Genome-Wide Identification and Analysis of the NF-Y Gene Family in Potato (Solanum tuberosum L.)

Zhen Liu1, Yuanming Li2, Jinyong Zhu3, Wenjing Ma3, Zhitao Li3, Zhenzhen Bi3, Chao Sun3, Jiangping Bai3, Junlian Zhang2 and Yuhui Liu1*

1 State Key Laboratory of Aridland Crop Science, Gansu Agricultural University, Lanzhou, China, 2 College of Horticulture, Gansu Agricultural University, Lanzhou, China, 3 College of Agronomy, Gansu Agricultural University, Lanzhou, China

Nuclear factor Y (NF-Y) is a ubiquitous transcription factor in eukaryotes, which is composed of three subunits (NF-YA, NF-YB, and NF-YC). NF-Y has been identified as a key regulator of multiple pathways in plants. Although the NF-Y gene family has been identified in many plants, it has not been reported in potato (Solanum tuberosum). In the present study, a total of 41 NF-Y proteins in potato (StNF-Ys) were identified, including 10 StNF-YA, 22 StNF-YB, and nine StNF-YC subunits, and their distribution on chromosomes, gene structure, and conserved motif was analyzed. A synteny analysis indicated that 14 and 38 pairs of StNF-Y genes were orthologous to Arabidopsis and tomato (Solanum lycopersicum), respectively, and these gene pairs evolved under strong purifying selection. In addition, we analyzed the expression profiles of NF-Y genes in different tissues of double haploid (DM) potato, as well as under abiotic stresses and hormone treatments by RNA-seq downloaded from the Potato Genome Sequencing Consortium (PGSC) database. Furthermore, we performed RNA-seq on white, red, and purple tuber skin and flesh of three potato cultivars at the tuber maturation stage to identify genes that might be involved in anthocyanin biosynthesis. These results provide valuable information for improved understanding of StNF-Y gene family and further functional analysis of StNF-Y genes in fruit development, abiotic stress tolerance, and anthocyanin biosynthesis in potato.

Keywords: potato (Solanum tuberosum), nuclear factor Y transcription factor, expression profiles, abiotic stress, anthocyanin biosynthesis

INTRODUCTION

Nuclear factor Y (NF-Y) transcription factor, also known as CCAAT-box binding factor (CBF) or heme-associated protein (HAP) (Mantovani, 1999), is a ubiquitous transcription factor in eukaryotes (Zhao et al., 2017). NF-Y is a trimeric transcription factor composed of three distinct subunits, namely, NF-YA, NF-YB, and NF-YC (Nardini et al., 2013). To form the NF-Y complex, the histone folding motifs of NF-YB and NF-YC interact in the cytoplasm to form heterodimer,
which is transferred to the nucleus. Upon arrival in the nucleus, the NF-YA subunit is recruited and combines with NF-YB/NF-YC heterodimer to produce mature NF-Y complex (Kahle et al., 2005; Laloun et al., 2013). In mature NF-Y complex, the NF-YA subunit provides a distinctive sequence that specifically binds to the CCAAT cis-element and regulates the expression of target genes (Hackenberg et al., 2012; Yan et al., 2013; Cao et al., 2014; Xu et al., 2016). In yeast and mammals, each NF-Y subunit is encoded by a single gene; however, three NF-Y subunits are encoded by multiple genes in plants (Li et al., 1992; Petroni et al., 2012).

In recent years, many NF-Ys were identified in Arabidopsis (Riechmann et al., 2000), rice (Oryza sativa) (Yang W. et al., 2017), tomato (Solanum lycopersicum) (Li et al., 2016), banana (Musa acuminata) (Yan et al., 2019), sorghum (Sorghum bicolor) (Malviya et al., 2016), watermelon (Citrus lanatus) (Yang J. et al., 2017), and canola (Brassica napus) (Liang et al., 2014). And, the functions of many NF-Y genes have been characterized to play multiple roles in plants, including embryogenesis (Kwong et al., 2003), seed germination (Liu et al., 2016), root growth (Sorin et al., 2014), flowering (Cao et al., 2014), fruit ripening (Li et al., 2016), as well as flavonoid biosynthesis (Wang et al., 2020) and response to various abiotic stresses (Li et al., 2008; Ni et al., 2013; Sato et al., 2014; Wang et al., 2018).

For example, AtLEC1 (AtNF-YB9) is a key regulator of late embryogenesis and seed development in Arabidopsis, and the ectopic expression of AtLEC1 caused abnormality in tobacco transgenic seedling (Guo et al., 2013). The overexpression of AtNF-YA1 and AtNF-YA9 significantly affects male gametophyte development, embryo development, seed morphology, and seed germination (Mu et al., 2013). The overexpression of AtNF-YA5, which is strongly induced under drought, increases drought tolerance of Arabidopsis plants (Li et al., 2008). The constitutive expression of TaNF-YA10 significantly increases the sensitivity of Arabidopsis plants to salinity (Ma et al., 2015). A recent report suggested that NF-Y complexes composed of NF-YB8, NF-YC1/9, and NF-YA1/9 play an important role in tomato flavonoid biosynthesis, and these complexes are involved in transcriptional regulation and H3K27me3 marking of the chalcone synthase (CHS1) locus (Wang et al., 2020). Although the NF-Y gene family has been extensively studied in plants, little report is yet available for potato.

Potato, which originated from the Andean regions of Peru and Bolivia (Spooner et al., 2005), is an important food crop worldwide. As a new kind of natural pigment and antioxidant resource, the pigmented potato has attracted more and more attention (Fossen and Andersen, 2000). Flavonoids not only resource, the pigmented potato has attracted more and more worldwide. As a new kind of natural pigment and antioxidant

1. http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml
2. http://www.Arabidopsis.org
3. http://pfam.xfam.org
4. http://hmmer.org/download.html
5. http://smart.embl-heidelberg.de
6. http://web.expasy.org/protparam
7. http://alternate.meme-suite.org/tools/meme
8. http://gsds.cbi.pku.edu.cn
Chromosomal Localization and Gene Duplication

All StNF-Y genes were mapped to 12 potato chromosomes based on physical location information from PGSC using MapChart software (Voorrips, 2002). MCScanX (Wang et al., 2012) was used to analyze the duplication events of the StNF-Y genes and identified the synteny of NF-Y genes between potato and Arabidopsis. Ka (non-synonymous) and Ks (synonymous) of each pair of duplicated NF-Y genes were calculated using KaKs Calculator 2.0 (Wang et al., 2010).

