phase with low yield~~,~~ and is a late-flowering cultivar (Wang et al. 2022; Wang ~~and~~ & Campbell 2007). Compared with MQ, KQ is an earlier flowering buckwheat. The two Tartary buckwheat seedlings were grown under natural field conditions at the experimental field of Chengdu University in Jianyang, Chengdu. The seeds were sown ~~at March 17th, 2023, and samples were collected at~~on March 17th, 2023, and samples were collected

on May 13th. Although the flowering time of KQ is earlier than that of MQ, they were almost at the same growth stage when samples were collected, because both the true leaf numbers were about twelve. The young floral bud and the top two fully expanded leaves of 3~5 plants were harvested at 09:00 with three biological replicates, frozen in liquid nitrogen, and stored at -80℃ for RNA extraction. Total RNA was extracted from various tissues using a Takara kit (Takara Biomedical Technology, Beijing) according to the instructionsAccording to the instructions, total RNA was extracted from various tissues using a Takara kit (Takara Biomedical Technology, Beijing). The RNA quantity and quality were measured using Scandrop (Jena, Germany). Approximately 3 3 μg of RNA was used for synthesizing the cDNA by using Prime Script RT reagent kit with gDNA Reaser (Trans Gene Biotech, Beijing), and the products were diluted by 10 foldsten-fold diluted the products for quantitative real-time PCR (qRT-PCR) analysis. Primers used (**Table S1**) for qRT-PCR were designed using the online tool (https://www.genscript.com/tools/real-time-pcr-taqman-primer-design-tool). The *FtH3* gene was used as the reference gene (Liu et al. 2019). Three replications for each group were used for qRT-PCR analysis. qRT-PCR reactions were performed on the qTOWER^3 Real-Time PCR Thermal Cycler (Jena, Germany) using THUNDERBIRD® SYBR® qPCR Mix (TOYOBO BIOTECH, Shanghai). Every qRT-PCR reaction (20 μL) included 10 μL of qPCR Mix, 2 μL of 50mM primers, 2 μL of cDNA and 6 μL of ddH_2O. The qRT-PCR program consists of 95℃ for 30 s, followed by 40 cycles of 95℃ for 5 s and 60℃ for 20 s. The $2^{-\Delta\Delta CT}$ method was used to determine the expression level (Livak and Schmittgen 2001).

Subcellular localization analysis

Due to the lack of a stable genetic transformation system and effective transient expression system, Tartary buckwheat gene functions were usually studied through the heterologous expression systems in Arabidopsis thaliana (Sun et al. 2023b), and subcellular localization can be investigated via tobacco (Sun et al. 2019). We observed the subcellular locations of FtPEBP proteins transiently expressed in tobacco (*Nicotiana tabacum* L.) leaves. The CDS sequences of Tartary buckwheat *PEBP* genes were amplified by PCR, and CDS fragments were inserted into the *KpnI* and *HindIII*

sites of binary vector pEZR(K)-LN to create the *35S*::*FtPEBP-GFP* proteins. The primer sequences for CDS amplification were: FtFT1-CDS-1F: ATTCACTGAAATCCCACAAAACA, FtFT1-CDS-1R: TCCCTCTGGCAGTTGAAGTAG; FtFT3-CDS-1F: ATGGCAAGATCGAGAGATCC, FtFT3-CDS-1R: CACAGATGGATCTGGATAACG; FtTFL1-CDS-1F: ATGTCCAGACAGGTCATAGAGC, FtTFL1-CDS-1R: TCTTCTTCTAGCAGCAGTTTCC. The vectors were transformed into *Agrobacterium tumefaciens* strain GV3101 by thermal shock transformation. The transformed Agrobacterium was inoculated in a 50 mL YEB liquid medium containing 50 mg/L Kanamycin~~,~~ and cultured at 28℃ until OD600=0.6-0.8. Centrifuge the cultured products for 5 minutes at 5000 g to discard the supernatant~~ and Agrobacterium pellet was resuspend with the same volume of infiltration solution (containing 10 mM MES,~~ and the Agrobacterium pellet was resuspended with the same volume of infiltration solution (containing 10 mM MES and 100 μM acetosyringone). The infiltration solution was injected ~~in to~~into the back of tobacco leaves with a 1 mL syringe. After injection for three days, the GFP fluorescence signal was observed by confocal microscopy.

Results

Identification, phylogenetic relationship analysis of *PEBPs* in Tartary buckwheat

We used HMMER and BLASTP searches to identify the *PEBP* genes in Tartary buckwheat, and all the candidate *PEBP* members in the whole genome of Tartary buckwheat were detected. Based on NCBI-CDD, the fourteen candidate genes were further verified to harbor specific PBEP domain (**Table 1**). The PEBP ~~proteins lengths were ranged from 120 to 194 amino acid~~protein lengths ranged from 120 to 194 amino acids (aa), with an average length of 176 aa. *FtFT1* had the longest coding sequence (CDS) length (585 bp), and the molecular weight and theoretical pI were 22166.48 Da and 9.27, respectively. *FtTFL5* had the shortest CDS length (363 bp), and the molecular weight and theoretical pI values were 13302.07 Da and 6.5, respectively. *In silico* subcellular localization, analysis showed that all the PEBP proteins are ~~located~~ in the cytoplasm and nucleus. To investigate the subcellular localizations of Tartary buckwheat PEBP proteins in plant cells, we constructed three *35S::FtPEBP-GFP* vectors, *35S*::*FtFT1-GFP*, *35S::FtFT3-GFP*, and *35S::FtTFL1-GFP*, and transiently expressed them in tobacco leaf cells. The GFP fluorescence signals were observed by confocal microscopy. The results showed that all ~~these three PEBP-GFP fusion proteins were localized in both~~ three PEBP-GFP fusion proteins were localized in both the nucleus and cytoplasm (**Figure 1**), consistent with the *in silico* prediction results.

