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ORIGINAL ARTICLE

Gene structure, expression pattern and interaction of Nuclear Factor-Y family in castor bean (*Ricinus communis*)

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Abstract

Main conclusion **Nuclear Factor-Y transcription factors, which function in regulating seed development (including storage reservoir accumulation) and responding to abiotic stresses, were identified and characterized in castor bean**.

Nuclear Factor-Y (NF-Y) transcription factors in plants contain three subunits (NF-YA, NF-YB and NF-YC), and function as a heterodimer or heterotrimer complex in regulating plant growth, development and response to stresses. Castor bean (*Ricinus communis*, Euphorbiaceae) one of the most economically important non-edible oilseed crops, able to grow in diverse soil conditions and displays high tolerance to abiotic stresses. Due to increasing demands for its seed oils, it is necessary to elucidate the molecular mechanism underlying the regulation of growth and development. Based on the available genome data, we identified 25 RcNF-Y members including six RcNF-YAs, 12 RcNF-YBs and seven RcNF-YCs, and characterized their gene structures. Yeast two-hybrid assays confirmed the protein–protein interactions among three subunits. Using transcriptomic data from different tissues, we found that six members were highly or specifically expressed in endosperms (in particular, two LEC1-type members *RcNF-YB2* and *RcNF-YB12*), implying their involvement in regulating seed development and storage reservoir accumulation. Further, we investigated the expression changes of *RcNF-Y* members in two-week-old seedlings under drought, cold, hot and salt stresses. We found that the expression levels of 20 *RcNF-Y* members tested were changed and three *RcNF-Y* members might function in response to abiotic stresses. This study is the first reported on genomic characterization of NF-Y transcription factors in the family Euphorbiaceae. Our results provide the basis for improved understanding of how *NF-Y* genes function in the regulation of seed development and responses to abiotic stresses in both castor bean and other plants in this family.

Keywords Abiotic stress · Castor bean · Expression profiles · NF-Y transcription factor · Protein interaction

Abbreviations

NF-Y	Nuclear Factor-F
Rc	*Ricinus communis*
LEC1	*Leafy Cotyledon 1*
CDS	Coding sequence
Y2H	Yeast two hybrid

Introduction

Transcription factors play a crucial role in numerous life activities and usually regulate gene expression by recognizing and binding to specific DNA elements in the promoter region of targeted genes. The Nuclear Factor-F (NF-Y) TF family, also called heme activator protein (HAP) or CCAAT

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binding factor (CBF), has been found in all the sequenced genomes of eukaryotes (Cao et al. 2011; Laloum et al. 2013; Cagliari et al. 2014). As a complex, the NF-Y protein consists of three distinct subunits, namely HapB (NF-YA), HapC (NF-YB) and HapE (NF-YC), and each subunit is encoded by one or two genes in yeast and mammals. To function for NF-F complexes, the translocation of NF-Y subunits into the nucleus is required. Due to lack of a nuclear localization signal, HapC usually interacts with HapE to form a tight heterodimer in the cytoplasm (Thön et al. 2010; Hackenberg et al. 2012a). HapB has a nuclear localization signal but needs the combined protein surface provided by dimerization of HapC and HapE as found in *Aspergillus nidulans*, rat and yeast (Sinha et al. 1995; McNabb and Pinto 2005; Thön et al. 2010). It has been found that there is a piggyback transport of complete heterotrimeric NF-Y complex in A*spergillus nidulans* (Steidl et al. 2004). It was recently proposed that AtNF-YA and AtNF-YC were imported into the nucleus while AtNF-YBs could be translocated into the nucleus after interaction with NF-YC (Hackenberg et al. 2012a) in *Arabidopsis*. Many studies have demonstrated that the NF-Y complex plays a critical role in regulating cell proliferation and early embryo development through interactions among subunits in yeasts and mammals (De Silvio et al. 1999; Siefers et al. 2009).

In plants, the distinctive character of the *NF-Y* gene family showed that each subunit was usually encoded by multiple members (Petroni et al. 2012). For example, there are 30 members (including 10 *NF-YAs*, 10 *NF-YBs* and 10 *NF-YCs*) in *Arabidopsis thaliana* (Siefers et al. 2009; Petroni et al. 2012) and 59 members (including 12 *NF-YAs*, 32 *NF-YBs* and 15 *NF-YCs*) in *Glycine max* (Quach et al. 2015). The expansion of *NF-Y* gene number in plants showed genetic redundancy or functional divergence, which might contribute to the combinatorial and flexible TF network, and therefore, regulate plant growth and development and response to environmental changes (Calvenzani et al. 2012; Petroni et al. 2012; Ren et al. 2016). Many studies have found that *NF-Y* genes are responsible for regulating diverse physiological processes related to plant growth and development, such as embryogenesis (Lotan et al. 1998; Kwong et al. 2003; Mu et al. 2013), photomorphogenesis (Myers et al. 2016), photoperiod dependent flowering (Ben-Naim et al. 2006; Hackenberg et al. 2012b; Petroni et al. 2012), seed germination (Petroni et al. 2012; Huang et al. 2015; Liu et al. 2016), and root elongation (Ballif et al. 2011; Sorin et al. 2014). In particular, *Arabidopsis Leafy Cotyledon 1* (*AtLEC1*, *AtNF-YB9*) gene, the first cloned and identified member in the NF-Y family, has been shown to play a role in regulating embryogenesis (West et al. 1994; Meinke et al. 1994; Lotan et al. 1998; Lee et al. 2003). Usually, *AtLEC1* is specifically expressed in the developing embryo. Loss-of-function of *AtLEC1* resulted in abnormal cotyledons and the end of embryo development (West et al. 1994) whereas over-expression of *AtLEC1* led to the formation of embryo-like structures in the leaf tissues of *Arabidopsis* (Lotan et al. 1998). (*AtLIL*, *AtNF-YB6*), a homolog of *LEC1*, shared high similarity to *LEC1* in sequences, but showed different expression patterns and could partly replace the function of LEC1 during embryo development (Kwong et al. 2003).

Previous studies have found evidence that NF-Y members such as LEC1 and L1L are involved in regulating fatty acid biosynthesis. Overexpression of *LEC1* could result in the increased accumulation of fatty acid and triacylglycerol (TAG), and increased seed oil content in *A. thaliana* (Mu et al. 2008), *Brassica napus* (Tan et al. 2011) and maize (Shen et al. 2010). NF-Y members can also interact with other transcription factors to regulate seed oil content or fatty acid composition. For example, NF-YA3 interacts directly with WRI1-1 (AP2/ERF), together with NF-YC2 and ABI5 (bZIP) to regulate the transcription of oil biosynthetic genes in oil palm (Yeap et al. 2017). *Arabidopsis* LEC1 and NF-YC interacts with other transcription factors such as LEC2 to activate the transcription of the *FAD3* gene that encodes an omega-3 fatty acid desaturase and catalyzes linolenic acid biosynthesis (Mendes et al. 2013). The mechanism of NF-Y in regulating the storage material accumulation in developing seeds needs to be further elucidated.

Recent studies have revealed that NF-Y members are involved in regulating physiological processes in response to various abiotic or biotic stresses (Nelson et al. 2007; Hackenberg et al. 2012b; Ni et al. 2013; Zhang et al. 2015a, 2016; Swain et al. 2016; Zhao et al. 2017). The over-expression of the *NF-YA5* (*AT1G54160*) and *NF-YB1* (*AT2G38880*) in *Arabidopsis* enhanced growth and survival under drought stress (Li et al. 2008). The heterologous expression of *GmNF-YA3* from soybean exhibited a stronger drought tolerance in *Arabidopsis* (Ni et al. 2013). Similarly, the heterologous expression of *Picea wilsonii PwNF-YA3* significantly enhanced the ability of *Arabidopsis* to tolerate salinity and drought stresses (Zhang et al. 2015b). These studies showed that the NF-Y family participated in various regulatory processes during plant growth and development with functional divergences among the members. However, only several genes were functionally documented in limited model plants such as *Arabidopsis* (Siefers et al. 2009), rice (Thirumurugan et al. 2008; Lee et al. 2015) and maize (Zhang et al. 2016).

The completion of genome sequences for several plants have allowed the comprehensive characterization and functional annotation of *NF-Y* genes in several species, such as *Arabidopsis* (Siefers et al. 2009), maize (Zhang et al. 2016), tomato (Li et al. 2016) and soybean (Quach et al. 2015). Castor bean (*Ricinus communis* L., Euphorbiaceae) is one of the most economically important non-edible oilseed crops and its seed oil is broadly used in industry due to its

high ricinoleic acid content, which makes castor oil an ideal feedstock for biochemical and biodiesel production (Akpan et al. 2006; Ogunniyi 2006; Scholza and da Silva 2008). Castor bean is also extremely hardy: it is drought-tolerant, has a small gestation period and can adapt to a wide variety of soil conditions, including high levels of soil salinity. Due to increasing demand for production of castor bean seed oil in many countries, the development of improved varieties is of great importance to breeders and producers (Qiu et al. 2010). Further efforts should be made to elucidate the molecular mechanisms underlying the regulation of growth and development. The available genome sequences (Chan et al. 2010) provide a great opportunity for comprehensive characterization of the NF-Y family in castor bean, which could provide useful information in understanding the molecular mechanism of the *NF-Y* genes underlying the regulation of growth and development of castor bean. In the present study, genomic identification and structural characterization of the NF-Y family in castor bean was performed. The interactions among subunits comprising the NF-Y complex were assayed. Global expression profiles of the castor bean *NF-Y* genes in different tissues were examined using high-throughput transcriptomic sequencing technology and expressional responses to different abiotic stresses were inspected using the quantitative real-time PCR method. The results obtained here provide a comprehensive profile of the molecular basis of the NF-Y family in castor bean and other plants in the family Euphorbiaceae.