Multiple Alignments, Phylogenetic Analysis, and Classification of NF-Ys

The multiple alignments were performed using ClustalW on the 41 StNF-Ys amino acid under default parameters. The full-length amino acid sequences of 41 StNF-Ys, 54 SlNF-Ys (tomato NF-Y), and 36 AtNF-Ys were used for phylogenetic analysis. The Molecular Evolutionary Genetics Analysis (MEGA) 7.0 software (Kumar et al., 2016) was used to construct an unrooted phylogenetic tree with the following parameters: Poisson model and 1,000 bootstrap replications.

Plant Materials and Treatments

Three potato cultivars, including “Xindaping” (XD—white skin and white flesh), “Lingtianhongmei” (LT—red skin and red flesh), and “Heimeiren” (HM—purple skin and purple flesh), were grown in a greenhouse at Gansu Agricultural University in Lanzhou, Gansu Province, China (Figure 1). Six fresh tubers (diameter = 4–5 cm) were collected from each cultivar for harvesting the skin tissue and flesh tissue. Skin tissues were carefully obtained from the cortical tissue using a scalpel. Flesh tissues were isolated at least 5-mm distance from the skin. The samples were immediately frozen in liquid nitrogen and stored in −80°C refrigerator for further use.

RNA-Seq Data Analysis

Total RNA of the aforementioned samples was chosen for further RNA-seq library construction. Each sample had three biological replications. Next-generation Illumina sequencing was performed by Sagene Biotech Corporation (Guangzhou, China). The raw RNA-seq data were uploaded to the NCBI (Project ID PRJNA541919). After RNA sequencing, the clean reads were obtained by trimming the raw reads and filtering out contaminant adapters, Phred scores less than 20, and uncertain bases. The cleaned data were aligned to PGSC_DM_v3.4 gene models downloaded from Solanaceae Genomics Resource at Michigan State University by Bowtie2 (v2.2.9). Only reads with a perfect match or one mismatch were further analyzed.

Differential Expression Genes Analysis

Differential genes were identified using fragments per kilobase of exon per million fragments mapped (FPKM). Genes with the absolute value of log2 fold change (FC) ≥ 1 and a false discovery rate (FDR) < 0.05 were considered significant differentially expressed genes (DEGs).

The DEGs were annotated against non-redundant database (Nr), SwissProt/UniProt Plant Proteins, Kyoto Encyclopedia of Genes and Genomes (KEGG), Cluster of Orthologous Groups of proteins (COG/KOG), and the potato protein database by BLASTX, with a cut-off E-value of 1e-5. Then, DEGs were subjected to enrichment analysis of GO functions and KEGG pathways.

RNA Isolation and Quantitative Real-Time PCR

Total RNA isolation was performed with the RNA extraction kit (Tiangen DP419, Beijing, China). The integrity of RNA was monitored by agarose gel electrophoresis, and the concentration was detected using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, United States). The cDNAs were synthesized using a FastKing RT kit with gDNAse (Tiangen KR116, Beijing, China). Quantitative real-time PCR (qPCR) was performed with SuperReal PreMix Plus kit (SYBR Green FP205, Tiangen, Beijing, China) in Bio-Rad CFX96 (Bio-Rad, Hercules, CA, United States). The PCR program was as follows: 30 s at 95°C, followed by 40 cycles of 5 s at 95°C and 30 s at 60°C, followed by 65–95°C melting curve detection. Three independent biological replicates were used for each gene.

FIGURE 1 | Phenotypes of three potato cultivars in (A) “Xindaping” (XD—white skin and white flesh), “Lingtianhongmei” (LT—red skin and red flesh), and “Heimeiren” (HM—purple skin and purple flesh). (B) The content of anthocyanin in three potato cultivars. Data represent the mean of three biological replicates ± standard error. Standard errors are shown as bars above the columns. Different letters above bars denote significant difference at P < 0.05.
were constructed using online STRING v11.0 software (http://string-db.org/) to predict the protein interaction network. The protein interactions among pigmented potato cultivars, we selected StNF-Ys with FPKM > 5 to predict the protein interaction network. Based on the RNA-seq dataset of pigmented potato cultivars (XD, LT, and HM) were analyzed based on RNA-seq. The Illumina sequencing was performed by Sagene Biotech Corporation (Guangzhou, China). The raw data were uploaded on NCBI (Project ID PRJNA541919).

The Interaction Network of NF-Y Proteins

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) is a database of known and predicted protein–protein interactions. Based on the RNA-seq dataset of pigmented potato cultivars, we selected StNF-Ys with FPKM > 5 to predict the protein interaction network. The protein interactions were constructed using online STRING v11.0 software with a combined score > 400 (medium confidence) (Szklarczyk et al., 2016). As active interaction sources, text mining, experiments, databases, co-expression, neighborhood, gene fusion, and co-occurrence were selected. The interaction network was visualized by Cytoscape v3.7.1 (Shannon et al., 2003).

Statistical Analysis

For qPCR analyses, data were presented as means (±SE) of three biological replicates. Statistical significance was determined by one-way ANOVA followed by the least significant difference (LSD) computed at P < 0.05.

RESULTS

Identification and Chromosomal Distribution of StNF-Ys

To obtain the NF-Y family members in potato, the HMM and BLASTP algorithm search were combined to analyze the potato genome. A total of 41 StNF-Y genes were identified and confirmed in the potato genome, including 10 StNF-YA, 22 StNF-YB, and 9 StNF-YC (Supplementary Table 2). The transcript IDs were converted to corresponding gene IDs and the “SC0003DMG40” in the gene ID was omitted for brevity. The bioinformatics data of StNF-Y members were analyzed, including the number of amino acid residues, theoretical molecular weight (MM), and theoretical isoelectric point (pl). The StNF-Y proteins varied in length and physicochemical properties, the number of amino acid residues of StNF-Y proteins ranged from 120 (PG0013302) to 311 (PG0021365), the molecular weights were between 13.82 kDa (PG0013302) and 33.97 kDa (PG0021365), and the pI values ranged from 4.64 (PG0023065) to 9.65 (PG0002484). The details are listed in Supplementary Table 2.

The identified 41 StNF-Y genes were distributed unevenly on 12 chromosomes (Figure 2A). Seven StNF-Y genes were located on chromosomes 1 and 5, which had the largest number of StNF-Y genes. In contrast, only one StNF-Y was distributed on chromosome 8, which had the least number of StNF-Y genes (Figure 2B). In addition, the high densities of StNF-Y genes were distributed at the proximal end of chromosome 1 and the distal end of chromosome 5.