MEGA 11.0 was used to perform sequence alignment. A phylogenetic tree was constructed. The tree was composed of fifty-eight PEBP-like protein sequences from four species, in which six *PEBP*s from *A. thaliana*, nineteen *PEBP*s from *Oryza sativa*, fourteen *PEBP*s from *Fagopyrum tataricum* and nineteen *PEBP*s from *Fagopyrum esculentum* (**Figure 2**). According to the phylogenetic relationships, these genes were clustered into ~~three groups, namely *FT-like*~~FT-like, *TFL1-like*, and *MFT-like* subfamily (**Figure 2**)~~, and were~~. They were named as *FtFT1*~ *FtFT6*, *FtTFL1*~ *FtTFL6* and *FtMFT1*~ *FtMFT2* which belonged to *FT-like*, *MFT* and *TFL1-like* subfamily, respectively (**Figure 2**).

Gene structure, conserved motifs, and amino acid alignment analysis —of *FtPEBPs*

Gene structure analysis showed that of the fourteen genes, most *FtPEBP*s contained four exons and three introns, with the exception that *FtTFL1* contained two exons and one intron (**Figure 3**). The motifs prediction results showed that a total of eight motifs were identified in all FtPEBP proteins~~, motif~~; motifs 1 to motif 5 were the most conserved motifs in all FtPEBP proteins, meaning that the structures of the *FtPEBP* members were highly conserved (**Figure 3**). Motif 6 was only detected in *FtTFL4* and *FtTFL5*. The varied motif structures may indicate the diverse roles of *FtPEBP* members from different subgroups. According to ~~the results of multiple amino acid sequence alignment~~multiple amino acid sequence alignment results, we found that FtFT had the key amino acid residue tyrosine (Y) at the 106 site~~, while~~. At the same time, it was replaced by histidine (H) and tryptophan (W) in FtTFL and FtMFT, which is in consistent with other plants (Hu et al. 2023) (Figure S2). In addition, all FtFT proteins contained Arginine (R) at position 148, whereas FtTFL proteins contained Lysine (K) and FtMFT had ~~Glutamicaci~~ Glutamic acid (E). Thus, we speculated that the site (R/K/E) ~~may a novel key site to distinguish the conserve functions of FT, TFL~~might be a novel key site to distinguish the conserved functions of FT, TFL, and MFT (Figure S2).

Chromosomal location, duplication, and synteny analysis

We mapped the physical locations of *FtPEBP*s on chromosomes by using TBtools. As shown in **Figure 4**, fourteen *FtPEBP* genes were unevenly distributed on ~~6~~ six chromosomes (Ft1, Ft2, Ft3, Ft4, Ft5 and Ft7). Moreover, ~~chromosomes Ft4 contain the most *PEBP* genes (4 *PEBP* genes), while chromosomes Ft2 had~~ chromosome Ft4 contains the most PEBP genes (4 PEBP genes), while chromosome Ft2 has the least *PEBP* genes (1 *PEBP* gene). Genome replication events have long been considered as the main driver for evolution (Ge et al. 2022). Gene duplication, tandem duplication, and ~~large~~ significant fragment duplication tend to trigger the creation of gene families (Ge et al. 2022; Xu et al. 2012). The chromosomal region within 200 kb containing more than two homologs is defined as a tandem duplication event (Holub 2001). Analysis of the gene duplication events of Tartary buckwheat showed that no segmental duplication occurred (**Figure S1**), but there were three gene pairs (*FtMFT1/2*, *FtFT5/6*,

FtTFL4/5) located in tandem repeats (**Table 1**, **Figure 4**). These results mean most of the *FtPEBP* genes might evolve independently, and tandem repeat plays a ~~major~~ significant role ~~for~~ in *FtPEBP* gene family expansion.

To further know the evolutionary history of *PEBP* genes between Tartary buckwheat and other species, collinearity analysis was performed between the genomes of Tartary buckwheat and three other plants including two model plants (Arabidopsis and rice), and a close relative of Tartary buckwheat (common buckwheat) (**Figure 5**). It was found that there was only one *PEBP* homologous gene pair between Tartary buckwheat *FtPEBP* genes and Arabidopsis *AtPEBP* genes, three *PEBP* homologous gene pairs with rice *OsPEBP* genes and five *PEBP* homologous gene pairs with common buckwheat (**Figure 5**). ~~Phylogenetic tree showed that *FtTFL4/5* was in the same clade with~~The phylogenetic tree showed that FtTFL4/5 was in the same clade as *FeTFL6* of common buckwheat (**Figure 2**). *FtTFL4/5* has a collinear relationship with *FeTFL6* (Figure 5), but we did not detect any tandem repeat around *FeTFL6* (Figure 5). Thus, we speculated that the tandem repeat *FtTFL4/5* may occur after Tartary buckwheat diverged from common buckwheat.

