



Article

Genome-Wide Characterization of Nuclear Factor Y (NF-Y) Transcription Factors Gene Family in Cabbage (*Brassica oleracea* var. *capitata* L.) Uncovers Their Critical Roles in Salt Stress Tolerance

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Abstract

Nuclear Factor Y (NF-Y) transcription factors play pivotal roles in plant adaptation to abiotic stress, yet their genomic landscape and functional mechanisms in cabbage (*Brassica oleracea* var. *capitata* L.) remain underexplored. Here, we performed a genome-wide identification of the NF-Ys in cabbage, identifying 53 BoNF-Ys classified into three subfamilies: 20 BoNF-YAs, 22 BoNF-YBs, and 11 BoNF-YCs. Phylogenetic clustering revealed evolutionary conservation with their Arabidopsis orthologs. Domain analysis revealed that all BoNF-YA members contain the CBF_NF-YA domain, while all BoNF-YB and BoNF-YC members possess the CBFD_NFYB_HMF conserved domain. The BoNF-Y genes were named according to their chromosomal locations. Bioinformatic analysis showed that BoNF-Y proteins range in size from 131 to 642 amino acids, with molecular weights of 14.82–73.18 kDa, theoretical pI values of 4.57–9.96, instability indices between 33.02 and 73.48, aliphatic indices of 45.3–86.26, and grand average of hydropathicity (GRAVY) values ranging from −1.139 to −0.367. Promoter cis-element profiling uncovered stress- and hormone-responsive motifs, including abscisic acid-responsive elements (ABREs), TC-rich repeats, and ethylene-responsive elements (EREs). RNA sequencing (RNA-seq) and quantitative reverse transcription polymerase chain reaction (qRT-PCR) conducted under salt stress (256 mM) identified three salt-responsive candidate genes (BoNF-YA14, BoNF-YB9, and BoNF-YC8). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses highlighted significantly expressed genes' roles in MAPK signaling, proline metabolism, and phytohormone transduction pathways. This study conducted a comprehensive survey of the BoNF-Y gene family in cabbage. It could serve as a theoretical foundation for further functional identification and utilization of BoNF-Y family members and their role in the interaction between cabbage and salt stress.

Keywords: salt stress; NF-Y gene family; RNA-seq; *Brassica oleracea*; expression analysis

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1. Introduction

Salt poses a threat to global agricultural lands, leading to declines in crop yield and quality. Gradual exposure of crops to increasing salt concentrations or prolonged exposure to even low salinity levels can trigger salt stress, which may ultimately result in crop death [1,2]. Cabbage (*Brassica oleracea* var. *capitata* L.), a globally cultivated vegetable, is valued for its distinctive flavor and nutrient density [3]. Through long-term evolutionary adaptation, plants have developed sophisticated regulatory networks to mitigate salt stress-induced damage, comprising three principal mechanisms: signal transduction cascades, transcriptional regulation, and post-translational modifications [4]. Transcription factors (TFs) are DNA-binding proteins that recognize and bind to cis-regulatory elements within promoter regions, mediating precise spatiotemporal regulation of target gene expression through transcriptional activation or repression [5]. Substantial research has established that Nuclear Factor Y (NF-Y) transcription factors play pivotal regulatory roles in plant responses to abiotic stress [6]. NF-Y is also known as Heme Activator Protein (HAP) or CCAAT-Binding Factor (CBF) due to its specific binding to the CCAAT cis-acting element [7].

NF-Y represents a heterotrimeric complex, consisting of the subunits NF-YA (CBF-B/HAP2), NF-YB (CBF-A/HAP3), and NF-YC (CBF-C/HAP5) [8–10]; these subunits are essential for binding to the promoters of the CCAAT target gene [11,12]. The NF-YA subunit localizes to the cell nucleus and features two conserved α -helical structural domains (A1 and A2), positioned at the N- and C-terminal regions flanking the central core domain [13]. The A1 domain mediates interactions with the NF-YB/NF-YC subunits, whereas the A2 domain selectively recognizes and binds to the cis-acting CCAAT box element; NF-YB is organized into three distinct structural modules: an N-terminal A domain, a central B domain harboring the core histone-fold motif, and a C-terminal C domain [14]. The B domain of LEC1-type NF-YB proteins harbors a conserved aspartic acid residue (Asp-55), which functions as a critical functional determinant governing seed embryogenesis [15]. The NF-Y trimer is recruited to the evolutionarily conserved CCAAT box motif within eukaryotic promoter regions, where it modulates transcriptional activation or repression depending on the cellular context [16]. The rapid advancements in sequencing technologies and bioinformatics have enabled the genome-wide identification and systematic characterization of NF-Y gene families across diverse plant species [17]. Based on the present research, 36 NF-Y transcription factors were identified (10 NF-YAs, 13 NF-YBs, and 13 NF-YCs) in *A. thaliana* [18]; 52 NF-Y transcription factors (including 16 NF-YAs, 19 NF-YBs, and 17 NF-YCs) were identified in *Zea mays* [19]; and 33 NF-Y transcription factors (including 8 NF-YAs, 11 NF-YBs, and 14 NF-YCs) were identified in *Sorghum bicolor* [20].

Recently, the functions of NF-Ys genes in responding to abiotic stresses have been elucidated across multiple species [21]. *ZmNF-YB10* enhances maize salt tolerance by scavenging reactive oxygen species (ROS) through elevated antioxidant enzyme activity [22]; *GmNF-YC9* exhibits strong induction under salt stress, and its overexpression increases biomass accumulation and proline levels in soybean under saline conditions, thereby enhancing osmotic adjustment and salt stress tolerance [23]; transgene-mediated overexpression of *Cdt-NF-YC1* in rice triggered significant upregulation of ABA biosynthesis genes, thus conferring enhanced salt tolerance through the activation of the ABA-dependent signaling pathway [24]. When *A. thaliana* experiences salt stress, *JAZ8* is degraded via the 26S proteasome pathway. This degradation releases the active AtNF-YA1-YB2-YC9 complex, which then activates the expression of salt-responsive genes, such as MYB75, thereby enhancing salt tolerance [25]. Collectively, these findings demonstrate that the NF-Y gene family plays a functionally conserved role in mediating salt stress responses across diverse crop species. Although the roles of NF-Y had been extensively studied in various plant species [26,27], the role of BoNF-Y family genes in cabbage salt

stress is still unclear. In this study, 53 BoNF-Y family genes have been identified in the cabbage genome, with analysis of their role in responding to salt stress. Our research provides a theoretical basis for subsequent salt stress-resistant breeding in cabbage.

2. Results

2.1. Identification and Characterization of BoNF-Ys in Cabbage

A total of 53 BoNF-Ys were identified by searching “CBF” domain in the brassica genome (Braol_JZS_V2.0). Based on the conserved structural domain analysis of 53 cabbage NF-Ys CDS, we found that 20 BoNF-YAs possess the CBF_NF-YA domain (Figure 1a), and 22 BoNF-YBs and 11 BoNF-YCs contain the CBFD_NFYB_HMF conserved domain (Figure 1b,c). According to their position on the genome chromosome, all BoNF-Ys were renamed. BoNF-Ys encoded proteins with a length of 131–642 amino acids, molecular weights of 14.82–73.18 kDa, pI values of 4.57–9.96, instability indices of 33.02–73.48, aliphatic indices of 45.3–86.26, and grand average of hydropathicity (GRAVY) values between −1.139 and −0.367; all GRAVY values are negative, indicating that these proteins are generally hydrophilic (Supplementary Table S1).

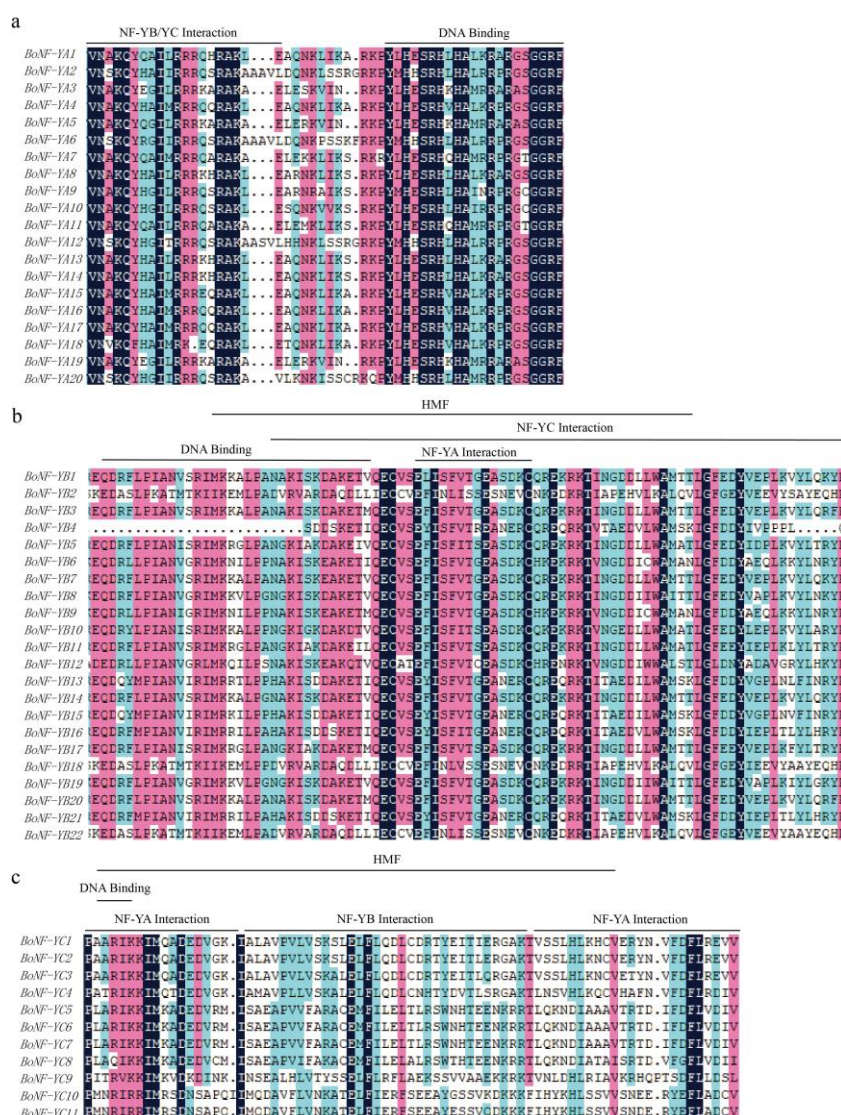


Figure 1. Multiple sequence alignment of BoNF-Y protein sequences in cabbage. (a–c) represent the sequence alignments of the highly conserved domains of BoNF-YA, BoNF-YB, and BoNF-YC proteins in cabbage, respectively.