Multiple Alignments, Phylogenetic Analysis, and Classification of StNF-Ys

Multiple sequence alignments showed that StNF-Y family proteins had conserved region domains as shown in Figure 3. The results showed that each member of StNF-Y contains a heterodimerization domain and a DNA-binding domain that recognizes the CCAAT site. The StNF-YA subunit contains two conserved domains (Figure 3A); one is the NF-YB and NF-YC interaction domain and the other is the DNA-binding domain. In this DNA-binding domain, three histidine (H) and three arginine (R) residues are absolutely conserved and essential (Xing et al., 1993). The conserved domain of StNF-YB subunit was about 93 AAs in length, which contained a NF-YA interaction region, a NF-YC interaction region, and a DNA-binding domain (Figure 3B). Previous studies have shown that the NF-YB subunits could be divided into LEC1 type and non-LEC1 type according to sequence similarity; the LEC1 type is composed of LEC1 and LEC1-like (L1L) (Lee et al., 2003; Siefers et al., 2009).

As shown in Figure 3B, the key amino acids that distinguish Lec1 from non-LEC1 were represented in the red box, and aspartate (D) at this site was considered to be specific for LEC1 type. The conserved domains of StNF-YC subunits contained three heterodimerization regions and one DNA-binding domain, which was about 79 AAs in length (Figure 3C).

To further investigate the phylogenetic relationships of NF-Ys among potato, tomato, and Arabidopsis, an unrooted phylogenetic tree of 41 StNF-Ys, 54 SinNF-Y, and 36 Arabidopsis protein sequences was constructed by MEGA 7.0 software, as shown in Figure 4. The 131 NF-Ys were divided into three distinct subunits (NF-YA, NF-YB, and NF-YC). Ten StNF-Ys, 10 SinNF-Ys, and 10 Arabidopsis-Ys belonged to the NF-YA subunit. Twenty-two StNF-Ys, 27 SinNF-Ys, and 13 Arabidopsis-Ys were assigned to the NF-YB subunit. Nine StNF-Ys, 17
SINF-Ys, and 13 AtNF-Ys belonged to the NF-YC subunit. Notably, a distinct cluster was identified as LECI-type group in NF-YB, including AtNF-YB6 (LEC1-like), AtNF-YB9 (LEC1), 13 SINF-Ys, and eight StNF-YBs (PG1013505, PG2013505, PG0013503, PG0013302, PF0013502, PG0030986, PG0022277, and PG0034306).

**Gene Structure and Conserved Motifs of StNF-Ys**

The full-length amino acid sequences of 41 StNF-Ys were used to construct an unrooted phylogenetic tree by MEGA 7.0 software. Based on phylogenetic analysis of potato, tomato, and Arabidopsis, 41 StNF-Ys were divided into three subunits (Figure 5A). A gene structure analysis can provide insight into the evolution of gene family. Therefore, we analyzed the organization of exons and introns (Figure 5B). The results showed that all of StNF-YA genes were separated by introns, of which eight StNF-YAs contained four introns, one StNF-YA contained three introns, and one StNF-YA contained two introns. Most of StNF-YBs and StNF-YCs did not have introns, including 14 StNF-YBs and six StNF-YCs; the rest of StNF-YBs and StNF-YCs contained 1–4 introns. In general, the gene structure of StNF-YA members was more conserved than that of StNF-YBs and StNF-YCs.

In order to study the diversification of StNF-Ys, we searched for conserved motifs of these proteins using the online MEME program. The details of the 20 motifs are in Supplementary Table 3. The results showed that the three StNF-Y subunits have a unique motif distribution (Figure 5C). Motifs 12, 17, and 18 were specifically present in the StNF-YA subunits. Motifs 1, 9, 10, 16, and 19 were unique to the StNF-YB subunits. Motifs 4, 9, 13, 15, and 20 were present only in StNF-YC subunits. Motif 2 was distributed in StNF-YA and StNF-YB subunits. Motif 3 was present in StNF-YB and StNF-YC subunits. Motif 6 was widely distributed in three StNF-Y subunits. Overall, each subunit had a conserved motif composition.

![FIGURE 2](#) Genomic distributions of 41 StNF-Y genes on 12 potato chromosomes. (A) “StNF-Y gene distribution map” on 12 potato chromosomes. Tandemly duplicated genes were marked by red boxes. (B) Numbers of StNF-Y genes on each chromosome in potato.
Gene Duplication and Genome Synteny

Tandem and segmental duplications are crucial for the extension of gene family members and the realization of new functions (Cannon et al., 2004). In the present study, three pairs of StNF-Y genes (5/41, 12.20%) were identified as tandemly duplicated genes, of which chromosome 3 had two pairs and chromosome 5 had one pair (Figure 2A and Supplementary Table 4). In addition to tandem duplication, six pairs (10/41, 24.39%) of segmental duplication events were identified (Figure 6 and Supplementary Table 4).

In order to further investigate the potential evolution processes of the NF-Y gene family, we analyzed the synteny relationship of NF-Ys among potato, Arabidopsis, and tomato. A total of 14 pairs of orthologs were identified between potato and Arabidopsis, and 38 pairs of orthologs between potato and tomato were identified (Figure 6 and Supplementary Tables 5, 6).

The substitution rate of non-synonymous (Ka) and synonymous (Ks) is the basis for evaluating the positive selection pressure of duplication events. Ka/Ks = 1 indicates neutral selection, Ka/Ks < 1 denotes purification selection, and Ka/Ks > 1 signifies positive selection. The Ka/Ks of duplication NF-Y genes was calculated using KaKs Calculator 2.0. The results showed that the Ka/Ks of tandem and segmental duplications ranged from 0.0787 to 0.9482, with a mean value of 0.3569 (Supplementary Table 4). The Ka/Ks of the orthologous relationship between potato and Arabidopsis ranged from 0.0305 to 0.3507, with a mean value of 0.1736 (Supplementary Table 5).

And, the Ka/Ks of the orthologous relationship between potato and tomato.
and tomato ranged from 0.001 to 0.9260, with a mean value of 0.2863 (Supplementary Table 6). The Ka/Ks values were < 1, suggesting that these genes had evolved under the effect of purifying selection.

Expression Profiles of StNF-Y Genes in Different Tissues

Based on transcriptome data downloaded from PGSC, the expression patterns of StNF-Y genes in 13 tissues (leaves, roots, shoots, tubers, sepals, stamens, stolons, mature flowers, petals, carpels, immature fruit, and mature fruit) of DM potato were analyzed (Figure 7 and Supplementary Table 7). The results showed that 14 StNF-Y genes (PG0001733, PG0003296, PG0003357, PG0004076, PG0004299, PG0006863, PG0007350, PG0008989, PG0101224, PG01024603, PG0026357, and PG0027390) were highly expressed in all tissues with FPKM > 5. Four StNF-Y genes (PG0011385, PG01013505, PG0039636, and PG004745) were not expressed in all tissues (FPKM = 0). In addition, some StNF-Y genes showed tissue-specific expression patterns, e.g., PG002136 was highly expressed in stolon and PG0044580 was highly expressed in the tuber. It is noteworthy that six StNF-YB genes (PG0013502, PG0013503, PG0015177, PG0022277, PG0030986, and PG0034306) were specifically expressed in immature fruit with FPKM > 5 and log2FC > 1, which were in the same cluster as AtNF-YB9 (LEC1) and AtNF-YB6 (LEC1-like).

