# Identification and expression analysis of maize NF-YA subunit genes

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## Abstract

NF-YA is subunits of Nuclear Factor-Y (NF-Y) gene family. NF-YA is a kind of conservative transcription factor in plants and involved in plant growth and development and resistance to biotic and abiotic stress. In this study, 16 maize NF-YA subunit genes were identified by bioinformatics methods, and they were divided into 3 categories by phylogenetic analysis. Conserved domain analysis showed that most of the them contained CBFB_NF-YA domain. Tissue expression analysis showed that maize NF-YA subunit genes showed very obvious tissue expression characteristics. The expression level of the NF-YA subunit genes showed significant changes under different abiotic stresses, *Fusarium graminearum* infection and salicylic acid (SA) or jasmonic acid (JA) treatments. The results showed that NF-YA subunit genes played an important role in maize, which was manifested in promoting maize growth and development and resistance to stress, and laid the foundation for clarifying the function and regulatory mechanism of NF-YA subunit genes in maize.


**Keywords**: *Zea mays*, NF-YA subunit gene, biological stress, abiotic stress, expression analysis

## Introduction

Nuclear Factor-Y (NF-Y) transcription factor widely exists in eukaryotes and also known as hemo-activator protein (HAP) (Thirumurugan et al. 2008; Petroni et al. 2012). NF-Y can bind to CCAAT-box in promoter sequence, so it is also called CCAAT-binding factor (CBF) (Laloum et al. 2013). NF-Y is a large gene family, which is composed of NF-YA (CBFB / HAP2), NF-YB (CBFA / HAP3) and NF-YC (CBFC / HAP5) subunits (Nardini et al. 2013). NF-Y is usually located in the nucleus and evolutionarily conserved (Mantovani 1999). In animals, NF-YA, NF-YB and NF-YC subunits are encoded by three single genes, and the three subunits function in the form of heterologous trimers (Benatti et al. 2008). In plants, three subunits are encoded by more than 10 genes, and they can perform their functions independently (Petroni et al. 2012). CBFB_NF-YA domain is the core conserved domain of NF-YA family. The N-terminal of this domain can bind to NF-YB and NF-YC subunits, and the C-terminal can bind to DNA CCAAT-box (Quach et al. 2015).

In recent years, studies have shown that the single subunit of NF-Y is widely involved in plant growth and development and stress response, such as controlling gametogenesis, embryo and plant development (Mu et al. 2013), abscisic acid (ABA) signal transduction (Yu et al. 2021), flowering cycle regulation (Hwang et al. 2019), primary root elongation (Ballif et al. 2011; Zhou et al. 2020), blue light response (Warpeha et al. 2007), photosynthesis (Tokutsu et al. 2019) and abiotic stress tolerance such as drought, high temperature and salt (Li et al. 2021). In *Arabidopsis*, *NF-YA3* and *NF-YA8* genes mediate cell differentiation and embryo formation through ABA signaling pathway at early embryonic development. *NF-YA1*, *NF-YA5*, *NF-YA6* and *NF-YA9* are involved in the development of gametes, embryos and seeds (Mu et al. 2013). In potato, NF-YA responded to drought by regulating the number of chlorophylls, stomatal conductance and photosynthesis (Li et al. 2021). *PtNF-YA9* plays an important role in drought resistance of *Populus trichocarpa* as a positive regulator of stress resistance (Lian et al. 2018). There are 5 NF-Y genes in tomato which play roles in tomato fruit ripening (Li et al. 2016). In soybean, GmNFYC14 forms heterotrimer with GmNF-YA16 and GmNFYB2, activates *GmPYR1* mediated abscisic acid signaling pathway and regulates soybean stress tolerance (Yu et al. 2021). In maize, *ZmNFYB16* can form heterotrimer with *ZmNFYC17* and *ZmNFYA01*, and heterotrimer binds to CCAAT cis-acting elements in the promoter region of stress response and growth-related genes through *ZmNFYA01* subunit, regulating the expression of multiple genes related to stress resistance and growth, thereby improving the drought resistance of plants (Wang et al. 2018).

So far, the overall study of maize NF-YA subunit gene family has not been reported, and the number, physicochemical properties and function of maize NF-YA subunit genes are not clear. In this study, the maize NF-YA subunit genes were identified by bioinformatics methods, and the phylogenetic relationship, conserved domains, tissue specificity, gene expression patterns of maize NF-YA subunit genes under biotic and abiotic stress and SA or JA treatments were clarified, which laid the foundation for elucidating the function and regulation mechanism of maize NF-YA subunit genes.

## Materials & Methods

### Plant materials and sources

The seeds of maize inbred line B73 were preserved in the Mycotoxin and Molecular Plant Pathology Laboratory, Hebei Agricultural University.

## Data sources and phylogenetic analysis of NF-YA subunit genes in different species

The information and amino acid sequences of NF-YA subunit genes in maize, rice and *Arabidopsis* were downloaded from MaizeGDB (http://www.maizegdb.org/), RGAP (http://rice.plantbiology.msu.edu/) and TAIR (https://www.arabidopsis.org/). The amino acid sequences were aligned by Clustal X software (Larkin et al. 2007). The aligned results were imported into MEGA 7.0 software (Kumar et al. 2016) and phylogenetic tree was constructed by maximum likelihood method.

## Chromosome localization analysis

Chromosome location information of maize NF-YA subunit genes were obtained from MaizeGDB, chromosome mapping of maize NF-YA subunit genes using RIdeogram software (https://cran.r-project.org/web/packages/RIdeogram/vignettes/RIdeogram.html).

## Domain analysis

The conserved domains of maize NF-YA subunit genes were analyzed by online software SMART (http://smart.embl-heidelberg.de/) and the domain analysis maps were drawn by IBS software (Liu et al. 2015).

## Gene structure analysis

The annotated information about the gene structure of maize NF-YA subunit genes were obtained from MaizeGDB, including gene length, 5′ UTR, 3′ UTR and the distribution of each intron and exon. The gene structure map was drawn by IBS software.

## Expression pattern of NF-YA subunit genes in maize

Transcriptome sequencing data of maize NF-YA subunit genes in different tissues, response to abiotic and biotic stresses were obtained through SRA database of NCBI. Heat map analysis of NF-YA subunit genes expression in maize using HemI 1.0 software (Deng et al. 2014). In this study, RNA-seq data of 32 maize tissues such as seed 5 days after pollination, endosperm 25 days after pollination, seed 10 days after pollination, 5 abiotic stresses such as heat, cold, salt, ultraviolet radiation (UV) and drought, and *F. graminearum* infection were selected.

## Subcellular localization prediction

According to the amino acid sequences of NF-YA subunit genes in maize, the subcellular localization was analyzed by Plant-mPLoc software (Chou & Shen 2010), and the prediction table of subcellular localization was summarized.

### Expression analysis of NF-YA subunit genes in maize under hormone treatment

1 L of salicylic acid (100 μM) and jasmonic acid (100 μM) were sprayed evenly on the aboveground parts of the two groups of maize B73 (V5 stage), respectively. Sampling at 0 h, 3 h, 9 h, and 24 h, frozen with liquid nitrogen and stored in a -80°C refrigerator.

The specific quantitative real-time PCR (qRT-PCR) primers of internal reference *UBQ9* and maize NF-YA subunit genes were designed online by NCBI (Table S1) and synthesized by Beijing Bomede Biotechnology Co., Ltd. The cDNAs of maize inbred line B73 plant samples collected at different times under the treatment of two hormones were used as templates for qRT-PCR. The reaction system was as follows: 2 × M5 HiPer SYBR Premix EsTaq (with Tli RNaseH) 7 μL, cDNA template 1 μL, Primer-F 0.5 μL, Primer-R 0.5 μL, dd$H_2O$ 5 μL. Each of the above treatment systems was repeated for three times, and the reaction was performed by fluorescence quantitative PCR instrument. Conditions: 95°C 30 s, 95°C 5 s, 60°C 30 s, 40 cycles. The 0 h after hormone spraying in B73 plant was used as the control of gene transcription level, and the Ct value ($2^{-\Delta\Delta Ct}$) method was used to analyze the relative expression levels of each gene at different time points after different hormone treatments in B73 maize plant.

