



**Full Length Article**

# Identification, Evolution and Expression Analysis of NF-YB Transcription Factor Family in Maize (*Zea mays*)

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## Abstract

In order to further understand the structure and function of NF-YB gene family, bioinformatics method was used to study the evolutionary relationship, amino acid sequence characteristics, physicochemical properties, subcellular localization prediction and expression analysis of NF-YB family genes in maize genome. The results showed that there were 17 NF-YB family genes in the maize genome, which were distributed in 9 chromosomes of maize, and each NF-YB family protein contained a CBF<sub>D</sub>\_NFYB\_HMF conserved domain, all of which were located in the nucleus. In different developmental stages, six NF-YB genes were highly expressed in different stages and tissues, one NF-YB gene was only highly expressed in leaves, and the other 10 NF-YB genes remained low at each stage. The results of this study promote the understanding of NF-YB family genes and lay a foundation for the functional study of NF-YB family genes at the molecular level. © 2020 Friends Science Publishers

**Key words:** Maize; NF-YB; Transcription factor; Gene; Bioinformatics

## Introduction

Maize (*Zea mays* L.) is the most important food, feed, industrial raw material and energy crop in the world today, which plays an important role in ensuring world food security, economic development and alleviating energy crisis (Zhao *et al.* 2016; Zhao *et al.* 2018). With the continuous improvement of people's living standards, maize breeding has gradually developed in the direction of high yield, high quality and stress resistance (Zhao *et al.* 2016). Therefore, increasing corn yield is of long-term significance to promote the safe and healthy development of grain in our country.

Nuclear factor NF-Y (Nuclearfactor-Y, NF-Y) is a ubiquitous transcription factor in eukaryotes, which is composed of NF-YA, NF-YB and NF-YC subunits and plays an important role in physiological processes such as plant development and environmental stress response (Yuan 2017). At present, reports on maize NF-Y transcription factors are rare, so it is necessary to analyze the transcription factor family to further understand the role of this transcription factor (Stelplflug *et al.* 2016). In this study, bioinformatics methods were used to analyze the NF-YB subfamily of nuclear factor NF-Y, including NF-YB expression analysis, evolution analysis, conservative domain analysis, conservative element prediction, gene structure prediction and gene location on chromosome, in

order to further understand the function and role of NF-YB transcription factors in maize. The bioinformatics analysis of maize NF-YB family can lay a foundation for in-depth analysis of the function of maize NF-YB transcription factors, and provide a theoretical basis for further using NF-YB transcription factors in maize breeding and improving maize yield.

## Materials and Methods

### Plant material

From the plant transcription factor database Plant TFDB (<http://plantfdb.cbi.pku.edu.cn/>), the nucleic acid and protein sequences of maize NF-YB transcription factor family gene and Arabidopsis NF-YB transcription factor family gene for correlation analysis of this study was downloaded (Liu *et al.* 2015). The name of maize was JKY818, which offered by Jilin Agricultural Science and Technology University, and its seed germination was 99%.

### Test method

**Acquisition of NF-YB transcription factor sequence:** NF-YB sequences of *Arabidopsis thaliana* and maize were downloaded from the plant transcription factor database Plant TFDB (<http://plantfdb.cbi.pku.edu.cn/>). The NF-YB

protein sequences of maize and *A. thaliana* obtained in the above database include proteins translated by multiple transcripts (transcription isoform) with different NF-YB genes. In this study, the longest transcripts (and their encoded proteins) of NF-YB genes with multiple transcripts were analyzed. Thus, thirteen NF-YB gene coding regions and their protein sequences of *A. thaliana* and nineteen NF-YB gene coding regions and protein sequences of maize were obtained.

**Construction of phylogenetic tree of transcription factor NF-YB-like gene:** Using Clustal X software to carry out multiple matching analyses on the protein sequences of maize and Arabidopsis. Based on the comparison results, using MEGA5.05 to build neighbor joining NJ, set Bootstrap as 1000 repetitions and other as default parameters (Zhao *et al.* 2015). The deletion data and Poisson model were used to analyze the NF-YB gene in maize.