Expression Profiles of StNF-Y Genes Under Abiotic Stresses and Hormone Treatments

The RNA-seq data was downloaded from the PGSC database to analyze the expression profiles of StNF-Y genes in DM potato under abiotic stresses. The results showed that six, nine, and 11 StNF-Y genes were differentially expressed...
(FPKM > 1 and | log₂FC| > 1) under salt, mannitol, and heat treatments, respectively. Among them, four, seven, and nine StNF-Y genes were upregulated under salt, mannitol, and heat treatments, respectively. In addition, two StNF-Y genes (PG2015259 and PG0021365) were differentially expressed under three stresses, five StNF-Y genes (PG0002484, PG0004299, PG0022172, PG0028144, and PG0044580) were differentially expressed under two stresses, and 10 genes (PG0004076, PG0008148, PG0008989, PG0011385, PG0014643, PG1021124, PG0021747, PG0022680, PG0023065, and PG0032552) were only differentially expressed under single abiotic stress (Figure 8A and Supplementary Table 8).

To further explore the expression changes in the StNF-Y genes under various hormone treatments (BAP, ABA, IAA, and GA3), the expression of 41 StNF-Y genes was analyzed using the RNA-seq data downloaded from the PGSC database. The results indicated that 22 and 10 StNF-Y genes responded to BAP and ABA treatments (FPKM > 1 and | log₂FC| > 1),
respectively. All of 22 StNF-Y genes were downregulated under BAP treatments, and all of 10 StNF-Y genes were upregulated under ABA treatments (FPKM > 1 and \(| \log_2 FC | > 1 \)). However, all of StNF-Y genes were not differentially expressed under IAA and GA3 treatments, implying that StNF-Y genes did not respond to IAA and GA3 treatments (Figure 8A and Supplementary Table 8).

Combined with RNA-seq data analysis under abiotic stress and hormone treatment, four StNF-Y genes (PG0002484, PG0008989, PG0023065, and PG0028144) were differentially expressed (FPKM > 1 and \(| \log_2 FC | > 1 \)) under mannitol and ABA treatments in DM potato. The results indicate that these four StNF-Y genes may be involved in drought stress response through an ABA-dependent pathway.
Expression Profiles of StNF-Y Genes in Pigmented Potato Cultivars

To identify the potential functions of StNF-Y genes in flavonoid biosynthesis of potato, the expression patterns of the StNF-Y genes in tuber tissues (skin and flesh) of tetraploid pigmented potato cultivars were analyzed according to RNA-seq dataset. The results showed that eight StNF-Y genes (PG0011385, PG0013502, PG0013503, PG0013302, PG0015260, PG0024745, PG0039636, and PG0022277) were not expressed (FPKM = 0) in the skin, and 12 StNF-Y genes were differentially expressed in pigmented skin compared with the white skin of XD (FPKM > 1 and |log₂FC| > 1). Among them, six genes (PG0002484, PG0008148, PG0008989, PG0010697, PG0028144, and PG0044580) were upregulated and four genes (PG0022680, PG0034306, PG1013505, and PG2013505) were downregulated in LTS and one gene (PG0002484) was upregulated and five genes (PG0003357, PG0026357, PG0034306, PG1013505, and PG2013505) were downregulated in HMS. In pigmented skin, one gene (PG0002484) was upregulated both in the skin of HM and LT,
Liu et al. NF-Y Gene Family in Potato (Solanum tuberosum L.)

FIGURE 8 | The expression profiles of StNF-Y genes. (A) The expression profiles of StNF-Y genes under abiotic stresses (salt, mannitol, and heat stress) and hormone treatments (BAP, ABA, IAA, and GA3) in DM potato. The color scale was plotted using the log2 FC of each gene. (B) The expression profiles of StNF-Y genes in white and pigmented potato tuber skin and flesh. XDS, LTS, HMS represent the white skin of white potato cultivar (Xindaping, XD), the red skin of red potato cultivar (Lingtianhongmei, LT), and the purple skin of purple potato cultivar (Heimeiren, HM), respectively. XDF, LTF, and HMF represent the white flesh of XD, the red flesh of LT, and the purple flesh of HM, respectively. The color in the heat map represents the FPKM value using logarithm with a base of 2. The heatmap was constructed by TBtools software. BAP, benzylaminopurine; ABA, abscisic acid; IAA, indole acetic acid; GA3, gibberellic acid.
and three genes (PG0034306, PG1013505, and PG2013505) were downregulated both in the skin HM and LT (Figure 8B and Supplementary Table 9).

In tuber flesh, eight StNF-Y genes were not expressed (FPKM = 0), and these eight genes also were not expressed in the skin. Compared with the white flesh of XD, a total of 17 StNF-Y genes were differentially expressed (FPKM > 1 and | log2FC| > 1) in pigmented flesh, of which eight genes (PG0001733, PG0002484, PG0003296, PG0004299, PG0006863, PG0021365, PG0021747, and PG0044580) were upregulated in both LTF and HMF and one gene (PG0034306) was downregulated in pigmented flesh (Figure 8B and Supplementary Table 9). It is noteworthy that one gene (PG0002484) was upregulated, and one gene (PG0034306) was downregulated in pigmented tissues (skin and flesh).

Eight StNF-Y genes with relatively high expression levels in pigmented tissues were selected as targets, and the reliability of RNA-seq dataset was verified by qPCR. The results confirmed that the qPCR expression patterns were in agreement with the RNA-seq dataset (Figure 9A), showing a high correlation (R² = 0.8393) between the RNA-seq dataset and the simple linear regression equation of qPCR (y = 0.6430x + 0.0924), indicating the consistency of the two analysis methods (Figure 9B).

The Interaction Network of StNF-Y Proteins

Previous studies have shown that NF-Y subunits are involved in flavonoid synthesis, and they play a role through interaction with each other (Hackenberg et al., 2012; Mu et al., 2013; Wang et al., 2020). We further investigated the interaction between StNF-Y proteins that might be involved in flavonoid biosynthesis. Based on the RNA-seq dataset of pigmented potato cultivars, we selected highly expressed StNF-Y (FPKM > 5) to predict the protein interaction network with median confidence (combination score > 400). The results showed that a total of 89 pairs of interacting proteins were predicted. Twenty-one StNF-Y proteins (eight StNF-YAs, 10 StNF-YBs, and three StNF-YCs) were included in the protein interaction network, of which, eight StNF-Y genes (PG0003296, PG0004299, PG0013135, PG0002484, PG0006863, PG0021747, PG0045580, and PG0034306) were differentially expressed in pigmented tissues compared to that in white tissues. These eight StNF-Ys contained four StNF-YA subunits, three StNF-YBs, and one StNF-YC. In addition, we found that StNF-YA subunits did not interact with each other at high confidence (combined score > 400), whereas they showed a direct interaction with StNF-YBs or StNF-YCs (Figure 10 and Supplementary Tables 10, 11).

