Received: 2 December 2021 | Revised: 22 February 2022 | Accepted: 8 March 2022

DOI: 10.1111/ppl.13668

Physiologia Plantarum

Genome-wide characterization of nuclear factor Y transcription factors in *Fagopyrum tataricum*

Ling Zhang[1] | **Wai-Shing Yung**[2] | **Wei Sun**[3] | **Man-Wah Li**[2] | **Mingkun Huang**[1,2]

[1]Lushan Botanical Garden Jiangxi Province and Chinese Academy of Sciences, Jiujiang, China

[2]Center for Soybean Research of the State Key Laboratory of Agrobiotechnology, School of Life Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

[3]Key Laboratory of Beijing for Identification and Safety Evaluation of Chinese Medicine, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

Correspondence
Mingkun Huang, Lushan Botanical Garden Jiangxi Province and Chinese Academy of Sciences, 9 Zhiqing Rd, Jiujiang, 332005 Jiangxi, China.
Email: huangmk@lsbg.cn

Funding information
Hong Kong Research Grants Council Area of Excellence Scheme, Grant/Award Number: AoE/M-403/16; Lushan Botanical Garden Research Funds, Grant/Award Number: 2021ZWZX31; 2022ZWZX01

Edited by S. Pelaz

Abstract

The nuclear factor Y (NF-Y) is an important transcription factor family that regulates plant developmental processes and abiotic stress responses. Currently, genome-wide studies of the NF-Y family are limited in *Fagopyrum tataricum*, an important economic crop. Based on the released genome assembly, we predicted a total of 38 NF-Y encoding genes (*FtNF-Ys*), including 12 *FtNF-YAs*, 18 *FtNF-YBs*, and eight *FtNF-YCs* subunits, in *F. tataricum*. Phylogenetic tree and sequence alignments showed that *FtNF-Ys* were conserved between *F. tataricum* and other species. Tissue expressions and network analyses suggested that *FtNF-Ys* might be involved in regulating developmental processes in different tissues. Several *FtNF-YAs* and *FtNF-Ybs* were also potentially involved in light response. In addition, *FtNF-YC-like1* and *FtNF-YC-like2* partially rescued the late flowering phenotype in *nf-yc1 nf-yc3 nf-yc4 nf-yc9* (*ycQ*) mutant in *Arabidopsis thaliana*, supporting a conserved role of *FtNF-Ys* in regulating developmental processes. Together, the genomic information provides a comprehensive understanding of the *NF-Y* transcription factors in *F. tataricum*, which will be useful for further investigation of their functions in *F. tataricum*.

1 | INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum*) is often regarded as a cereal, although it is an annual dicot. *Fagopyrum tataricum* originated in southwest China and is now widely grown in Japan, Korea, Canada, and several regions in Europe (Zhang et al., 2017). Among these areas, China has the largest cultivation area with the highest production volume of *F. tataricum*. The edible seed of *F. tataricum* is rich in primary nutrients such as proteins and fatty acids (Costantini et al., 2014; Zhu, 2016). Besides, *F. tataricum* seeds also contain high contents of secondary metabolites such as flavonoids (Nam et al., 2015), which are often consumed as dietary antioxidants and considered to be beneficial to health (Gimenez-Bastida & Zielinski, 2015; Kreft, 2016). In addition, compared to other crops, *F. tataricum* has the advantage to grow on infertile soil and in harsh environments (Gao et al., 2016; Tsurunaga et al., 2013; Yokosho et al., 2016). Therefore, *F. tataricum* was considered as an economic crop for agricultural production especially in western China.

Nuclear factor Y (NF-Y) transcription factors (TFs) are rapidly emerging as crucial regulators in numerous plant developmental processes such as flowering and embryogenesis, and abiotic stress responses such as drought and salt stresses (Kumimoto et al., 2010; Laloum et al., 2013; Li et al., 2021; Petroni et al., 2012; Quach et al., 2015; Yu et al., 2021; Zhou et al., 2020). In addition, recent studies showed that NF-Ys might play an important role in fruit development in *F. tataricum* (Yan et al., 2021). NF-Y consist of three subunits: NF-YA, NF-YB, and NF-YC, which contain conserved interaction domains to interact with each other and form heterotrimeric protein complexes (Calvenzani et al., 2012). The NF-Y heterotrimeric complexes are able to bind to the CCAAT cis-element in the promoters to regulate gene expression (Cao et al., 2014; Wenkel et al., 2006). Interestingly, each NF-Y subunit is encoded by a single gene in animals (Laloum et al., 2013), whereas each NF-Y subunit is encoded by multiple genes in plants. For example, the model plant *Arabidopsis thaliana* has 10 NF-YAs, 13 NF-YBs, and 13 NF-YCs

encoding genes in the genome (Siefers et al., 2009). The large volume of genome assembly resource has enabled the characterization of the genomic features of NF-Y in plants, such as rice (Laloum et al., 2013), soybean (Quach et al., 2015), grape (Ren et al., 2016), sorghum (Malviya et al., 2016), petunia (Wei et al., 2020), potato (Liu, 2021a), and Populus (Liu et al., 2021b).

The released high-quality reference grade *F. tataricum* genome assembly (Zhang et al., 2017) facilitates our comprehensive analysis of the NF-Y family in *F. tataricum*. In total, we have predicted 38 FtNF-Y encoding genes. Sequence alignment and phylogenetic analysis suggested that the FtNF-Ys were conserved in *F. tataricum*. Most of the *FtNF-Ys* displayed broad expression patterns in eight tissues. By calculating the Pearson correlation coefficient (PCC) between the expression levels of *FtNF-Ys* and other TFs, a potential FtNF-Y/TF interacting network was predicted. The expressions of two *FtNF-YAs* and eight *FtNF-YBs* were found to be altered in different light/dark treatments. Finally, ectopic expression of *FtNF-YC-like1* and *FtNF-YC-like2*, the homologs of Arabidopsis *NF-YC1/3/4/9*, partially rescued the flowering phenotype of the *nf-yc1 nf-yc3 nf-yc4 nf-yc9* (*ycQ*) mutant, suggesting a conserved role of these *FtNF-YCs* in regulating flowering.

2 | MATERIALS AND METHODS

2.1 | FtNF-Y prediction, alignment, and phylogenetic tree construction

The genome assembly, the general feature format (GFF), coding genes sequences, and so forth of *F. tararicum* were downloaded from the MBKBASE (http://mbkbase.org/Pinku1) database. The transcription factors including NF-Ys were identified using the iTAK program (Zheng et al., 2016). The *FtNF-Y* gene structures were displayed using TBtools (https://github.com/CJ-Chen/TBtools). The CLC Sequence Viewer software (https://www.qiagenbioinformatics.com/products/clc-sequence-viewer/) was used for sequence alignment. The phylogenetic tree was constructed using the MEGA7 (Kumar et al., 2016) and was processed by the iTOL software (http://itol.embl.de).

2.2 | RNA-seq data analysis and network construction

The RNA-seq data for each tissue were downloaded from the previous studies (Zhang et al., 2017). Reads were mapped to the reference genome (http://mbkbase.org/Pinku1) using HISAT2 (http://ccb.jhu.edu/software/hisat2). The expression level was calculated using Fragments Per Kilobase of exon model per Million mapped fragments (FPKM) via cuffnorm (http://cole-trapnell-lab.github.io/cufflinks/cuffnorm/). The heatmaps were constructed in R language using the Pheatmap package (https://cran.rproject.org/web/packages/pheatmap).

For co-expression network construction between *FtNF-Y*s and other *TF*s, genes with a FPKM > 1 in any of the samples were used for the calculation of PCC using a Pearson calculator, a subfunction from Scipy package in Python. Only the absolute PCC value > 0.9 was considered as potential interaction. The potential network was visualized using the Cytoscape software (http://www.cytoscape.org).

2.3 | Subcellular localization

The full-length CDSs excluding the stop codon of *FtNF-YC*s were cloned into the 35S:GFP pGreen vector in-frame with the CDS of GFP at the C-terminal to generate the *FtNF-YC-GFP* fusion constructs. The resulting vectors were then transformed into tobacco leaf epidermal cells by infiltration as previously described (Sparkes et al., 2006). The fluorescence signal was determined using a fluorescence microscope (Leica). Primers for vector construction are listed in the Table S5.

2.4 | *Arabidopsis thaliana* transformation

The full length CDSs of *FtNF-YCs* were cloned into the pGreen vector downstream of a 35S promoter. Arabidopsis transformation was performed using the floral dip method (Clough & Bent, 1998) and transgenic seeds were selected with Basta. Primers used for vector construction are listed in Table S5.

2.5 | Quantitative polymerase chain reaction (qPCR)

Total RNA of plant samples, including the 7-day-old dark-grown *F. tataricum* seedlings, 7-day-old dark-grown *F. tataricum* seedlings exposed to light for 6 h, 10-day-old wild-type Arabidopsis, 10-day-old transgenic Arabidopsis and the 10-day-old *ycQ* mutant were extracted using the Plant RNA Kit (TianGen) and reverse transcribed using the M-MLV reverse transcriptase (TransGen). qPCR was performed in triplicates using the TB Green Premix ExTaq Mix (TaRaKa). Primers used for gene expression analysis are listed in Table S5.