**Analysis of conserved domains of amino acid sequences of NF-YB transcription factor family proteins:** Protective domains of amino acid sequences of NF-YB proteins in maize were predicted by using Pfam domain pattern redrawer function in tbtools software.

**Location of NF-YB Gene on chromosome:** According to the published information of maize genome B73 RefGen\_v4, the distribution of 17 NF-YB transcription factor genes on 10 maize chromosomes was determined. The specific location of NF-YB gene on chromosome was found by BLSAT comparison in Maze GDB database (<https://www.maizegdb.org/>).

**Analysis of physico-chemical properties of amino acids:** The number of amino acids, molecular weight, theoretical isoelectric point, number of aliphatic amino acids and hydrophobicity of proteins were analyzed by online ProtParam tool provided by ExPASy (<https://web.expasy.org/protparam/>) (Xie *et al.* 2014).

**Sub-cellular localization of NF-YB:** Plant-mPLOC ([www.csbio.sjtu.edu.cn](http://www.csbio.sjtu.edu.cn)) was used to predict the location of 17 NF-YB transcription factor family proteins in cells (Zhu *et al.* 2012).

**Expression analysis of NF-YB family gene:** Using the published transcriptome data of maize tissues at different developmental stages, the expression patterns of 17 NF-YB transcription factor family genes in different maize tissues and developmental stages were analyzed. Heat map through the Amazing Heatmap function in TB tools was drawn.

## Results

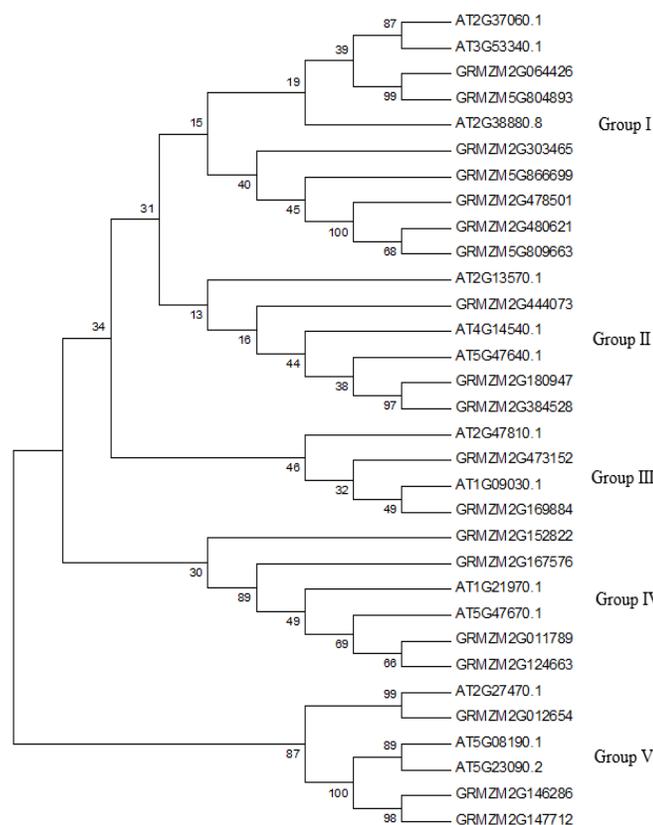
### Identification of NF-YB transcription factors

According to the NF-YB proteins identified in the plant transcription factor database Plant TFDB, for the proteins translated by different transcripts of the same NF-YB gene, the protein with the longest amino acid sequence was selected for the following analysis. Thirteen NF-YB proteins of *A. thaliana* were obtained, which were