### Protein–Protein interaction network analysis

For the protein–protein interaction (PPI) predicted analysis, all of the NF-YA amino acid sequences were searched by the STRING database version 11.5 (https://cn.string-db.org/) (Szklarczyk et al. 2021). The “confidence score” of STRING was used, and a confidence score of > 0.7 (high confidence) between proteins was used(Mei et al. 2021). The interaction networks of protein-protein generated by STRING were used to show the relationship of proteins with NF-YA.

## Results

### Phylogenetic analysis and chromosomal localization of NF-YA subunit genes in maize

The number of amino acids encoded by CDS of 16 maize NF-YA subunit genes was obtained from MaizeGDB. The molecular weight and isoelectric point of the proteins encoded by

maize NF-YA subunit genes were analyzed and named *ZmNFYA01* to *ZmNFYA16* according to their distribution on chromosomes. The results showed that the 16 proteins were different in amino acid number, relative molecular mass and isoelectric point. The lengths of the 16 NF-YA amino acid sequences are between 90 ~ 742 aa, and most of the length is about 300 aa. From the predicted isoelectric point data of NF-YA subunit gene proteins, it was found that most of these proteins were alkaline, and only the proteins encoded by *ZmNFYA15* and *ZmNFYA16* were acidic (Table 1). In order to clarify the phylogenetic relationship between maize NF-YA subunit genes, 16 maize NF-YA subunit genes, 10 *Arabidopsis* NF-YA subunit genes and 11 rice NF-YA subunit genes were analyzed. The results showed that the NF-YA family could be divided into three groups: I, II and III. In maize, there were 6, 6 and 4 NF-YA subunit genes in I, II and III, respectively. Among them, *ZmNFYA01* is orthologous to *OsNF-YA2*, *ZmNFYA06* is orthologous to *OsNF-YA5*, *ZmNFYA09* is orthologous to *OsNF-YA6*, *ZmNFYA10* is orthologous to *OsNF-YA4*, *ZmNFYA14* is orthologous to *OsNF-YA1* (Figure 1).

The chromosome location information of 16 maize NF-YA subunit genes was obtained from MaizeGDB. It was found that the location of maize NF-YA subunit genes on chromosomes was unevenly distributed. Among them, chromosome 1 contains the largest number (7) of maize NF-YA subunit genes, including *ZmNFYA01*, *ZmNFYA02*, *ZmNFYA03*, *ZmNFYA04*, *ZmNFYA05*, *ZmNFYA06* and *ZmNFYA07*. Chromosome 5 contains 4 maize NF-YA subunit genes: *ZmNFYA11*, *ZmNFYA12*, *ZmNFYA13* and *ZmNFYA14*. Chromosome 2 containes 2 maize NF-YA subunit genes, namely *ZmNFYA08* and *ZmNFYA09*. Chromosomes 3, 7 and 10 contained only 1 maize NF-YA subunit gene, while chromosomes 4, 6, 8 and 9 contained no maize NF-YA subunit gene (Figure 2).

## Domain analysis of maize NF-YA subunit genes

The conserved domains of NF-YA subunit genes in maize were analyzed by using the online software SMART. The results showed that among the 16 genes in the NF-YA family, 11 genes contained CBFB_NF-YA domain, and *ZmNFYA03*, *ZmNFYA07*, *ZmNFYA11*, *ZmNFYA13* and *ZmNFYA16* had no conserved domain (Figure 3). This domain plays a key role in binding to NF-YB subunit and specifically binds to CCAAT box.

## Gene structure analysis of maize NF-YA subunit genes

The gene information of maize NF-YA subunit genes was obtained from MaizeGDB, and the gene structure map was drawn by IBS software. The analysis showed that the lengths of NF-

YA subunit gene sequence were quite different, among which *ZmNFYA03*, *ZmNFYA07*, *ZmNFYA11* and *ZmNFYA13* had no 5′ UTR and 3′ UTR structures, which might be related to their absence of CBFB_NF-YA domain. Introns exist in the 5′ UTR of 10 genes except *ZmNFYA02* and *ZmNFYA14* of 12 NF-YA subunit genes (Figure 4).

## Tissue expression analysis of maize *NF-YA* subunit genes

Using the data from NCBI SRA database, the tissue expression levels of 16 maize *NF-YA* subunit genes obtained in the experiment were analyzed. The results showed that the expression of 16 maize *NF-YA* subunit genes in the same tissue at different developmental stages had obvious temporality, and the gene expression levels showed significant differences. The expression of the same gene was different in different tissues at different stages. *ZmNFYA01* was highly expressed at 25 d of embryonic development, and its expression in most tissues was significantly higher than that of other genes, indicating that it plays an important role in maize growth and development. *ZmNFYA14* was highly expressed at 16 d and 25 d of embryonic development. *ZmNFYA08* was highly expressed during embryonic development, endosperm development and seed germination. Low expression of *ZmNFYA02*, *ZmNFYA07* and *ZmNFYA13* was observed in all tissues at all stages (Figure 5).

## Expression pattern of maize *NF-YA* subunit genes under abiotic stress

The expression patterns of *NF-YA* subunit gene in maize under heat, cold, salt, UV and drought were analyzed by NCBI SRA database. The results showed that the gene expression of *ZmNFYA08* was significantly up-regulated under salt and drought stress. The gene expression level of *ZmNFYA11* was significantly up-regulated under salt and drought treatment. *ZmNFYA10* was up-regulated under heat and salt stress. *ZmNFYA02* was significantly down-regulated under heat, salt and UV stress; The expression of *ZmNFYA05*, *ZmNFYA12* and *ZmNFYA14* decreased significantly under heat stress; The expression of *ZmNFYA07* did not change significantly under various stresses, and maintained a low level of expression (Figure 6).

## Expression pattern of maize *NF-YA* subunit genes under biological stress

The expression pattern of *NF-YA* subunit genes in maize during *F. graminearum* infection was analyzed by NCBI SRA database. The results showed that the expression levels of *ZmNFYA01* and *ZmNFYA15* were the highest at 0 h after infection, and then decreased gradually. The expression levels of *ZmNFYA02*, *ZmNFYA04*, *ZmNFYA05*, *ZmNFYA06*, *ZmNFYA08*, *ZmNFYA09*, *ZmNFYA11*, *ZmNFYA12* and *ZmNFYA14* all showed a trend of first decreasing and

then increasing. The expression of *ZmNFYA03* and *ZmNFYA10* increased first, then decreased and then increased. The expression of *ZmNFYA16* did not change significantly at 0-48 h after infection, but decreased at 72 h after infection (Figure 7). The results showed that the expression levels of *NF-YA* subunit genes in maize were varied during *F. graminearum* infection, indicating that *NF-YA* subunit genes were involved and played an important role in maize disease resistance.

## Subcellular localization prediction of maize NF-YA subunit genes

Using the results of subcellular localization prediction obtained by Plant-mPLoc software, the following subcellular localization prediction tables are summarized. The results showed that ZmNFYA07 was located in chloroplast and cytoplasm and ZmNFYA16 was located in mitochondrion and nucleus. In addition, all 14 maize NF-YA subunit genes were located in the nucleus, indicating that most transcription factors were located in the nucleus, and revealing that transcription factors also play an important role in mitochondrion and chloroplast (Table 2).