Materials and methods

Identification of RcNF-Y family members in castor bean

According to the updated classification criteria of NF-Y transcription factors in *A. thaliana* (Petroni et al. 2012), 30 amino acid sequences including 10 NF-YA, 10 NF-YB and 10 NF-YC subunits were retrieved from the TAIR (http://www.arabidopsis.org/). An initial search for NF-Y transcription factors in castor bean was performed using the protein sequences of *Arabidopsis* NF-Ys as queries against the protein database downloaded from the website http://castorbean.jcvi.org/index.php using the local software Blast + (2.2.24 +). Hits with significant *E* value ($E \leq 10^{-10}$) were extracted from the castor bean database using a custom perl script. Subsequently, the protein sequences were analyzed with the online SMART tool (http://smart.embl-heidelberg.de/) to confirm the presence of conserved domains in the NF-Y family. The resulting sequences were considered as NF-Y candidates in castor bean. The identified NF-Ys were named according to their order in the scaffold from top to bottom.

Alignments, phylogenies and gene structure analysis of RcNF-Ys

The multiple sequence alignment of each RcNF-Y family was carried out using full-length protein sequences in Clustal X software (Larkin et al. 2007). The protein alignments were imported into MEGA7 (Kumar et al. 2016) to construct phylogenetic trees using the neighbor-joining (NJ) method with 1000 bootstrap replicates. The gene structures of NF-Y members of castor bean and *A. thaliana* were created by comparing the full-length CDS (coding sequence) with the corresponding genomic DNA sequence, using the Gene Structure Display Server (GSDS, http://gsds.cbi.pku.edu.cn/) (Hu et al. 2015). In addition, the conserved motifs of all NF-Y members were investigated using the online MEME tool (http://meme.nbcr.net/meme/tools/meme). Parameters were set with optimum width of 10–200 amino acids, a motif with any number of repetitions, and the maximum number of motifs at 10.

Interactions between of RcNF-Y members by Y2H assay

To determine the protein–protein interaction between RcNF-YB and RcNF-YC or RcNF-YA members, we performed the yeast two hybrid (Y2H) assays. In this study, the constructs pDest22 and pDest32 (Invitrogen) were used for Y2H assays, which include transcription activating domain (AD) and DNA binding domain (BD), respectively. The CDS of each *RcNF-YB* gene was cloned into prey vector pDest22 through BP and LR recombination reactions, while the CDS of a single *RcNF-YA* or *RcNF-YC* gene was cloned into bait vector pDest32 in the same way. Two constructs of different combinations were co-transformed in yeast host strain Y2Hgold (Clontech) according to the previously reported protocol (Gietz and Schiest 2007). After co-transformation, yeast cultures were selected for leucine and tryptophan in synthetic drop-out medium (SD-Leu-Trp) (Clontech). Protein–protein interactions were screened by growth of at least three independent yeast colonies on SD medium without leucine, tryptophan and histidine (SD-Leu-Trp-His) and on SD medium without leucine, tryptophan, histidine and adenine (SD-Leu-Trp-His-Ade). All primers used in this experiment are listed in Table S1.

Gene expression analysis of *RcNF-Ys* in different tissues of castor bean

The RNA-seq data of five tissues types from the castor bean plant were downloaded from NCBI SRA under the accession ERA047687, including male developing flower, leaf, germinating seed and developing endosperms at stage II/III (free nuclear, early stage) and V/VI (later stage, cellular).

The expression levels of *RcNF-Ys* were normalized to fragments per kilobase million (FPKM) and the heat map of gene expression was visualized by MeV 4.0 (http://www.tm4.org/mev.html). In addition, our previous transcriptomic data were also used to analyze *RcNF-Y* expression (Xu et al. 2013).

Stress responses of *RcNF-Ys* in castor bean

We treated two-week seedlings of castor bean ZB306 (provided kindly by Zibo Academy of Agricultural Sciences, Shandong, China) with four abiotic stresses: drought, cold, heat and salt for 12 h. The detailed procedures for each stress treatment were the same as described in the previous report (Xu et al. 2016). After treatment, the seedlings were immediately frozen in liquid nitrogen and used for RNA extraction. Total RNAs from different treatments were isolated using Tiangen RNAprep Pure Plant Kit (Beijing, China) according to the manufacturer's instructions. The cDNAs were synthesized using PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara, Dalian, China) with DNase treatment. Quantitative real-time PCR (qRT-PCR) was performed with SYBR Green I fluorescent dye (Transgene, Beijing, China) in Bio-Rad CFX machine (Bio-Rad, Hercules, CA, USA). The PCR program was as followed: 95 °C for 2 min, 40 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s with a final dissociation stage. The internal reference gene, *RcACTIN2*, was used to normalize the expression levels of the tested genes. The primers used in qRT-PCR are listed in Table S2. Three independent biological replications were performed. Statistical analyses were carried out using the *t* test.

Results

The RcNF-Y family consists of 25 members

Through an extensive search, a total of 25 RcNF-Y members were identified from the castor bean genome, such as six RcNF-YAs, 12 RcNF-YBs and seven RcNF-YCs. They were designated as RcNF-YA1-6, RcNF-YB1-12 and RcNF-YC1-7 according to the recently suggested nomenclature rule for the NF-Y family (Siefers et al. 2009). All protein sequences contained the typically conserved domains of NF-Ys confirmed by SMART and Pfam online tools. Although additional six members had little similarities with NF-Y family, further analysis found that they should be classified as NC2s or Dbp3/4s, excluded in this analysis.

The basic information of RcNF-Ys and the corresponding homologs in *Arabidopsis* are shown in Table 1. RcNF-YA genomic sequences ranged in size from 2405 to 6870 bp and their coding sequence (CDS) lengths varied from 642 to 1053 bp, which resulted in amino acid numbers from 117 to 706. Their molecular weights (MW) were 23.44–38.14 kD and isoelectric points were 7.0–9.27. The genomic DNA sequences of RcNF-YBs varied greatly in size from 354 to 3808 bp when their CDS lengths were between 354 and 759 bp. RcNF-YB proteins had 117–252 AAs with MW of 13.34–27.97 and lower pI of 5.15–7.01. The sizes of RcNF-YC genomic DNA sequences were 354–6556 bp and the CDS lengths were 354–2121 bp. The RcNF-YC proteins had 117–706 AAs with pI of 5.26–8.37 and MW of 12.86–81.96. In addition, the locus of RcNF-YB11 contained two isoforms with variation in CDS length or gene structure. The primary form of RcNF-YB11.1 contained one intron within the gene regions and the CDS without the intron sequence encoded a protein with 117 AAs. The RcNF-YB11.2 isoform was intronless and the CDS which retained the intron sequence encoded a relatively large protein with 131 AAs.

Phylogenetic analyses revealed the existence of three different clades in the RcNF-Ys family

To investigate the phylogenetic relationship of RcNF-Ys, the protein sequences of 25 members were aligned using Clustal X and phylogenetic trees were created by MEGA7 software with the neighbor-joining (NJ) criteria. Highly conserved domains were found among the members of each subunit as shown in Fig. 1, while N-terminal and C-terminal regions of RcNF-Y proteins showed more variable AAs. The core conserved domain of RcNF-YAs had about 53 AAs (Fig. 1a), which included two subdomains. Each subdomain contained 22 AAs, one for interaction with NF-YB/C and another for binding DNA. The central domain of RcNF-YBs had 95 AAs (Fig. 1b) and there were responding subdomains for DNA binding and for interactions of NF-YB/YC and NF-YB/YA. According to the distinct 16 AAs previously reported in the NF-YB conserved domain (Lee et al. 2003), RcNF-YB2 and RcNF-YB12 had obviously different residues from other 12 RcNF-YB members, and as a result two types of RcNF-YBs can be distinguished (LEC1 type and non-LEC1 type). The aspartate at D71 site was present in LEC1-type RcNF-YBs and was thought to be for protein interactions (Siefers et al. 2009). The central domain of RcNF-YCs contained about 80 AAs for interactions between subunits (Fig. 1c).

The phylogenetic relationships of the members of each RcNF-Y subunit were analyzed and each subunit could be further clustered into two to three clades (Fig. 2). Among RcNF-YAs (Fig. 2a), four members formed a large group, whereas RcNF-YA1 and RcNF-YA6 were relatively distant to them. RcNF-YA2 and RcNF-YA3 showed the strongest similarities to RcNF-YA4 and RcNF-YA6, respectively. The members of RcNF-YBs can be divided into three groups (Fig. 2b) where the largest group consisted of seven members. RcNF-YB2 and RcNF-YB12 closely clustered