2.2. Phylogenetic Analysis, Protein Motifs and Gene Structure of BoNF-Ys

Existing studies have demonstrated that conserved motifs in transcription factor protein sequences can serve as DNA-binding sites. The motif patterns, conserved domain architecture and gene structure of the BoNF-Ys were analyzed and presented based on the above results from the cabbage genome database (Figure 2). A comprehensive phylogenetic analysis of 53 BoNF-Y protein sequences was conducted using MEGA7, and a total of 10 conserved motifs were identified in the BoNF-Y proteins. The distribution of motifs in three BoNF-Y subunits was found to be unique: all BoNF-YAs contained three conserved motifs (Motifs 2, 3 and 9), among which Motifs 3 and 9 were exclusively present in the BoNF-YA subfamily. Motifs 1, 2 and 4 were highly conserved in BoNF-YBs. Motif 5 is highly conserved in all BoNF-YCs, but Motif 1 and Motif 6 are only conserved in partial BoNF-YCs; BoNF-YC1, 2, 3 and 11 harbored Motif 6, whereas BoNF-YC4, 5, 6, 7, 8, 9 and 10 possessed Motif 1 (Figure 2a). Taken together, each subfamily had evolved a set of distinct conserved motifs that were characteristic of its own clade. All BoNF-Ys contained a conserved CBF domain, but there were also some unique domains among BoNF-YAs, BoNF-YBs and BoNF-YCs (Figure 2b). BoNF-YB11 and BoNF-YB16 each contain two distinct domains: BoNF-YB11 possesses a BAG domain and a Biotin/lipoyl domain superfamily domain, while BoNF-YB16 contains a DUF5401 superfamily domain and a MYB DNA-binding domain. The number of exons varies among members within the BoNF-YA, BoNF-YB, and BoNF-YC subfamilies, suggesting possible functional diversification of BoNF-Y family genes during evolution (Figure 2c).

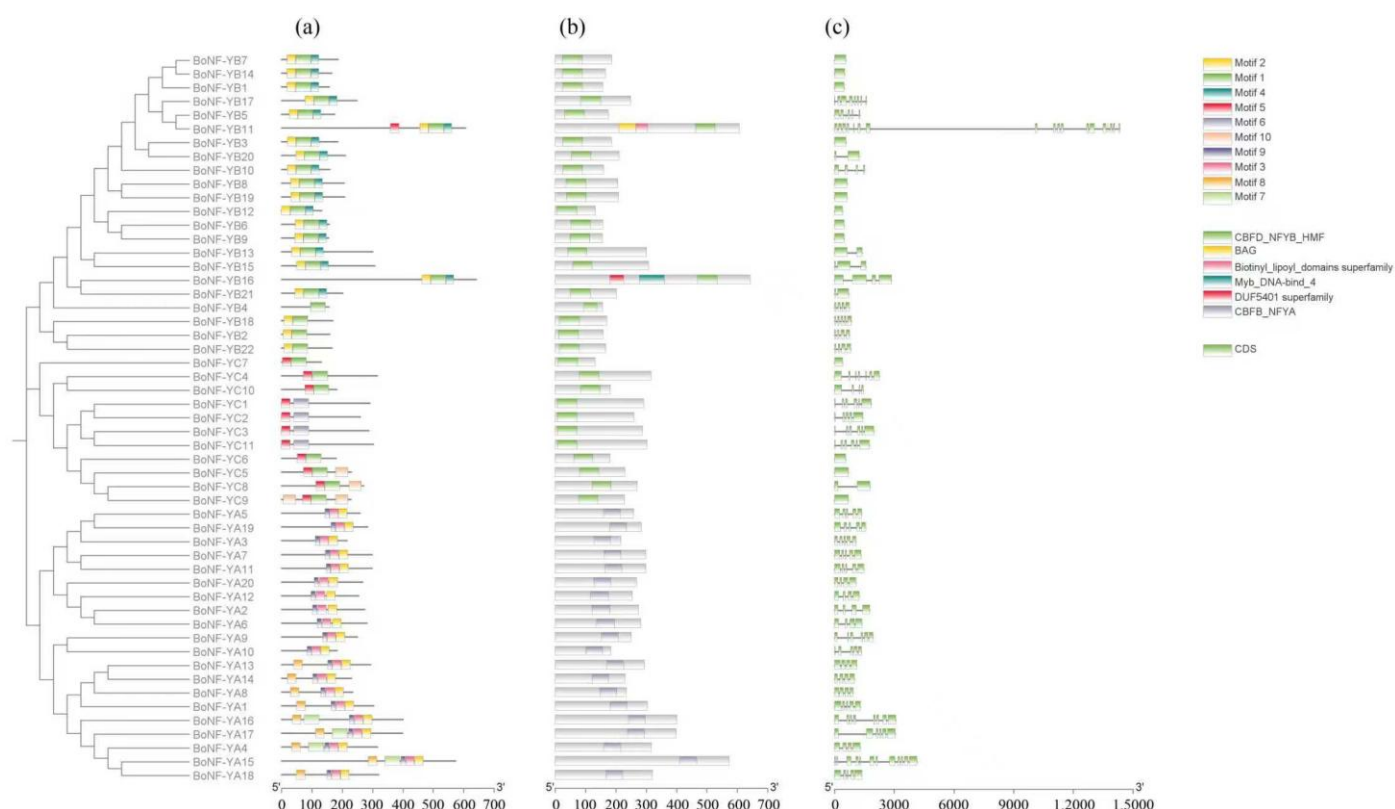


Figure 2. Characterization and phylogenetic analysis of the identified BoNF-Ys in cabbage. (a) Distribution of the motif patterns of BoNF-Ys proteins. (b) Conserved domain of BoNF-Ys proteins. (c) Exon–intron structure of BoNF-Ys.

2.3. Chromosomal Localization of BoNF-Ys

To analyze the chromosomal localization of BoNF-Y family genes, they were mapped onto the cabbage chromosomes (Figure 3). All 53 BoNF-Y genes were distributed across

the nine chromosomes of cabbage (Figure 3). Notably, there were nine BoNF-Y genes located on chromosome C03, while only three BoNF-Y genes (*BoNF-YB15*, *BoNF-YB16*, and *BoNF-YC7*) were mapped to chromosome C07. Interestingly, some genes exhibited clustered localization, which presumably formed gene clusters during the evolutionary process, such as *BoNF-YB3/4*, *BoNF-YC5/BoNF-YB12*, *BoNF-YA16/17*, *BoNF-YC9/10* and *BoNF-YB22/BoNF-YC11/BoNF-YA20*. These clustered genes may have a functional resemblance, forming functional redundancy.

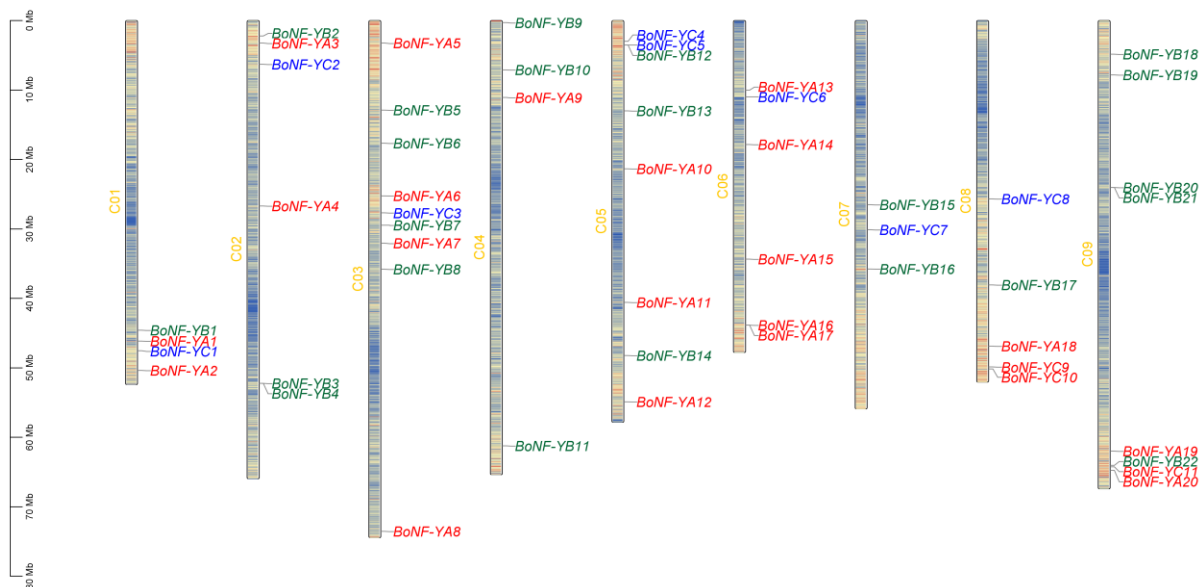


Figure 3. Chromosomal distribution of *BoNF-Ys* genes in cabbage.