3 | RESULTS

3.1 | Prediction of *NF-Y* genes in the *F. tataricum* genome

To predict the *NF-Y* genes in the *F. tataricum* genome, the deduced protein sequences from the *F. tataricum* database (http://mbkbase.org/Pinku1) were analyzed using the iTAK program (Zheng et al., 2016). A total of 38 *FtNF-Y* (Figure S1; Table S1) genes were

distributed in eight chromosomes, including 12 *FtNF-YAs*, 18 *FtNF-YBs*, and eight *FtNF-YCs* with the average gene length of 3277, 1421, and 2060 bp, respectively (Table 1).

The exon-intron structures of *FtNF-Y* genes were also analyzed (Figure 1; Tables 1 and S1). The average exon/intron lengths of *FtNF-YAs*, *FtNF-YBs*, and *FtNF-YCs* were 1008/2277, 644/778, and 908/1155 bp, respectively, while the average exon/intron numbers were 5.83/4.83, 2.28/1.28, and 3.25/2.25, respectively (Tables 1 and S1). Among them, eight out of 38 *FtNF-Ys* were intron-free, which all belonged to the *FtNF-YB* subfamily (Figure 1; Table S1). The *FtNF-YAs* had the longest average gene length, likely because of the higher intron number as well as the longer intron length compared to the other two *FtNF-Y* subunits. The intron–exon structure was thought to influence gene transcription and splicing (Koralewski & Krutovsky, 2011; Le Hir et al., 2003; Maity & de Crombrugghe, 1998). However, in the *F. tataricum* genome, only three *FtNF-Y* genes were predicted to have alternative splicing isoforms, including *FtPinG0000686200.01* (*FtNF-YA*), *FtPinG0003810700.01* (*FtNF-YB*) and *FtPinG0005857800.01* (*FtNF-YC*; Figure S2). In addition, the average molecular weights of the deduced amino acid sequences of FtNF-YAs, FtNF-YBs, and FtNF-YCs were around 27, 20, and 26 kDa, respectively (Table 1).

3.2 | Multiple sequence alignment and phylogenetic analysis

As reported previously, the NF-Y family contains conserved protein domains for protein–protein or protein–DNA interaction (Calvenzani et al., 2012; Chaves-Sanjuan et al., 2021; Gnesutta et al., 2017; Laloum et al., 2013). As expected, FtNF-YAs contained the highly conserved DNA-binding domain and the NF-YB/YC interacting domain, which were separated by a conserved linker sequence (Figure 2A). Similar to FtNF-YAs, FtNF-YBs consisted of a DNA-binding domain and the NF-YC/NF-YA interaction domain (Figure 2B). FtNF-YCs

TABLE 1 Statistics of the *FtNF-Y* gene family

FtNF-Y	GN	AGL	AEL	AEN	AIL	AIN	AAL	AMW
YA	12	3277.08	1008.17	5.83	2277.58	4.83	244.67	27.04
YB	18	1421.17	644.39	2.28	778.78	1.28	180.56	19.99
YC	8	2060.00	908.38	3.25	1155.13	2.25	231.75	25.62

Abbreviations: AAL, average amino acid length (aa); AEL, average exon length (bp); AEN, average exon number; AGL, average gene length (bp); AIL, average intron length (bp); AIN, average intron number; AMW, average molecular weight (kDa); GN, gene number.

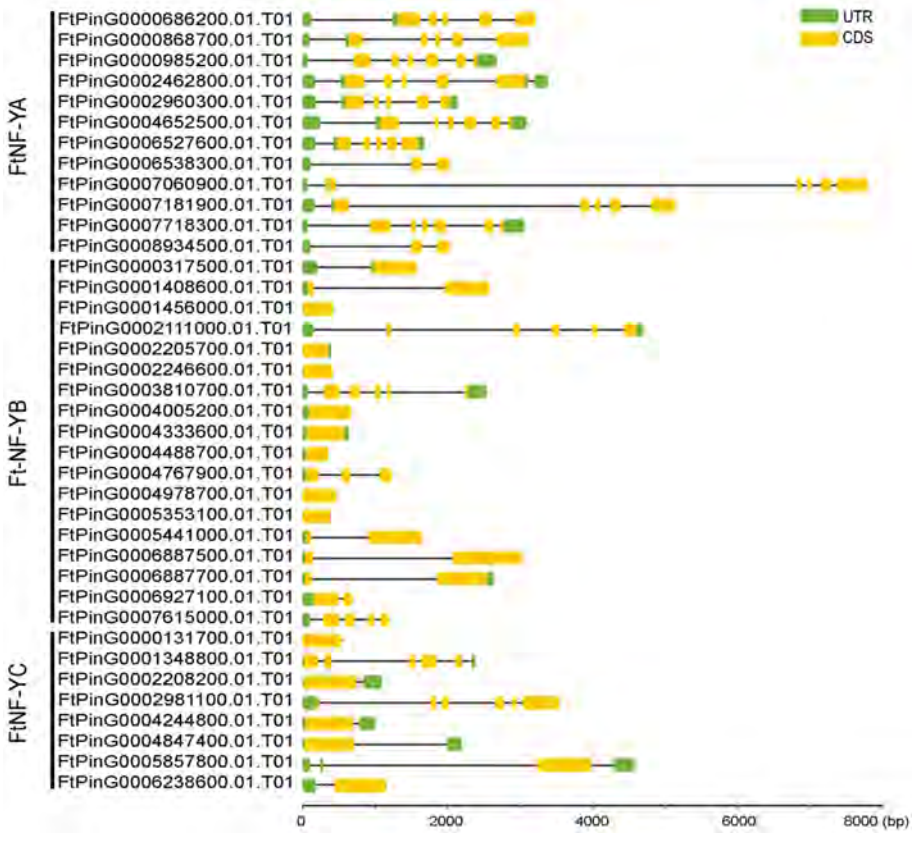

FIGURE 1 The gene structures of *FtNF-Ys* in the *Fagopyrum tartaricum* genome. Yellow color indicates coding sequences (CDS). Green color indicates untranslated regions (UTR). The scale bar indicates the gene length (bp)

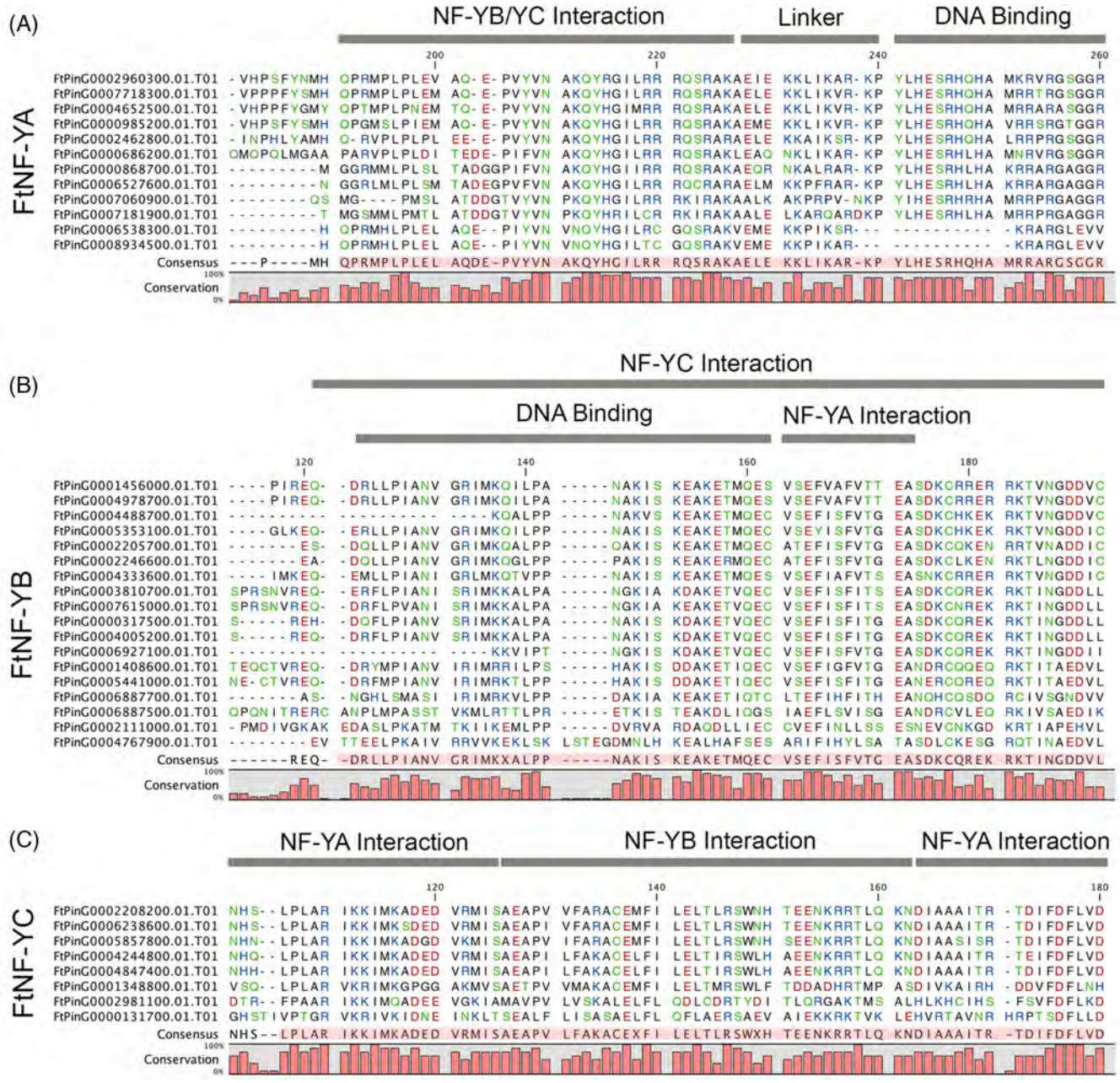

FIGURE 2 The conserved domains of the FtNF-Y gene family. The deduced amino acid sequences of FtNF-YAs (A), FtNF-YBs (B), and FtNF-YCs (C) were aligned and conserved domains among members of the subfamilies were identified. FtNF-YAs contained an NF-YB/YC interaction domain, DNA-binding domain and a linker. FtNF-YBs contained NF-YC, NF-YA interaction and a DNA-binding domain. FtNF-YCs contained NF-YA/NF-YB interaction domains

contained the conserved NF-YA and NF-YB interaction domain but lacked a DNA-binding domain (Figure 2C).