Table 1 Basic information of 25 RcNF-Y members

Name	Gene ID	Putative *Arabidopsis* orthologs	CDS length	DNA length	No of AA	pI	MW (kD)
NF-YA subunit							
RcNF-YA1	29333.m001059	AtNF-YA10, 2	945	6363	314	9.27	34.46
RcNF-YA2	29706.m001307	AtNF-YA3, 8	1008	2773	335	8.61	36.86
RcNF-YA3	29864.m001503	AtNF-YA1, 9	1011	6870	336	9.13	35.36
RcNF-YA4	29912.m005288	AtNF-YA3, 5	984	4618	327	9.22	36.09
RcNF-YA5	30075.m001182	AtNF-YA7, 4	642	5580	213	9.22	23.44
RcNF-YA6	30169.m006445	AtNF-YA1, 9	1053	2405	350	7.00	38.14
NF-YB subunit							
RcNF-YB1	27699.m000212	AtNF-YB3, 2	549	1590	182	6.13	19.35
RcNF-YB2	29629.m001369	AtNF-YB6, 9	759	2802	252	6.90	27.97
RcNF-YB3	29629.m001382	AtNF-YB3, 2	543	543	180	5.15	19.39
RcNF-YB4	29719.m000316	AtNF-YB3, 2	663	663	220	7.01	24.19
RcNF-YB5	29726.m004023	AtNF-YB5, 3	477	477	158	6.63	18.0
NF-YB6	29805.m001490	AtNF-YB8, 10	543	2424	180	5.82	19.74
RcNF-YB7	29923.m000817	AtNF-YB8, 1	525	3808	174	5.65	18.84
RcNF-YB8	29966.m000228	AtNF-YB3, 2	594	594	197	5.98	21.21
RcNF-YB9	29992.m001420	AtNF-YB7, 3	702	702	233	5.81	25.77
RcNF-YB10	30128.m008801	AtNF-YB4, 5	477	477	158	6.83	17.67
RcNF-YB11.1	30129.m000351	AtNF-YB5, 2	396	396	131	6.17	15.18
RcNF-YB11.2	30129.m000351	AtNF-YB5, 2	354	354	117	6.15	13.34
RcNF-YB12	57991.m000014	AtNF-YB6, 9	519	519	173	5.63	19.78
NF-YC subunit							
RcNF-YC1	29586.m000615	AtNF-YC2, 4	714	2672	237	5.53	27.10
RcNF-YC2	29656.m000483	AtNF-YC9, 3	2121	6556	706	8.37	81.96
RcNF-YC3	29844.m003332	AtNF-YC9, 3	741	2071	246	5.31	27.52
RcNF-YC4	30128.m008750	AtNF-YC9, 3	744	1782	247	7.06	27.59
RcNF-YC5	30147.m014493	AtNF-YC1, 4	810	1488	269	5.26	29.22
RcNF-YC6	30169.m006305	AtNF-YC2, 3	819	3345	272	5.61	30.56
RcNF-YC7	30190.m011186	AtNF-YC3, 9	354	354	117	7.71	12.86

The RcNF-Ys was ordered according to the scaffold from top to the bottom within each subunit. Each RcNF-Y protein was used to BLAST against AtNF-Ys and two best matches were considered as *Arabidopsis* orthologs

together as LEC1-type. The RcNF-YCs formed three clusters (Fig. 2c). A further phylogenetic analysis was performed to explore the evolutionary relationships between NF-Y members from six species including moss (*Physcomitrella patens*), three dicotyledonous species (*Phaseolus vulgaris*, *Arabidopsis*, and castor bean) and two monocotyledonous species (*Oryza sativa* and *Setaria italica*). RcNF-Y members were clustered with their homologs from other species, suggesting that RcNF-Y members were conserved in evolutionary lines (Fig. S1). Notably, a distinct clade was identified as LEC1-type group in NF-YB, including RcNF-YB2, 12, AtNF-YB6, 9, PvNF-YB12, OsNF-YB7, 9 and SiNF-YB6, 9 (Fig. S1b). Based on previous studies (West et al. 1994; Lotan et al. 1998; Kwong et al. 2003; Mu et al. 2008; Tan et al. 2011), the members within this clade might be functionally conserved in plants, involved in regulating seed development and storage reservoir accumulation in developing seeds. It should be noted that the partial NF-YB and NF-YC members identified from moss (Zhang et al. 2015a) and *Setaria italica* (Feng et al. 2015), classified as NC2 and Dpb3/4, were included in this phylogenetic analysis (see Fig. S1b, c).

Characterization of gene structures and motifs

To identify potential functional regions in the NF-Ys protein, MEME tool was used to find out the conserved protein motifs of NF-Ys from castor bean and *Arabidopsis*. As a result, we found that the closely relevant NF-Y members between castor bean and *Arabidopsis* had highly conserved motifs and similar arrangements (Fig. 3). For 16 NF-YA proteins, all of them shared motif 4 and motif 5, and 12

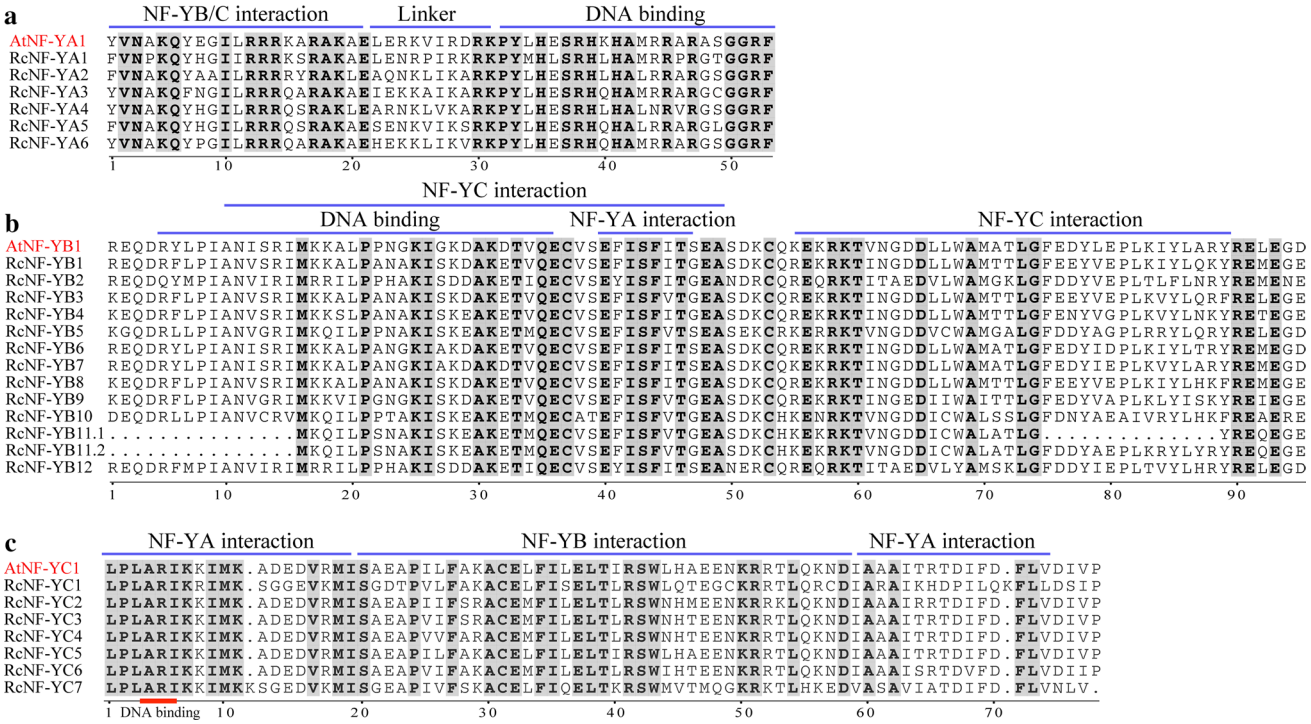

Fig. 1 Multiple sequence alignments of castor bean NF-Y TF members. **a** Multiple alignment of RcNF-YA proteins. **b** Multiple alignment of RcNF-YB proteins. **c** Multiple alignments of RcNF-YC proteins. One member from *Arabidopsis thaliana* (AtNF-YA1, AtNF-YB1, AtNF-YC1) was included, respectively. Multiple alignments of NF-Y protein sequences were carried out with ClustalX and MEGA7. The identical AAs are marked with black shadows. The AA numbers of the conserved domains and the functional regions are marked

members contained motif 9. In the case of 22 NF-YB members, three motifs (motif 1-3) were widely distributed. Four LEC1-type NF-YB members (including RcNF-YB2, 12 and AtNF-YB6, 9) shared the highly conserved motif 10. All of NF-YC members had the motif 1, and 15 NF-YC members contained motif 4, 6 and 7. We also observed that motif 8 was limited to a clade containing 10 members with abundant residues of glutamine (Q) in protein sequences, which was thought to play a critical role in transcription activation (Li et al. 2016).

In additions, we compared the exon–intron structures of *NF-Y* genes of castor bean with *Arabidopsis* (Fig. 3). The gene structures of *NF-YA* members were highly conserved, consistent with previous studies of other species (Gusmaroli et al. 2001; Zhang et al. 2015a). For example, the *NF-YA1* gene in castor bean contained three introns and its homologues in *Arabidopsis* had the same number of introns. The five *RcNF-YA* genes and their homologs in *Arabidopsis* shared four introns (except *AtNF-YA5*, which contained two introns). For *NF-YB* genes, 13 members were intronless, including five from *Arabidopsis* and eight from castor bean, whereas other members had variable numbers of introns from one to four. Among 17 NF-YC members, 12 members (including nine from *Arabidopsis* and three from castor bean) had no intron, and the number of introns within the other five members varied from one to five. The *RcNF-YC2* member contained up to 15 introns. Although the intron length was variable between castor bean and *Arabidopsis*, five out of seven *RcNF-Y* genes and their homologs in *Arabidopsis* had the same intron numbers, suggesting that *NF-Y* genes were highly conserved in evolution (Liang et al. 2014).