2.4. Analysis of Cis-Elements in the Promoter of *BoNF-Ys*

The genomic region spanning −2000 bp upstream of the transcription start site of all *BoNF-Ys* is considered as the potential promoter sequence for subsequent analyses (Figure 4). We found that there were multiple cis-acting elements related to stress responses and plant hormone responses within the promoter region of *BoNF-Ys*. The LTR elements were involved in low-temperature response and the abscisic acid-responsive elements (ABREs) were involved in abscisic acid response. A total of 175 ABREs, 131 AREs (antioxidant/auxin response elements), and 105 Box-4 elements were identified in the promoter regions of *BoNF-Ys*. Additionally, 25 types of light-responsive elements were detected. Furthermore, several cis-acting elements linked to plant growth and development were discovered, including CAT-box (associated with meristem-specific expression) and GCN4 motif (involved in endosperm expression regulation). These findings provided critical clues for elucidating the functional roles of *BoNF-Ys* in stress adaptation and developmental processes.

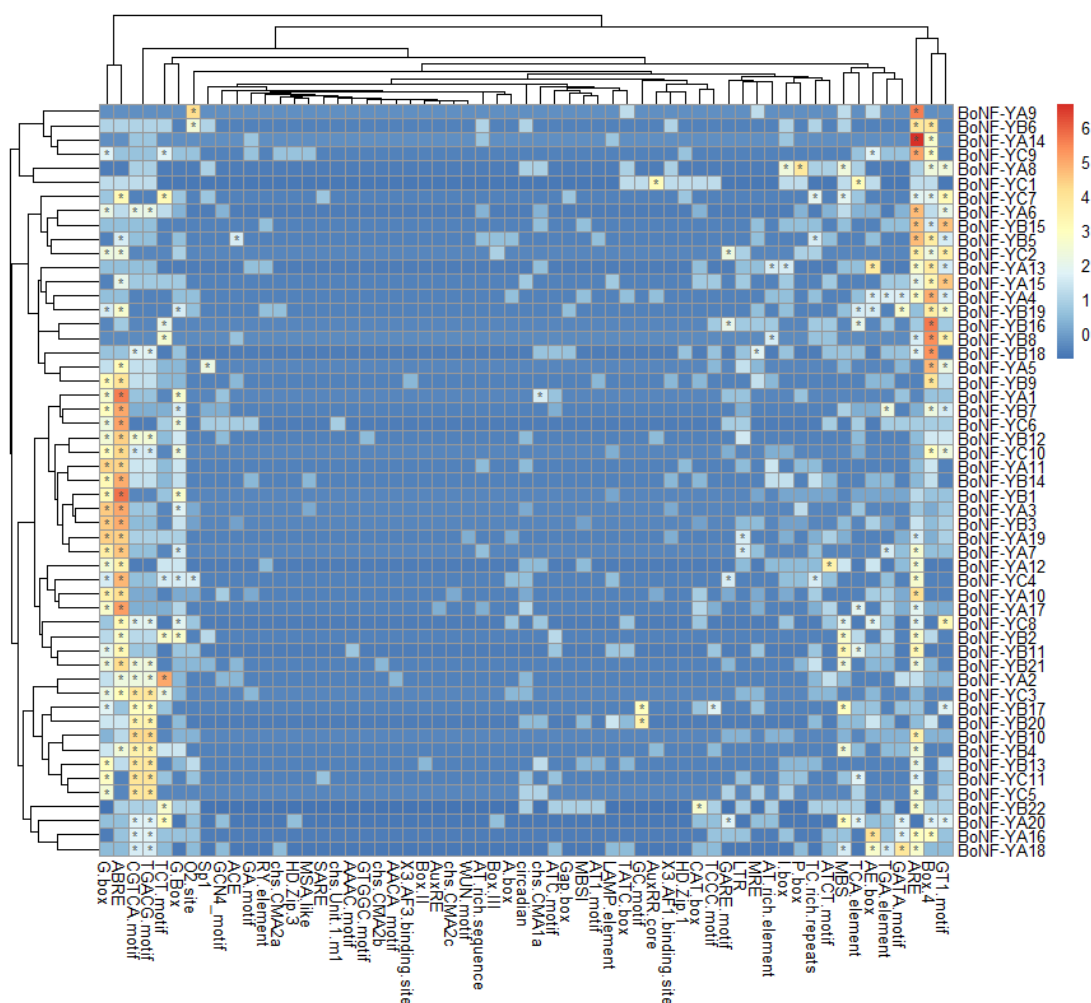


Figure 4. Analysis of cis-acting elements in the promoter regions of *BoNF-Ys* genes. Horizontal axis: Different types of cis-acting elements. Vertical axis: Individual genes of the *BoNF-Y* family. The darker red color indicates a greater number or higher enrichment level of such cis-acting elements in the gene. The asterisk (*) indicates that the enrichment of the corresponding cis-acting element in the target promoter region is statistically significant ($p < 0.05$).

2.5. Phylogenetic Analysis of BoNF-Ys and AtNF-Ys

Protein sequences of the *BoNF-Ys* were retrieved from the cabbage genome and combined with orthologous *AtNF-Y* sequences. A phylogenetic tree was constructed based on these protein sequences (Figure 5). All *BoNF-Ys* and *AtNF-Ys* were clustered into three groups. Group I comprises 20 *BoNF-YA* genes and 10 *AtNF-YA* genes; Group II contains 22 *BoNF-YB* genes and 13 *AtNF-YB* genes; Group III harbors 11 *BoNF-YC* genes and 24 *AtNF-YC* genes. Overall, multiple NF-Y genes from *B. oleracea* and *A. thaliana* are clustered on the same sub-branches of the phylogenetic tree, which indicate a high degree of homology between *BoNF-Y* and *AtNF-Y* proteins. The phylogenetic relationships suggest that NF-Y genes from different species may have originated from a common domain, and NF-Y genes within the same group may perform similar biological functions but are subject to differential expression regulation.

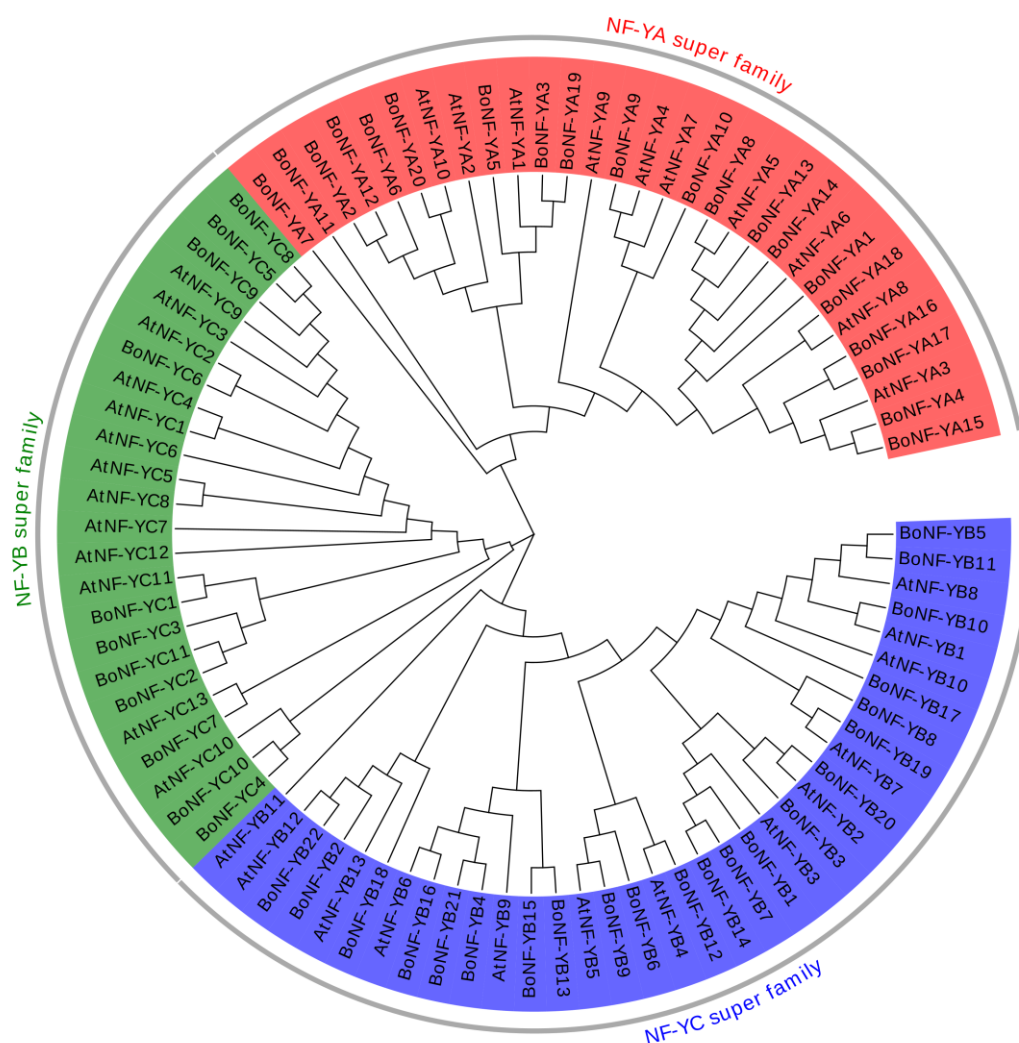


Figure 5. Phylogenetic tree of NF-Y protein sequences in *B. oleracea* and *A. thaliana* constructed by the Maximum Likelihood (ML) method.