The *cis*-acting element of *FtPEBPs*

Cis-acting elements in gene ~~promoter have important roles on mediating transcriptional activation and repression, and numerous *cis*-acting elements controlling specific progresses~~promoters have important roles in mediating transcriptional activation and repression, and numerous cis-acting elements controlling specific progress have been reported (Hernandez-Garcia and Finer 2014). In order to explore and understand the potential molecular function of ~~*FtPEBP* family, the 2000 bp promoter sequences upstream of *FtPEBP* genes were analyzed to detect the various *cis*-acting elements on~~ *the FtPEBP family, the 2000 bp promoter sequences upstream of FtPEBP genes were analyzed to detect the various cis-acting elements on the* Plant CARE website. The results suggested that many *cis*-acting elements were involved in the processes of light, phytohormone (auxin, abscisic acid, gibberellin, Methyl jasmonate, and salicylic acid), stress (anaerobic induction, drought-inducibility, defense and stress and low-temperature responsiveness) (**Figure 6**), ~~this~~ these findings are similar with that in

several other plants (Zhong et al. 2022; Zhang et al., 2023). Of these *cis*-acting elements, G-box, ABRE ~~and ARE take the most proportions among light, phytohormone and stress responsive~~, and ARE take the most proportions among light, phytohormone, and stress-responsive elements. ABRE was the most abundant element distributed in all *PEBP* promoters, except for the promoter of *FtTFL1* (**Figure 6C**). Some *cis*-acting elements showed gene-specific distribution patterns. More Abscisic and responsive elements (ABREs) were presented in the promoters of *FtFT3, FtFT6, FtTFL2* ~~and *TFL6* (**Figure 6A, B**), indicating these four genes might related to in~~ *, and TFL6 (Figure 6A, B), indicating these four genes might be related to* ABA signaling. Low-temperature responsive elements (LTRs) were mainly distributed in the *FT-like* subfamily~~, while~~. In contrast, the elements of the MYB binding site involved in drought-inducibility (MBS) were mainly detected in the *MFT-like* subfamily (**Figure 6**). In addition, we noticed that the *cis*-acting elements composition of *FtTFL4* are similar to *FtTFL5* for their similar location in the genome, which may result from the tandem repeat. These findings revealed that the *FtPEBP*s could respond to light, hormones, and stress to affect the development of Tartary buckwheat.

Expression analysis of *FtPEBPs* during the floral transition of Tartary buckwheat

To investigate the relationship between *PEBP* genes with the flowering time of Tartary buckwheat, we tested the expression levels of *FtPEBP*s in two cultivars ~~that have varied flowering time~~with varied flowering times. Compared with the cultivar KQ, MQ-a rice Tartary buckwheat ~~which~~ had a later flowering time (Figure 7). ~~Yet~~However, they are nearly at the same growth stage because ~~both of~~ the true leaf numbers were about twelve (Figure 7). As the flowering genes are usually expressed in leaf and floral organs to activate downstream signal cascade, we detect the expression of *FtPEBP* genes in leaf and inflorescence at a floral transition time in those cultivars. Among the fourteen genes, three were detected in either leaf or inflorescence tissues (Figure 8). As ~~showed~~ shown in Figure 8, *FtFT1* had the most abundant expression level in the leaf and inflorescence of KQ, whereas it was almost not detected in late-flowering MQ. The expression level of *FtFT3* was higher in the leaf and inflorescence of KQ than in MQ. The expressions of *FtTFL1* were similar in both samples of all cultivars. *FtFT1*/*FtFT3* were ~~higher~~

~~expressed~~ expressed more strongly in KQ (the early-flowering type cultivar) than in late-flowering MQ. Therefore, we speculated that *FtFT1*/*FtFT3* ~~may be the florigen-encoding genes positively controlling floral transition in Tartary buckwheat..~~might be the florigen-encoding genes positively controlling floral transition in Tartary buckwheat.

Discussion

PEBP genes play ~~important~~ essential roles in regulating flowering time, inflorescence morphology, and the formation of tubers (Karlgren et al. 2011; Susila ~~and~~ & Purwestri 2023; Putterill and Varkonyi-Gasic 2016; eGuo et al. 2014). The *PEBP* gene family has been isolated and identified from many plants, such as *A. thaliana* (six members) (Hedman et al. 2009; Carmona et al. 2007), *O. sativa* (nineteen members) (Chardon and Damerval 2005), and *Solanum lycopersicum* (twelve members) (Sun et al. 2023c). Gene family is a group of genes originating from the same ancestor, produced two or more copies of one gene through gene duplication, and they are similar in gene structure and function (Xu et al. 2012). In this study, a total of fourteen *FtPEBP* genes were identified from the Tartary buckwheat genome by bioinformatics methods. We found that the exon-intron and motif structure were comparable among those *PEBP* genes. Collinearity analysis between *FtPEBP*s in the Tartary buckwheat genome showed ~~that there were~~ no segmental repeated events in *FtPEBP* genes, indicating that the *FtPEBP*s might evolve independently. Phylogenetic analysis of fourteen *FtPEBP* genes was performed with model plants (~~Arabidopsisand~~ Arabidopsis and rice) and common buckwheat, a related species of Tartary buckwheat. In the evolutionary relationship, one pair of homologous ~~gene~~ genes was found between Tartary buckwheat and Arabidopsis, and three pairs of homologous genes were found between Tartary buckwheat and rice~~, while~~. In contrast, the most homologous gene pairs (five) were found between Tartary buckwheat and common buckwheat. We speculated that this may be due to the ~~closet~~ closest relationship between Tartary buckwheat and common buckwheat.