Interactions between RcNF-Ys by yeast two hybrid assay

It was thought that NF-YB and NF-YC formed a tight dimer, which resulted in a new conformation that facilitated binding with NF-YA (Sinha et al. 1995; McNabb and Pinto 2005). Using Y2H assays, we detected the possible interactions between some RcNF-Y members. The constitutively expressed genes (such as RcNF-YB2 and RcNF-YC1) and most specifically expressed genes (such as RcNF-YB1, 7 and RcNF-YC4) were selected for further Y2H experiments. Each CDS of five *RcNF-YAs* and two *RcNF-YCs* were cloned into bait vector which contained DNA binding domain (BD) while each of six selected *RcNF-YBs* were cloned into prey vector which had activation domain (AD). As shown in Fig. 4, on SD-L-T-H, most of the combinations between RcNF-YB and RcNF-YC were interacted except RcNF-YA2 or RcNF-YA5, which

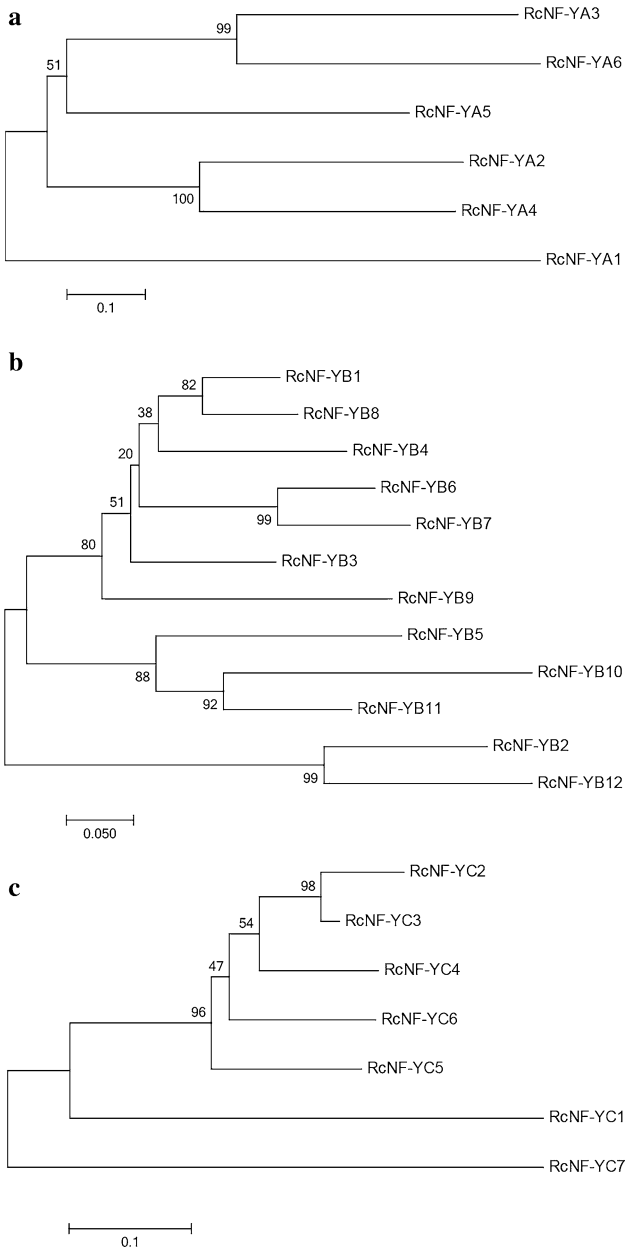

Fig. 2 The phylogenetic trees of RcNF-YAs, RcNF-YBs and RcNF-YCs constructed by MEGA7 with NJ method and a bootstrap of 1000 replicates. **a** Phylogenetic tree of RcNF-YA members. **b** Phylogenetic tree of RcNF-YB members. **c** Phylogenetic tree of RcNF-YC members

were conjuncted with BD. On SD-L-T-H-A medium, five stronger interactions were observed in combinations between RcNF-YBs and RcNF-YCs and three interactions were found between RcNF-YAs and RcNF-YBs. No or less interactions were found in RcNF-YB3 or RcNF-YB4. Indeed, these results suggested that most of the RcNF-YBs and RcNF-YCs could interact each other.

Overlapped and exclusive expression patterns of *RcNF-Y* members

To identify the potential functions of *RcNF-Ys* in the development of castor bean, we investigated the gene expression levels in five tissue types: developing endosperms at early (II/III) and later (V/VI) stages, germinating seed, leaf and male flower (Brown et al. 2012). The results showed that 22 of 25 *RcNF-Ys* were expressed in at least one of the five tissues, while three members including *RcNF-YB5*, *RcNF-YB10*, and *RcNF-YB11* could not be detected (Fig. 5). There were 17 *RcNF-Ys* transcribed in leaf tissue, 14 in germinating seed, 16 in male flower, 18 in early endosperm (II/III) and 17 in later endosperm (V/VI) (Fig. S2). The transcription levels of each *RcNF-Y* gene varied across the different tissues. Among them, 13 genes comprising five *RcNF-YAs*, three *RcNF-YBs* and five *RcNF-YCs* were constitutively expressed in all tissues, nine genes were highly expressed in leaf tissue (*RcNF-YA2*, *RcNF-YB8* and *RcNF-YC5*), and six genes (*RcNF-YA5*, *6*, *RcNF-YB9* and *RcNF-YC4*, *5*, *6*) were relatively abundant in the male flower. Notably, the gene *RcNF-YB9* was abundantly and specifically expressed in the male flower. During endosperm development, as many as 17 *RcNF-Y* genes showed higher expression levels at the early stage than at the later stage. Six members (*RcNF-YA2*, *RcNF-YA4*, *RcNF-YB2*, *RcNF-YB12*, *RcNF-YC4* and *RcNF-YC6*) were highly or specifically expressed in endosperms, and *RcNF-YB2* and *RcNF-YB12* belonged to LEC1-type members.

We further analyzed *RcNF-Y* expression patterns from the transcriptomic data of Xu et al. (2013), in which root tissues, seed1 (15 days after pollination) and seed2 (35 days after pollination) were included (Table S3). In total, 20 *RcNF-Ys* were found to be expressed in one or more tissues, including 16 in leaf, 14 in root, 14 in seed1, 15 in seed2 and 16 in endosperm (Fig. S2). There were 19 members that could be detected in both data sets, while*RcNF-YB10*) and three genes (*RcNF-YB8*, *RcNF-YB9*, and *RcNF-YC7*) were missing in the data of Xu et al. (2013) and Brown et al. (2012), respectively (Fig. S2). Two genes (*RcNF-YB5* and *RcNF-YB11*) could not be found in both sets of gene expression data. Most of the *RcNF-Ys* showed similar expression patterns where the same tissue was tested in both data. For most of the *RcNF-Y* genes, there were more transcripts in seed1 than seed2. Notably, *RcNF-YB2* and *RcNF-YB12* were only found in developing seeds and endosperm. Collectively, more than half of the *RcNF-Y* members were widely expressed in various tissues and several members were tissue-specifically expressed, such as seed-specific *RcNF-YB2* and *RcNF-YB12*. As mentioned above, NF-Y members usually form a tight heterodimer or trimmer to activate the expression of targeted genes. To inspect whether the *RcNF-Y* genes interacted

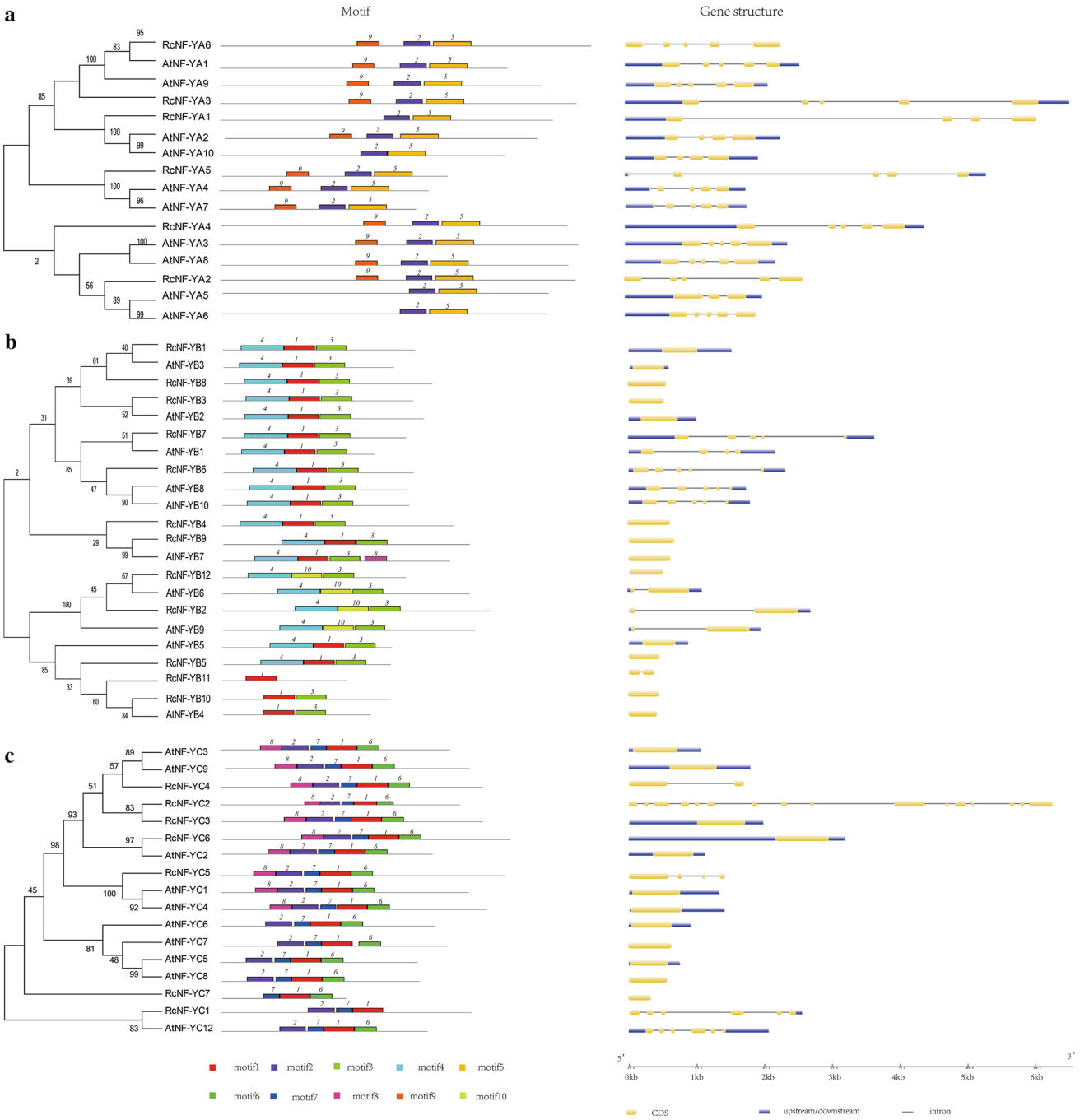

Fig. 3 Gene structures and conserved motifs of RcNF-Y and AtNF-Y proteins. Ten motifs were identified through MEME tool search and indicated with different colors. The blue box denotes untranslated region (UTR), yellow box represents exons within CDS while the line between them indicates intron. The length represents the corresponding size of exon and intron