2.6. RNA-Seq Revealed the Expression Patterns of BoNF-Y Family Genes Under Salt Stress

Our previous results showed that 256 mM NaCl was optimal for inducing salt stress responses in *B. oleracea* seedlings (unpublished data). To elucidate the underlying regulatory mechanisms, RNA sequencing was conducted on roots of the moderately tolerant line HB70 under 256 mM NaCl at 0 h and 12 h (versus water-treated controls). Using stringent thresholds ($|\log_2FC \text{ (fold change)}| > 1$ and $p\text{-value} < 0.05$), we identified a total of 5037 differently expressed genes (DEGs). As illustrated in the volcano plot (Figure 6), among these DEGs, 2961 genes were upregulated (represented by red dots) and 2076 were downregulated (represented by blue dots).

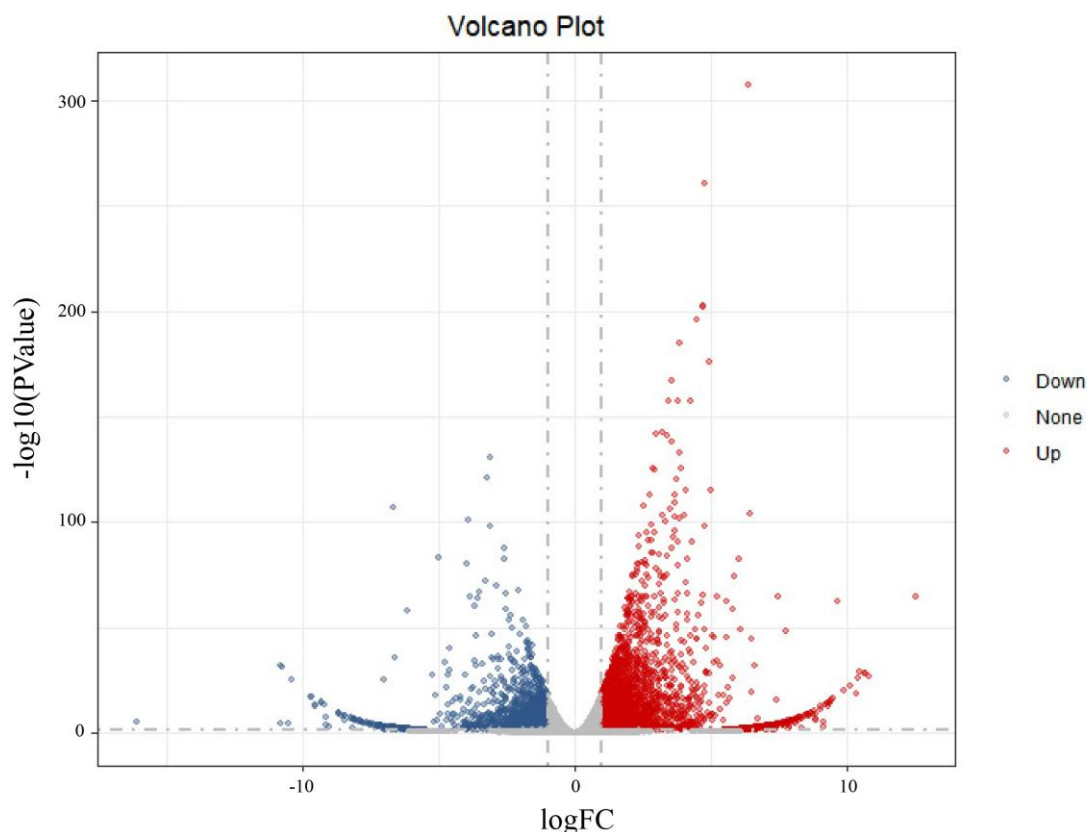


Figure 6. Volcano plot of differentially expressed genes. Selected genes and the transcriptome data confirmed the reliability of the transcriptome dataset.

To clarify the transcriptional regulatory mechanism of NF-Ys, we conducted KEGG pathway enrichment analysis on differentially expressed genes (DEGs) and constructed KEGG enrichment bubble plots (Figure 7). Specifically, Figure 7a presents upregulated DEGs, while Figure 7b displays downregulated DEGs. The results revealed significant enrichment pathways including the MAPK signaling pathway–plant, starch and sucrose metabolism, and phenylpropanoid biosynthesis. The MAPK signaling pathway likely functions as a central hub for stress signal transduction, activating the expression of a suite of defense-related genes [28]. Enhanced starch and sucrose metabolism not only provide energy for stress responses but also facilitate the accumulation of soluble sugars as osmo-protectants to maintain cellular osmotic balance [29]. These enriched pathways reflected global transcriptomic responses to salt stress, and the differential expression of BoNF-Y genes (identified via RNA-seq and qRT-PCR) suggested their potential participation in these pathways. Additionally, phenylpropanoid biosynthesis promotes the production of flavonoids, which help alleviate salt-induced oxidative stress through their antioxidant activity. The protein processing pathway in the endoplasmic reticulum had the largest gene count, indicating that the most differentially expressed genes were involved, although its enrichment score was relatively low and the *p*-value was moderately significant. This suggests an intensive regulation in protein folding, modification, and transport within the endoplasmic reticulum. Salt stress affects protein folding in the endoplasmic reticulum, and correct protein folding can effectively alleviate the toxicity caused by salt stress, thereby enhancing plant salt tolerance.

To further link global transcriptomic changes to the regulatory roles of BoNF-Ys, we integrated the DEG enrichment pathways with the known functional characteristics of NF-Y family proteins. Previous studies have demonstrated that NF-Y factors typically ex-

ert regulatory effects by forming trimers and binding to CCAAT cis-elements in the promoters of target genes [11,16]. In the current study, promoter cis-element analysis revealed that 78.9% (42/53) of BoNF-Y genes contain ABRE, TC-rich repeats, or ERE motifs (Figure 4), which are closely associated with the top enriched pathways (plant hormone signal transduction, MAPK signaling pathway) in DEGs. Additionally, GO terms related to “reactive oxygen species (ROS) response” and “osmotic adjustment” (Figure 8) overlap with the functional roles of NF-Y genes reported in other species (e.g., *ZmNF-YB10* scavenges ROS [22], *GmNF-YC9* enhances osmotic adjustment [23]). These observations imply that BoNF-Ys may directly regulate key DEGs in these pathways by recognizing CCAAT elements, or indirectly modulate pathway activity through interactions with other transcription factors (e.g., MYB75 [25]). However, the specific target genes of the BoNF-Y trimer and their binding specificity still need to be further verified through experiments.

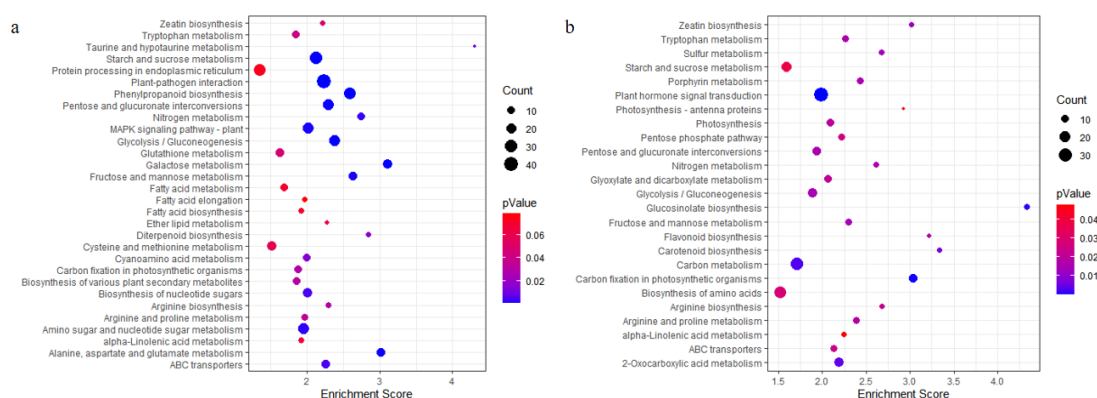


Figure 7. KEGG analysis of differentially expressed genes. (a) The KEGG enrichment map of upregulated genes; (b) the KEGG enrichment bubble chart of downregulated genes.