Furthermore, using the deduced amino acid sequences of FtNF-Ys, Arabidopsis NF-Ys (AtNF-Ys) as well as the rice NF-Ys, a phylogenetic tree was constructed using the Neighbor-joining algorithm. These NF-Ys were clustered into three major clusters: NF-YA, NF-YB, and NF-YC, which were in line with previous reports (Figure 3; Siefers et al., 2009). According to previous reports in Arabidopsis, *AtNF-YB6* and *AtNF-YB9* played essential roles in developmental processes such as embryogenesis (Huang et al., 2015a, 2015b; Kumimoto et al., 2010; West et al., 1994), while *AtNF-YC1*, *AtNF-YC3*, *AtNF-YC4*, and *AtNF-YC9* were involved in flower initiation (Cao et al., 2014; Hou et al., 2014; Kumimoto et al., 2010; Lv et al., 2021; Mach, 2017). In the phylogenetic tree, *FtPinG0005441000.01.T01* and *FtPinG0001408600.01.T01* were in the same clade as *AtNF-YB6* and *AtNF-YB9*, while *FtPinG0004847400.01.T01*, *FtPinG0004244800.01.T01*, *FtPinG0005857800.01.T01*, *FtPinG0002208200.01.T01*, and *FtPinG0006238600.01.T01* formed a clade with *AtNF-YC1/3/4/9*. These data together suggested that FtNF-Ys might have similar functions to their homologs in Arabidopsis.

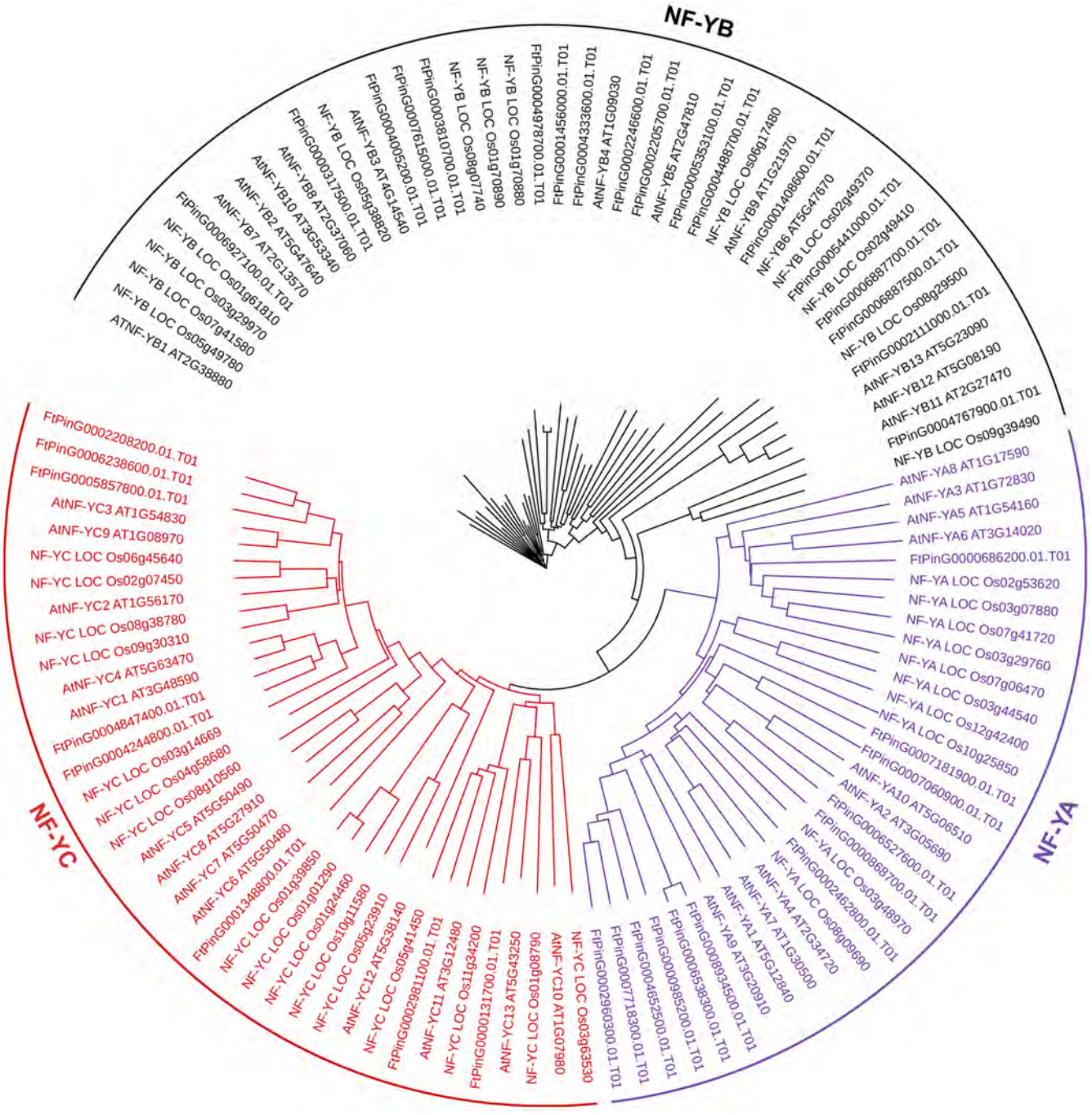

FIGURE 3 The phylogenetic tree of the FtNF-Y gene family. The deduced amino acid sequences of FtNF-Ys were used for neighbor-joining tree construction. FtNF-YAs, FtNF-YBs, and FtNF-YCs were highlighted in purple, black and red, respectively. The Arabidopsis and rice (*Oryza sativa subsp. Japonica*) NF-Y amino acid sequences were used for comparison in this analysis

3.3 | Expression patterns of *FtNF-Ys* in different tissues

Plant *NF-Y* genes usually display functional redundancy, and the tissue-specific expression patterns may help us understand how *FtNF-Ys* participate in specific developmental processes (Siefers et al., 2009). Using the released RNA-seq data (Zhang et al., 2017), we measured the FPKM of *FtNF-Ys* in eight tissues, including flower (FL), leaf (LE), root (RO), young stem (YST), young seed (YSE), seed at pre-filling stage (PSS), seed at filling stage (FSS), and seed at mature stage (MSS).

Most of the *FtNF-YAs* displayed broad expression patterns in all eight tissues, except several cases (Figure 4A). For example, *FtPinG0008934500.01.T01* was expressed higher in leaf (LE) than other tissues, while *FtPinG0006527600.01.T01* was only expressed highly in root (RO; Figure 4A), indicating their specific expression in