## Expression analysis of maize *NF-YA* subunit genes under hormone treatment

qRT-PCR was used to detect the expression of *NF-YA* subunit genes in maize during salicylic acid and jasmonic acid treatment. The results showed that under salicylic acid treatment, the expression changes of maize *NF-YA* subunit genes were mainly divided into 4 categories. The first type was that the expression level increased first and then decreased. The expression levels of *ZmNFYA01*, *ZmNFYA02* and *ZmNFYA15* increased to the highest at 3 h after treatment, and then decreased gradually. The expression of *ZmNFYA03*, *ZmNFYA11*, *ZmNFYA13* and *ZmNFYA14* increased to the highest level at 9 h and then decreased. The second type showed that the expression level decreased first and then increased. The expression levels of *ZmNFYA04* and *ZmNFYA05* reached the lowest at 9 h after treatment and then increased slightly. The expression of *ZmNFYA12* reached the lowest level at 3 h after treatment and then increased to the initial level. The third type was that after salicylic acid treatment, gene expression continued to increase and maintained at high levels such as *ZmNFYA08* and *ZmNFYA16*. The fourth type was the expression level fluctuated after salicylic acid treatment. For example, the expression of *ZmNFYA06* and *ZmNFYA09* decreased first, then increased and then decreased. The expression of *ZmNFYA10* increased first and then decreased to the lowest and then increased (Figure 8A).

Under jasmonic acid treatment, the expression changes of maize *NF-YA* subunit genes were mainly divided into three categories. The first type was that after jasmonic acid treatment, the gene expression content increased to the highest level at 3 h and then gradually decreased, Such as *ZmNFYA01*, *ZmNFYA02*, *ZmNFYA04*, *ZmNFYA09*, *ZmNFYA12*, *ZmNFYA13*, *ZmNFYA15* and *ZmNFYA16*. The second type showed that the expression level increased first, then decreased and then increased after treatment. The expression of *ZmNFYA03*, *ZmNFYA06*, *ZmNFYA08* and *ZmNFYA10* reached the highest level at 3 h, decreased to the lowest level at 9 h, and then increased. The third type was that the expression of *ZmNFYA05* and *ZmNFYA11* decreased after jasmonic acid treatment. In addition, the expression of *ZmNFYA14* decreased first, then increased and then decreased after treatment (Figure 8B). It is speculated that *NF-YA* subunit genes were involved in salicylic acid and jasmonic acid signaling pathways and plays an important role in these two signaling pathways.

### Prediction of maize NF-YA protein interaction network

In order to identify the function of NF-YA gene, PPI network of NF-YA proteins were constructed by STRING database. All NF-YA proteins were predicted to interact with proteins encoded by GRMZM2G180947_P01, GRMZM2G473152_P01 and GRMZM2G444073_P01 (Figure 9). In addition, it was predicted that the GRMZM2G143450_P01 and GRMZM2G099628_P01 might interact with ZmNFYA16. These two proteins are probable methionine-tRNA ligase, so ZmNFYA16 may play a role in translation.

## Discussion

NF-Y family widely exist in various organisms, such as animals, plants and microorganisms. Compared with mammalian and yeast NF-Y protein research progress is rapid, plant NF-Y family research is slow (Liang et al. 2012). Up to now, the research on NF-Y family is limited to the preliminary bioinformatics comparison between *Arabidopsis thaliana* and related plants, as well as the gene expression analysis and function research based on it. The role of NF-YA subunit genes in the molecular mechanism of maize response to pathogen infection is rarely reported. In this study, we obtained 16 NF-YA subunit genes from MaizeGDB and divided into 3 categories by phylogenetic analysis.

NF-YA subunit genes are involved in multiple processes of plant life. *AtNF-YA5* is regulated by miR169, thereby improving the resistance of *Arabidopsis* to drought stress (Li et al.

2008). Overexpression of *OsNF-YA7* can improve drought tolerance of rice through an ABA-independent pathway (Lee et al. 2015). ZmNFYA03 could promote early flowering by binding to the *FT-like12* promoter in maize (Su et al. 2018). In this study, we found that maize NF-YA subunit genes *ZmNFYA01*, *ZmNFYA08* and *ZmNFYA14* were highly expressed during embryonic development (Figure 5), suggesting that these three genes play an important role in maize growth and development. Different maize NF-YA subunit genes showed different expression patterns under heat, cold, salt, UV and drought stress (Figure 6) and *F. graminearum* infection (Figure 7), and showed obvious gene expression changes after SA and JA treatment (Figure 8), indicating that maize NF-YA subunit genes may be involved in maize resistance to abiotic and biotic stress.

PPI network predicted analysis indicated all 16 NF-YA proteins interact with GRMZM2G180947_P01, GRMZM2G473152_P01 and GRMZM2G444073_P01 (Figure 9). These 3 proteins were identified and named as ZmNF-YB4 (GRMZM2G180947_P01), ZmNF-YB6 (GRMZM2G473152_P01) and ZmNF-YB9 (GRMZM2G444073_P01) (Zhang et al. 2016). It is consistent with the binding of CBFB_NF-YA, a conserved domain in NF-YA subunit gene mentioned above, to NF-YB subunit. These results laid a foundation for elucidating the function and regulation mechanism of maize NF-YA subunit genes. However, the specific function of NF-YA subunit genes and their relationship with NF-YBs and NF-YCs still need further study.

## Conclusions

In summary, a total of 16 NF-YA subfamily genes in maize were identified, and the evolutionary relationship, chromosome localization, conserved domains, tissue expression characteristics, subcellular localization, gene expression under biological and abiotic stresses, gene expression under hormone SA and JA treatments were analyzed, and PPI network was predicted. The results were preliminarily determined that maize NF-YA subunit genes play an important role in maize growth and development as well as resistance to biotic and abiotic stresses.

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## Figure Legends

**Figure 1** Phylogenetic relationship of NF-YAs in *Arabidopsis*, rice and maize.

**Figure 2** Chromosomal localization of NF-YAs in maize. Different genes are represented by dots of different colors. Stripes represent gene density.

**Figure 3** Phylogenetic analysis and domain analysis of NF-YAs in maize. Green box: CBFB_NF-YA domain.

**Figure 4** Exon–intron organization of NF-YAs in maize. Blue box: UTRs, green box: Exons, black lines: introns.

**Figure 5** Hierarchical clustering of expression level of *ZmNFYAs* in 32 tissues. Deep color indicates high expression, light color indicates low expression level.

**Figure 6** Hierarchical clustering of expression level of *ZmNFYAs* under abiotic stress. Deep color indicates high expression, light color indicates low expression level.

**Figure 7** Hierarchical clustering of expression level of *ZmNFYAs* under biotic stress. Deep color indicates high expression, light color indicates low expression level.

**Figure 8** Expression changes of *ZmNFYAs* under hormone treatment. Expression changes under SA (A) and JA (B) treatment. Horizontal coordinates represent processing time.

**Figure 9** The PPI network of ZmNFYAs detected by STRING. Empty nodes: proteins of unknown 3D structure, filled nodes: some 3D structure is known or predicted.