DISCUSSION

NF-Ys are important transcription factors in plants, which play critical roles in plant growth, development, and stress responses (Yan et al., 2013; Liu et al., 2016; Xu et al., 2016; Yu et al., 2020). However, little is known about NF-Ys in potato. In the present study, a total of 41 StNF-Y genes were identified, including 10 StNF-YAs, 22 StNF-YBs, and nine StNF-YCs. The bioinformatics analysis included a study of the phylogenetic relationships between potato and Arabidopsis NF-Y proteins and an analysis of chromosomal location, gene structures, encoded protein conserved motifs, and gene duplication events of StNF-Y genes. In addition, the expression pattern of StNF-Y genes in different tissues and their response to various abiotic stresses and hormone treatments were analyzed. This study provided comprehensive information for further investigation of their biological functions and the evolution of StNF-Y gene family.

Expansions of StNF-Y Gene Family in Potato

Gene duplication plays a major role in the expansion of gene families and may be an important driver of evolutionary diversification (Lawton-Rauh, 2003). In the present study, nine pairs of duplication genes were identified in potato, of which the number of StNF-Y genes arranged in tandem duplications contributed to 12.20% (5/41) and segmental duplication events contributed to 24.39% (10/41). These results suggested that tandem and segmental duplication events were the main mechanisms of StNF-Y gene family evolution and that some of the StNF-Y genes were possibly generated by gene duplication events. Duplication events often lead to increased functional diversity between duplicated gene pairs, and some new members may acquire new functions or lose their original functions (Dias et al., 2003; Singh et al., 2012). In our study, three StNF-Y genes (PG0039636, PG0015260, and PG2015259) were tandem duplication, of which PG2015259 was downregulated under various abiotic stresses (salt, mannitol, and heat) and highly expressed in 13 tissues (FPKM > 5), while the PG0039636 and PG0015260 were not expressed (FPKM = 0), indicating that PG0039636 and PG0015260 may become pseudogenes during duplication events and evolution. In addition, some duplication gene pairs were expressed consistently, but some were expressed inconsistently, such as PG0021747/PG0002484 and PG0021365/PG0028144 that were segmental duplication gene pairs. Among them, PG0021747 and PG0002484 were upregulated in pigmented flesh; however, PG0021365 was downregulated and PG28144 was upregulated under salt and mannitol stresses. In conclusion, gene duplication provided a large number of functional or non-functional genes for the potato genome.

Phylogenetic Analysis and Evolution of StNF-Ys

To investigate the relationship of NF-Y proteins among potato, tomato, and Arabidopsis, an unroot phylogenetic tree was constructed using complete NF-Y protein sequences. The results showed that there were 10 NF-YA in all three plants (potato, tomato, and Arabidopsis). However, the amounts of NF-YB and NF-YC varied widely; there are 13 YBs and 13 YCs in Arabidopsis, 22 YBs and 9 YCs in potato, and 27 YBs and 17 YCs in tomato, respectively, suggesting that NF-YB and NF-YC play an important role in the expansion of NF-Y gene family.
FIGURE 9 | The qPCR expression analyses of eight StNF-Y genes in (A) the skin/flesh (S/F) of white (XD), purple (HM), and red (LT) cultivars. (B) The correlation between qPCR and RNA-seq analysis. Error bars represent the means ± SE of three biological replicates. Different letters above bars denote significant difference at \( p < 0.05 \). Column graphs represent the relative expression levels of genes determined by qPCR; the values are shown on the left \( y \)-axis, and line graphs represent the FPKMs obtained by RNA-seq; the values are shown on the right \( y \)-axis.

Studies have shown that AtNF-YC1/2/3/4/9 controls flowering time (Siefers et al., 2009; Kumimoto et al., 2010; Hackenberg et al., 2012). Three StNF-YCs (PG0003357, PG0008989, and PG2015259) were clustered with AtNF-YC1/2/3/4/9, indicating that these StNF-Ys may have a similar function as AtNF-YC1/2/3/4/9. Previous studies have reported that the AtLEC1 and AtLEC1-like play an important role in embryo development and fruit ripening (Kwong et al., 2003; Li et al., 2016; Gago et al., 2017). It is noteworthy that AtLEC1 (AtNF-YB9) and AtLEC1-like (AtNF-YB6) formed a distinct cluster in NF-YB (Figure 4). Our study found that eight StNF-YB genes were clustered with AtLEC1 and AtLEC1-like in one cluster, of which six StNF-YB genes were specifically expressed in immature fruits. It is suggesting that LEC1 and LEC1-like genes were extensively extended during potato evolution and that these genes may be associated with embryo development and fruit ripening.

Expression Profile Analysis of StNF-Y Genes Under Abiotic Stress and Hormone Treatment

Previous studies have shown that the NF-Y family is involved in various abiotic stresses (Li L. et al., 2013; Li Y. J. et al., 2013; Alam et al., 2015; Feng et al., 2015). In Arabidopsis, AtNF-YA1 (At5G12840) is significantly induced by NaCl, mannitol, PEG, and ABA, and it is involved in the regulation of post-germination growth arrest under salt stress (Li Y. J. et al., 2013). PG0006863 was homologous to AtNF-YA1, and it was highly

\[ y = 0.6430x + 0.0924 \]

\[ R^2 = 0.8393 \]
expressed under mannitol treatment and upregulated under ABA treatment in DM potato, indicating that PG0006863 may function in a similar way as AtNF-YA1 under mannitol treatment. Li et al. (2008) found that overexpression of AtNF-YA5 (AT1G54160) in Arabidopsis can increase the tolerance of plants to drought stress through the ABA-dependent pathway. PG0002484 was homologous to AT1G54160, which was upregulated under salt, mannitol, and ABA treatments in DM potato, suggesting that PG0002484 may have similar functions to AtNF-YA5. Overexpression of AtNF-YA7 (AT1G30500) enhanced tolerance to various abiotic stresses in Arabidopsis (Leyva-González et al., 2012). PG1021124, homologous to AtNF-YA7, was upregulated under heat stress and differentially expressed under various hormone treatments in DM potato, suggesting that PG1021124 may have similar functions to AtNF-YA7 under abiotic stresses. Overexpression of NF-YA10 can improve tolerance to drought and salt stress in Arabidopsis (Ma et al., 2015; Yu et al., 2020). PG0028144 and PG0021365 were a pair of segmental duplication genes, which were clustered with AtNF-YA10 (AT6G06510). PG0021365 was differentially expressed under mannitol and heat treatments, while PG0028144 was upregulated under salt, mannitol, and ABA treatments. The results indicated that PG0028144 may be induced by drought stress in an ABA-dependent manner, while PG0021365 was not. Sato et al. (2019) found that overexpression of AtNF-YB2 (AT5G47640) and AtNF-YB3 (AT4G14540) specifically enhances drought and heat stress tolerance, respectively. PG0004299, PG0044580, and PG0022172 were clustered with AtNF-YB2 and AtNF-YB3 in