AT2G37060.1, AT3G53340.1, AT2G38880.8, AT2G13570.1, AT4G14540.1, AT5G47640.1, AT2G47810.1, AT1G09030.1, AT1G21970.1, AT5G47670.1, AT2G27470.1, AT5G08190.1 and AT5G23090.2, respectively. Nineteen maize NF-YB proteins were obtained, which were GRMZM2G064426, GRMZM5G804893, GRMZM2G303465, GRMZM5G866699, GRMZM2G478501, GRMZM2G480621, GRMZM5G809663, GRMZM2G444073, GRMZM2G180947, GRMZM2G384528, GRMZM2G473152, GRMZM2G169884, GRMZM2G152822, GRMZM2G167576, GRMZM2G11789, GRMZM2G124663, GRMZM2G012654, GRMZM2G146286 and GRMZM2G147712, respectively. The NF-YB genes of the above 17 maize were named NF-YB-1, NF-YB-2, NF-YB-3, NF-YB-4, NF-YB-5, NF-YB-6, NF-YB-7, NF-YB-8, NF-YB-9, NF-YB-10, NF-YB-11, NF-YB-12, NF-YB-13, NF-YB-14, NF-YB-15, NF-YB-16 and NF-YB-17, respectively.

### Phylogenetic analysis of NF-YB Gene in Maize and *A. thaliana*

Twenty eight *A. thaliana* NF-YB genes and 49 maize NF-YB genes were selected and the protein sequences of NF-YB transcription factor family were compared by ClustalW and the phylogenetic tree was established. NF-YB transcription factors in maize and Arabidopsis could be divided into five groups, which were named Group I, Group II, Group III, Group IV and Group V respectively (Fig. 1). We found that there were 7 members of maize in Group I, which were GRMZM2G064426, GRMZM5G804893, GRMZM2G303465, GRMZM5G866699, GRMZM2G478501, GRMZM2G480621 and GRMZM5G809663. *A. thaliana* had three members, including AT2G37060.1, AT3G53340.1 and AT2G38880.8. In Group II, maize and *A. thaliana* each had three members, and maize were GRMZM2G444073, GRMZM2G180947 and GRMZM2G384528 respectively. *A. thaliana* were AT2G13570.1, AT4G14540.1 and AT5G47640.1. In Group III, maize and *A. thaliana* had 2 members respectively, maize were GRMZM2G473152 and GRMZM2G169884 respectively. *A. thaliana* had two members, which were AT2G47810.1 and AT1G09030.1. In Group IV, maize had four members, which were GRMZM2G152822, GRMZM2G167576, GRMZM2G11789 and GRMZM2G124663. *A. thaliana* had two members, which were AT1G21970.1 and AT5G47670.1 respectively. In Group V, maize had three members and *A. thaliana* had three members respectively. Maize were GRMZM2G012654, GRMZM2G146286 and GRMZM2G147712 respectively. *A. thaliana* were AT2G27470.1, AT5G08190.1 and AT5G23090.2 respectively. Among the entire group, maize gene encoded protein accounted for the largest proportion in Group I.



**Fig. 1:** Phylogenetic evolution of NF-YB genes in maize and *A. thaliana*

### Analysis of conserved domains of NF-YB transcription factor family protein sequences

Conservative domain refers to a kind of highly conserved domain in the process of biological evolution. Analysis of the conserved domain of the amino acid sequence of NF-YB protein in maize showed that all NF-YB transcription factors in maize contained a CBF<sub>2</sub>D\_NFYB\_HMF domain, in which GRMZM2G011789, GRMZM2G167576 and GRMZM2G012654 contained a Histone domain, which coincided with their CBF<sub>2</sub>D\_NFYB\_HMF domain (Fig. 2), while Histone represented a histone binding domain, which may be related to the epigenetic signals such as histone modification read by the above NF-YB transcription factors. GRMZM5G866699 contains a CCT domain. In the CCT domain, it contains a nuclear localization signal and a zinc finger structure that mediates the interaction between the protein and the protein, which means that the protein may form a complex with other transcriptional regulatory factors.