with each other, were validated by Y2H experiments and exhibited co-expression patterns among different tissues, we carried out analysis using the transcriptomic data of Xu et al. (2013). As shown in Fig. S3, the co-expressions of the *RcNF-Y* genes with protein–protein interactions were weak, suggesting the transcriptional regulations of different members might be independent from each other.

RcNF-Y members behaved differently in response to abiotic stresses

An increasing number of studies have demonstrated that members of *NF-Y* gene family are involved in plant responses to various abiotic stresses. In the current study, we examined changes in the gene expression levels of *RcNF-Ys*

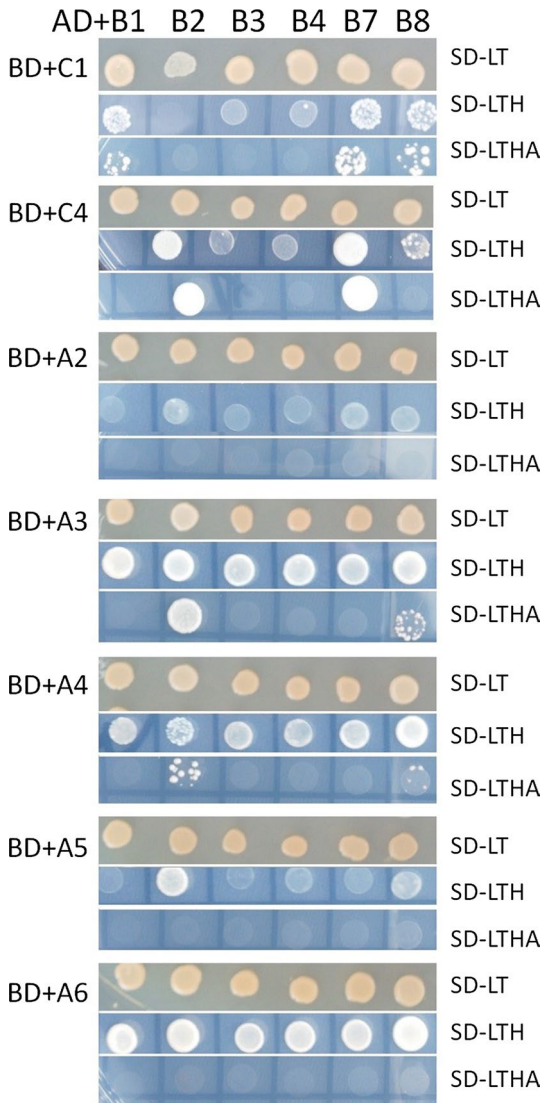

Fig. 4 Protein–protein interactions between RcNF-Y subunits by Y2H. *RcNF-YB* genes were fused to GAL4 Activation Domain (AD) and *RcNF-YC* or *RcNF-YA* genes were fused to GAL4 DNA-binding Domain (BD). SD-LT, SD-LTH and SD-LTHA stands for SD-Leu-Trp, SD-Leu-Trp-His and SD-Leu-Trp-His-Ade medium, respectively

in two-week-old seedlings that were exposed to environmental stresses including drought, salt, cold and heat using the qRT-PCR method. The results showed that 19 *RcNF-Ys* were responsive to one or more abiotic stresses and *RcNF-YC5* was little affected by any of the treatments tested (Fig. 6). Similarly, we found that five members (*RcNF-YA1*, *RcNF-YB4*, *RcNF-YB5*, *RcNF-YB10* and *RcNF-YC1*) showed very low or no expression in the transcriptomic data, which could not be detected in two-week seedlings under the various abiotic stresses we tested.

After drought treatment, the expression levels of *RcNF-YB12* decreased by up to 70%, while the expression levels of 13 *RcNF-Y* genes were induced. Four members (*RcNF-YB1*, *RcNF-YB2*, *RcNF-YB8*, and *RcNF-YC6*) were up-regulated and in particular, the expression level of *RcNF-YC6* increased up to 16-folds (*t* test, $P < 0.05$). The expression levels of seven *RcNF-Y* members were up-regulated under the salt stress treatment, of which the largest changes were observed for *RcNF-YB3*, and *RcNF-YC6*. The expression levels of seven *RcNF-Y* members were down-regulated under the salt stress treatment. Cold stress increased the expression levels of six *RcNF-Y* members (especially *RcNF-YC6*), and decreased the expression levels of 9 genes (notably, *RcNF-YC7*). When castor bean seedlings were imposed to heat stress, the expression levels of 12 *RcNF-Y* members were elevated while those of five genes decreased. The expression level of RcNF-YB6 and RcNF-YC6 increased by up to a factor of 3, whereas the expression levels of *RcNF-YB11* and *RcNF-YC2* showed 90% decreases under heat stress (*t* test, $P < 0.05$). In addition, we noted that the expression levels of *RcNF-YA2* and *RcNF-YC6* were up-regulated while that of *RcNF-YB12* gene was down-regulated by all four treatments. These results indicated that *RcNF-Y* genes were broadly involved in responses to various abiotic stresses.

Discussion

Although NF-Y transcription factors have been broadly studied in several plant species, the current study is the first report on genomic identification and characterization of the NF-Y transcription factors based on the genome of castor bean in the family Euphorbiaceae, an important group of resource plants. In total, according to the updated classification criteria of NF-Y transcription factors in *A. thaliana* (Petroni et al. 2012), 25 NF-Y genes were identified based on the castor bean genome sequences. Compared with the numbers of NF-Y members, such as 30 in *Arabidopsis* (Siefers et al. 2009), 33 in canola (Liang et al. 2014), 28 in rice (Thirumurugan et al. 2008; Petroni et al. 2012) and 30 in common bean (Rípodas et al. 2015), castor bean (genome size 310 Mb) harbored a comparable number of genes. However, soybean possessed as many as 66 (Quach et al. 2015) NF-Y members. These variations in gene numbers might be associated with variations in genome size. Also, these variations in gene numbers are related to the classification criteria, which include or exclude the partial NC2 and Dpb3/4 members based on the identification of *Arabidopsis* NF-Y (which included 36 or 30 members), e.g., maize and tomato had 50 (Zhang et al. 2016) and 59 (Li et al. 2016) NF-Y members, respectively, both of which include several NC2 and Dpb3/4 members.

The 25 RcNF-Y members identified in castor bean were divided into three subunits: RcNF-YA, RcNF-YB and RcNF-YC. Notably, *RcNF-YB* genes were separated into LEC1-type and non-LEC1-type, as found in both monocotyledons

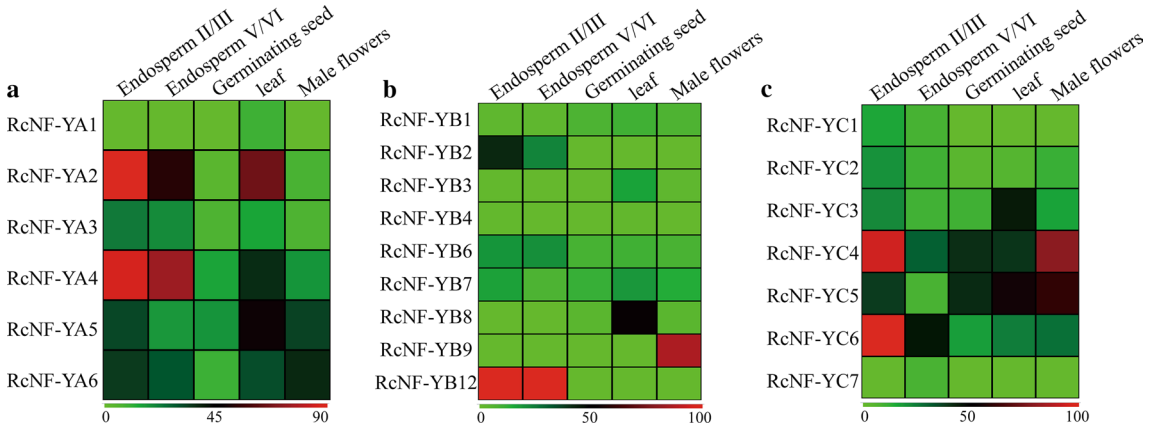

Fig. 5 Expression patterns of *RcNF-Ys* in five tissues of castor bean. **a** Expression levels of *RcNF-YAs*. **b** Expression levels of *RcNF-YBs*. **c** Expression levels of *RcNF-YCs*. The heat maps were created according to transcriptomic data of five tissues, including leaf, male flowers, germinating seed and two endosperms at two different stages (II/III and V/VI) (Brown et al. 2012). Red represents higher expression levels while blue color indicates no transcript detected