~~Gene expression is often regulated by *cis*-acting elements in the promoter region~~Cis-acting elements in the promoter region often regulate gene expression. By analyzing the *cis*-acting elements in the promoter region of the *FtPEBP* genes of Tartary buckwheat, it was found that all fourteen *FtPEBP* promoters contained ~~light~~

~~responsive~~light-responsive elements, which was consistent with the previous research conclusion that photoperiod is involved in the regulation of *FT* and *TFL1* (Wanhui et al. 2013; Pearce et al. 2017). ABRE elements are widely found in each *FtPEBP*, and some gene promoter regions also contain other hormone elements, such as auxin, methyl- jasmonate, salicylic acid, and gibberellin. These results indicated that the *FtPEBP* genes may be involved in the growth and development of Tartary buckwheat. ~~LTRs elements mainly exist in *FT-like* subfamily, while MBS elements mainly exist in~~ LTR elements mainly exist in the FT-like subfamily, while MBS elements mainly exist in the *TFL1-like* subfamily, indicating the diverse functions between *FT*- and *TFL*-like subfamilies. The spatiotemporal-specific expression of genes may suggest the specific regulatory roles in the development of plants (Sonawane et al. 2017). In the present study, only three *FtPEBPs* out of fourteen genes were ~~found to be~~ expressed in leaf and inflorescence. *FtPEBP* genes were differentially expressed in different flowering types of Tartary buckwheat. *FtFT1* was only expressed in the inflorescence and leaf of ~~early-flowering~~early-flowering KQ. *FtFT3* was more enriched in the leaf and inflorescence of early-flowering type KQ ~~while~~, *while it* was expressed at very low levels in late-flowering type MQ. The correlation between the expression levels of *FtFT1*/*FtFT3* and the flowering time of buckwheat ~~suggest~~ suggests they may be the candidate florigen-encoding genes in Tartary buckwheat~~, and so~~. So, we think *FtFT1*/*FtFT3* could be used for yield improvement, especially for rice Tartary buckwheat, by molecular breeding approaches in the future.

Conclusions

~~To sum up, in this study~~In this study, we identified and comprehensively analyzed fourteen putative *FtPEBP* genes. The evolutionary relationships, gene structure, and gene duplication among *FtPEBP*s were performed. The correlations between *FtPEBP* gene expression levels and the flowering time of early- and late-flowering cultivars ~~indicates that, *FtFT1*/*FtFT3* may be involved in~~ indicate that FtFT1/FtFT3 may be involved in Tartary buckwheat's flowering time and yield regulation~~flowering time and yield regulation of Tartary buckwheat~~. ~~In all, this~~This study lays a foundation for further elucidating the potential roles of *FtPEBP* genes in Tartary buckwheat.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This work was supported by Natural Science Foundation of Sichuan Province (Grant 2022NSFSC1773, 2022NSFSC1725, 2023ZHCG0091, 2023NSFSC1177), National Natural Sciences Foundation of China (No. 32301850), the Open Project Program of State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China (Grant No. SKL-KF202302), China Agriculture Research System (Grant CARS07-B-1) and Ganzi Science and Technology Program (Grant 220015).

Grant Disclosures

The following grant information was disclosed by the authors:

Natural Science Foundation of Sichuan Province: 2022NSFSC1773, 2022NSFSC1725, 2023ZHCG0091, 2023NSFSC1177.

National Natural Sciences Foundation of China: 32301850.

The Open Project Program of State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China: SKL-KF202302.

China Agriculture Research System: CARS07-B-1.

Ganzi Science and Technology Program: 220015.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

Qi Wu conceived, designed the project and revised the manuscript.

Mengping Nie performed most of the bioinformatics analysis, experiments and wrote the manuscript.

Li Li participated in gene expression and evolutionary analysis.

Jing Lu participated in gene expression and evolutionary analysis.

Cailin He participated in gene expression and evolutionary analysis.

Wenjun Sun participated in gene expression and evolutionary analysis.

Junjie Yin participated in subcellular localization analysis and gene expression analysis.

Huihui Guo participated in cultivating, observing and screening the different flowering-type materials for gene expression analysis.

Xiao'an Li participated in cultivating, observing and screening the different flowering-type materials for gene expression analysis.

Mi Jiang participated in cultivating, observing and screening the different flowering-type materials for gene expression analysis.

Ruiling Zhan participated in cultivating, observing and screening the different flowering-type materials for gene expression analysis.

Supplemental Information

Supplemental information for this article can be found online at.

Figure legends

Figure 1 Subcellular localization of empty vector and three PEBP-GFP proteins.

Figure 2 Phylogenetic tree of PEBPs from *Fagopyrum tataricum* (fourteen genes), *Fagopyrum esculentum* (nineteen genes), *Oryza sativa* (nineteen genes) and *A. thaliana* (six genes). The proteins from each species are labeled with different graphics and colors (blue triangle: *A. thaliana*, yellow star: *Oryza sativa*, green circle: *Fagopyrum tataricum*, red check: *Fagopyrum esculentum*). A total of fifty-eight protein sequences were aligned using CLUSTALW in MEGA 11.0. The tree was constructed by MEGA 11.0 using the Neighbor-Joining method with a bootstrap of 1000. Bootstrap values are shown on branches. Three subgroups were colored with different colors (MFT-like is colored in sky blue, TFL1-like is colored in purple and FT-like is colored in orange).