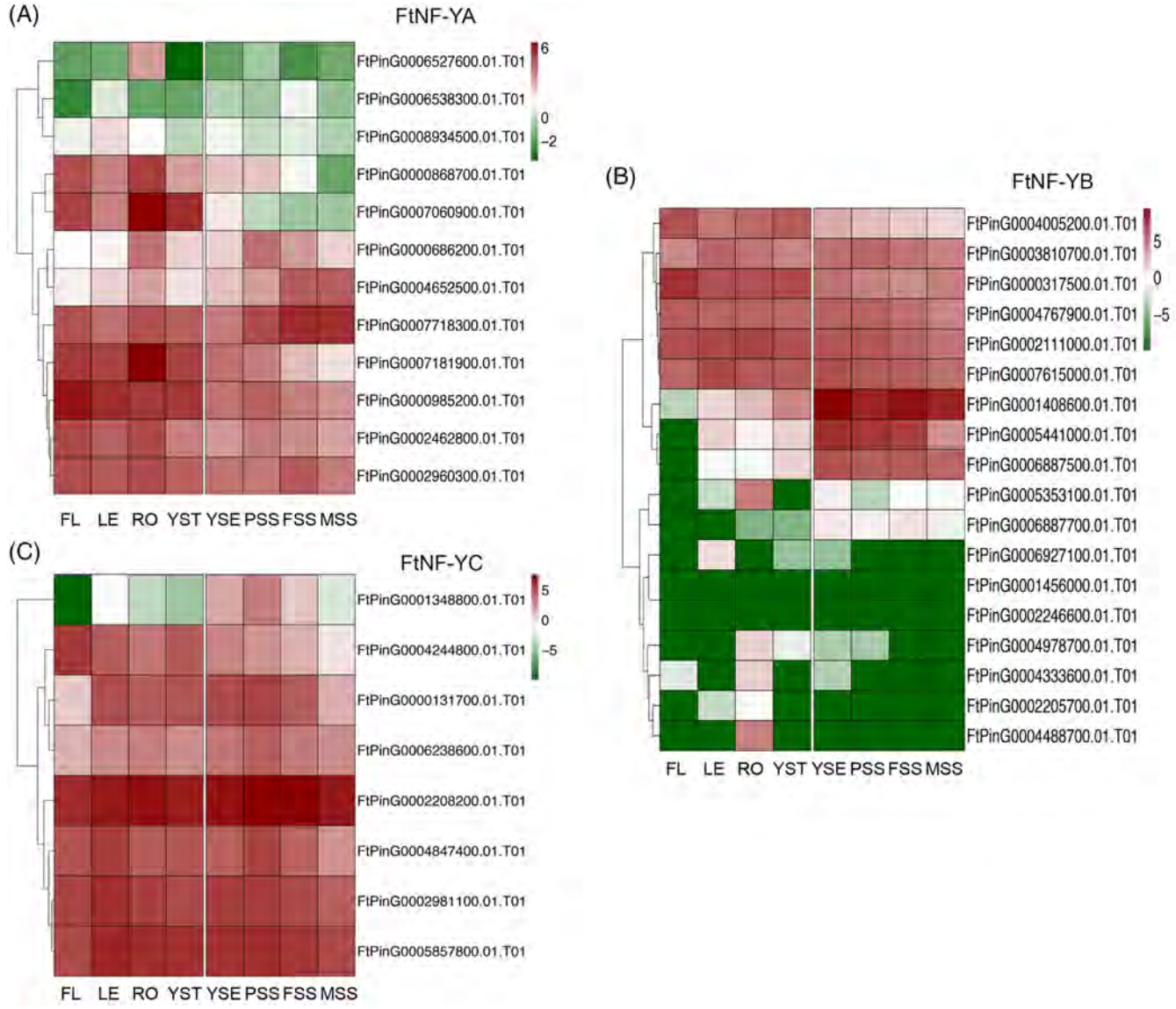

FIGURE 4 Heatmaps showing the expression levels of *FtNF-Ys* in eight tissues. The expression levels of *FtNF-YA*s (A), *FtNF-YB*s (B), and *FtNF-YC*s (C) in eight tissues are shown. The $\log_2$(FPKM values) were used to generate the heatmaps. FL, flower; FSS, seed in filling stage; LE, leaf; MSS, seed in mature stage; PSS, seed in prefilling stage; RO, root; YSE, young seed; YST, young stem

leaf or root. Unlike *FtNF-YA*s, the expressions of *FtNF-YB*s could be roughly grouped into two clusters in the heatmap. The upper cluster, including nine *FtNF-YB*s, such as *FtPinG0004005200.01.T01* and *FtPinG0003810700.01.T01*, displayed broad expression patterns in all tissues. The lower cluster of the heatmap, including the other nine *FtNF-YB*s, showed extremely low expression levels (e.g. *FtPinG0001456000.01.T01*) or more specific expression in only a few tissues, such as *FtPinG0004488700.01.T01* which was expressed in root. In addition, the *AtNF-YB6/9* homologs, including *FtPinG0005441000.01.T01* and *FtPinG0001408600.01.T01*, were highly expressed in seed tissues (e.g. YSE, PSS, FSS, and MSS) compared to other developmental stages (e.g. FL), indicating that these two *FtNF-YB*s played important roles in developing seeds similar to *AtNF-YB6/9*. Most of the *FtNF-YC*s were highly expressed in all eight tissues except *FtPinG0001348800.01.T01*.

3.4 | The potential co-expression network between FtNF-Ys and other TFs

NF-Y subunits are able to cooperate with other TFs to regulate gene expression (Swain et al., 2017; Chaves-Sanjuan et al., 2021). To predict the potential regulatory partners of FtNF-Ys, we calculated the PCC between the expression levels of *FtNF-Ys* and other TFs in eight tissues (Tables S2–S4). The FtNF-Y/TFs pairs with PCC values over 0.9 were considered as the potential FtNF-Y interacting partners. In total, 1681 TFs from 73 TFs families, such as MYB, ERF, HB, bHLH, bZIP, NAC, WRKY, and C2C2-Dof, were predicted to be the potential interacting partners of FtNF-Ys (Figure 5A). Next, a potential FtNF-Y/TF interacting network was constructed using these candidates. Several FtNF-Y/TF groups were clustered together in this network. For example, the B1/B5/B8-TFs group, the A7/A8-TFs group and the

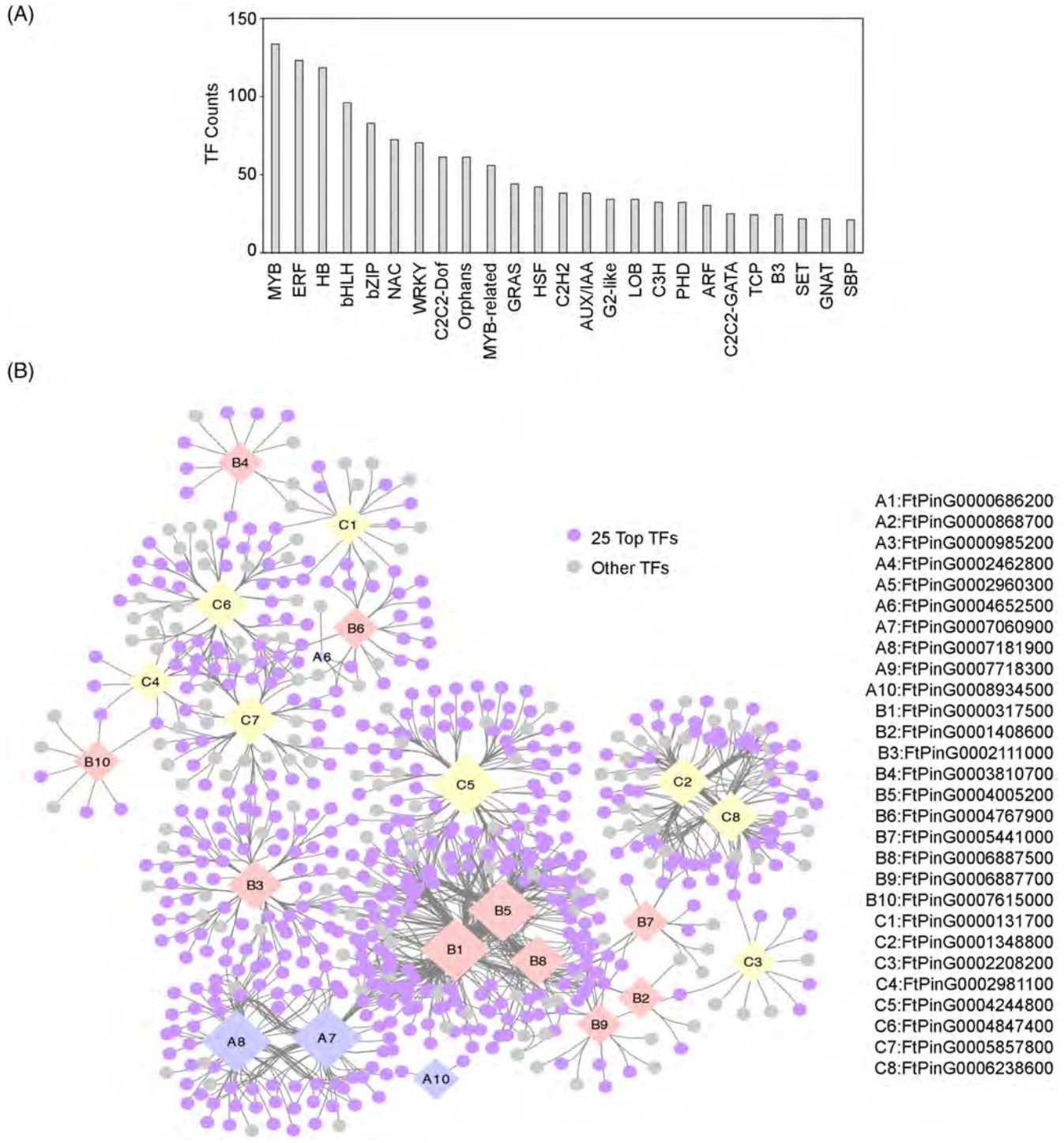

FIGURE 5 Interactions between FtNF-Ys and other transcription factors (TFs). (A) The top 25 TF families with the highest number of members co-expressed with *FtNF-Ys*. (B) The potential interacting network between FtNF-Ys and other TFs. Pearson correlation coefficient (PCC) values were calculated between the expression values of *FtNF-Ys* and other TF genes in different tissues. A PCC value over 0.9 was used to determine potential interactions