(Cao et al. 2011; Zhang et al. 2016) and dicotyledons (Siefers et al. 2009; Liang et al. 2014; Quach et al. 2015), suggesting the emergence of LEC1-type genes before the monocot–dicot split during plant evolution. Also, our results support the previous hypothesis that LEC1-type genes might originate from the non-LEC1 type (Cagliari et al. 2014). Each of the identified RcNF-Y subunits contained a conserved core domain which is responsible for DNA binding at CCAAT site (Fig. 1), suggesting that RcNF-Y transcription factors might share a similar regulatory pathway when they activate gene expression. Usually, the AA residues within NF-Y proteins are necessary to function. While comparing the AA residues for each subunit (RcNF-YA, RcNF-YB and RcNF-YC) based on amino acid sequences among castor bean, *Arabidopsis* (Siefers et al. 2009), canola (Liang et al. 2014) and maize (Zhang et al. 2016), we found that the AA residues were quite conserved in length (53 AAs for RcNF-YA, 100 AAs for RcNF-YB and 80 AAs for RcNF-YC). Previous studies have found that most of the members within NF-YAs possess 3–6 introns in genomes, whereas the intron/exon organization seems to be rather variable within NF-YBs and NF-YCs (Liang et al. 2014; Rípodas et al. 2015; Li et al. 2016; Ren et al. 2016; Zhang et al. 2016). Similarly, our current study found that most of the *RcNF-YAs* possessed four introns, while the number of introns between *NF-YBs* and *NF-YCs* was highly variable. In addition, based on analysis of gene structure and phylogenetic relationships, RcNF-YB2 and RcNF-YB12 were most likely to be the two LEC1-type orthologs of AtLEC1 and AtL1L that are pivotal in regulating the embryogenesis and seed development in *Arabidopsis*. Thus, we infer that RcNF-YB2 and RcNF-YB12 might share similar functions in regulating embryogenesis and seed development in castor bean, if these orthologs of LEC1-type genes are functionally conserved in plants.

A distinctive feature of *NF-Y* genes is that they usually function by formation of a heterodimeric or heterotrimeric complex. Our yeast two-hybrid experiments demonstrated that there were strong interactions between RcNF-YB and RcNF-YC members, whereas the interaction between RcNF-YA and RcNF-YB members was weak, consistent with the findings in *Arabidopsis* (Calvenzani et al. 2012; Hackenberg et al. 2012a). Since many heterogeneous combinations exist among NF-Y members in theory, only limited RcNF-Y complexes were verified in this study. In particular, we found that two *NF-Y* members (*RcNF-YC4* and *RcNF-YA2*) were specifically or highly co-expressed in endosperm (Fig. 5), which exhibited strong interactions with another member RcNF-YB2 (specifically expressed in seed) in vitro, strongly implying that these factors most likely co-acted to participate in regulation of endosperm development and storage reservoir accumulation in castor bean seeds. Another constitutively expressed member RcNF-YB1 interacted with RcNF-YC1, which were co-expressed in diverse tissues including root, leaf, seed and male flower. In addition, previous research has shown that NF-Y protein can form diverse complexes with other proteins, such as basic region/leucine zipper motif (bZIP) bZIP67 (Mendes et al. 2013). The NF-YB/NF-YC dimers have been known to interact with a master regulator CONSTANS for regulating flowering (Ben-Naim et al. 2006). These findings indicate that fully investigating the heterogeneous protein interactions of the NF-Y family is a highly complex task.

NF-Y genes are functionally involved in regulating diverse physiological processes in plant growth and development (Zhao et al. 2017). Here, the expression level of more than half of the *RcNF-Y* members were identified from five tissues tested, suggesting that *RcNF-Y*s functionally participate in regulating the growth and development of different

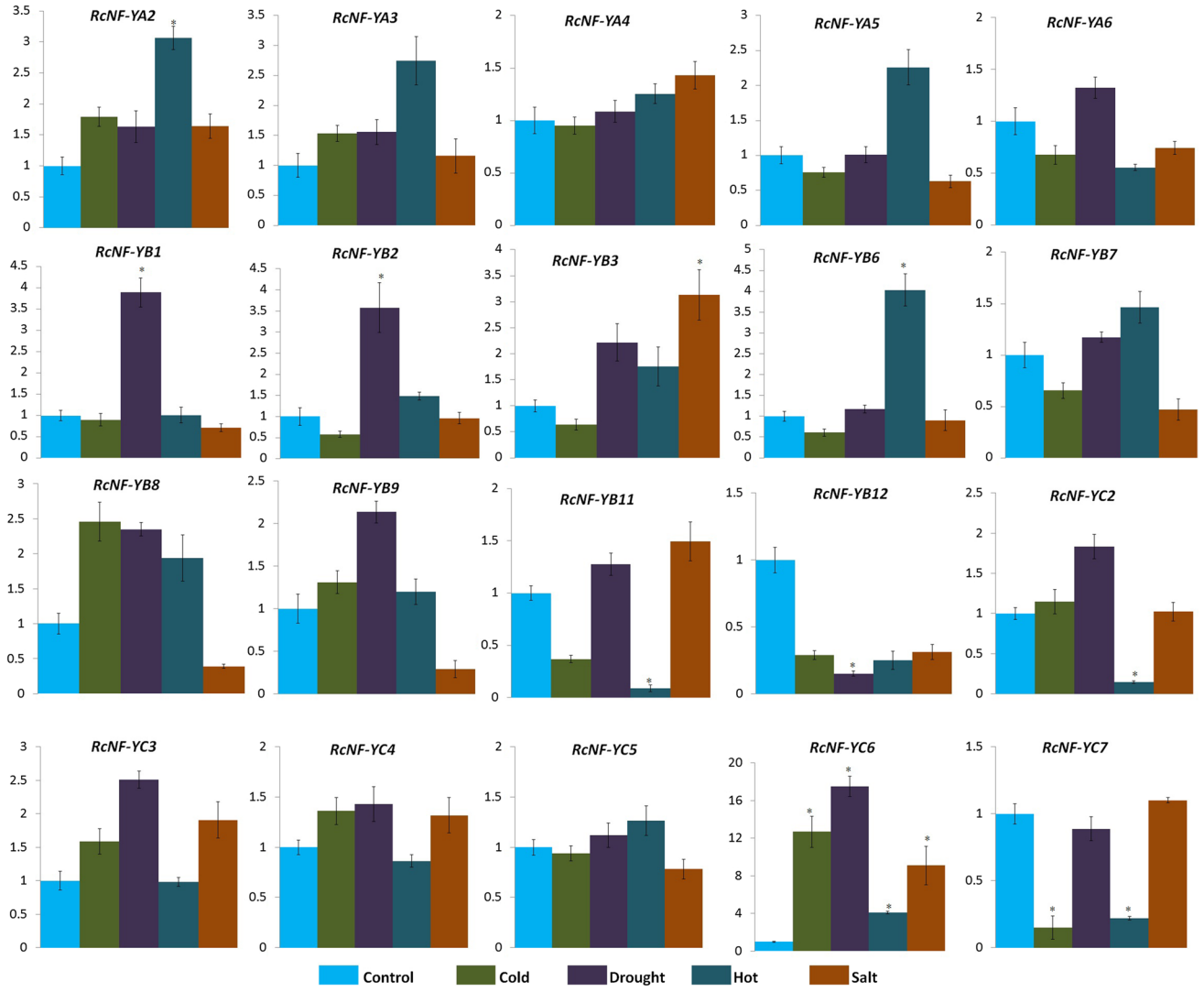

Fig. 6 Relative expression levels of *RcNF-Y*s in response to four abiotic stresses. The relative expression levels of five *RcNF-YA*s, nine *RcNF-YB*s and six *RcNF-YC*s were analyzed by qRT-PCR and *RcActin2* was used as internal reference. The expression level of control without any treatment was set as 1.0. Each sample was performed with three repeats. The values represent mean value ± SD and the column with asterisk refers to statistical difference at $P < 0.05$

tissues. Various expression profiles of *RcNF-Y* members indicated their functional divergence. In this study, one of the main objectives was to identify the potential RcNF-Y members that regulate endosperm development and storage reservoir accumulation in developing castor bean seeds. In particular, we found that *RcNF-YA2*, *RcNF-YA4*, *RcNF-YB12* and *RcNF-YC6* were highly and specifically expressed in the early (endosperm II/III) and later (endosperm V/VI) stages of developing endosperm. This strongly suggests that these RcNF-Y members might participate in regulation of endosperm development and storage reservoir accumulation. Interestingly, *RcNF-YB12*, one of the *AtLEC1* orthologs, as mentioned above, was highly and specifically expressed in the developing endosperm of castor bean. It has been well documented both that the gene *AtLEC1* was specifically expressed in the developing embryo and that it acts to regulate embryogenesis (Lotan et al. 1998; Kwong et al. 2003; Mu et al. 2013). Our study adds to scientific understanding of the function of *LEC1* in regulating not only embryogenesis but also endosperm development in the developing seed. Previous studies suggested that LEC1-type genes participated in regulating oil accumulation in developing seeds (Mu et al. 2008; Shen et al. 2010; Tan et al. 2011). In the present study, since the endosperm development and storage reservoir accumulation are an integrated process, it is difficult to separate the potential functions of *RcNF-YA2*, *RcNF-YA4*, *RcNF-YB12* and *RcNF-YC6* in regulating endosperm genesis or oil accumulation, although they were highly and

specifically expressed in developing endosperm. Previous reports have shown that NF-Y members could interact with some transcriptional regulators such as bZIP67 or LEC2, which regulates fatty acid biosynthesis in *Arabidopsis* (Yamamoto et al. 2009; Mendes et al. 2013; Baud et al. 2016). It is, therefore, possible that these RcNF-Y members, which are characterized by high and specific expression in the developing endosperms, functionally participate in regulating oil accumulation in castor bean seeds. Further research is required to dissect the functions of these RcNF-Y members in regulating endosperm genesis or oil accumulation in castor bean. In addition, *RcNF-YB9*, an ortholog of *AtNF-YB7* which usually functions in regulating flower development in *Arabidopsis*, was specifically expressed in male flowers. This is an indication of a promising direction for future studies of the function of *RcNF-YB9* in regulating the flower development in castor bean.