FIGURE 10 | Protein interaction network predicted for StNF-Y (combined score of 400, medium confidence). The purple lines represent the highest confidence (combined score > 900), the green lines indicate high confidence (combined score > 700), and the gray lines indicate medium confidence (combined score > 400). NF-YA subunits are highlighted in purple, NF-YB subunits are highlighted in blue, and NF-YC subunits are highlighted in yellow, respectively. The color scale was plotted using the log2 FC of each gene.
Expression of StNF-Y Genes in Pigmented Tuber Tissue

The expression profiles of StNF-Y genes in different tuber tissues (skin and flesh) of tetraploid pigmented potato cultivars were analyzed based on the RNA-seq dataset. Studies have shown that the NF-Y genes play crucial roles in flavonoid biosynthesis, e.g., NF-YB8 interacts with NF-YC1 and then recruits NF-YA1/9 to form NF-Y complexes that bind the CCAAT element in the CHS1 promoter to regulate the flavonoid biosynthesis in tomato (Wang et al., 2020). PG0001733 was homologous to AtNF-YB8 (AT2G37060) and clustered with StNF-YB8c in one cluster, which was upregulated in pigmented flesh (LTF and HMF). PG2015259 was clustered with AtNF-YC1 (AT3G48590) and StNF-YC1 (Soly03g110860) in one cluster and was highly expressed in LTF and upregulated in HMF. PG0006863 and PG0008148 were homologous to SINF-YA9 (Soly01g087240), of which PG006863 was upregulated in LTF and HMF and PG0008148 was upregulated in LTS and LTF. In addition, PG0010697 was homologous to SINF-YA1a (Soly01g008490) and was highly expressed in pigmented tissues and upregulated in LTS. These results indicated that these five genes may have similar functions to SINF-YA1/9, SINF-YB8c, and SINF-YC1a in flavonoid biosynthesis. Furthermore, six genes were upregulated in pigmented tissues (LTF and HMF), of which three genes (PG0002484, PG0021365, and PG0021747) belonged to StNF-YA subunit, two genes (PG0004299 and PG0044580) were assigned to StNF-YB subunit, and one gene (PG0003296) was grouped into StNF-YC subunit.

In addition, we analyzed the correlation coefficient (CC) between differentially expressed StNF-Ys of potato tuber skin and flesh in RNA-seq data and anthocyanin content. The results showed that the expression of some StNF-Y genes was highly correlated to anthocyanin content in tuber flesh, such as PG0044580 and PG0021747 with CC > 0.7; PG0004299, PG0002484, and PG0010697 with CC > 0.8; and PG2015259 (CC = 0.8) (Supplementary Table 12), suggesting that these StNF-Y genes may be involved in the anthocyanin biosynthesis in potato tuber flesh, and their functions require further investigation.

Through the prediction of StNF-Y protein interaction network, we found that there were interaction relationships (high confidence with combined score > 700) between these differentially expressed genes (Figure 10 and Supplementary Table 10), i.e., two StNF-YAs (PG0021747 and PG0021365) directly interacted with one StNF-YB (PG0004299), and one StNF-YB (PG0004299) and one StNF-YA (PG0021365) interacted with one StNF-YC (PG2021259). These StNF-Ys may be involved in the biosynthesis of anthocyanins by interacting with each other to form the completed NF-Y complex, and their functions need to be further studied.

CONCLUSION

In this study, we identified a total of 41 StNF-Y genes from the potato genome, including 10 StNF-YA, 22 StNF-YB, and nine StNF-YC, and their distribution on chromosomes, multiple alignments, gene structure, conserved motifs, and phylogenetic relationships were analyzed. Synteny analysis showed that nine pairs of duplication genes were identified in the potato genome, which played an important role in the expansion of the StNF-Y gene family. Synteny analysis indicated that 14 and 38 pairs of StNF-Y genes were orthologous to Arabidopsis and tomato, respectively. Six StNF-YB genes were specifically expressed in immature fruit, which may be involved in fruit ripening. In addition, the StNF-Y genes displayed different expression patterns under the salt, mannitol, heat, and hormone treatments in DM potato. Furthermore, we analyzed the expression of StNF-Y genes in tetraploid pigmented potato cultivars and the protein interaction networks that were highly expressed in anthocyanin biosynthesis. The results of this study provide valuable information for further studies on the functions of the StNF-Y gene family.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

YHL, JB, and JLZ conceptualized the study. ZL, YML, JYZ, WM, ZB, and ZTL curated the data. ZL and CS conducted the formal analysis. ZL, YHL, and JLZ acquired funding. ZL contributed the software and wrote the original draft. YHL wrote, reviewed, and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by the Scientific Research Start-up Funds for Openly-recruited Doctors of Gansu Agricultural University (GAU-KYQD-2020-11), the Program for Innovation Ability Improvement of the Higher Education Institutions of Gansu Province (2020A-056), the Program for Longyuan Youth Innovation Team of Gansu Province (LRYCT-2020-1), the National Natural Science Foundation of China (31601356 and 31860398), the Gansu Science Foundation for Distinguished
Young Scholars (17JR5RA138), the Fuxi Talent Project of Gansu Agricultural University (Ganfx-02Y04), and the China Agriculture Potato Research System of MOF and MARA (CARS-09-P14).

ACKNOWLEDGMENTS

We would like to thank Yuanyuan Pu of Gansu Agricultural University for her help in the improvement of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2021.739989/full#supplementary-material

Supplementary Table 1 | Primers used for qPCR.

Supplementary Table 2 | Characteristic features of 41 StNF-Y genes.

Supplementary Table 3 | Conserved motifs identified by MEME among StNF-Y genes.

Supplementary Table 4 | Ka/Ks ratios of tandemly and segmentally duplicated StNF-Y genes.

Supplementary Table 5 | Ka/Ks ratios of the syntenic relationships for NF-Y genes between potato and Arabidopsis.

Supplementary Table 6 | Ka/Ks ratios of the syntenic relationships for NF-Y genes between potato and tomato.

Supplementary Table 7 | RNA-seq data of StNF-Y genes in different tissues.

Supplementary Table 8 | RNA-seq data of StNF-Y genes under abiotic stress and hormone treatment.