## Table Legends

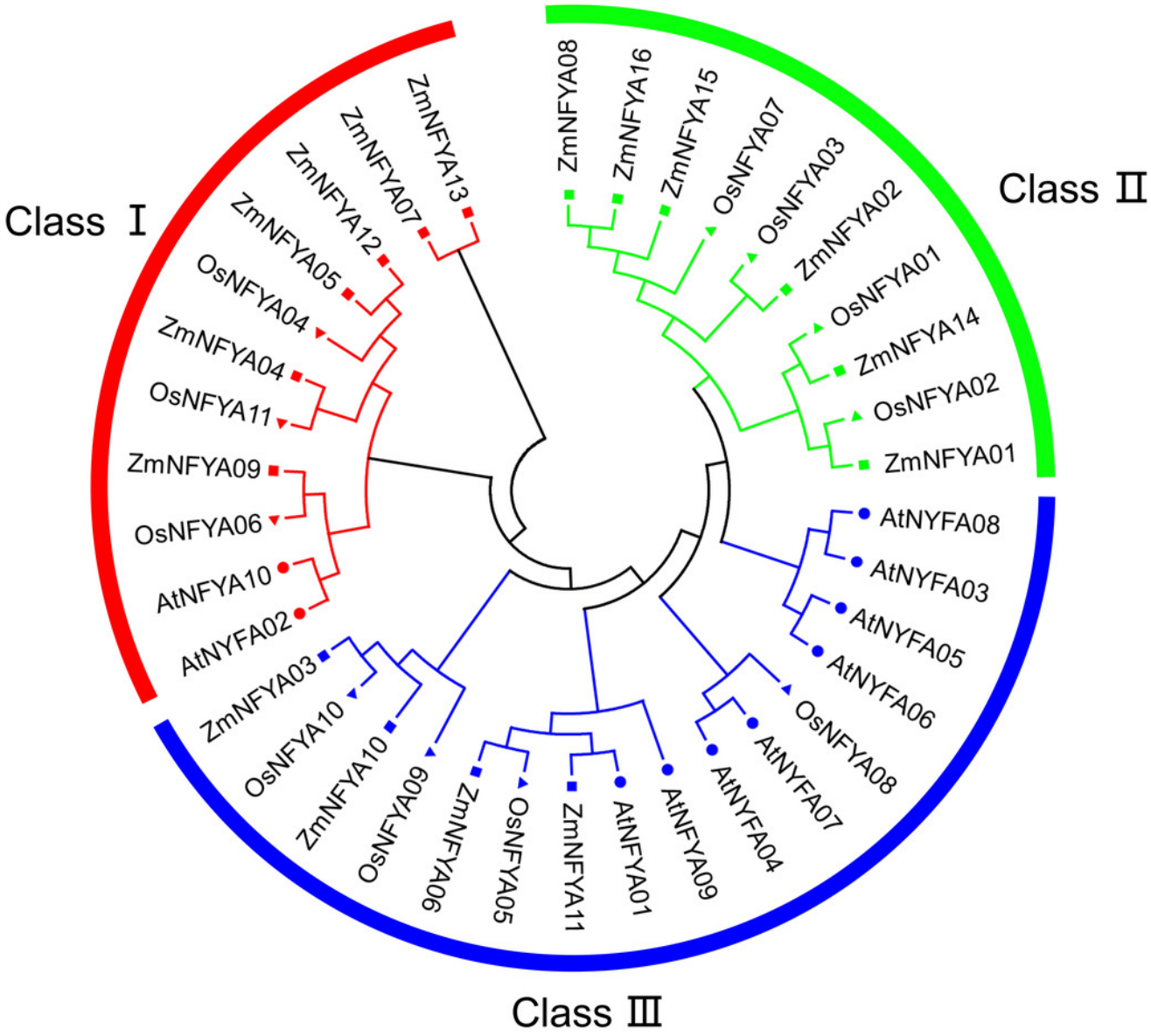
**Table 1** Physicochemical properties of NF-YA subunit genes in maize.

**Table 2** Subcellular localization prediction table of maize NF-YA subunit genes.

**Table S1** qRT-PCR primers for maize *NF-YA* subunit genes.

# Figure 1

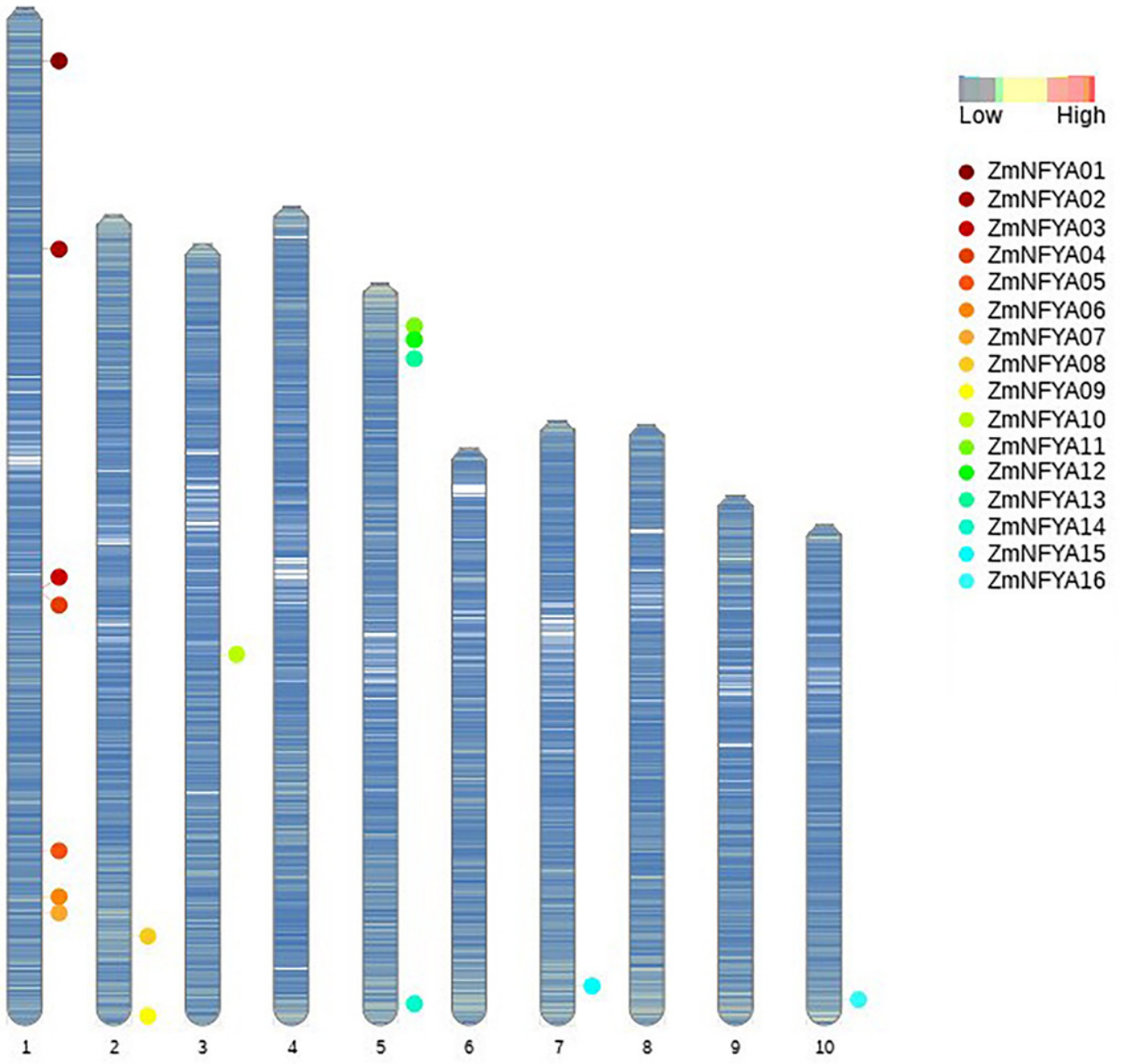
Phylogenetic relationships of NF-YA in *Arabidopsis*, rice and maize.



# Figure 2

Chromosomal localization of NF-YA in maize.