As mentioned above, *NF-Y* genes were functionally involved in regulating plant responses to stress (Hackenberg et al. 2012a; Junker and Bäumlein 2012; Laloum et al. 2013; Petroni et al. 2012; Zhang et al. 2016; Swain et al. 2016; Zhao et al. 2017). Castor bean is highly tolerant of abiotic stresses (Qiu et al. 2010), but the potential molecular basis remains unknown. In this study, we showed that more than 50% of *RcNF-Y* members were responsive to abiotic stresses at the transcriptional levels, meaning that their functions might be involved in regulating plant responses to stresses. More studies are required to dissect the functions of *RcNF-Y* members in responses to stresses. In particular, expression levels of *RcNF-YA2* and *RcNF-YC6* were up-regulated, and that of *RcNF-YB12* was down-regulated under all four treatments. When comparing changes in expression levels of *NF-Y* members in response to abiotic stresses between *Arabidopsis* (Hackenberg et al. 2012a) and castor bean, we noted that most of the *NF-Y* orthologs exhibited different or divergent expression profiles, though a few orthologs such as *AtNF-YC2*, *RcNF-YB12* and *AtNF-YB6* shared similar expression patterns. Not surprisingly, the functional divergence of most of the *NF-Y* orthologs in response to abiotic stresses varies widely across different species due to the variety of strategies and tolerance abilities of different plants to abiotic stresses. There is also evidence that *NF-Y* members function as a heterotrimeric complex in regulating plant response to abiotic stresses (Sato et al. 2014). It, therefore, seems important to identify the potential members that can form the heterotrimeric complex in regulating castor bean responses to abiotic stresses. The results of the current study provide a good opportunity for future studies to further identify potential heterotrimeric members according to the co-expression patterns with different abiotic stresses.

In conclusion, a comprehensive genomic characterization for the RcNF-Y family in castor bean was performed. The potential *RcNF-Y* members functionally involved in regulating the seed development and responses to abiotic stresses were characterized. The present study not only provides informative data to understand the regulation of seed development and storage reservoir accumulation in developing castor seeds, but also indicates potential *RcNF-Y* genes involved in regulating castor bean responses to abiotic stresses.

Author contribution statement AL conceived and designed the study. YW, WX performed experiments and analyzed the data. ZC and BH participated in the data analysis. YW and AL wrote the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Akpan U, Jimoh A, Mohammed A (2006) Extraction, characterization and modification of castor seed oil. Leonardo J Sci 8:43–52

Ballif J, Endo S, Kotani M, Macadam J, Wu Y (2011) Over-expression of *HAP3b* enhances primary root elongation in *Arabidopsis*. Plant Physiol Biochem 49:579–583. https://doi.org/10.1016/j.plaphy.2011.01.013

Baud S, Kelemen Z, Thévenin J, Boulard C, Blanchet S, To A et al (2016) Deciphering the molecular mechanisms underpinning the transcriptional control of gene expression by master transcriptional regulators in *Arabidopsis* seed. Plant Physiol 171:1099–1112. https://doi.org/10.1104/pp.16.00034

Ben-Naim O, Eshed R, Parnis A, Teper-Bamnolker P, Shalit A, Coupland G, Samach A, Lifschitz E (2006) The CCAAT binding factor can mediate interactions between CONSTANS-like proteins and DNA. Plant J 46:462–476

Brown AP, Kroon JT, Swarbreck D, Febrer M, Larson TR, Graham IA, Caccamo M, Slabas AR (2012) Tissue-specific whole transcriptome sequencing in castor, directed at understanding triacylglycerol lipid biosynthetic pathways. PLoS One 7(2):e30100. https://doi.org/10.1371/journal.pone.0030100

Cagliari A, Turchetto-Zolet AC, Korbes AP, dos Santos Maraschin F, Margis R, Margis-Pinheiro M (2014) New insights on the evolution of Leafy cotyledon1 (LEC1) type genes in vascular plants. Genomics 103:380–387. https://doi.org/10.1016/j.ygeno.2014.03.005

Calvenzani V, Testoni B, Gusmaroli G, Lorenzo M, Gnesutta N, Petroni K, Mantovani R, Tonelli C (2012) Interactions and CCAAT-binding of *Arabidopsis thaliana* NF-Y subunits. PLoS One 7(8):e42902. https://doi.org/10.1371/journal.pone.0042902

Cao S, Kumimoto RW, Siriwardana CL, Risinger JR, Holt BF III (2011) Identification and characterization of NF-Y transcription factor families in the monocot model plant *Brachypodium*

distachyon. PLoS One 6(6):e21805. https://doi.org/10.1371/journal.pone.0021805
Chan AP, Crabtree J, Zhao Q, Lorenzi H, Orvis J, Puiu D et al (2010) Draft genome sequence of the oilseed species *Ricinus communis*. Nat Biotechnol 28(9):951–956. https://doi.org/10.1038/nbt.1674
De Silvio A, Imbriano C, Mantovani R (1999) Dissection of the NF-Y transcriptional activation potential. Nucleic Acids Res 27(13):2578–2584
Feng Z-J, He G-H, Zheng W-J, Lu P-P, Chen M, Gong Y-M, Ma Y-Z, Xu Z-S (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. https://doi.org/10.3389/fpls.2015.01142
Gietz RD, Schiest RH (2007) Large-scale high-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. Nat Protoc 2(1):38–41
Gusmaroli G, Tonelli C, Mantovani R (2001) Regulation of the CCAAT-binding NF-Y subunits in *Arabidopsis thaliana*. Gene 264:173–185
Hackenberg D, Keetman U, Grimm B (2012a) Homologous NF-YC2 subunit from *Arabidopsis* and tobacco is activated by photooxidative stress and induces flowering. Int J Mol Sci 13:3458–3477. https://doi.org/10.3390/ijms13033458
Hackenberg D, Wu Y, Voigt A, Adams R, Schramm P, Grimm B (2012b) 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. https://doi.org/10.1093/mp/ssr107
Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G (2015) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31(8):1296–1297. https://doi.org/10.1093/bioinformatics/btu817
Huang M, Hu Y, Liu X, Li Y, Hou X (2015) *Arabidopsis* LEAFY COTYLEDON1 mediates postembryonic development via interacting with PHYTOCHROME-INTERACTING FACTOR4. Plant Cell 27:3099–3111. https://doi.org/10.1105/tpc.15.00750
Junker A, Bäumlein H (2012) Multifunctionality of the LEC1 transcription factor during plant development. Plant Signal Behav 7(12):1718–1720. https://doi.org/10.4161/psb.22365
Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33(7):1870–1874
Kwong RW, Bui AQ, Lee H, Kwong LW, Fischer RL, Goldberg RB, Harada JJ (2003) LEAFY COTYLEDON1-LIKE defines a class of regulators essential for embryo development. Plant Cell 15(1):5–18
Laloum T, De Mita S, Gamas P, Baudin M, Niebel A (2013) CCAAT-box binding transcription factors in plants: Y so many? Trends Plant Sci 18(3):157–166. https://doi.org/10.1016/j.tplants.2012.07.004
Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and clustal X version 2.0. Bioinformatics 23(21):2947–2948
Lee H, Fischer RL, Goldberg RB, Harada JJ (2003) *Arabidopsis* LEAFY COTYLEDON1 represents a functionally specialized subunit of the CCAAT binding transcription factor. Proc Natl Acad Sci USA 100(4):2152–2156
Lee DK, Kim HI, Jang G, Chung PJ, Jeong JS, Kim YS, Bang SW, Jung H, Choi YD, Kim JK (2015) The NF-YA transcription factor *OsNF-YA7* confers drought stress tolerance of rice in an abscisic acid independent manner. Plant Sci 241:199–210. https://doi.org/10.1016/j.plantsci.2015.10.006
Li W, Oono Y, Zhu J, He X, Wu J, Iida K, Lu XY, Cui X, Jin H, Zhu JK (2008) The *Arabidopsis* NF-YA5 transcription factor is regulated transcriptionally and posttranscriptionally to promote drought resistance. Plant Cell 20:2238–2251. https://doi.org/10.1105/tpc.108.059444
Li S, Li K, Ju Z, Cao D, Fu D, Zhu H, Zhu B, Luo Y (2016) Genome-wide analysis of tomato NF-Y factors and their role in fruit ripening. BMC Genom 17:36. https://doi.org/10.1186/s12864-015-2334-2
Liang M, Yin X, Lin Z, Zheng Q, Liu G, Zhao G (2014) Identification and characterization of NF-Y transcription factor families in canola (*Brassica napus* L.). Planta 239:107–126. https://doi.org/10.1007/s00425-013-1964-3
Liu X, Hu P, Huang M, Tang Y, Li Y, Li L, Hou X (2016) The NF-YC-RGL2 module integrates GA and ABA signalling to regulate seed germination in *Arabidopsis*. Nat Commun 7:12768. https://doi.org/10.1038/ncomms12768
Lotan T, Ohto M, Yee KM, West MAL, Lo R, Kwong RW, Yamagishi K, Fischer RL, Goldberg RB, Harada JJ (1998) *Arabidopsis* LEAFY COTYLEDON1 is sufficient to induce embryo development in vegetative cells. Cell 93:1195–1205
McNabb DS, Pinto I (2005) Assembly of the Hap2p/Hap3p/Hap4p/Hap5p-DNA complex in *Saccharomyces cerevisiae*. Eukaryot Cell 4:1829–1839
Meinke DW, Franzmann LH, Nickle TC, Yeung EC (1994) Leafy cotyledon mutants of *Arabidopsis*. Plant Cell 6:1049–1064
Mendes A, Kelly AA, van Erp H, Shaw E, Powers SJ, Kurup S, Eastmond PJ (2013) bZIP67 regulates the omega-3 fatty acid content of *Arabidopsis* seed oil by activating *FATTY ACID DESATURASE3*. Plant Cell 25:3104–3116. https://doi.org/10.1105/tpc.113.116343
Mu J, Tan H, Zheng Q, Fu F, Liang Y, Zhang J, Yang X, Wang T, Chong K, Wang XJ, Zuo J (2008) *LEAFY COTYLEDON1* is a key regulator of fatty acid biosynthesis in Arabidopsis. Plant Physiol 148:1042–1054. https://doi.org/10.1104/pp.108.126342
Mu J, Tan H, Hong S, Liang Y, Zuo J (2013) *Arabidopsis* transcription factor genes *NF-YA1*, *5*, *6*, and *9* play redundant roles in male gametogenesis, embryogenesis, and seed development. Mol Plant 6(1):188–201. https://doi.org/10.1093/mp/sss061
Myers ZA, Kumimoto RW, Siriwardana CL, Gayler KK, Risinger JR, Pezzetta D, Holt BF III (2016) NUCLEAR FACTOR Y, subunit C (NF-YC) transcription factors are positive regulators of photomorphogenesis in *Arabidopsis thaliana*. PLoS Gene 12(9):e1006333. https://doi.org/10.1371/journal.pgen.1006333
Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC et al (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. Proc Natl Acad Sci USA 104(42):16450–16455
Ni Z, Hu Z, Jiang Q, Zhang H (2013) *GmNFYA3*, a target gene of miR169, is a positive regulator of plant tolerance to drought stress. Plant Mol Biol 82:113–129. https://doi.org/10.1007/s11103-013-0040-5
Ogunniyi DS (2006) Castor oil: a vital industrial raw material. Bioresour Technol 97(9):1086–1091
Petroni K, Kumimoto RW, Gnesutta N, Calvenzani V, Fornari M, Tonelli C, Holt BF III, Mantovani R (2012) The promiscuous life of plant NUCLEAR FACTOR Y transcription factors. Plant Cell 24:4777–4792. https://doi.org/10.1105/tpc.112.105734
Qiu L, Yang C, Tian B, Yang JB, Liu A (2010) Exploiting EST databases for the development and characterization of EST-SSR markers in castor bean (*Ricinus communis* L.). BMC Plant Biol 10:278. https://doi.org/10.1186/1471-2229-10-278
Quach TN, Nguyen HTM, Valliyodan B, Joshi T, Xu D, Nguyen HT (2015) Genome-wide expression analysis of soybean *NF-Y* genes reveals potential function in development and drought response. Mol Genet Genomics 290:1095–1115. https://doi.org/10.1007/s00438-014-0978-2
Ren C, Zhang Z, Wang Y, Li S, Liang Z (2016) Genome-wide identification and characterization of the *NF-Y* gene family in grape