Supplementary Table 9 | The FPKM value of StNF-Y genes in white and pigmented tissues.

Supplementary Table 10 | Protein interaction network of StNF-Ys.

Supplementary Table 11 | The FPKM value of genes in protein interaction networks.

Supplementary Table 12 | The correlation between the expression of StNF-Ys and anthocyanin content in potato tuber (skin and flesh).

REFERENCES

Alam, M. M., Tanaka, T., Nakamura, H., Ikikawa, H., Kobayashi, K., Yaeno, T., et al. (2015). Overexpression of a rice heme activator protein gene (OsHAP2E) confers resistance to pathogens, salinity and drought, and increases photosynthesis and tiller number. *Plant Biotechnol. J.* 13, 85–96. doi: 10.1111/pbi.12239

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., et al. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402. doi: 10.1093/nar/25.17.3389

Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., et al. (2009). MEME Suite: tools for motif discovery and searching. *Nucleic Acids Res.* 37, W202–W208.

Cannon, S. B., Mitra, A., Baumgarten, A., Young, N. D., and May, G. (2004). The roles of segmental and tandem gene duplication in the evolution of large gene families in *Arabidopsis thaliana*. *BMC Plant Biol.* 4:10. doi: 10.1186/1471-2229-4-10

Cao, S., Kumimoto, R. W., Gnesutta, N., Calogero, A. M., Mantovani, R., and Holt, B. F. III (2015). 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, 1009–1017. doi: 10.1105/tpc.113.120352

Castañeda-Ovando, A., Pacheco-Hernández, M. D. L., Páez-Hernández, M. E., Rodríguez, J. A., and Galán-Vidal, C. A. (2009). Chemical studies of dietary flavonoids in man. *Annu. Rev. Food Sci. Technol.* 1, 163–187. doi: 10.1146/annurev.food.080708.100757

Chen, C., Xia, H., Bi, Y., Zhao, C., Zhao, S., et al. (2013). Induced expression and hormone treatment. *Front. Plant Sci.* 6:1142. doi: 10.3389/fpls.2015.01142

Feng, Z.-F., He, G.-H., Zheng, W.-J., Lu, P.-, Chen, M., Gong, Y.-M., et al. (2015). Foxtail millet NF-Y families: genome-wide survey and evolution analyses identified two functional genes important in abiotic stresses. *Front. Plant Sci.* 6:1142. doi: 10.3389/fpls.2015.01142

Fossen, T., and Andersen, ØM. (2000). Anthocyanins from tubers and shoots of the purple potato, *Solanum tuberosum*. *J. Hortic. Sci. Biotechnol.* 75, 360–363. doi: 10.1080/14620316.2000.1151125

Gago, C. D., Paschalidis, K., Guerreiro, A., Miguel, G., Antunes, D., and Hilioti, Z. (2017). Targeted gene disruption coupled with metabolic screen approach to uncover the LEAFY COTYLEDON1-LIKE4 (L1L4) function in tomato fruit metabolism. *Plant Cell Rep.* 36, 1065–1082. doi: 10.1007/s00299-017-1379-7

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S. E., Wilkins, M. R., Appel, R. D., et al. (2005). “Protein identification and analysis tools on the ExPaSy server,” in *The Proteomics Protocols Handbook*, ed. J. M. Walker (Totowa, NJ: Humana Press), 571–607. doi: 10.1385/1-59259-890-0:571

Guo, A. Y., Zhu, Q. H., and Xin, C. (2007). GSDS:a gene structure display server. *Hereditas* 29, 1023–1026. doi: 10.1360/yyc-007-1023

Guo, F., Liu, C., Xia, H., Bi, Y., Zhao, C., Zhao, S., et al. (2013). Induced expression of AtLEC1 and AtLEC2 differentially promotes somatic embryogenesis in transgenic tobacco plants. *PLoS One* 8:e71714. doi: 10.1371/journal.pone.0071714

Hackenberg, D., Wu, Y., Voigt, A., Adams, R., Schramm, P., and Grimm, B. (2012). Studies on differential nuclear translocation mechanism and assembly of the three subunits of the *Arabidopsis thaliana* transcription factor NF-Y. *Mol. Plant* 5, 876–888. doi: 10.1093/mp/sst117

He, J., and Giusti, M. M. (2010). Anthocyanins: natural colorants with health-promoting properties. *Annu. Rev. Food Sci. Technol.* 1, 163–187. doi: 10.1146/annurev.food.080708.100757

Hollman, P., and Katan, M. B. (1997). Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed. Pharmacother.* 51, 305–310. doi: 10.1016/0753-3322(97)80405-6

Kahle, J., Baake, M., Doenecke, D., and Albig, W. (2005). Subunits of the heterotrimeric transcription factor NF-Y are imported into the nucleus by distinct pathways involving importin beta and importin 13. *Mol. Cell Biol.* 25, 5339–5354. doi: 10.1128/mcb.25.13.5339-5354.2005

Kim, J., Lee, W. J., Vu, T. T., Jeong, C. Y., Hong, S. W., and Lee, H. (2017). High accumulation of anthocyanins via the ectopic expression of *AtDFR* confers significant salt stress tolerance in *Brassica napus*. *Plant Cell Rep.* 36, 1215–1224. doi: 10.1007/s00299-017-2147-7

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874.

Kumimoto, R. W., Zhang, Y., Siefers, N., and Holt, B. F. III (2010). NF-YC3, NF-YC4 and NF-YC9 are required for CONSTANS-mediated, photoperiod-dependent flowering in *Arabidopsis thaliana*. *Plant J.* 63, 379–391. doi: 10.1111/j.1365-313x.2010.04247.x
Kwong, R. W., Bui, A. Q., Lee, H., Kwong, L. W., Fischer, R. L., Goldberg, R. B., et al. (2003). LEAFY COTYLEDON1-LIKE defines a class of regulators essential for embryo development. *Plant Cell* 15, 5–18. doi: 10.1105/tpc.00 6973

Laloum, T., De Mita, S., Gamas, M. B., and Niebel, A. (2013). CCAAT-box binding transcription factors in plants: so many? *Trends Plant Sci.* 18, 157–166. doi: 10.1016/j.pt.2012.07.004

Lawton-Rauh, A. (2003). Evolutionary dynamics of duplicated genes in plants. *Mol. Phylogenet. Evol.* 29, 396–409. doi: 10.1016/j.ympev.2003.07.004

Lee, H., Fischer, R. L., Goldberg, R. B., and Harada, J. J. (2003). *Arabidopsis* LEAFY COTYLEDON1 represents a functionally specialized subunit of the CCAAT binding transcription factor. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2152–2156. doi: 10.1073/pnas.0437991100