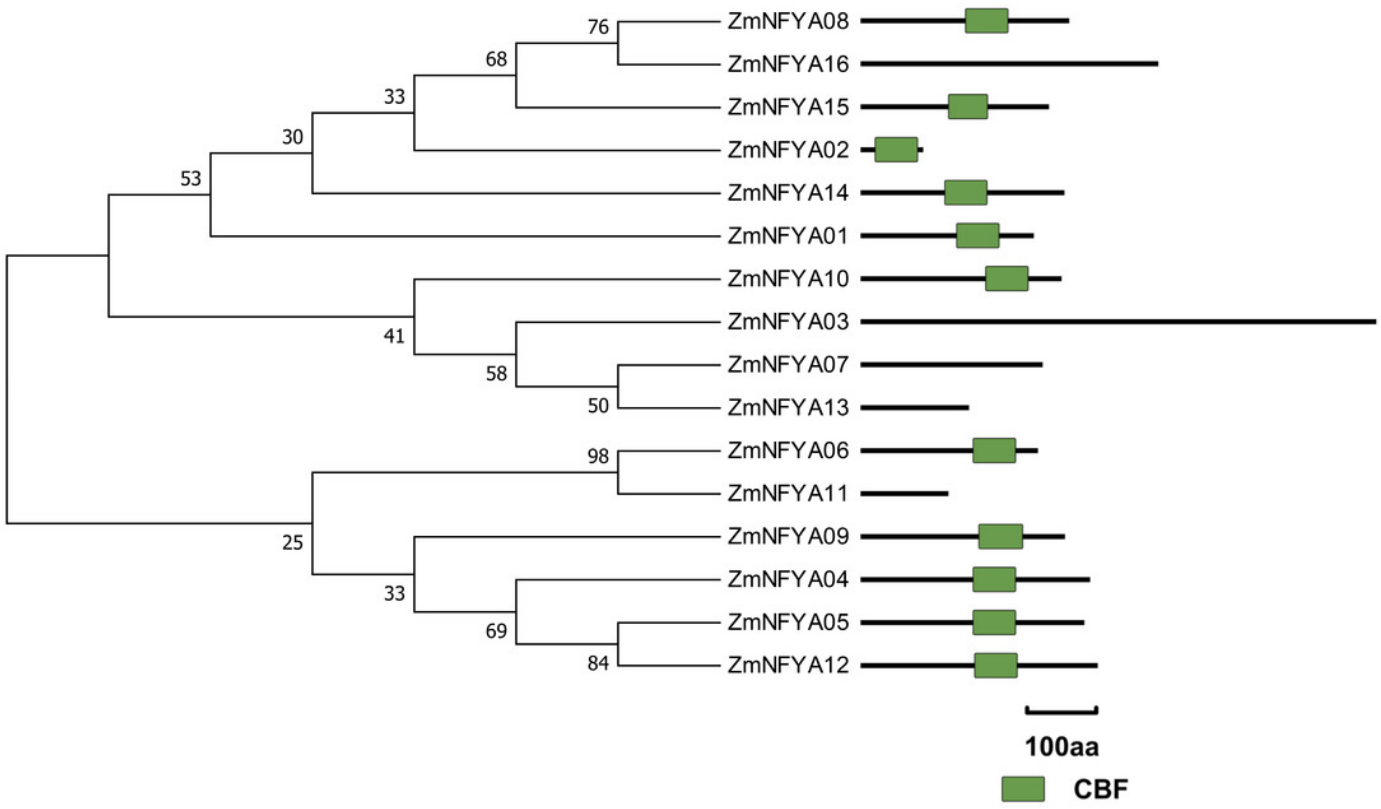
Different genes are represented by dots of different colors. Stripes represent gene density.



# Figure 3

Phylogenetic analysis and domain analysis of NF-YA in maize.

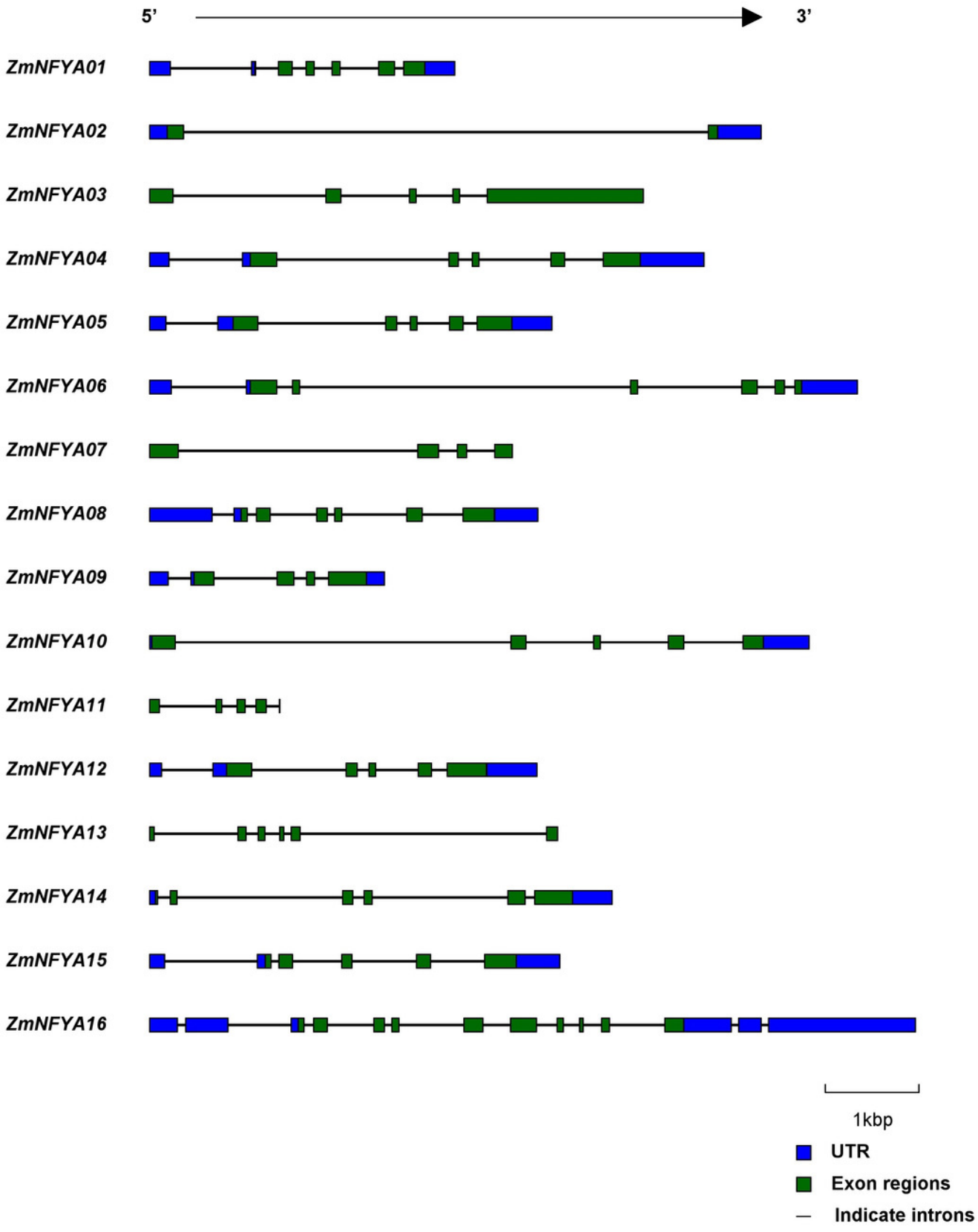
Green box: CBFB_NF-YA domain.



# Figure 4

Exon–intron organization of NF-YA in maize.