(*Vitis vinifera* L.). BMC Genom 17:605. https://doi.org/10.1186/s12864-016-2989-3

Rípodas C, Castaingts M, Clúa J, Blanco F, Zanetti ME (2015) Annotation, phylogeny and expression analysis of the nuclear factor Y gene families in common bean (*Phaseolus vulgaris*). Front Plant Sci 5:761. https://doi.org/10.3389/fpls.2014.00761

Sato H, Mizoi J, Tanaka H, Maruyama K, Qin F, Osakabe Y, Morimoto K, Ohori T, Kusakabe K, Nagata M, Shinozaki K, Yamaguchi-Shinozaki K (2014) *Arabidopsis* DPB3-1, 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. https://doi.org/10.1105/tpc.114.132928

Scholza V, da Silva JN (2008) Prospects and risks of the use of castor oil as a fuel. Biomass Bioenerg 32:95–100

Shen B, Allen WB, Zheng P, Li C, Glassman K, Ranch J, Nubel D, Tarczynski MC (2010) Expression of *ZmLEC1* and *ZmWRI1* increases seed oil production in maize. Plant Physiol 153:980–987. https://doi.org/10.1104/pp.110.157537

Siefers N, Dang KK, Kumimoto RW, Bynum WE IV, Tayrose G, Holt BF III (2009) Tissue-specific expression patterns of Arabidopsis NF-Y transcription factors suggest potential for extensive combinatorial complexity. Plant Physiol 149:625–641. https://doi.org/10.1104/pp.108.130591

Sinha S, Maity SN, Lu J, de Crombrugghe B (1995) Recombinant rat CBF-C, the third subunit of CBF/NFY, allows formation of a protein-DNA complex with CBF-A and CBF-B and with yeast HAP2 and HAP3. Proc Natl Acad Sci USA 92:1624–1628

Sorin C, Declerck M, Christ A, Blein T, Ma L, Lelandais-Brière C, Njo MF, Beeckman T, Crespi M, Hartmann C (2014) A miR169 isoform regulates specific NF-YA targets and root architecture in *Arabidopsis*. New Phytol 202:1197–1211

Steidl S, Tüncher A, Goda H, Guder C, Papadopoulou N, Kobayashi T, Tsukagoshi N, Kato M, Brakhage AA (2004) A single subunit of a heterotrimeric CCAAT-binding complex carries a nuclear localization signal: piggyback transport of the pre-assembled complex to the nucleus. J Mol Biol 342:515–524

Swain S, Myers ZA, Siriwardana CL, Holt BF III (2016) The multifaceted roles of NUCLEAR FACTOR-Y in *Arabidopsis thaliana* development and stress responses. Biochim Biophys Acta. https://doi.org/10.1016/j.bbagrm.2016.10.012

Tan H, Yang X, Zhang F, Zheng X, Qu C, Mu J, Fu F, Li J, Guan R, Zhang H, Wang G, Zuo J (2011) Enhanced seed oil production in canola by conditional expression of *Brassica napus LEAFY COTYLEDON1* and *LEC1-LIKE* in developing seeds. Plant Physiol 156:1577–1588. https://doi.org/10.1104/pp.111.175000

Thirumurugan T, Ito Y, Kubo T, Serizawa A, Kurata N (2008) Identification, characterization and interaction of *HAP* family genes in rice. Mol Genet Genomics 279(3):279–289. https://doi.org/10.1007/s00438-007-0312-3

Thön M, Al Abdallah Q, Hortschansky P, Scharf DH, Eisendle M, Haas H, Brakhage AA (2010) The CCAAT-binding complex coordinates the oxidative stress response in eukaryotes. Nucleic Acids Res 38:1098–1113

West MAL, Yee KM, Danao J, Zimmerman JL, Fischer RL, Goldberg RB, Harada JJ (1994) LEAFY COTYLEDONl is an essential regulator of late embryogenesis and cotyledon identity in *Arabidopsis*. Plant Cell 6:1731–1745

Xu W, Li F, Ling L, Liu A (2013) Genome-wide survey and expression profiles of the AP2/ERF family in castor bean (*Ricinus communis* L.). BMC Genom 14:785. https://doi.org/10.1186/1471-2164-14-785

Xu W, Chen Z, Ahmed N, Han B, Cui Q, Liu A (2016) Genome-wide identification, evolutionary analysis, and stress responses of the *GRAS* gene family in castor beans. Int J Mol Sci 17:1004. https://doi.org/10.3390/ijms17071004

Yamamoto A, Kagaya Y, Toyoshima R, Kagaya M, Takeda S, Hattori T (2009) Arabidopsis NF-YB subunits LEC1 and LEC1-LIKE activate transcription by interacting with seed-specific ABRE-binding factors. Plant J 58:843–856. https://doi.org/10.1111/j.1365-313X.2009.03817.x

Yeap WC, Lee FC, Shabari Shan DK, Musa H, Appleton DR, Kulaveerasingam H (2017) WRI1-1, ABI5, NF-YA3 and NF-YC2 increase oil biosynthesis in coordination with hormonal signaling during fruit development in oil palm. Plant J 91:97–113. https://doi.org/10.1111/tpj.13549

Zhang F, Han M, Lv Q, Bao F, He Y (2015a) Identification and expression profile analysis of NUCLEAR FACTOR-Y families in *Physcomitrella patens*. Front Plant Sci 6:642. https://doi.org/10.3389/fpls.2015.00642

Zhang T, Zhang D, Liu Y, Luo C, Zhou Y, Zhang L (2015b) Overexpression of a *NF-YB3* transcription factor from *Picea wilsonii* confers tolerance to salinity and drought stress in transformed *Arabidopsis thaliana*. Plant Physiol Biochem 94:153–164. https://doi.org/10.1016/j.plaphy.2015.05.001

Zhang Z, Li X, Zhang C, Zou H, Wu Z (2016) Isolation, structural analysis, and expression characteristics of the maize nuclear factor Y gene families. Biochem Biophys Res Commun 478:752–758. https://doi.org/10.1016/j.bbrc.2016.08.020

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