Subsequently, we performed a detailed comparison of the screened differentially expressed genes with the GO database (Figure 8). In the Biological Process (BP) category, the significant enrichment of terms such as “response to hypoxia,” “response to decreased oxygen levels,” and “cellular response to hypoxia” indicated that cabbage perceives and responded to salt stress as a hypoxic environment to mitigate its damage. Furthermore, enrichment was observed for “response to salicylic acid” and “response to reactive oxygen species”, which were pathways known to activate ROS homeostasis and enhanced antioxidant enzyme activities under salt stress. In the Molecular Function (MF) category, the enrichment of terms such as “sugar transmembrane transporter activity,” “inorganic anion transmembrane transporter activity,” and “carbohydrate binding” enhanced cabbage salt tolerance by maintaining osmotic balance, preserving ion homeostasis, and strengthening cellular integrity. Enrichment was also observed for “ADP binding,” which may provide energy for salt tolerance-related processes by regulating energy synthesis and supply. In the Cellular Component (CC), the Casparian strip and xylem terms were significantly enriched. Under salt stress, these two components may collaborate by regulating ion uptake and transport. This joint action helped reduce ionic toxicity and osmotic damage caused by salt stress to plants.

To identify salt-responsive BoNF-Y genes, we overlapped the differentially expressed BoNF-Ys with the GO enrichment results of global DEGs, and found seven BoNF-Y genes (*BoNF-YC5*, *BoNF-YC6*, *BoNF-YB9*, *BoNF-YB12*, *BoNF-YA10*, *BoNF-YA13*, *BoNF-YA14*) whose expression dynamics were correlated with the enriched GO terms under salt stress. Analyzing the functions of these genes, we found that *AtNF-YC9*, the Arabidopsis ortholog of *BoNF-YC5*, can form a complex (*AtNF-YA1-YB2-YC9*) with *NF-YA1* (*BoNF-YA19*) and *NF-YB2* (*BoNF-YB20*), and this complex served as the core regulatory complex

that mediates *Arabidopsis*' salt stress response through the JA signaling pathway. Based on this result, we hypothesized that a similar salt stress-responsive complex may also exist in *Brassica oleracea*. Furthermore, we wonder whether other BoNF-Y genes might mediate the salt stress response of *Brassica oleracea* by regulating other signaling pathways and growth responses.

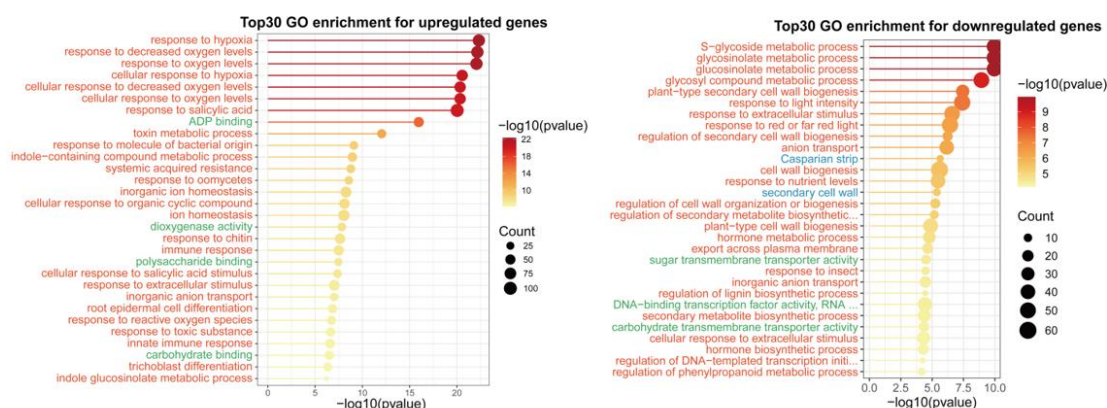


Figure 8. GO analysis of differentially expressed genes. The red part on the y-axis represents BP, the green part represents MF, and the blue part represents CC.

2.7. Screening and Expression Profiling of Salt-Responsive BoNF-Ys Candidate Genes

Based on our RNA-seq results, we focused on analyzing the differential expression of BoNF-Y genes under salt stress and control conditions. This heatmap used a color gradient to represent expression intensity: red denotes relatively high expression, while blue denotes relatively low expression. The left column corresponds to the control group (CK: 0 h) and the right column to the experimental group (256 mM NaCl 10 h), with each row representing a single BoNF-Y gene. Among the differentially expressed genes, nine were highly expressed (red) in the 256 mM NaCl treatment group compared to the control group, indicating that their expression was induced by salt stress; four were weakly expressed (blue) in the treatment group, indicating that their expression was repressed (Figure 9).

To verify the accuracy of RNA-seq data and investigate the differential expression patterns of BoNF-Y genes under salt stress more precisely, we performed quantitative real-time PCR (qRT-PCR) analysis to measure their expression levels after 2 h, 4 h, 10 h, and 12 h of salt treatment (as shown in Figure 10). At 2 h of salt treatment, seven genes were significantly downregulated, including *BoNF-YA2*, *BoNF-YA10*, *BoNF-YA14*, *BoNF-YA17*, *BoNF-YA19*, *BoNF-YB7*, and *BoNF-YB9*; six genes showed no significant change in expression, namely *BoNF-YA3*, *BoNF-YA4*, *BoNF-YA8*, *BoNF-YA13*, *BoNF-YB3*, and *BoNF-YC8*. At 4 h of salt treatment, three genes were significantly downregulated (*BoNF-YA3*, *BoNF-YA10*, and *BoNF-YA19*), and another six genes were significantly downregulated, including *BoNF-YA8*, *BoNF-YA13*, *BoNF-YA14*, *BoNF-YB3*, *BoNF-YB7*, and *BoNF-YC8*; four genes exhibited no significant expression change (*BoNF-YA2*, *BoNF-YA4*, *BoNF-YA17*, and *BoNF-YB9*). This result indicated that early salt stress inhibits the expression of most genes. At 10 h of salt treatment, the significantly upregulated genes included *BoNF-YA4*, *BoNF-YA8*, *BoNF-YA13*, *BoNF-YA14*, *BoNF-YB3*, *BoNF-YB9*, and *BoNF-YC8*, with *BoNF-YB9* showing more than a 4-fold increase in expression. The downregulated genes were *BoNF-YA10* and *BoNF-YA17*, while *BoNF-YA2*, *BoNF-YA3*, *BoNF-YA19*, and *BoNF-YB7* had no significant expression changes. At 12 h of salt treatment, two genes (*BoNF-YA14* and *BoNF-YC8*) showed a continuously significant upward trend in expression. The ex-

pression of four genes (*BoNF-YA2*, *BoNF-YA3*, *BoNF-YA10*, and *BoNF-YA17*) was significantly downregulated, and *BoNF-YA13* had significantly upregulated expression. The expression levels of seven genes (*BoNF-YA4*, *BoNF-YA8*, *BoNF-YA19*, *BoNF-YB3*, *BoNF-YB7*, and *BoNF-YB9*) did not change significantly. In conclusion, based on the expression magnitude and temporal induction patterns identified by qRT-PCR analysis, we proposed that *BoNF-YA14*, *BoNF-YB9*, and *BoNF-YC8* are salt-responsive candidate genes potentially involved in the salt stress response of cabbage, as they exhibited sustained or strong upregulation during salt stress.

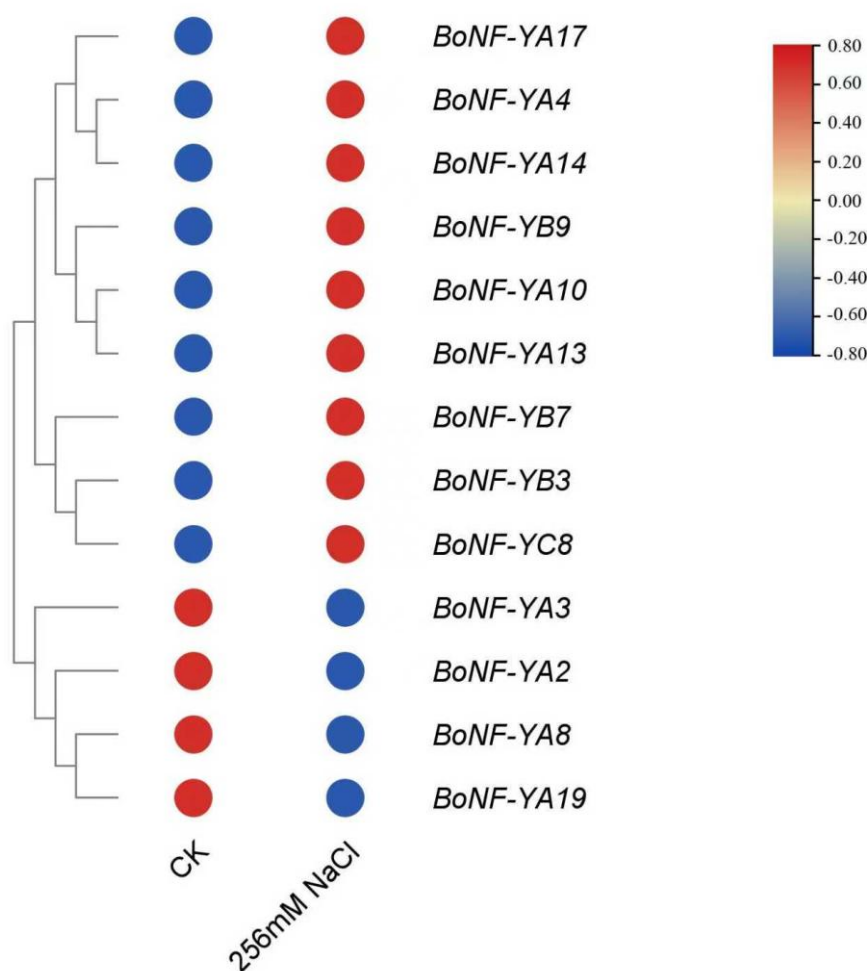


Figure 9. Heatmap of relative expression levels of differentially expressed *BoNF-Y* genes. The color scale (right) represents relative expression intensity: blue indicates low relative expression and red indicates high relative expression. Columns represent experimental groups.