Figure 3 The motifs and exon-intron structures of *PEBP* genes in Tartary buckwheat. A total of eight conserved motifs were discovered among all *PEBP* genes identified by using MEME, and different motifs are showed in different colored boxes. *FT-like*, *TFL1-like* and *MFT-like* sub-clade genes are colored in orange, sky blue and purple. Exons, introns and UTRs of *PEBP* genes are represented by yellow boxes, dark lines and green boxes, respectively.

Figure 4 Distribution of *PEBP* genes on Tartary buckwheat chromosomes. The names of fourteen Tartary buckwheat *PEBP* genes are shown at the right side of each chromosome. Gene positions and chromosome size can be measured using the scale on the left side in mega bases (Mb). Black characters represent chromosome names and

red characters represent gene names. Chromosome segments were colored in red and blue indicating high and low gene densities.

Figure 5 Collinearity analysis of *PEBP* genes between Tartary buckwheat and three other plant species. Red lines indicate the intergenomic collinearity and red characters represent homologous genes. (A) Syntenic relationships between the homologous *PEBP*s of Tartary buckwheat and Arabidopsis. (B) Syntenic relationships between the homologous *PEBP*s of Tartary buckwheat and rice. (C) Syntenic relationships between the homologous *PEBP*s of Tartary buckwheat and common buckwheat.

Figure 6 Regulatory elements in the promoter regions of *FtPEBP* genes. (A) The number of *cis*-acting elements in *FtPEBP* promoter region. (B) The *cis*-acting elements distributions in *FtPEBP* promoters. (C) The pie charts showed the proportion of each *cis*-acting elements of light, phytohormone and stress response elements.

Figure 7 Different flowering time type Tartary buckwheat cultivars at 55 days after sowing and statistics of true leaf numbers at sample-harvesting time.

Figure 8 Real-time PCR analysis of *FtPEBP*s in the inflorescence and leaf of two Tartary buckwheat cultivars with different flowering time.

Figure S1 Synteny analysis of *FtPEBP*s in Tartary buckwheat genome.

Figure S2 Multiple sequence alignment of PEBP protiens. The red arrow indicated the key amino acids distinguishing FT-like (Y), TFL1-like (H), and MFT-like (W) functions. The blue arrow indicated the other key amino acids distinguishing FT-like (R), TFL1-like (K), and MFT-like (E) functions.

Table S1 The qRT-PCR primers used in this study.

Dataset 1 The raw data sequences used for phylogenetic tree construct.

Reference

Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS (2009) MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res 37 (Web Server issue):W202-208. doi:10.1093/nar/gkp335

Bauer AM, Derbyshire M, Gonzales N, Bryant SH (2014) Cdd: NCBI's conserved domain database.

Bi Z, Li X, Huang H, Hua Y (2016) Identification, Functional Study, and Promoter Analysis of HbMFT1, a Homolog of MFT from Rubber Tree (Hevea brasiliensis). Int J Mol Sci 17 (3):247. doi:10.3390/ijms17030247

Carmona MJ, Calonje M, Martinez-Zapater JM (2007) The FT/TFL1 gene family in grapevine. Plant Mol Biol 63 (5):637-650. doi:10.1007/s11103-006-9113-z

Chardon F, Damerval C (2005) Phylogenomic Analysis of the PEBP Gene Family in Cereals. Journal of Molecular Evolution 61 (5):579-590. doi:10.1007/s00239-004-0179-4

Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R (2020) TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. Mol Plant 13 (8):1194-1202. doi:10.1016/j.molp.2020.06.009

Chen Y, Xu X, Chen X, Chen Y, Zhang Z, Xuhan X, Lin Y, Lai Z (2018) Seed-Specific Gene MOTHER of FT and TFL1（MFT） Involved in Embryogenesis, Hormones and Stress Responses in Dimocarpus longan Lour. Int J Mol Sci 19 (8). doi:10.3390/ijms19082403

Chengming Fan, Ruibo Hu, Xiaomei Zhang, Xu Wang, Wenjing Zhang, Qingzhe Zhang, Jinhua Ma, Fu Y-F (2014) Conserved CO-FT regulons contribute to the photoperiod flowering control in soybean. BMC Plant Biology 14:1-14. doi:10.1186/1471-2229-14-9.Danilevskaya ON, Meng X, Hou Z, Ananiev EV, Simmons CR (2008) A genomic and expression compendium of the expanded PEBP gene family from maize. Plant Physiol 146 (1):250-264. doi:10.1104/pp.107.109538

eGuo Y, eHarloff H-J, eJung C, eMolina C (2014) Mutations in single FT- and TFL1-paralogs of rapeseed (Brassica napus L.) and their impact on flowering time and yield components Frontiers in Plant Science 5

Fryxell KJ (1996) The coevolution of gene family trees. Trends in Genetics 12 (9):364-369. doi:10.1016/s0168-9525(96)80020-5

Ge H, Xu J, Hua M, An W, Wu J, Wang B, Li P, Fang H (2022) Genome-wide identification and analysis of ACP gene family in Sorghum bicolor (L.) Moench. BMC Genomics 23 (1). doi:10.1186/s12864-022-08776-2

He Q, Ma D, Li W, Xing L, Zhang H, Wang Y, Du C, Li X, Jia Z, Li X, Liu J, Liu Z, Miao Y, Feng R, Lv Y, Wang M, Lu H, Li X, Xiao Y, Wang R, Liang H, Zhou Q, Zhang L, Liang C, Du H (2023) High-quality Fagopyrum esculentum genome provides insights into the flavonoid accumulation among different tissues and self-incompatibility. J Integr Plant Biol. 00 (00):1-19. doi:10.1111/jipb.13459