C2/C8-TFs group formed tightly interacting networks, indicating that the development processes might be regulated by different FtNF-Y/TFs regulatory modules in *F. tataricum*. In addition, among these putative networks, several types of TFs were previously reported to interact with NF-Ys in other species. For example, AtNF-YB9 interacted with bHLH, MYB (e.g. TCL), and GRAS (e.g. DELLA) to regulate photomorphogenesis, trichome formation, and seed development (Hu et al., 2018; Huang et al., 2015a, 2015b). Also, NF-YCs interacted with GRAS (e.g. DELLA)

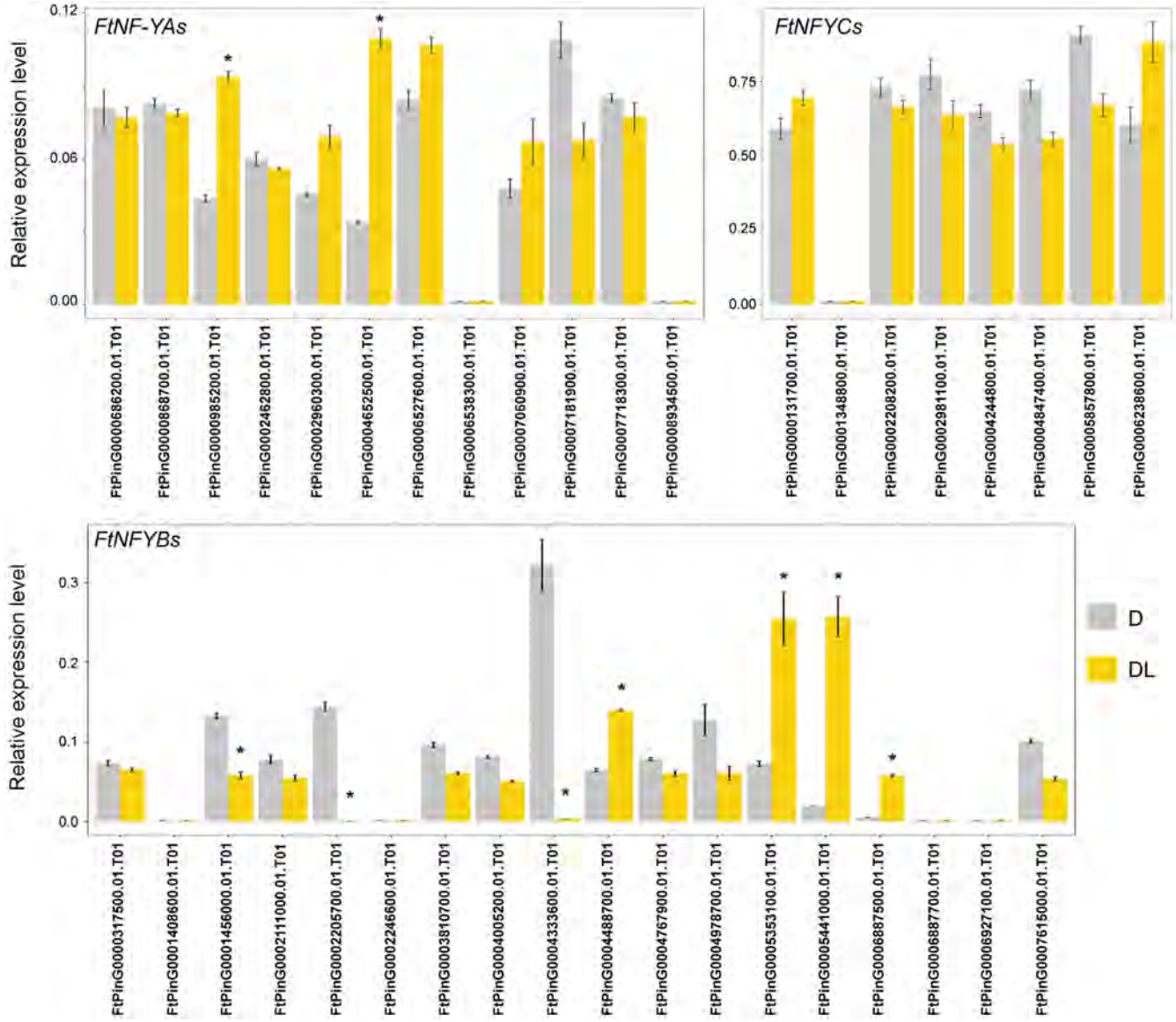

FIGURE 6 Expression pattern of *FtNF-Ys* in response to light in *Fagopyrum tataricum* seedlings. The expression levels of *FtNF-Ys* were analyzed using qPCR. D, dark-grown seedlings; DL, dark-grown seedlings transferred to light condition for 6 h. Asterisk (*) indicates a significant difference between D and DL condition (|Mean fold change|>1.5 and $p < 0.05$, Student's *t* test). The error bar indicates the mean ± SD of three replicates. The expression level was normalized to an internal control gene *FtHistone3* (NCBI accession number, JF769134)

and zinc-finger transcription factors (e.g. CO) to regulate seed germination, flowering, and hypocotyl growth (Hou et al., 2014; Kumimoto et al., 2010; Liu et al., 2016; Lv et al., 2021; Zhang et al., 2021). Therefore, these putative interacting networks provide useful information for further investigation on how FtNF-Ys interact with other TFs.

3.5 | FtNF-Ys were involved in light response

NF-Ys were reported to play an important role in response to light (Huang et al., 2015b; Tang et al., 2017; Zhang et al., 2021). To investigate whether FtNF-Ys were involved in the light signaling pathway, we compared the expression levels of *FtNF-Ys* between the dark-grown (D) seedlings and the dark-grown seedlings transferred to light conditions (DL) for 6 h. Only two *FtNF-YAs* (*FtPinG000985200*, *FtPinG0004652500*) showed significant up-regulation in the DL treatment (Figure 6). Among the 18 *FtNF-YBs*, seven *FtNF-YBs* were differentially expressed in the DL treatment. Three *FtNF-YBs* (*FtPinG0001456000*, *FtPinG0002205700*, and *FtPinG0004333600*) were downregulated while the other four (*FtPinG0004488700*, *FtPinG0005353100*, *FtPinG0005441000*, and *FtPinG0006887500*) were upregulated in DL compared to D seedlings. Changes in the expressions of NF-YAs and NF-YBs have been observed in Arabidopsis under different light conditions. It was proposed that NF-YAs and NF-YBs were involved in the cross-talk with hormone signaling

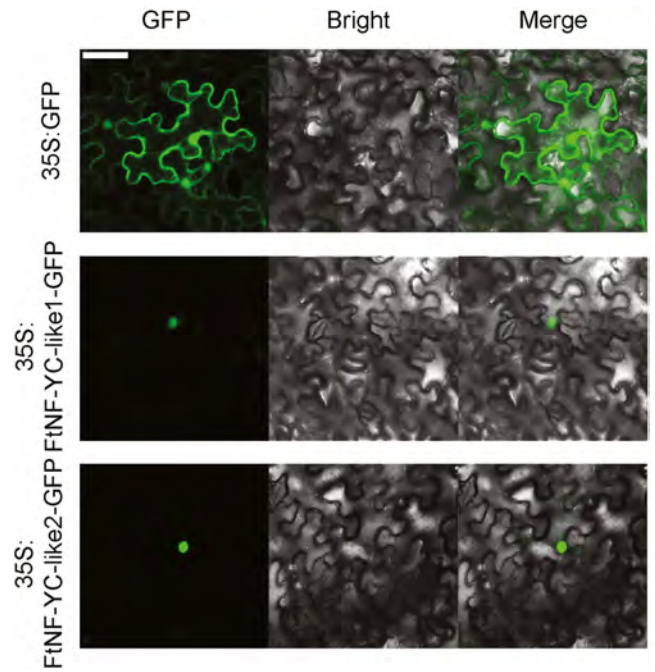

FIGURE 7 Subcellular localization of two FtNF-YCs. Transient expression in tobacco leaves indicated that FtNF-YC-like1 and FtNF-YC-like2 were localized in the nuclei. The 35S:GFP empty vector was used as the control. Scale bar indicates 50 μm. Merge, merge of GFP and bright-field

pathways (e.g. abscisic acid) to regulate plant development in response to light (Warpeha et al., 2007). Interestingly, there is no significant change in the expression of FtNF-YCs between these two conditions (Figure 6), indicating a different regulatory manner of *FtNF-YC* in light response in *F. tataricum* compared to Arabidopsis (Siefers et al., 2009), which needs further investigation.