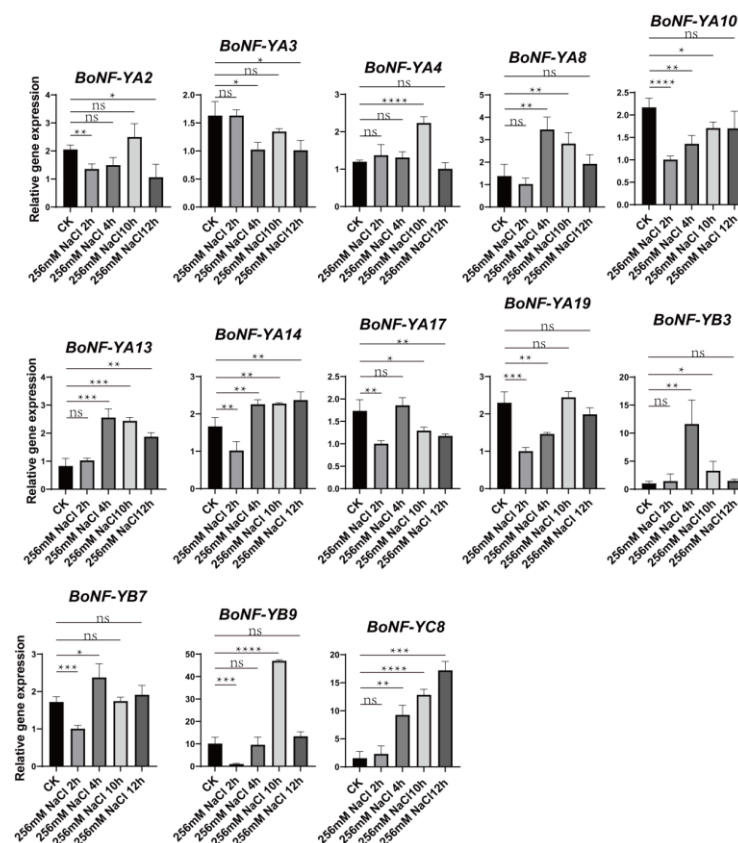


Figure 10. The analysis of differentially expressed genes at different time points under salt stress. A single star (*) represents a significant level ($p < 0.05$), two stars (**) indicate a significant level ($p < 0.01$), three stars (***) denote a highly significant level ($p < 0.001$), and four stars (****) represent an extremely significant level ($p < 0.0001$). “ns” indicates no significant difference.

3. Discussion

In this study, we conducted a genome-wide identification and analysis of the NF-Y family in cabbage to elucidate their functional roles and molecular mechanisms. A total of 53 NF-Y family members were identified, comprising 20 *BoNF-YA*x, 22 *BoNF-YB*x, and 11 *BoNF-YC*x subfamily members. Comprehensive analyses were performed to characterize their gene structures, chromosomal localizations, and physicochemical properties (e.g., amino acid length, molecular weight, isoelectric point). Additionally, we investigated the differential expression profiles of *BoNF-Y*s under salt stress, providing critical evidence for a comprehensive understanding of the *BoNF-Y*s family in *B. oleracea*.

The NF-Y family has been identified across diverse plant species, including potato (*Solanum tuberosum*) [30], alfalfa (*Medicago truncatula*) [31], poplar (*Populus trichocarpa*) [32], and petunia (*Petunia hybrida*) [33]. However, the biological functions and regulatory mechanisms of NF-Y genes remain largely unexplored in many species. The NF-Y family exhibits variable member numbers across plant species, with significant divergence observed between different lineages. However, the genomic architecture is generally conserved during plant evolution [34,35]. Through sequence alignment, we confirmed the conserved domains of all members: CBF_NF-YA for NF-YA subunits and CBFD_NF-YB_HMF for both NF-YB and NF-YC subunits. Recently, it was reported that the *AtNF-YA1/AtNF-YB2/AtNF-YC9* complex can positively regulate the salt tolerance of *A. thaliana* [36]. Through sequence comparison with the NF-Y family genes of *A. thaliana*, we found that in *Brassica oleracea*, three *BoNF-YA* genes, two *BoNF-YB* genes, and three *BoNF-YC* genes are respectively in the same clade as the *AtNF-YA1*, *AtNF-YB2*, and *AtNF-YC9* genes. Among them, the amino acid sequence similarity between the protein *BoNF-YA19*

and *AtNF-YA1* reaches 80.63%, which is higher than that of *BoNF-YA5* and *BoNF-YA3* (Figure 11a); the amino acid sequence similarity between the proteins *BoNF-YB3*, *BoNF-YB20* and *AtNF-YB2* reaches 82.41% (Figure 11b); the amino acid sequence similarity between the proteins *BoNF-YC5*, *BoNF-YC5* and *AtNF-YC9* reaches 95.55% (Figure 11c). It is speculated that *BoNF-YA19*, *BoNF-YA5*, *BoNF-YA3*, and *BoNF-YC5* may be potential salt-responsive genes, given their high amino acid sequence similarity to *AtNF-YA1/AtNF-YC9* (known to mediate salt tolerance in Arabidopsis) [37]. Previous studies have shown that the NF-Y family is involved in plant abiotic stress responses, though the specific regulatory pathways in cabbage require further validation [38]. NF-Y factors function as trimeric complexes, and their regulatory effects depend on the combination mode of specific NF-YA/YB/YC subunits [8,12]. In this study, the candidate salt-responsive genes (*BoNF-YA14*, *BoNF-YB9*, *BoNF-YC8*) exhibited upregulated expression patterns (Figure 10), which may facilitate the formation of functional trimers to further regulate downstream target differentially expressed genes (DEGs).

In maize [39], *ZmNF-YA8* can scavenge reactive oxygen species (ROS) by transcriptionally activating the expression of the *PEROXIDASE1* gene, thereby improving the salt tolerance of maize. In rapeseed [40], *BnaA9.NF-YA7* indirectly inhibits the expression of *BnaABF3/4s* by suppressing *Bna.ASHH4s* to reduce the *H3K36me3* level, thus negatively regulating the drought tolerance of *Brassica napus*. Through the analysis of NF-Y promoter elements, some stress-related regulatory elements have been identified. For example, MBS is involved in the regulation of drought stress; TC-rich repeats are widely involved in stress responses; LTR is used for coping with low-temperature stress; and the ABRE element regulates the ABA response, etc. These findings provide evidence that *BoNF-Ys* may have potential abiotic stress resistance capabilities.

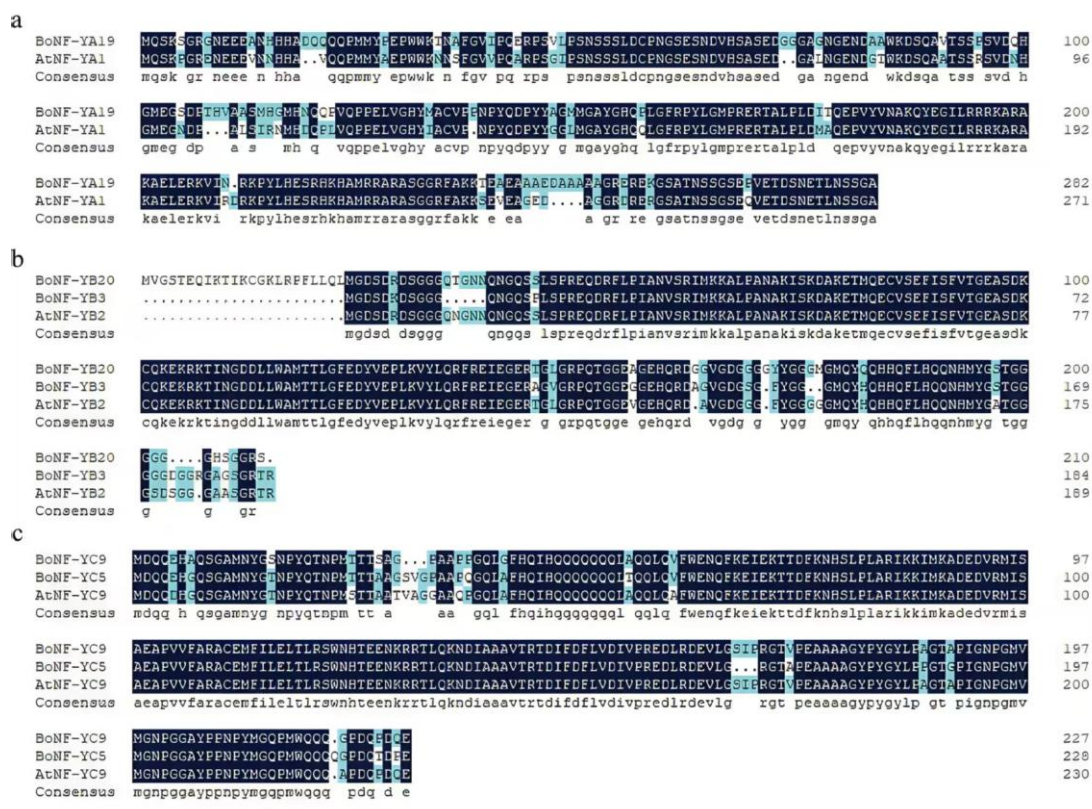


Figure 11. Sequence alignment of the amino acid sequences of proteins *BoNF-Ys* and *AtNF-Ys*. (a) Sequence alignment of the amino acid sequences of proteins *BoNF-YA19* and *AtNF-YA1*. (b) Sequence alignment of the amino acid sequences of proteins *BoNF-YA20*, *BoNF-YA3*, and *AtNF-YB2*.