Hedman H, Kallman T, Lagercrantz U (2009) Early evolution of the MFT-like gene family in plants. Plant Mol Biol 70 (4):359-369. doi:10.1007/s11103-009-9478-x

Hemming MN, Peacock WJ, Trevaskis DB (2008) Low-temperature and daylength cues are integrated to regulate FLOWERING LOCUS T in barley. Plant Physiology 147 (1):355-366

Hernandez-Garcia CM, Finer JJ (2014) Identification and validation of promoters and cis-acting regulatory elements. Plant Sci 217-218:109-119. doi:10.1016/j.plantsci.2013.12.007

Holub EB (2001) The arms race is ancient history in Arabidopsis, the wildflower. Nature Reviews Genetics volume 2:516–527. doi:10.1038/35080508

Huang X, Yao J, Zhao Y, Xie D, Jiang X, Xu Z (2016) Efficient Rutin and Quercetin Biosynthesis through Flavonoids-Related Gene Expression in Fagopyrum tataricum Gaertn. Hairy Root Cultures with UV-B Irradiation. Front Plant Sci 7:63. doi:10.3389/fpls.2016.00063

Jaina M, Sara C, Lowri W, Matloob Q, Gustavoa S, Sonnhammer ELL, Tosatto SCE, Lisanna P, Shriya R, Richardson LJJNAR (2020) Pfam: The protein families database in 2021.

Jiang B, Zhang S, Song W, Khan MAA, Sun S, Zhang C, Wu T, Wu C, Han T (2019) Natural variations of FT family genes in soybean varieties covering a wide range of maturity groups. BMC Genomics 20 (1):230. doi:10.1186/s12864-019-5577-5

Karlgren A, Gyllenstrand N, Kallman T, Sundstrom JF, Moore D, Lascoux M, Lagercrantz U (2011) Evolution of the PEBP gene family in plants: functional diversification in seed plant evolution. Plant Physiol 156 (4):1967-1977. doi:10.1104/pp.111.176206

Laurent Corbesier CV, Seonghoe Jang,Fabio Fornara, Qingzhi Fan,Iain Searle,, Antonis Giakountis SF, Lionel Gissot,Colin Turnbull,George Coupland (2007) FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science 316(5827):1030-3

Lee R, Baldwin S, Kenel F, McCallum J, Macknight R (2013) FLOWERING LOCUS T genes control onion bulb formation and flowering. Nat Commun 4:2884. doi:10.1038/ncomms3884

Li H-Y, Wu C-X, Lv Q-Y, Shi T-X, Chen Q-J, Chen Q-F (2020a) Comparative cellular, physiological and transcriptome analyses reveal the potential easy dehulling mechanism of rice-tartary buckwheat (Fagopyrum Tararicum). BMC Plant Biology 20 (1). doi:10.1186/s12870-020-02715-7

Li X, Sathasivam R, Park NI, Wu Q, Park SU (2020b) Enhancement of phenylpropanoid accumulation in tartary buckwheat hairy roots by overexpression of MYB transcription factors. Industrial Crops and Products 156. doi:10.1016/j.indcrop.2020.112887

Liu C, Xiang D, Wu Q, Ye X, Yan H, Zhao G, Zou L (2021) Dynamic transcriptome and co-expression analysis suggest the potential roles of small secreted peptides from Tartary buckwheat (Fagopyrum tataricum) in low nitrogen stress response. Plant Science 313. doi:10.1016/j.plantsci.2021.111091

Liu M, Wang X, Sun W, Ma Z, Zheng T, Huang L, Wu Q, Tang Z, Bu T, Li C, Chen H (2019) Genome-wide investigation of the ZF-HD gene family in Tartary buckwheat (Fagopyrum tataricum). BMC Plant Biol 19 (1):248. doi:10.1186/s12870-019-1834-7

Liu W, Jiang B, Ma L, Zhang S, Zhai H, Xu X, Hou W, Xia Z, Wu C, Sun S, Wu T, Chen L, Han T (2018) Functional diversification of Flowering Locus T homologs in soybean: GmFT1a and GmFT2a/5a have opposite roles in controlling flowering and maturation. The New phytologist 217 (3):1335-1345. doi:10.1111/nph.14884

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. METHODS 25:402–408. doi:10.1006/meth.2001.1262

Ma Z, Liu M, Sun W, Huang L, Wu Q, Bu T, Li C, Chen H (2019) Genome-wide identification and expression analysis of the trihelix transcription factor family in tartary buckwheat (Fagopyrum tataricum). BMC Plant Biol 19 (1):344. doi:10.1186/s12870-019-1957-x

Magali Lescot PD, Gert Thijs, Kathleen Marchal, Yves Moreau, Yves Van de Peer, Pierre Rouzé, Stephane Rombauts (2002) a database of plant cis-acting regulatory elements and a portal.