### Location of NF-YB gene on chromosomes

Based on the information of maize genomic B73\_ref\_v4, we determined the length of 17 NF-YB transcription factor

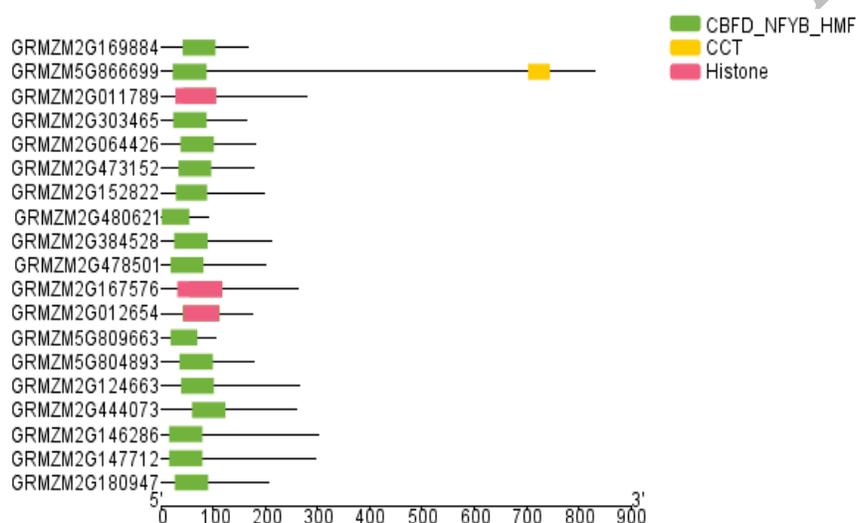
family genes (Table 1), and analyzed their distribution on 10 maize chromosomes. The 17 NF-YB transcription factor family members are unevenly distributed on 10 chromosomes of maize. Chromosome 1 contains NF-YB-5 and NF-YB-16; Chromosome 2 contains NF-YB-10 and NF-YB-14; Chromosome 3 contains NF-YB-9, NF-YB-11 and NF-YB-13; Chromosome 4 contains NF-YB-4 and NF-YB-6; Chromosome 5 contains NF-YB-1; Chromosome 6 contains NF-YB-15; Chromosome 7 contains NF-YB-2, NF-YB-7 and NF-YB-12; Chromosome 8 contains NF-YB-3 and NF-YB-17; Chromosome 9 contains NF-YB-8; While chromosome 10 does not contain this transcription factor family gene (Fig. 3).

### Analysis of physicochemical properties of NF-YB transcription factor family proteins

The amino acid composition and physicochemical properties of different NF-YB transcription factor family proteins are different, and the amino acid number, molecular weight, theoretical isoelectric point, fat coefficient and hydrophilic average coefficient are quite different among different NF-YB transcription factors. As shown in Table 2, on the whole, NF-YB transcription factor family proteins in

**Table 1:** Length of NF-YB gene

Gene	Locus	Gene location
NF-YB-1	GRMZM2G011789	Chr5: 204332559..204333737
NF-YB-2	GRMZM2G012654	Chr7:68977358..68979646
NF-YB-3	GRMZM2G064426	Chr8:117802830..117806690
NF-YB-4	GRMZM2G124663	Chr4:164097202..164098323
NF-YB-5	GRMZM2G146286	Chr1:219379297..219385327
NF-YB-6	GRMZM2G147712	Chr4:72846053..72852624
NF-YB-7	GRMZM2G152822	Chr7:16191777..16192698
NF-YB-8	GRMZM2G167576	Chr9:36849525..36850583
NF-YB-9	GRMZM2G169884	Chr31:54415632..154416136
NF-YB-10	GRMZM2G180947	Chr22:10388035..210389391
NF-YB-11	GRMZM2G303465	Chr3:182417996..182420272
NF-YB-12	GRMZM2G384528	Chr7:164694041..164695458
NF-YB-13	GRMZM2G478501	Chr3:21149128..21167654
NF-YB-14	GRMZM2G480621	Chr2:565394..566331
NF-YB-15	GRMZM5G804893	Chr6:152187992..152193678
NF-YB-16	GRMZM5G809663	Chr1:106417592..106419191
NF-YB-17	GRMZM5G866699	Chr8:169431514..169439728