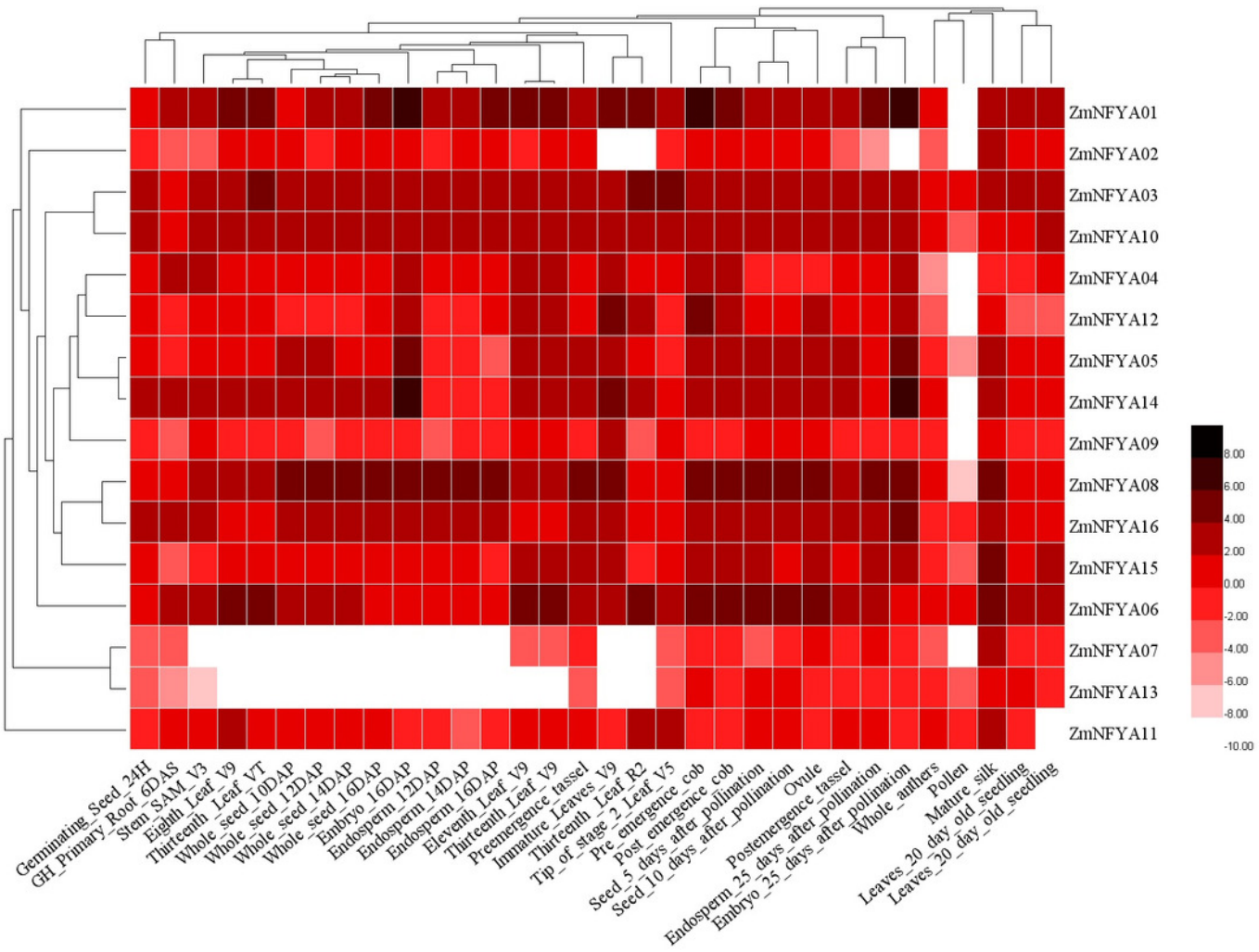
Blue box: UTRs, green box: Exons, black lines: introns.


5'
3'
ZmNFYA01
ZmNFYA02
ZmNFYA03
ZmNFYA04
ZmNFYA05
ZmNFYA06
ZmNFYA07
ZmNFYA08
ZmNFYA09
ZmNFYA10
ZmNFYA11
ZmNFYA12
ZmNFYA13
ZmNFYA14
ZmNFYA15
ZmNFYA16
1kbp
UTR
Exon regions
Indicate introns

# Figure 5

Hierarchical clustering of expression level of *ZmNFYAs* in 32 tissues.

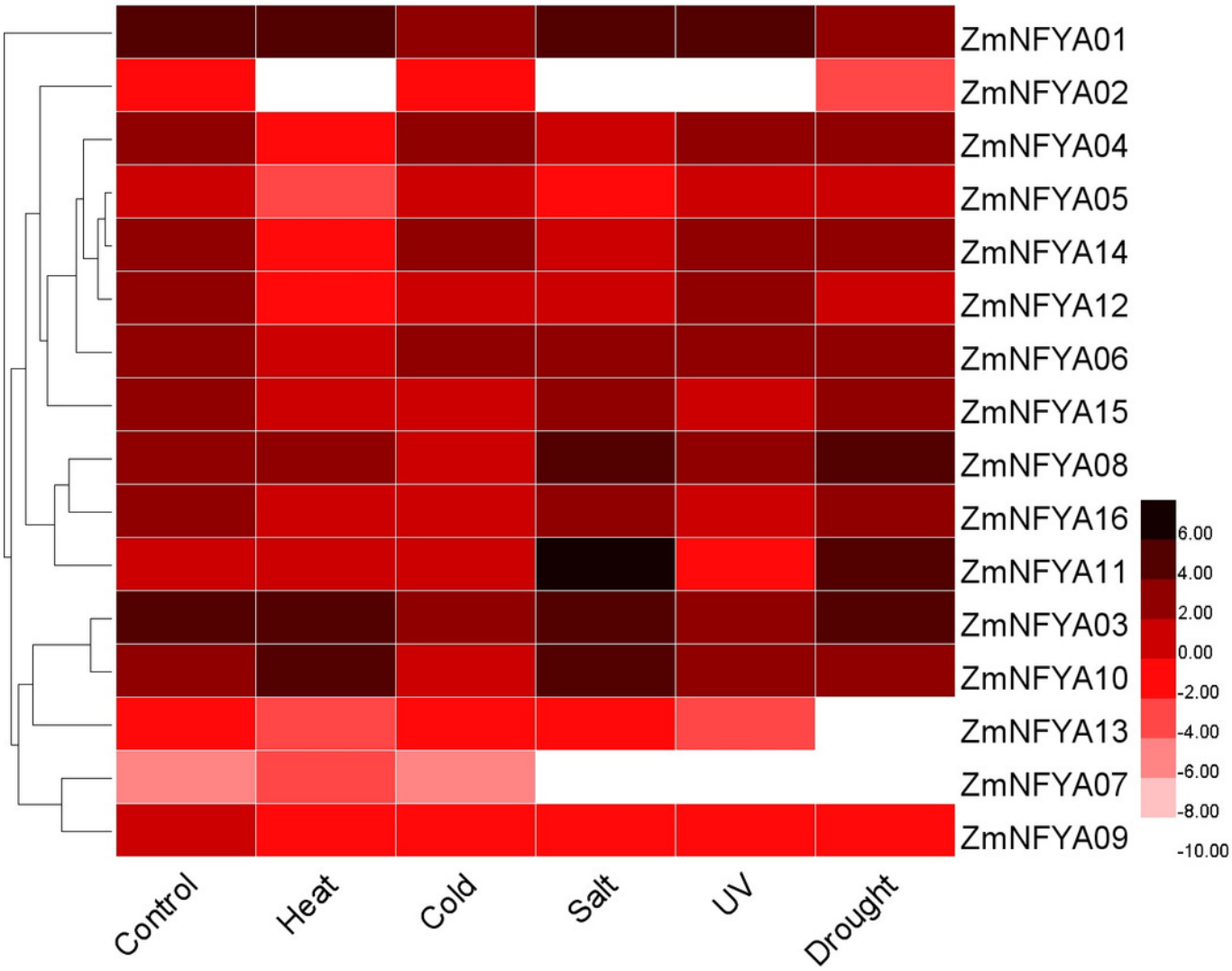
Deep color indicates high expression, light color indicates low expression level.



# Figure 6

Hierarchical clustering of expression level of *ZmNFYAs* under abiotic stress.