3.6 | Functional complementation of an Arabidopsis *ycQ* mutant by two FtNF-YCs

As mentioned above, five *FtNF-YCs* (*FtPinG0004847400.01.T01*, *FtPinG0004244800.01.T01*, *FtPinG0005857800.01.T01*, *FtPinG 0002208200.01.T01*, and *FtPinG0006238600.01.T01*), which were clustered with *AtNF-YC1/3/4/9*, might function in regulating flowering. The *nf-yc1 nf-yc3 nf-yc4 nf-yc9* (*ycQ*) quadruple mutant (Tang et al., 2017) displayed a late flowering phenotype in Arabidopsis (Kumimoto et al., 2010). To confirm whether the *FtNF-YCs* function in regulating flowering, we selected *FtPinG0004244800.01.T01* from the NF-YC1/4 subclade and *FtPinG0006238600.01.T01* from the NF-YC3/9 subclade for further analysis. We renamed *FtPinG0004244800.01.T01* and *FtPinG0006238600.01.T01* as *FtNF-YC-like1* and *FtNF-YC-like2*, respectively. To confirm the subcellular localization of these two proteins, *FtNF-YC-like1* and *FtNF-YC-like2* fused with a GFP tag were transiently expressed in tobacco leaves. Fluorescent signals of FtNF-YC-like1-GFP and FtNF-YC-like2-GFP were detected solely in the nucleus in contrast to the GFP, whose signals were detected in both cytosol and nucleus, suggesting that FtNF-YC-like1 and FtNF-YC-like2 were targeted to the nucleus (Figure 7; Hackenberg et al., 2012). Next, these two *FtNF-YCs* were overexpressed in the Arabidopsis *ycQ* mutant which displayed a late flowering phenotype. Overexpression of *FtNF-YC-like1* and *FtNF-YC-like2* partially rescued the late flowering phenotype of the *ycQ* mutant (Figure 8A). Furthermore, the expression levels of several flowering related genes, including *AtFT* (Cao et al., 2014; Kumimoto et al., 2010), *AtSOC1* (Hou et al., 2014) and *AtAP1* (Ye et al., 2016), were analyzed in the two *FtNF-YC-like* transgenic plants, *ycQ* mutant and wild-type (WT). Ectopic expression of *FtNF-YC-like1* and *FtNF-YC-like2* restored the expressions of the flowering related genes, which were downregulated in the *ycQ* mutant, to the levels similar to those in wild-type (Figure 8B). NF-YC could directly bind to the conserved CCAAT box in the promoters of *FT* and *SOC1* to regulate flowering in Arabidopsis (Cao et al., 2014; Hou et al., 2014). Two *SOC1-like* genes and four *FT-like* genes were predicted in the *F. tataricum* genome. Among these six genes, except the FT-like gene *FtPinG0006092200.01.T01*, the remaining five genes harbored at least one CCAAT-box in their promoters (Figure S3). These data suggested that *FtNF-YC-like1/2* might also regulate the *FT* and *SOC1-like* genes by direct binding to the *cis*-elements in *F. tataricum* to regulate flowering.

4 | DISCUSSION

TFs play essential roles in developmental processes and stress responses in plants. To date, several *F. tataricum* TFs have been cloned and functionally characterized in *A. thaliana* or *F. tataricum* using the hairy root system. For example, *FtNAC16* was reported to play a role in regulating pod cracking and salinity tolerance in Arabidopsis (Wang et al., 2021). *FtMYB9* (Gao et al., 2017), *FtbZIP5* (Li et al., 2020), *FtbZIP83* (Li et al., 2019), *FtbHLH2* (Yao et al., 2018), and *FtbHLH3* (Yao et al., 2017) enhanced tolerance to abiotic stresses, such as cold, drought, or salt, in Arabidopsis. In addition, *FtMYB6* and *FtMYB116* regulated the flavonoid accumulation through regulating the genes related to flavonoid biosynthesis (Yao et al., 2020; Zhang et al., 2019). Besides, genome-wide characterization studies of NAC (Liu et al., 2019), trihelix (Ma et al., 2019), and WRKY (Sun et al., 2020) have also been reported in *F. tataricum*. These studies have revealed the potential roles of TFs in crop improvement. The NF-Y family is widespread in higher plants and plays crucial roles in plant development and stress responses (Laloum et al., 2013; Swain et al., 2017; Li et al., 2021; Yu et al., 2021). In this study, 38 FtNF-Ys encoding genes were identified in the *F. tataricum* genome (Zhang et al., 2017). Sequence alignment, phylogenetic tree and functional analysis in Arabidopsis suggested a conserved role of *FtNF-Y* in developmental processes and light responses in *F. tataricum*. Our results provide a comprehensive understanding of the genomic features of *FtNF-Ys*, which facilitates further investigations of their functions in *F. tataricum*.

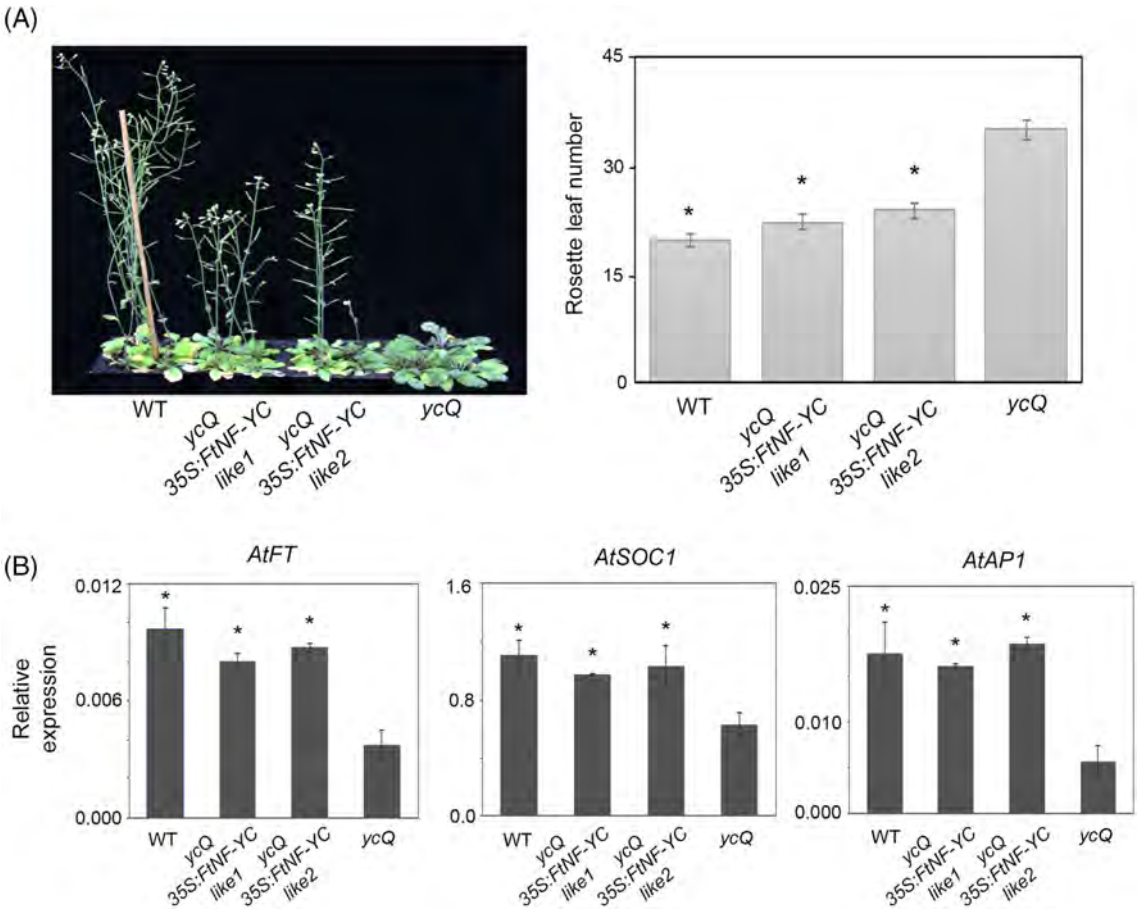

FIGURE 8 Two FtNF-YCs rescued the late flowering phenotype in Arabidopsis *ycQ* quadruple mutant. (A) The overexpression of *FtNF-YC-like1* and *FtNF-YC-like2* rescued the flowering defect in *ycQ* mutant. Left panel, the flowering phenotype of wild-type (WT), *ycQ 35S:FtNF-YC-like1*, *ycQ 35S:FtNF-YC-like2*, and *ycQ* mutant. Right panel, the rosette leaf number of wild-type (WT), *ycQ 35S:FtNF-YC-like1*, *ycQ 35S:FtNF-YC-like2*, and *ycQ* mutant. Asterisk (*) indicates a significant difference compared to *ycQ* mutant ($p < 0.05$, Student's *t* test). The error bar indicates the mean SD of 20 plants. (B) The expressions of flowering-related genes including *AtFt*, *AtSOC1*, and *AtAP1* were upregulated in WT and *ycQ 35S:FtNF-YCs* transgenic plants compared to the *ycQ* mutant. Asterisk (*) indicates a significant difference compared to *ycQ* mutant ($p < 0.05$, Student's *t* test). The error bar indicates the mean ± SD of three replicates

As mentioned above, most of the functional analyses of the TFs in *F. tataricum* were done using the model plant Arabidopsis or the convenient hairy root system. However, reports of transgenic plants or CRISPR-Cas9 knockout mutants of the TFs in *F. tataricum* are limited. Indeed, the plantlet regeneration of *F. tataricum* has been reported, and the Agrobacterium-mediated transformation method in buckwheat has also been established (Suvorova, 2016; Wang et al., 2016). Thus, with the development of transgenic methods in *F. tataricum*, characterization of TFs using genome-wide prediction, expression analysis and functional validation in Arabidopsis or *F. tataricum* will provide valuable genomic information and candidates for future crop improvement.