**Fig. 2:** Conserved domains of NF-YB amino acid sequence in maize

maize are rich in acidic amino acids, most of the isoelectric points are in the acidic or weakly acidic range, and only the isoelectric points of NF-YB-3, NF-YB-9 and NF-YB-15 are in the alkaline range. The average hydrophilic coefficient of NF-YB transcription factor family proteins in maize is only negative but not positive, indicating that the transcription factor family proteins are hydrophilic proteins, not hydrophobic proteins. Fat coefficient can be used as an index of protein stability. Generally, the higher the fat coefficient is, the higher the protein stability is. In this study, it was found that the basic fat coefficients of maize NF-YB transcription factors were all in a high range (Table 2), which inferred that the stability of maize NF-YB transcription factor family proteins was good.

#### Subcellular localization of NF-YB transcription factor

We use the online tool Plant-mPLOC

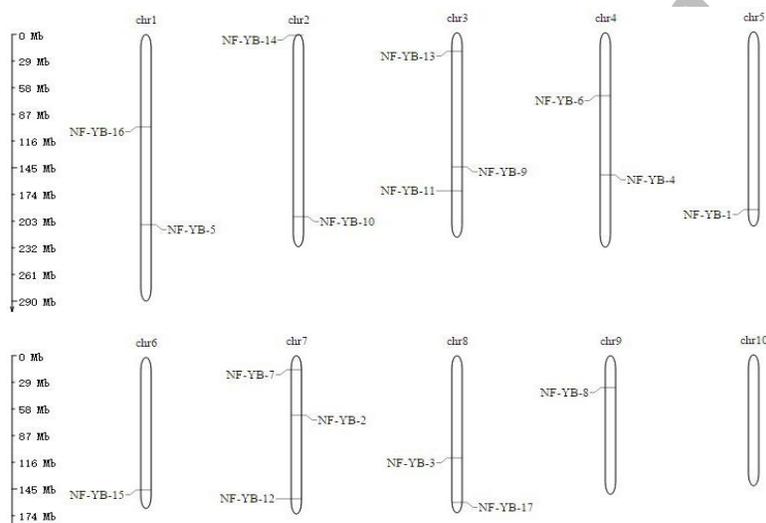
(<http://www.csbio.sjtu.edu.cn/bioinfo/plant-multi/>) to predict subcellular localization (Zhu *et al.* 2012). NF-YB transcription factor family proteins are located in the nucleus (Table 2). Results show that NF-YB transcription factor family proteins are all concentrated in the nucleus and may have the function of transcriptional regulation.

#### Expression analysis of NF-YB in maize

Using the transcriptome sequencing data of maize tissues at different developmental stages published by Stelpflug *et al.* (2016), we analyzed the expression of 17 NF-YB genes in different developmental stages of maize, and drew a heat map (heatmap) based on the FPKM values of each gene at each developmental stage. The tissues analyzed included germinated seeds at different stages, different regions of roots, seedlings, stems in different positions, apical meristems of stems, leaves, internodes, spikes, anthers and

**Table 2:** Analysis of physical and chemical properties and subcellular localization of NF-YB transcription factor family proteins