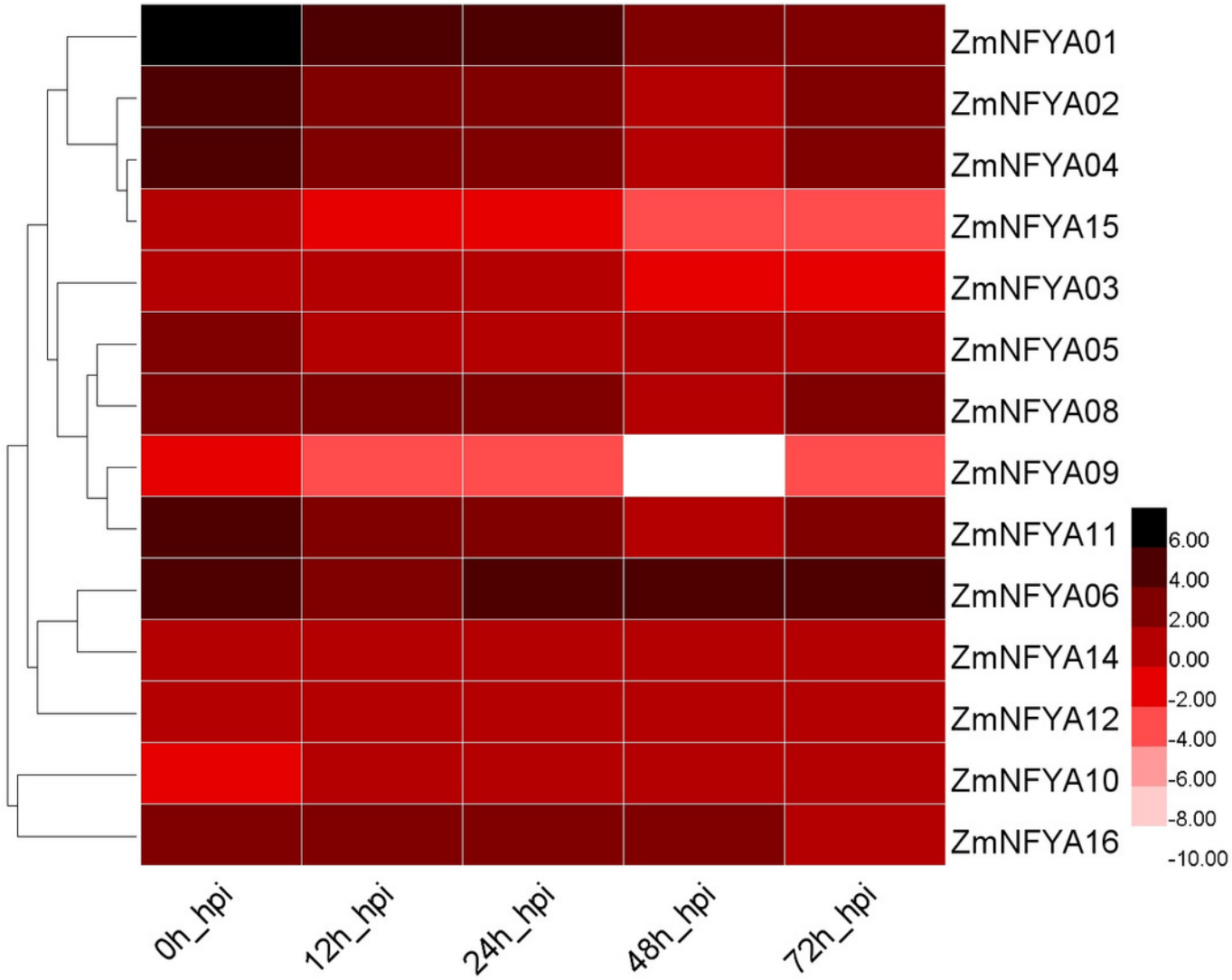
Deep color indicates high expression, light color indicates low expression level.



# Figure 7

Hierarchical clustering of expression level of *ZmNFYAs* under biotic stress.

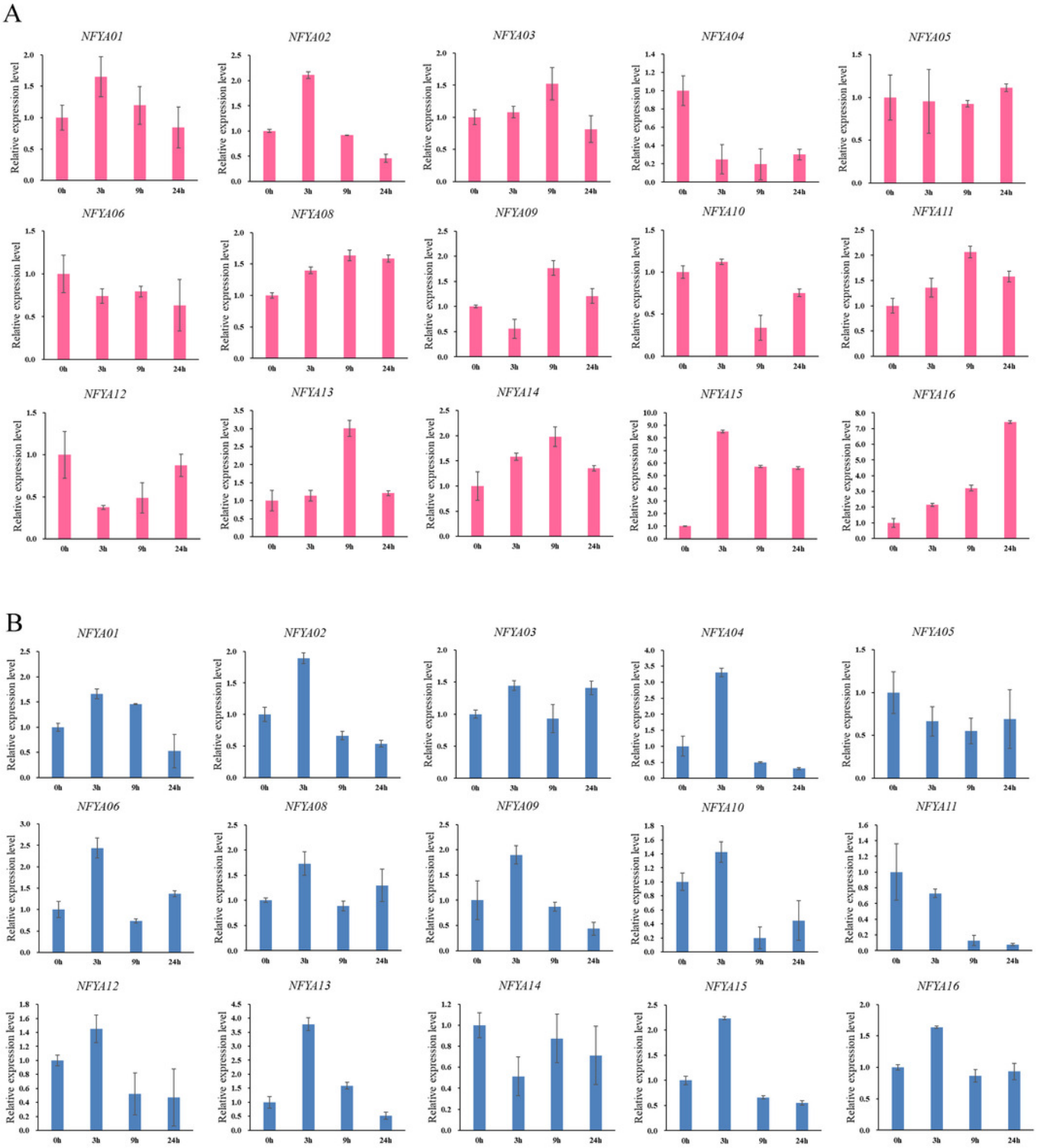
Deep color indicates high expression, light color indicates low expression level.



# Figure 8

Expression changes of *ZmNFYAs* under hormone treatment.