ACKNOWLEDGMENTS

This study was supported by the Hong Kong Research Grants Council Area of Excellence Scheme (AoE/M-403/16) and the Lushan Botanical Garden Research Funds (2021ZWZX31 and 2022ZWZX01). The authors would like to thank Xingliang Hou for providing the *ycQ* mutant. Any opinions, findings, conclusions or recommendations expressed in this publication do not reflect the views of the Government of Hong Kong Special Administrative Region or the Innovation and Technology Commission.

AUTHOR CONTRIBUTIONS

Mingkun Huang and Ling Zhang conceived and coordinated this research and designed the experimental details. Ling Zhang and Wai-Shing Yung performed the wet-lab experiments and data analyses. All authors wrote the manuscript and read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The raw data of the RNA-seq in this study were downloaded from NCBI with the SRA accession number as follows: Flower, SRR5433732; Leaf, SRR5433730; Root, SRR5433734; YST, SRR5433731; YSE, SRR5433733; PSS, SRR5006769; FSS, SRR5006770; MSS, SRR5006766.

ORCID

Wai-Shing Yung https://orcid.org/0000-0001-8299-7374
Mingkun Huang https://orcid.org/0000-0001-9501-1797

REFERENCES

Calvenzani, V., Testoni, B., Gusmaroli, G., Lorenzo, M., Gnesutta, N., Petroni, K. et al. (2012) Interactions and CCAAT-binding of *Arabidopsis thaliana* NF-Y subunits. *PLoS One*, 7(8), e42902.

Cao, S., Kumimoto, R.W., Gnesutta, N., Calogero, A.M., Mantovani, R. & Holt, B.F., 3rd. (2014) A distal CCAAT/NUCLEAR FACTOR Y complex promotes chromatin looping at the *FLOWERING LOCUS T* promoter and regulates the timing of flowering in Arabidopsis. *Plant Cell*, 26(3), 1009–1017.

Chaves-Sanjuan, A., Gnesutta, N., Gobbini, A., Martignago, D., Bernardini, A., Fornara, F. et al. (2021) Structural determinants for NF-Y subunit organization and NF-Y/DNA association in plants. *The Plant Journal*, 105(1), 49–61.

Clough, S.J. & Bent, A.F. (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant Journal*, 16(6), 735–743.

Costantini, L., Luksic, L., Molinari, R., Kreft, I., Bonafaccia, G., Manzi, L. et al. (2014) Development of gluten-free bread using Tartary buckwheat and chia flour rich in flavonoids and omega-3 fatty acids as ingredients. *Food Chemistry*, 165, 232–240.

Gao, F., Yao, H., Zhao, H., Zhou, J., Luo, X., Huang, Y. et al. (2016) Tartary buckwheat *FtMYB10* encodes an R2R3-MYB transcription factor that acts as a novel negative regulator of salt and drought response in transgenic Arabidopsis. *Plant Physiology and Biochemistry*, 109, 387–396.

Gao, F., Zhou, J., Deng, R.Y., Zhao, H.X., Li, C.L., Chen, H. et al. (2017) Overexpression of a Tartary buckwheat R2R3-MYB transcription factor gene, *FtMYB9*, enhances tolerance to drought and salt stresses in transgenic Arabidopsis. *Journal of Plant Physiology*, 214, 81–90.

Gimenez-Bastida, J.A. & Zielinski, H. (2015) Buckwheat as a functional food and its effects on health. *Journal of Agricultural and Food Chemistry*, 63(36), 7896–7913.

Gnesutta, N., Saad, D., Chaves-Sanjuan, A., Mantovani, R. & Nardini, M. (2017) Crystal structure of the *Arabidopsis thaliana* L1L/NF-YC3 histone-fold dimer reveals specificities of the LEC1 family of NF-Y subunits in plants. *Molecular Plant*, 10(4), 645–648.

Hackenberg, D., Wu, Y.F., Voigt, A., Adams, R., Schramm, P. & Grimm, B. (2012) Studies on differential nuclear translocation mechanism and assembly of the three subunits of the *Arabidopsis thaliana* transcription factor NF-Y. *Molecular Plant*, 5(4), 876–888.

Hou, X., Zhou, J., Liu, C., Liu, L., Shen, L. & Yu, H. (2014) Nuclear factor Y-mediated H3K27me3 demethylation of the *SOC1* locus orchestrates flowering responses of Arabidopsis. *Nature Communications*, 5, 4601.

Hu, Y., Zhou, L., Huang, M., He, X., Yang, Y., Liu, X. et al. (2018) Gibberellins play an essential role in late embryogenesis of Arabidopsis. *Nature Plants*, 4(5), 289–298.

Huang, M., Hu, Y., Liu, X., Li, Y. & Hou, X. (2015a) Arabidopsis LEAFY COTYLEDON1 controls cell fate determination during post-embryonic development. *Frontiers in Plant Science*, 6, 955.

Huang, M., Hu, Y., Liu, X., Li, Y. & Hou, X. (2015b) Arabidopsis LEAFY COTYLEDON1 mediates postembryonic development via interacting with PHYTOCHROME-INTERACTING FACTOR4. *Plant Cell*, 27(11), 3099–3111.

Koralewski, T.E. & Krutovsky, K.V. (2011) Evolution of exon-intron structure and alternative splicing. *PLoS One*, 6(3), e18055.

Kreft, M. (2016) Buckwheat phenolic metabolites in health and disease. *Nutrition Research Reviews*, 29(1), 30–39.

Kumar, S., Stecher, G. & Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7), 1870–1874.

Kumimoto, R.W., Zhang, Y., Siefers, N. & Holt, B.F., 3rd. (2010) NF-YC3, NF-YC4 and NF-YC9 are required for CONSTANS-mediated, photoperiod-dependent flowering in *Arabidopsis thaliana*. *The Plant Journal*, 63(3), 379–391.

Laloum, T., De Mita, S., Gamas, P., Baudin, M. & Niebel, A. (2013) CCAAT-box binding transcription factors in plants: Y so many? *Trends in Plant Science*, 18(3), 157–166.

Le Hir, H., Nott, A. & Moore, M.J. (2003) How introns influence and enhance eukaryotic gene expression. *Trends in Biochemical Sciences*, 28(4), 215–220.

Li, Q., Wu, Q., Wang, A., Lv, B., Dong, Q., Yao, Y. et al. (2019) Tartary buckwheat transcription factor FtbZIP83 improves the drought/salt tolerance of Arabidopsis via an ABA-mediated pathway. *Plant Physiology and Biochemistry*, 144, 312–323.

Li, S., Zhang, N., Zhu, X., Ma, R., Liu, S., Wang, X. et al. (2021) Genome-wide analysis of *NF-Y* genes in potato and functional identification of *StNF-YC9* in drought tolerance. *Frontiers in Plant Science*, 12, 749688.

Li, Q., Zhao, H., Wang, X., Kang, J., Lv, B., Dong, Q. et al. (2020) Tartary buckwheat transcription factor FtbZIP5, regulated by FtSnRK2.6, can improve salt/drought resistance in transgenic Arabidopsis. *International Journal of Molecular Sciences*, 21(3), 1123.

Liu, X., Hu, P., Huang, M., Tang, Y., Li, Y., Li, L. et al. (2016) The NF-YC–RGL2 module integrates GA and ABA signalling to regulate seed germination in Arabidopsis. *Nature Communications*, 7(1), 12768.

Liu, Z., Li, Y., Zhu, J., Ma, W., Li, Z., Bi, Z. et al. (2021a) Genome-wide identification and analysis of the *NF-Y* gene family in potato (*Solanum tuberosum* L.). *Frontiers in Genetics*, 12, 739989.

Liu, M., Ma, Z., Sun, W., Huang, L., Wu, Q., Tang, Z. et al. (2019) Genome-wide analysis of the NAC transcription factor family in Tartary buckwheat (*Fagopyrum tataricum*). *BMC Genomics*, 20(1), 113.

Liu, R., Wu, M., Liu, H.L., Gao, Y.M., Chen, J., Yan, H.W. et al. (2021b) Genome-wide identification and expression analysis of the NF-Y transcription factor family in Populus. *Physiologia Plantarum*, 171(3), 309–327.

Lv, X., Zeng, X., Hu, H., Chen, L., Zhang, F., Liu, R. et al. (2021) Structural insights into the multivalent binding of the Arabidopsis *FLOWERING LOCUS T* promoter by the CO-NF-Y master transcription factor complex. *Plant Cell*, 33(4), 1182–1195.

Ma, Z., Liu, M., Sun, W., Huang, L., Wu, Q., Bu, T. et al. (2019) Genome-wide identification and expression analysis of the trihelix transcription factor family in Tartary buckwheat (*Fagopyrum tataricum*). *BMC Plant Biology*, 19(1), 344.

Mach, J. (2017) CONSTANS companion: CO binds the NF-YB/NF-YC dimer and confers sequence-specific DNA binding. *The Plant Cell*, 29(6), 1183.

Maity, S.N. & de Crombrugghe, B. (1998) Role of the CCAAT-binding protein CBF/NF-Y in transcription. *Trends in Biochemical Sciences*, 23(5), 174–178.