Gene	Locus	Amino acid number	Molecular weight	Theoretical iso electric point	Fat coefficient	Average coefficient of hydrophobicity	Predicted location
NF-YB-1	GRMZM2G011789	303	31934.87	6.39	50.46	-0.552	Nucleus
NF-YB-2	GRMZM2G012654	200	22131.34	5.29	65.05	-0.071	Nucleus
NF-YB-3	GRMZM2G064426	205	21873.84	7.90	53.85	-0.678	Nucleus
NF-YB-4	GRMZM2G124663	289	30935.69	6.42	50.10	-0.656	Nucleus
NF-YB-5	GRMZM2G146286	326	36720.67	5.60	66.07	-0.898	Nucleus
NF-YB-6	GRMZM2G147712	322	36489.49	5.35	69.07	-0.878	Nucleus
NF-YB-7	GRMZM2G152822	222	24065.38	4.97	66.85	-0.526	Nucleus
NF-YB-8	GRMZM2G167576	287	30875.87	6.39	51.71	-0.673	Nucleus
NF-YB-9	GRMZM2G169884	191	21329.08	7.96	54.76	-0.879	Nucleus
NF-YB-10	GRMZM2G180947	230	24652.02	6.08	45.48	-0.690	Nucleus
NF-YB-11	GRMZM2G303465	189	20647.56	6.13	54.23	-0.735	Nucleus
NF-YB-12	GRMZM2G384528	237	24987.31	6.59	43.76	-0.635	Nucleus
NF-YB-13	GRMZM2G478501	225	25177.29	4.51	67.64	-0.762	Nucleus
NF-YB-14	GRMZM2G480621	116	13232.26	5.56	58.88	-0.825	Nucleus
NF-YB-15	GRMZM5G804893	203	21699.68	7.90	54.43	-0.667	Nucleus
NF-YB-16	GRMZM5G809663	130	14752.12	5.58	74.31	-0.580	Nucleus
NF-YB-17	GRMZM5G866699	855	94913.87	5.09	61.04	-0.694	Nucleus

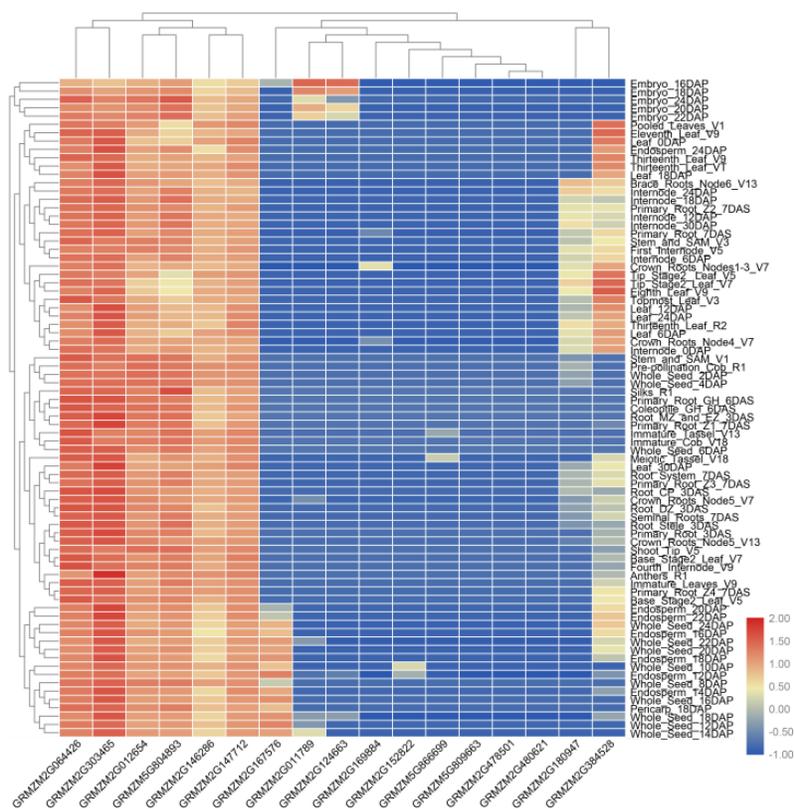
**Fig. 3:** Location of NF-YB transcription factor family genes on different chromosomes of maize