Nucleic Acids Res 30(1):325-7
Manoharan RK, Han JS, Vijayakumar H, Subramani B, Thamilarasan SK, Park JI, Nou IS (2016) Molecular and Functional Characterization of FLOWERING LOCUS T Homologs in Allium cepa. Molecules 21 (2):1-14. doi:10.3390/molecules21020217
Matthias B, Hsin-Yu C, Sara C, Tiago G, Swaathi K, Alex M, Gift N, Typhaine PL, Matloob Q, Shriya RJNAR (2020) The InterPro protein families and domains database: 20 years on. (D1):D1
Meng X, Muszynski MG, Danilevskaya ON (2011) The FT-like ZCN8 Gene Functions as a Floral Activator and Is Involved in Photoperiod Sensitivity in Maize. Plant Cell 23 (3):942-960. doi:10.1105/tpc.110.081406
Nakagawa M, Shimamoto K, Kyozuka J (2002) Overexpression of RCN1 and RCN2, rice TERMINAL FLOWER 1/CENTRORADIALIS homologs, confers delay of phase transition and altered panicle morphology in rice. Plant J 29 (6):743-750. doi:10.1046/j.1365-313x.2002.01255.x
Nan H, Cao D, Zhang D, Li Y, Lu S, Tang L, Yuan X, Liu B, Kong F (2014) GmFT2a and GmFT5a redundantly and differentially regulate flowering through interaction with and upregulation of the bZIP transcription factor GmFDL19 in soybean. PLoS One 9 (5):e97669. doi:10.1371/journal.pone.0097669
Navarro C, Abelenda JA, Cruz-Oro E, Cuellar CA, Tamaki S, Silva J, Shimamoto K, Prat S (2011) Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature 478 (7367):119-122. doi:10.1038/nature10431
Park JS, Park SJ, Kwon SY, Shin AY, Moon KB, Park JM, Cho HS, Park SU, Jeon JH, Kim HS, Lee HJ (2022) Temporally distinct regulatory pathways coordinate thermo-responsive storage organ formation in potato. Cell Rep 38 (13):110579. doi:10.1016/j.celrep.2022.110579
Pearce S, Shaw LM, Lin H, Cotter JD, Li C, Dubcovsky J (2017) Night-Break Experiments Shed Light on the Photoperiod1-Mediated Flowering. Plant Physiol 174 (2):1139-1150. doi:10.1104/pp.17.00361
Putterill J, Varkonyi-Gasic E (2016) FT and florigen long-distance flowering control in plants. Curr Opin Plant Biol 33:77-82. doi:10.1016/j.pbi.2016.06.008
Rashid MHA, Cheng W, Thomas B (2019) Temporal and Spatial Expression of Arabidopsis Gene Homologs Control Daylength Adaptation and Bulb Formation in Onion (Allium cepa L.). Sci Rep 9 (1):14629. doi:10.1038/s41598-019-51262-1
So Yeon Yoo IK, Igor Kardailsky, Jong Seob Lee, Detlef Weigel, Ji Hoon Ahn (2004) Acceleration of flowering by overexpression of MFT (MOTHER OF FT AND TFL1). Mol Cells 29;17(1):95-101
Sonawane AR, Platig J, Fagny M, Chen CY, Paulson JN, Lopes-Ramos CM, DeMeo DL, Quackenbush J, Glass K, Kuijjer ML (2017) Understanding Tissue-Specific Gene Regulation. Cell Rep 21 (4):1077-1088. doi:10.1016/j.celrep.2017.10.001
Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T (2015) Photoperiodic flowering: time measurement mechanisms in leaves. Annu Rev Plant Biol 66:441-464. doi:10.1146/annurev-arplant-043014-115555
Sun J, Bie XM, Chu XL, Wang N, Zhang XS, Gao XQ (2023a) Genome-edited TaTFL1-5 mutation decreases tiller and spikelet numbers in common wheat. Front Plant Sci 14:1142779. doi:10.3389/fpls.2023.1142779
Sun W, Chen Y, Zeng J, Li C, Yao M, Liu M, Ma Z, Huang L, Yan J, Zhan J, Chen H, Bu T, Tang Z, Li Q, Wu Q, Hou J, Huang Y (2023b) The Tartary buckwheat bHLH gene ALCATRAZ contributes