Malviya, N., Jaiswal, P. & Yadav, D. (2016) Genome-wide characterization of Nuclear factor Y (NF-Y) gene family of sorghum [*Sorghum bicolor* (L.) Moench]: a bioinformatics approach. *Physiology and Molecular Biology of Plants*, 22(1), 33–49.

Nam, T.G., Lee, S.M., Park, J.H., Kim, D.O., Baek, N.I. & Eom, S.H. (2015) Flavonoid analysis of buckwheat sprouts. *Food Chemistry*, 170, 97–101.

Petroni, K., Kumimoto, R.W., Gnesutta, N., Calvenzani, V., Fornari, M., Tonelli, C. et al. (2012) The promiscuous life of plant NUCLEAR FACTOR Y transcription factors. *Plant Cell*, 24(12), 4777–4792.

Quach, T.N., Nguyen, H.T., Valliyodan, B., Joshi, T., Xu, D. & Nguyen, H.T. (2015) Genome-wide expression analysis of soybean NF-Y genes reveals potential function in development and drought response. *Molecular Genetics and Genomics*, 290(3), 1095–1115.

Ren, C., Zhang, Z., Wang, Y., Li, S. & Liang, Z. (2016) Genome-wide identification and characterization of the NF-Y gene family in grape (*Vitis vinifera* L.). *BMC Genomics*, 17(1), 605.

Siefers, N., Dang, K.K., Kumimoto, R.W., Bynum, W.E., Tayrose, G. & Holt, B.F., 3rd. (2009) Tissue-specific expression patterns of Arabidopsis NF-Y transcription factors suggest potential for extensive combinatorial complexity. *Plant Physiology*, 149(2), 625–641.

Sparkes, I.A., Runions, J., Kearns, A. & Hawes, C. (2006) Rapid, transient expression of fluorescent fusion proteins in tobacco plants and generation of stably transformed plants. *Nature Protocols*, 1(4), 2019–2025.

Sun, W., Ma, Z., Chen, H. & Liu, M. (2020) Genome-wide investigation of WRKY transcription factors in Tartary buckwheat (*Fagopyrum tataricum*) and their potential roles in regulating growth and development. *PeerJ*, 8, e8727.

Suvorova, G. (2016) Chapter twenty nine - buckwheat tissue cultures and genetic transformation. In: Zhou, M., Kreft, I., Woo, S.-H., Chrungoo, N. & Wieslander, G. (Eds.) *Molecular breeding and nutritional aspects of buckwheat*. Cambridge: Academic Press, pp. 365–375.

Swain, S., Myers, Z.A., Siriwardana, C.L. & Holt, B.F., 3rd. (2017) The multifaceted roles of NUCLEAR FACTOR-Y in *Arabidopsis thaliana* development and stress responses. *Biochimica et Biophysica Acta*, 1860(5), 636–644.

Tang, Y., Liu, X., Liu, X., Li, Y., Wu, K. & Hou, X. (2017) Arabidopsis NF-YCs mediate the light-controlled hypocotyl elongation via modulating histone acetylation. *Molecular Plant*, 10(2), 260–273.

Tsurunaga, Y., Takahashi, T., Katsube, T., Kudo, A., Kuramitsu, O., Ishiwata, M. et al. (2013) Effects of UV-B irradiation on the levels of anthocyanin, rutin and radical scavenging activity of buckwheat sprouts. *Food Chemistry*, 141(1), 552–556.

Wang, C.L., Dong, X.N., Ding, M.Q., Tang, Y.X., Zhu, X.M., Wu, Y.M. et al. (2016) Plantlet regeneration of Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) in vitro tissue cultures. *Protein and Peptide Letters*, 23(5), 468–477.

Wang, J., Ma, Z., Tang, B., Yu, H., Tang, Z., Bu, T. et al. (2021) Tartary buckwheat (*Fagopyrum tataricum*) NAC transcription factors FtNAC16 negatively regulates of pod cracking and salinity tolerant in Arabidopsis. *International Journal of Molecular Sciences*, 22(6), 3197.

Warpeha, K.M., Upadhyay, S., Yeh, J., Adamiak, J., Hawkins, S.I., Lapik, Y.R. et al. (2007) The GCR1, GPA1, PRN1, NF-Y signal chain mediates both blue light and abscisic acid responses in Arabidopsis. *Plant Physiology*, 143(4), 1590–1600.

Wei, Q., Wen, S., Lan, C., Yu, Y. & Chen, G. (2020) Genome-wide identification and expression profile analysis of the NF-Y transcription factor gene family in *Petunia hybrida*. *Plants (Basel)*, 9(3), 336.

Wenkel, S., Turck, F., Singer, K., Gissot, L., Le Gourrierec, J., Samach, A. et al. (2006) CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of Arabidopsis. *Plant Cell*, 18(11), 2971–2984.

West, M., Yee, K.M., Danao, J., Zimmerman, J.L., Fischer, R.L., Goldberg, R. B. et al. (1994) LEAFY COTYLEDON1 is an essential regulator of late embryogenesis and cotyledon identity in Arabidopsis. *Plant Cell*, 6(12), 1731–1745.

Yan, H., Liu, C., Zhao, J., Ye, X., Wu, Q., Yao, T. et al. (2021) Genome-wide analysis of the *NF-Y* gene family and their roles in relation to fruit development in Tartary buckwheat (*Fagopyrum tataricum*). *International Journal of Biological Macromolecules*, 190, 487–498.

Yao, P., Huang, Y., Dong, Q., Wan, M., Wang, A., Chen, Y. et al. (2020) FtMYB6, a light-induced SG7 R2R3-MYB transcription factor, promotes Flavonol biosynthesis in Tartary buckwheat (*Fagopyrum tataricum*). *Journal of Agricultural and Food Chemistry*, 68(47), 13685–13696.

Yao, P.F., Li, C.L., Zhao, X.R., Li, M.F., Zhao, H.X., Guo, J.Y. et al. (2017) Overexpression of a Tartary buckwheat gene, *FtbHLH3*, enhances drought/oxidative stress tolerance in transgenic Arabidopsis. *Frontiers in Plant Science*, 8, 625.

Yao, P., Sun, Z., Li, C., Zhao, X., Li, M., Deng, R. et al. (2018) Overexpression of *Fagopyrum tataricum FtbHLH2* enhances tolerance to cold stress in transgenic Arabidopsis. *Plant Physiology and Biochemistry*, 125, 85–94.

Ye, L.L., Wang, B., Zhang, W.G., Shan, H.Y. & Kong, H.Z. (2016) Gains and losses of cis-regulatory elements led to divergence of the Arabidopsis *APETALA1* and *CAULIFLOWER* duplicate genes in the time, space, and level of expression and regulation of one paralog by the other. *Plant Physiology*, 171(2), 1055–1069.

Yokosho, K., Yamaji, N., Mitani-Ueno, N., Shen, R.F. & Ma, J.F. (2016) An aluminum-inducible *IREG* gene is required for internal detoxification of aluminum in buckwheat. *Plant & Cell Physiology*, 57(6), 1169–1178.

Yu, T.F., Liu, Y., Fu, J.D., Ma, J., Fang, Z.W., Chen, J. et al. (2021) The NF-Y-PYR module integrates the abscisic acid signal pathway to regulate plant stress tolerance. *Plant Biotechnology Journal*, 19(12), 2589–2605.

Zhang, D., Jiang, C., Huang, C., Wen, D., Lu, J., Chen, S. et al. (2019) The light-induced transcription factor FtMYB116 promotes accumulation of rutin in *Fagopyrum tataricum*. *Plant, Cell & Environment*, 42(4), 1340–1351.

Zhang, L., Li, X., Ma, B., Gao, Q., Du, H., Han, Y. et al. (2017) The Tartary buckwheat genome provides insights into Rutin biosynthesis and abiotic stress tolerance. *Molecular Plant*, 10(9), 1224–1237.

Zhang, W., Tang, Y., Hu, Y., Yang, Y., Cai, J., Liu, H. et al. (2021) Arabidopsis NF-YCs play dual roles in repressing brassinosteroid biosynthesis and signaling during light-regulated hypocotyl elongation. *Plant Cell*, 33(7), 2360–2374.

Zheng, Y., Jiao, C., Sun, H., Rosli, H.G., Pombo, M.A., Zhang, P. et al. (2016) iTAK: a program for genome-wide prediction and classification of plant transcription factors, transcriptional regulators, and protein kinases. *Molecular Plant*, 9(12), 1667–1670.

Zhou, Y., Zhang, Y., Wang, X., Han, X., An, Y., Lin, S. et al. (2020) Root-specific NF-Y family transcription factor, PdNF-YB21, positively regulates root growth and drought resistance by abscisic acid-mediated indoylacetic acid transport in Populus. *The New Phytologist*, 227(2), 407–426.

Zhu, F. (2016) Chemical composition and health effects of Tartary buckwheat. *Food Chemistry*, 203, 231–245.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Zhang, L., Yung, W.-S., Sun, W., Li, M.-W., Huang, M. (2022) Genome-wide characterization of nuclear factor Y transcription factors in *Fagopyrum tataricum*. *Physiologia Plantarum*, 174(2), e13668. Available from: https://doi.org/10.1111/ppl.13668