filaments (maize whiskers). The selective expression of NF-YB transcription factor family genes in different tissues and developmental stages in maize (Fig. 4). It has been found that overexpression of GRMZM2G012654 (NF-YB-2) can accelerate cell division and elongation, and promote the growth of taproot (Ballif *et al.* 2011). In the analysis, it was found that the expression of GRMZM2G064426 (NF-YB-3), GRMZM2G303465 (NF-YB-11), GRMZM5G804893 (NF-YB-15), GRMZM2G146286 (NF-YB-5) and GRMZM2G147712 (NF-YB-6) was very similar to that of GRMZM2G012654 (NF-YB-2), so we speculated that the function of these five NF-YB transcription factor family members was similar to that of GRMZM2G012654 (NF-YB-2). What is worth our most attention is that the expressions of GRMZM2G011789 (NF-YB-1) and GRMZM2G124663 (NF-YB-4) are relatively low during the whole growth and development period, and only at a high level at the early stage of embryo formation, indicating

that these two family members play a certain role in embryo formation. In addition, we also found that the overall expression of the six family members of GRMZM2G169884, GRMZM2G152822, GRMZM5G866699, GRMZM5G809663, GRMZM2G478501 and GRMZM2G480621 is relatively low, we speculate that these six members are highly expressed under biotic or abiotic stress, thus regulating the growth and development of maize under stress.

## Discussion

Transcription factors played an important role in the growth and development of plants and had response to changes in the external environment, which were also the key links in the regulation of various physiological activities (Yu *et al.* 2016; Yu *et al.* 2018). Nuclear factor Y (Nuclearfactor-Y), referred to as NF-Y, is a transcription factor that binds to the



**Fig. 4:** Expression map of NF-YB gene in different tissues of maize

cis-acting element of CCAAT-box and regulates the expression of target genes (Zhuang *et al.* 2009; Yuan, 2017). It is common in eukaryotes such as yeast, plants, animals and so on (Liu *et al.* 2016). Plant nuclear transcription factors are encoded by multiple genes, mainly including nuclear transcription factor A subunit family (NF-YA) (CBF-B or HAP2), B subunit family (NF-YB) (CBF-A or HAP3) and C subunit family (NF-YC) (CBF-C or HAP5) (Romier *et al.* 2003). Existing studies have shown that plant NF-Y transcription factors play an important role in plant embryonic development, photosynthesis, flowering time regulation and stress response, but the related studies are mainly carried out in *A. thaliana*, the function of this transcription factor in maize has not been reported (Cai *et al.* 2007; Chen *et al.* 2007).

In this study, the characteristics of NF-YB family genes in maize were analyzed by bioinformatics analysis. We screened nineteen NF-YB sequences from maize NF-YB transcription factor family, which is more than the number of *A. thaliana*, indicating that the expansion of the number of NF-YB genes may be the evolutionary characteristics of maize to better adapt to the environment. All 17 transcription factors contain the same conserved domain Cbfd\_NFYB\_HMF, and individual sequences also contain CTT domain and histones, indicating that the function of this transcription factor family is diverse.

Comparing the physical and chemical properties of NF-YB transcription factor family proteins, the transcription factor family proteins in maize are rich in acidic amino acids, most of the isoelectric points are in the acidic range, and the average hydrophilic coefficient of transcription factor family proteins is only negative but not positive, indicating that the NF-YB transcription factor family proteins are not very complex. In subcellular localization, it is found that NF-YB transcription factors are mostly located in the nucleus like traditional transcription factors. There is a close relationship between gene expression pattern and function. Different genes in NF-YB family are expressed differently in different tissues and periods, in which GRMZM2G064426 and GRMZM2G303465 are highly expressed in different tissues and periods, indicating that they play an important role in the whole growth and development of maize. However, GRMZM2G011789 and GRMZM2G124663 are only highly expressed during embryonic development, indicating that they play an important role in the regulation of embryonic development and maturation. In summary, this study laid a theoretical foundation for further exploring the gene function of maize NF-YB family.

Thus, the results of this study promote the understanding of NF-YB family genes and lay a foundation for the functional study of NF-YB family genes at the molecular level.

## Conclusion

The NF-YB transcription factor family in maize was identified by bioinformatics tools, which provided some basic data for further utilization of NF-FB transcription factors in maize breeding.

## Acknowledgements

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