to silique dehiscence in Arabidopsis thaliana. Plant Science 333. doi:10.1016/j.plantsci.2023.111733
Sun Y, Jia X, Yang Z, Fu Q, Yang H, Xu X (2023c) Genome-Wide Identification of PEBP Gene Family in Solanum lycopersicum. Int J Mol Sci 24 (11). doi:10.3390/ijms24119185
Sun Z, Linghu B, Hou S, Liu R, Wang L, Hao Y, Han Y, Zhou M, Liu L, Li H (2019) Tartary Buckwheat FtMYB31 Gene Encoding an R2R3-MYB Transcription Factor Enhances Flavonoid Accumulation in Tobacco. Journal of Plant Growth Regulation 39 (2):564-574. doi:10.1007/s00344-019-10000-7
Susan Shannon, Meeks-Wagner OR (1991) A Mutation in the Arabidopsis TFL1 Gene Affects Inflorescence Meristem Development. The Plant Cell 3:877-892. doi:10.1105/tpc.3.9.877
Susila H, Purwestri YA (2023) PEBP Signaling Network in Tubers and Tuberous Root Crops. Plants (Basel) 12 (2). doi:10.3390/plants12020264
Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K (2007) Hd3a protein is a mobile flowering signal in rice. Science 316 (5827):1033-1036. doi:10.1126/science.1141753
Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38 (7):3022-3027. doi:10.1093/molbev/msab120
Tao Huang HBh, Sven Eriksson,Franc¸ois Parcy, Ove Nilsson (2005) The mRNA of the Arabidopsis gene FT moves from leaf to shoot apex and induces flowering. Science 309(5741):1694-6
Thompson JD, Gibson TJ, Higgins DG (2002) Multiple sequence alignment using ClustalW and ClustalX. Curr Protoc Bioinformatics:1-22. doi:10.1002/0471250953.bi0203s00
Tsuji H, Tachibana C, Tamaki S, Taoka K, Kyozuka J, Shimamoto K (2015) Hd3a promotes lateral branching in rice. Plant J 82 (2):256-266. doi:10.1111/tpj.12811
Wang Y, Campbell CG (2007) Tartary buckwheat breeding (Fagopyrum tataricum L. Gaertn.) through hybridization with its Rice-Tartary type. Euphytica 156 (3):399-405. doi:10.1007/s10681-007-9389-3
Wang Y, Guan Z, Liang C, Liao K, Xiang D, Huang J, Wei C, Shi T, Chen QJSr (2022) Agronomic and metabolomics analysis of rice-Tartary buckwheat (Fagopyrum tataricum Gaertn) bred by hybridization. 12 (1):11986
Wang Y, Tang H, Debarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H, Kissinger JC, Paterson AH (2012) MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res 40 (7):e49. doi:10.1093/nar/gkr1293
Wang Z, Zhou Z, Liu Y, Liu T, Li Q, Ji Y, Li C, Fang C, Wang M, Wu M, Shen Y, Tang T, Ma J, Tian Z (2015) Functional evolution of phosphatidylethanolamine binding proteins in soybean and Arabidopsis. Plant Cell 27 (2):323-336. doi:10.1105/tpc.114.135103
Wanhui K, Im PT, Jeon YS, Rim JA, Hoon AJ (2013) Generation and analysis of a complete mutant set for the Arabidopsis FT/TFL1 family shows specific effects on thermo-sensitive flowering regulation. Journal of experimental botany 64 (6)
Wen W, Li Z, Shao J, Tang Y, Zhao Z, Yang J, Ding M, Zhu X, Zhou M (2021) The Distribution and Sustainable Utilization of Buckwheat Resources under Climate Change in China. Plants (Basel) 10 (10). doi:10.3390/plants10102081
Wickland DP, Hanzawa Y (2015) The FLOWERING LOCUS T/TERMINAL FLOWER 1 Gene Family: Functional Evolution and Molecular Mechanisms. Mol Plant 8 (7):983-997. doi:10.1016/j.molp.2015.01.007
Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of

689 spatial and temporal information during floral induction in Arabidopsis. Science 312
690 ((5780):1600):1056-1059. doi:10.1126/science.1114358
691 Wu Q, Bai X, Nie M, Li L, Luo Y, Fan Y, Liu C, Ye X, Zou L (2022) Genome-wide identification and
692 expression analysis disclose the pivotal PHOSPHATIDYLETHANOLAMINE BINDING
693 PROTEIN members that may be utilized for yield improvement of Chenopodium quinoa. Front
694 Plant Sci 13:1119049. doi:10.3389/fpls.2022.1119049
695 Wu Q, Bai X, Zhao W, Xiang D, Wan Y, Yan J, Zou L, Zhao G (2017) De Novo Assembly and Analysis
696 of Tartary Buckwheat (Fagopyrum tataricum Garetn.) Transcriptome Discloses Key Regulators
697 Involved in Salt-Stress Response. Genes 8 (10). doi:10.3390/genes8100255
698 Xi W, Liu C, Hou X, Yu H (2010) MOTHER OF FT AND TFL1 regulates seed germination through a
699 negative feedback loop modulating ABA signaling in Arabidopsis. Plant Cell 22 (6):1733-1748.
700 doi:10.1105/tpc.109.073072
701 Xu G, Guo C, Shan H, Kong H (2012) Divergence of duplicate genes in exon-intron structure. Proc Natl
702 Acad Sci U S A 109 (4):1187-1192. doi:10.1073/pnas.1109047109
703 Zhai H, Lu S, Liang S, Wu H, Zhang X, Liu B, Kong F, Yuan X, Li J, Xia Z (2014) GmFT4, a homolog
704 of FLOWERING LOCUS T, is positively regulated by E1 and functions as a flowering repressor
705 in soybean. PLoS One 9 (2):e89030. doi:10.1371/journal.pone.0089030
706 Zhang G, Jin X, Li X, Zhang N, Li S, Si H, Rajora OP, Li XQ (2022) Genome-wide identification of
707 PEBP gene family members in potato, their phylogenetic relationships, and expression patterns
708 under heat stress. Mol Biol Rep 49 (6):4683-4697. doi:10.1007/s11033-022-07318-z
709 Zhang M-M, Zhao X, He X, Zheng Q, Huang Y, Li Y, Ke S, Liu Z-J, Lan S (2023) Genome-Wide
710 Identification of PEBP Gene Family in Two Dendrobium Species and Expression Patterns in
711 Dendrobium chrysotoxum. International Journal of Molecular Sciences 24 (24).
712 doi:10.3390/ijms242417463
713 Zhao C, Zhu M, Guo Y, Sun J, Ma W, Wang X (2022) Genomic Survey of PEBP Gene Family in Rice:
714 Identification, Phylogenetic Analysis, and Expression Profiles in Organs and under Abiotic
715 Stresses. Plants (Basel) 11 (12). doi:10.3390/plants11121576
716 Zhong C, Li Z, Cheng Y, Zhang H, Liu Y, Wang X, Jiang C, Zhao X, Zhao S, Wang J, Zhang H, Liu X,
717 Yu H (2022) Comparative Genomic and Expression Analysis Insight into Evolutionary
718 Characteristics of PEBP Genes in Cultivated Peanuts and Their Roles in Floral Induction.
719 International Journal of Molecular Sciences 23 (20). doi:10.3390/ijms232012429

720