(c) Sequence alignment of the amino acid sequences of proteins *BoNF-YC9*, *BoNF-YC5*, and *AtNF-YC9*.

4. Materials and Methods

4.1. Plant Materials and Salt Stress Treatment

Cabbage (*Brassica oleracea* L.) cultivar HB70 was used as the experimental material. Prior to the formal experiment, a salt tolerance screening assay was conducted on cabbage germplasm to determine the appropriate salt stress concentration. Briefly, five gradient concentrations of NaCl solution were set, and the growth performance of HB70 and other cabbage accessions was systematically evaluated. The results showed that 214 mM NaCl could already significantly inhibit the growth of cabbage. Since HB70 was identified as a salt-tolerant material during the screening, 256 mM NaCl (a slightly higher concentration than 214 mM) was selected for the formal salt stress treatment. This concentration was chosen to balance two core objectives: avoiding excessive plant mortality and inducing distinct salt stress responses in HB70.

4.2. Genome-Wide Identification of *BoNF-Ys* in Cabbage

The genomic data of cabbage (BraOL_JZS_V2.0) used in this study were obtained from the Brassica Genome Database (<http://www.brassicadb.cn/#/Download/>, accessed on 4 March 2023) [41]. To comprehensively identify NF-Y gene family members in cabbage, we performed a systematic analysis combining BLAST 2.16.0 and HMMER 3.4 [42] approaches. A total of 36 NF-Y protein sequences of *Arabidopsis thaliana* (ATNF-Ys) were used to blast in the cabbage genome [43]. Subsequently, Hidden Markov Models (HMMs) for the NF-Y domains (PF02045, PF00808) were downloaded from the Pfam database (<http://pfam.xfam.org/>, accessed on 10 March 2023) [44]. Candidate *BoNF-Y* proteins were subsequently identified through genome-wide screening of cabbage protein sequences using HMMER 3.4 software.

4.3. Prediction of Basic Information of *BoNF-Ys*

Physicochemical characterization of *BoNF-Y* proteins was performed using TBtools (v2.0) to calculate amino acid composition, isoelectric point (pI), and molecular weight [45]. Protein sequences were subsequently submitted to ProtParam (<https://web.expasy.org/protparam/>, accessed on 10 March 2023) for computational prediction of instability index, aliphatic index, and grand average of hydropathicity (GRAVY) [46].

4.4. Analysis of the Conserved Domains and Phylogenetic Evolution of *BoNF-Ys*

Conserved motif analysis of *BoNF-Y* proteins was conducted using MEME Suite (<http://alternate.meme-suite.org/tools/meme>, accessed on 13 March 2023) [47] with parameters set to allow 0 or 1 occurrences per sequence, predict 10 motifs, and a maximum motif width of 50 residues. The resulting motif distribution patterns were visualized using TBtools (v2.0). Protein sequences of NF-Y members from *A. thaliana* and cabbage (*B. oleracea*) were aligned using MAFFT (v7.505) [48] under Linux, followed by phylogenetic tree construction via the Maximum Likelihood (ML) [49] method in MEGA7 [50].

4.5. Analysis of Chromosomal Localization, Promoter and Gene Structure of BoNF-Ys

The number, length, and genomic positions of introns and exons of BoNF-Ys were extracted from the GFF3 file of the *B. oleracea* genome database. The gene structures (exon–intron organization) were visualized using the Gene Structure View function in TBtools. Subsequently, the Gtf/Gff3 Sequences Extract module in TBtools was utilized to obtain 2000 bp upstream sequences (relative to the transcription start site) of all BoNF-Ys as putative promoter regions. These promoter sequences were analyzed for potential cis-acting elements (e.g., transcription factor binding sites, stress-responsive motifs, and core promoter elements) using the PlantCARE database [51]. The identified cis-acting elements were statistically categorized and visualized through TBtools.

4.6. Plant Materials and Treatments

The experimental material used in this study was the *B. oleracea* line HB70, provided by our lab. Seeds of HB70 were sown in 32-cell trays and cultivated in a seedling nursery greenhouse. One month after germination, the plants were subjected to salt stress by irrigation with 256 mM NaCl solution. Root samples were collected at 0, 2, 4, 10, and 12 h (h) post-treatment (0 h as the CK). The harvested roots were immediately frozen in liquid nitrogen and stored at -80°C for subsequent analysis.

4.7. RNA-Seq Experimental Design and Data Quality Control

For RNA-seq analysis, root samples of cabbage cultivar HB70 were collected from both the control group (CK, no salt stress) and the treatment group (256 mM NaCl salt stress). Each group included 2 biological replicates, with each replicate consisting of pooled root tissues from 3 individual plants to minimize biological variation.

Library construction and paired-end sequencing were performed on an Illumina NovaSeq 6000 platform, achieving an average sequencing depth of $30\times$ per sample. Raw sequencing data quality was evaluated using FastQC, with key metrics summarized in (Supplementary Table S1): all samples yielded 33.81–39.30 million raw reads, with total bases ranging from 10.14 Gb to 11.79 Gb. The GC content was consistently between 46.36% and 46.61%, consistent with the typical range for plant genomes and indicating no significant contamination. Base quality was high across all samples, with Q20 values (proportion of bases with $\geq 99\%$ accuracy) exceeding 95% and Q30 values (proportion of bases with $\geq 99.9\%$ accuracy) above 86% for both read pairs, confirming the reliability of the raw sequencing data (Supplementary Table S1 and Supplementary Figure S1).

4.8. Expression Analysis of BoNF-Ys Using RNA-Seq Data

The root samples of untreated and salt-treated HB70 materials were sequenced by GenePlus Biotechnology Co., Ltd (Beijing, China). After the data were downloaded, a Linux system was used to perform reference genome alignment, format conversion, and expression level calculation on the data. R language [52] was used to conduct Gene Ontology (GO) [53] and Kyoto Encyclopedia of Genes and Genomes (KEGG) [54] enrichment analyses on the differentially expressed genes.

4.9. Total RNA Extraction, cDNA Synthesis, and qRT-PCR Analysis

Total RNA was extracted from cabbage samples according to the instructions of the RNA prep Pure Plant Total RNA Extraction Kit from Tiangen Biotech (Beijing, China) Co., Ltd. The purity and quality of the RNA were evaluated using a spectrophotometer (BioDrop, Cambridge, UK) and 1% formaldehyde gel electrophoresis. First-strand cDNA was synthesized following the operating instructions of the FastKing RT Kit (Tiangen). Specific primers for the BoNF-Ys were designed using Premier 3.0 (<https://primer3.org/>, accessed

on 10 March 2023) software (Supplementary Table S3). qRT-PCR reactions were carried out on a CFX96 Real-Time Fluorescent Quantitative PCR System (Bio-Rad, Hercules, California, USA.) using the TransStart Top Green qPCR SuperMix Kit (Beijing TransGen Biotech Co., Ltd, Beijing, China.). Three technical replicates and biological replicates were set for each reaction.

5. Conclusions

In this study, 53 NF-Y members were identified in the *Brassica oleracea* genome, including 20 BoNF-YAx, 22 BoNF-YBx and 11 BoNF-YCx subfamily members. Through transcriptome data and qRT-PCR analysis after salt stress treatment, three genes with significantly upregulated expression were *BoNF-YA14*, *BoNF-YB9* and *BoNF-YC8*, respectively. In this study, the gene structures, phylogenetic relationships, chromosomal locations and promoter element analysis of *BoNF-Ys* genes were examined.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ijms27052256/s1>.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2025) in the National Genomics Data Center (Nucleic Acids Res 2025 [55]), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA034440), and are publicly accessible at <https://ngdc.cncb.ac.cn/gsa> (accessed on 10 March 2023) [56].

Conflicts of Interest: The authors declare that they have no competing interests.

References

1. Hassani, A.; Azapagic, A.; Shokri, N. Global predictions of primary soil salinization under changing climate in the 21st century. *Nat. Commun.* **2021**, *12*, 6663.
2. Shavrukov, Y. Salt stress or salt shock: Which genes are we studying? *J. Exp. Bot.* **2013**, *64*, 119–127.
3. Wu, Y.; Zhang, B.; Yang, L.; Zhuang, M.; Lv, H.; Wang, Y.; Ji, J.; Hou, X.; Zhang, Y. Fine mapping and identification of the downy mildew resistance gene BoDMR2 in Cabbage (*Brassica oleracea* L. var. capitata). *BMC Plant Biol.* **2024**, *24*, 987.
4. Lu, H.P.; Liu, X.H.; Wang, M.J.; Zhu, Q.Y.; Lyu, Y.S.; Xu, J.H.; Liu, J.X. The NAT1-bHLH110-CER1/CER1L module regulates heat stress tolerance in rice. *Nat. Genet.* **2025**, *57*, 427–440.
5. Weidemuller, P.; Kholmatov, M.; Petsalaki, E.; Zaugg, J.B. Transcription factors: Bridge between cell signaling and gene regulation. *Proteomics* **2021**, *21*, e2000034.