Leyva-Gonzalez, M. A., Ibarra-Laclette, E., Cruz-Ramirez, A., and Herrera-Lee, H., Fischer, R. L., Goldberg, R. B., and Harada, J. J. (2003). *Arabidopsis* LEAFY COTYLEDON1 represents a functionally specialized subunit of the CCAAT binding transcription factor. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2152–2156. doi: 10.1073/pnas.0437991100

Petroni, K., Kumimoto, R. W., Gnesutta Calvenzani, N., Fornari, M., Tonelli, C., Holt, B. F., et al. (2012). The promiscuous life of plant nuclear factor Y transcription factors. *Plant Cell* 24:4777. doi: 10.1105/tpc.112.10 5734

Riechmann, J. L., Heard, J., Martin, G., Reuber, L., Jiang, C., Keddie, J., et al. (2000). *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 290, 2105–2110. doi: 10.1126/science.290.5499.2105

Sato, H., Mizoi, J., Tanaka, H., Maruyama, K., Qin, F., Osakabe, Y., et al. (2014). *Arabidopsis* DBP8-1, a DREB2A interactor, specifically enhances heat stress-induced gene expression by forming a heat stress-specific transcriptional complex with NF-Y subunits. *Plant Cell* 26, 4954–4973. doi: 10.1105/tpc.114.132928

Sato, H., Suzuki, T., Takahashi, F., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2019). NF-YB and NF-YB3 have functionally diverged and differentially induce drought and heat stress-specific genes. *Plant Physiol.* 180, 1677–1690. doi: 10.1104/pp.19.00391

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498–2504.

Siefers, N., Dang, K. K., Kumimoto, R. W., Bynum, W. E. T., Tayrose, G., and Holt, B. F. III (2009). Tissue-specific expression patterns of *Arabidopsis* NF-Y transcription factors suggest potential for extensive combinatorial complexity. *Plant Physiol.* 149, 625–641. doi: 10.1104/pp.108.130591

Singh, A., Pandey Baranwal, A., Kapoor, S., and Pandey, G. K. (2012). Comprehensive expression analysis of rice phospholipase D gene family during abiotic stresses and development. *Plant Signal. Behav.* 7, 847–855. doi: 10.4161/psb.23835

Sorin, C., Declerck, M., Christ, A., Blein, T., Ma, L., Lelandais-Briée, C., et al. (2014). A miR169 isoform regulates specific NF-YA targets and root architecture in *Arabidopsis*. *New Phytol.* 202, 1197–1211. doi: 10.1111/nph. 12735

Spooner, D. M., McLean, K., Ramsay, G., Waugh, R., and Bryan, G. J. (2005). A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proc. Natl. Acad. Sci. U.S.A.* 102, 1494–1499. doi: 10.1073/pnas.0507400102

Szkarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., et al. (2016). The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. *Nucleic Acids Res.* 45, D362–D368.

Tang, X., Zhang, N., Si, H., and Calderón-Urrea, A. (2017). Selection and validation of reference genes for RT-qPCR analysis in potato under abiotic stress. *Plant Methods* 13:85.

The Potato Genome Sequencing Consortium, Xu X., Pan, S., Cheng, S., Zhang, B., Mu, D., et al. (2011). Genome sequence and analysis of the tuber crop potato. *Nature* 475:189.

Voorrips, R. E. (2002). MapChart: software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93, 77–78. doi: 10.1093/jhered/93.1.77

Wang, D., Zhang, Y., Zhang, Z., Zhu, J., and Yu, J. (2010). KaXs: Calculator 2.0: a toolkit incorporating gamma-series methods and sliding window strategies. *Genome. Proteom. Bioinform.* 8, 77–80. doi: 10.1672/1672-0229(2010)008-00608-3

Wang, J., Li, G., Li, C., Zhang, C., Cui, L., Ai, G., et al. (2020). NF-Y plays essential roles in flavonoid biosynthesis by modulating histone modifications in tomato. *New Phytol.* 229, 3237–3252. doi: 10.1111/nph.17112

Wang, Y., Tang, H., DeBarry, J. D., Tan, X., Li, J., Wang, X., et al. (2012). McScanX:a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res.* 40:e49. doi: 10.1093/nar/gkr1293

Wang, Y., Xue, W., Chen, Z., Han, B., Haque, M. E., and Liu, A. (2018). Gene sequence and analysis of the tuber crop potato. *Nature* 475:189.

Xing, Y., Fikes, J. D., and Guarente, L. (1993). Mutations in yeast HAP2/HAP3 define a hybrid CCAAT box binding domain. *EMBO J.* 12, 4647–4655. doi: 10.1002/j.1460-2075.1993.tb01653.x

Xu, J. J., Zhang, X. F., and Xue, H. W. (2016). Rice aleurone layer specific OsNF-YB1 regulates grain filling and endosperm development by interacting with an ERF transcription factor. *J. Exp. Bot.* 67, 6399–6411. doi: 10.1093/jxb/erv409

Yan, D. H., Xia, X., and Yin, W. (2013). *Arabidopsis* NF-YB family genes identified in a poplar genome-wide analysis and expressed in populus euphratica are responsive to drought stress. *Plant Mol. Biol.* 81, 363–370. doi: 10.1007/s11105-012-0508-5
Yan, H., Wu, F., Jiang, G., Xiao, L., Li, Z., Duan, X., et al. (2019). Genome-wide identification, characterization and expression analysis of NF-Y gene family in relation to fruit ripening in banana. *Postharvest Biol. Technol.* 151, 98–110. doi: 10.1016/j.postharvbio.2019.02.002

Yang, J., Zhu, J., and Yang, Y. (2017). Genome-wide identification and expression analysis of NF-Y transcription factor families in watermelon (*Citrullus lanatus*). *J. Plant Growth Regul.* 36, 590–607. doi: 10.1007/s00344-017-9670-1

Yang, W., Lu, Z., Xiong, Y., and Yao, J. (2017). Genome-wide identification and co-expression network analysis of the OsNF-Y gene family in rice. *Crop J.* 5, 21–31. doi: 10.1016/j.cj.2016.06.014

Yu, Y., Bai, Y., Wang, Y., Wang, W., Liu, C., and Ni, Z. (2020). Soybean nuclear factor YA10 positively regulates drought resistance in transgenic *Arabidopsis thaliana*. *Environ. Exp. Bot.* 180:104249. doi: 10.1016/j.envexpbot.2020.10.024

Zhao, H., Wu, D., Kong, F., Lin, K., Zhang, H., and Li, G. (2017). The *Arabidopsis thaliana* nuclear factor Y transcription factors. *Front. Plant Sci.* 7:2045. doi: 10.3389/fpls.2016.02045

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Liu, Li, Ma, Li, Bi, Sun, Bai, Zhang and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.