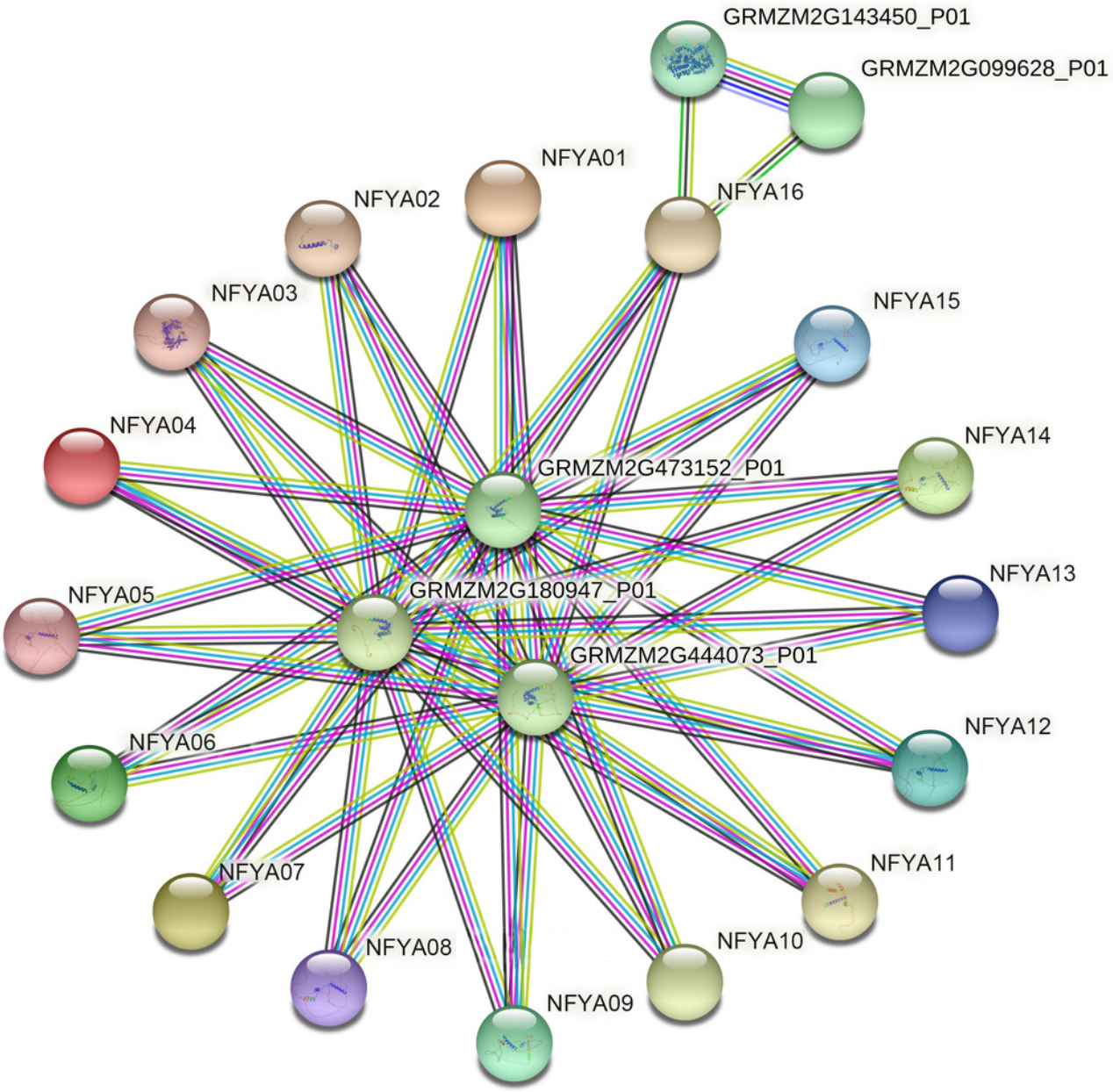
Expression changes under SA (A) and JA (B) treatment. Horizontal coordinates represent processing time.


A
NFYA01
NFYA02
NFYA03
NFYA04
NFYA05
NFYA06
NFYA08
NFYA09
NFYA10
NFYA11
NFYA12
NFYA13
NFYA14
NFYA15
NFYA16
Relative expression level
0h
3h
9h
24h
B
NFYA01
NFYA02
NFYA03
NFYA04
NFYA05
NFYA06
NFYA08
NFYA09
NFYA10
NFYA11
NFYA12
NFYA13
NFYA14
NFYA15
NFYA16
Relative expression level
0h
3h
9h
24h

# Figure 9

The PPI network of ZmNFYAs detected by STRING.

Empty nodes: proteins of unknown 3D structure, filled nodes: some 3D structure is known or predicted.



## Table 1(on next page)

Physicochemical properties of NF-YA subunit genes in maize.

| Gene ID | Gene name | Chr | Strat | End | AA | MW (Da) | pI |
|---|---|---|---|---|---|---|---|
| Zm00001d027874 | *ZmNFYA01* | 1 | 16042002 | 16038734 | 249 | 27207.16 | 8.96 |
| Zm00001d029489 | *ZmNFYA02* | 1 | 72880539 | 72887083 | 90 | 10062.62 | 11.87 |
| Zm00001d031063 | *ZmNFYA03* | 1 | 175269072 | 175263789 | 742 | 85041.59 | 9.42 |
| Zm00001d031092 | *ZmNFYA04* | 1 | 176875893 | 176869959 | 330 | 35231.81 | 8.93 |
| Zm00001d033215 | *ZmNFYA05* | 1 | 254416539 | 254420845 | 322 | 34122.58 | 9.61 |
| Zm00001d033602 | *ZmNFYA06* | 1 | 268308237 | 268315814 | 255 | 26775.89 | 9.52 |
| Zm00001d033773 | *ZmNFYA07* | 1 | 273183116 | 273187001 | 262 | 29076.26 | 9.80 |
| Zm00001d006835 | *ZmNFYA08* | 2 | 217600202 | 217595882 | 300 | 32759.35 | 9.02 |
| Zm00001d007882 | *ZmNFYA09* | 2 | 241675483 | 241672970 | 294 | 31336.04 | 9.78 |
| Zm00001d041491 | *ZmNFYA10* | 3 | 123767258 | 123760201 | 289 | 31009.56 | 9.39 |
| Zm00001d013501 | *ZmNFYA11* | 5 | 12927450 | 12924588 | 126 | 14415.92 | 10.90 |
| Zm00001d013676 | *ZmNFYA12* | 5 | 17050121 | 17054266 | 341 | 35736.46 | 9.40 |
| Zm00001d013856 | *ZmNFYA13* | 5 | 22800236 | 22795868 | 156 | 16876.48 | 9.64 |
| Zm00001d018255 | *ZmNFYA14* | 5 | 217466991 | 217462041 | 293 | 31907.09 | 10.31 |
| Zm00001d022109 | *ZmNFYA15* | 7 | 170564261 | 170559870 | 271 | 29607.50 | 6.63 |
| Zm00001d026305 | *ZmNFYA16* | 10 | 143264094 | 143272292 | 428 | 48253.63 | 6.16 |

## Table 2(on next page)

Subcellular localization prediction table of maize NF-YA subunit genes.

1

| Gene name | Predicted location(s) |
|---|---|
| *ZmNFYA01* | Nucleus |
| *ZmNFYA02* | Nucleus |
| *ZmNFYA03* | Nucleus |
| *ZmNFYA04* | Nucleus |
| *ZmNFYA05* | Nucleus |
| *ZmNFYA06* | Nucleus |
| *ZmNFYA07* | Chloroplast, Cytoplasm |
| *ZmNFYA08* | Nucleus |
| *ZmNFYA09* | Nucleus |
| *ZmNFYA10* | Nucleus |
| *ZmNFYA11* | Nucleus. |
| *ZmNFYA12* | Nucleus. |
| *ZmNFYA13* | Nucleus |
| *ZmNFYA14* | Nucleus |
| *ZmNFYA15* | Nucleus |
| *ZmNFYA16* | Mitochondrion, Nucleus |

2