6. Tian, Y.; Song, K.; Li, B.; Song, Y.; Zhang, X.; Li, H.; Yang, L. Genome-wide identification and expression analysis of NF-Y gene family in tobacco (*Nicotiana tabacum* L.). *Sci. Rep.* **2024**, *14*, 5257.
7. Siriwardana, C.L. Plant nuclear factor y (NF-Y) transcription factors: Evolving insights into biological functions and gene expansion. *Int. J. Mol. Sci.* **2024**, *26*, 38.
8. Oldfield, A.J.; Henriques, T.; Kumar, D.; Burkholder, A.B.; Cinghu, S.; Paulet, D.; Bennett, B.D.; Yang, P.; Scruggs, B.S.; Lavender, C.A.; et al. NF-Y controls fidelity of transcription initiation at gene promoters through maintenance of the nucleosome-depleted region. *Nat. Commun.* **2019**, *10*, 3072.
9. Pan, W.; Li, J.; Du, Y.; Zhao, Y.; Xin, Y.; Wang, S.; Liu, C.; Lin, Z.; Fang, S.; Yang, Y.; et al. Epigenetic silencing of callose synthase by VIL1 promotes bud-growth transition in lily bulbs. *Nat. Plants* **2023**, *9*, 1451–1467.
10. Zheng, L.; Gao, S.; Bai, Y.; Zeng, H.; Shi, H. NF-YC15 transcription factor activates ethylene biosynthesis and improves cassava disease resistance. *Plant Biotechnol. J.* **2024**, *22*, 2424–2434.
11. Myers, Z.A.; Holt, B.R. NUCLEAR FACTOR-Y: Still complex after all these years? *Curr. Opin. Plant Biol.* **2018**, *45*, 96–102.
12. Nardone, V.; Chaves-Sanjuan, A.; Nardini, M. Structural determinants for NF-Y/DNA interaction at the CCAAT box. *Biochim. Biophys. Acta Gene Regul. Mech.* **2017**, *1860*, 571–580.
13. Chaves-Sanjuan, A.; Gnesutta, N.; Gobbini, A.; Martignago, D.; Bernardini, A.; Fornara, F.; Mantovani, R.; Nardini, M. Structural determinants for NF-Y subunit organization and NF-Y/DNA association in plants. *Plant J.* **2021**, *105*, 49–61.
14. Holdsworth, M.J.; Bentsink, L.; Soppe, W. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. *New Phytol.* **2008**, *179*, 33–54.
15. Lee, H.; Fischer, R.L.; Goldberg, R.B.; Harada, J.J. Arabidopsis LEAFY COTYLEDON1 represents a functionally specialized subunit of the CCAAT binding transcription factor. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 2152–2156.
16. Dolfini, D.; Zambelli, F.; Pavesi, G.; Mantovani, R. A perspective of promoter architecture from the CCAAT box. *Cell Cycle* **2009**, *8*, 4127–4137.
17. Quach, T.N.; Nguyen, H.T.; Valliyodan, B.; Joshi, T.; Xu, D.; Nguyen, H.T. Genome-wide expression analysis of soybean NF-Y genes reveals potential function in development and drought response. *Mol. Genet. Genom.* **2015**, *290*, 1095–1115.
18. Siefers, N.; Dang, K.K.; Kumimoto, R.W.; Bynum, W.T.; Tayrose, G.; Holt, B.R. Tissue-specific expression patterns of Arabidopsis NF-Y transcription factors suggest potential for extensive combinatorial complexity. *Plant Physiol.* **2009**, *149*, 625–641.
19. Cao, L.; Ma, C.; Ye, F.; Pang, Y.; Wang, G.; Fahim, A.M.; Lu, X. Genome-wide identification of NF-Y gene family in maize (*Zea mays* L.) and the positive role of ZmNF-YC12 in drought resistance and recovery ability. *Front. Plant Sci.* **2023**, *14*, 1159955.
20. Malviya, N.; Jaiswal, P.; Yadav, D. Genome-wide characterization of Nuclear Factor Y (NF-Y) gene family of sorghum [*Sorghum bicolor* (L.) Moench]: A bioinformatics approach. *Physiol. Mol. Biol. Plants* **2016**, *22*, 33–49.
21. Zhang, H.; Liu, S.; Ren, T.; Niu, M.; Liu, X.; Liu, C.; Wang, H.; Yin, W.; Xia, X. Crucial abiotic stress regulatory network of NF-Y transcription factor in plants. *Int. J. Mol. Sci.* **2023**, *24*, 4426.
22. Wang, Y.; Jiao, P.; Wu, C.; Wang, C.; Shi, K.; Gao, X.; Guan, S.; Ma, Y. ZmNF-YB10, a maize NF-Y transcription factor, positively regulates drought and salt stress response in *Arabidopsis thaliana*. *GM Crops Food* **2025**, *16*, 28–45.
23. Yu, T.F.; Hou, Z.H.; Wang, H.L.; Chang, S.Y.; Song, X.Y.; Zheng, W.J.; Zheng, L.; Wei, J.T.; Lu, Z.W.; Chen, J.; et al. Soybean steroids improve crop abiotic stress tolerance and increase yield. *Plant Biotechnol. J.* **2024**, *22*, 2333–2347.
24. Chen, M.; Zhao, Y.; Zhuo, C.; Lu, S.; Guo, Z. Overexpression of a NF-YC transcription factor from bermudagrass confers tolerance to drought and salinity in transgenic rice. *Plant Biotechnol. J.* **2015**, *13*, 482–491.
25. Li, X.; Li, C.; Shi, L.; Lv, G.; Li, X.; Liu, Y.; Jia, X.; Liu, J.; Chen, Y.; Zhu, L.; et al. Jasmonate signaling pathway confers salt tolerance through a NUCLEAR FACTOR-Y trimeric transcription factor complex in Arabidopsis. *Cell Rep.* **2024**, *43*, 113825.
26. Petroni, K.; Kumimoto, R.W.; Gnesutta, N.; Calvenzani, V.; Fornari, M.; Tonelli, C.; Holt, B.R.; Mantovani, R. The promiscuous life of plant NUCLEAR FACTOR Y transcription factors. *Plant Cell* **2012**, *24*, 4777–4792.
27. Thirumurugan, T.; Ito, Y.; Kubo, T.; Serizawa, A.; Kurata, N. Identification, characterization and interaction of HAP family genes in rice. *Mol. Genet. Genom.* **2008**, *279*, 279–289.
28. Liu, X.; Zhao, M.; Gu, C.; Jiang, H.; Sun, J.; Li, J. Genome-wide identification of MAPK family genes and their response to abiotic stresses in tea plant (*Camellia sinensis*). *Open Life Sci.* **2022**, *17*, 1064–1074.
29. Chen, L.; Meng, Y.; Bai, Y.; Yu, H.; Qian, Y.; Zhang, D.; Zhou, Y. Starch and sucrose metabolism and plant hormone signaling pathways play crucial roles in aquilegia salt stress adaption. *Int. J. Mol. Sci.* **2023**, *24*, 3948.
30. Liu, Z.; Li, Y.; Zhu, J.; Ma, W.; Li, Z.; Bi, Z.; Sun, C.; Bai, J.; Zhang, J.; Liu, Y. Genome-Wide Identification and Analysis of the NF-Y Gene Family in Potato (*Solanum tuberosum* L.). *Front. Genet.* **2021**, *12*, 739989.

31. An, Y.; Uo, X.; Niu, Q.; Yin, S.; Chen, L. Genome-Wide identification and analysis of the NF-Y transcription factor family reveal its potential roles in salt stress in alfalfa (*Medicago sativa* L.). *Int. J. Mol. Sci.* **2022**, *23*, 6426.
32. Liu, R.; Wu, M.; Liu, H.L.; Gao, Y.M.; Chen, J.; Yan, H.W.; Xiang, Y. Genome-wide identification and expression analysis of the NF-Y transcription factor family in *Populus*. *Physiol. Plant* **2021**, *171*, 309–327.
33. Wei, Q.; Wen, S.; Lan, C.; Yu, Y.; Chen, G. Genome-Wide identification and expression profile analysis of the NF-Y transcription factor gene family in *Petunia hybrida*. *Plants* **2020**, *9*, 336.
34. Fedorov, A.; Merican, A.F.; Gilbert, W. Large-scale comparison of intron positions among animal, plant, and fungal genes. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 16128–16133.
35. Rogozin, I.B.; Wolf, Y.I.; Sorokin, A.V.; Mirkin, B.G.; Koonin, E.V. Remarkable interkingdom conservation of intron positions and massive, lineage-specific intron loss and gain in eukaryotic evolution. *Curr. Biol.* **2003**, *13*, 1512–1517.
36. Zhao, H.; Wu, D.; Kong, F.; Lin, K.; Zhang, H.; Li, G. The *Arabidopsis thaliana* Nuclear Factor Y Transcription Factors. *Front. Plant Sci.* **2017**, *7*, 2045.
37. Zanetti, M.E.; Ripodas, C.; Niebel, A. Plant NF-Y transcription factors: Key players in plant-microbe interactions, root development and adaptation to stress. *Biochim. Biophys. Acta Gene Regul. Mech.* **2017**, *1860*, 645–654.
38. Chouaibi, Y.; Ben Romdhane, W.; Wiszniewska, A.; Baazaoui, N.; Gamas, N.; Bouteraa, M.T.; Ben Akacha, B.; Ka Ániová, M.; Ben Hsouna, A.; Garzoli, S.; Ben Saad, R. NF-Y transcription factors: Key players in biotic and abiotic stress tolerance in plants. *BioEssays News Rev. Mol. Cell. Dev. Biol.* **2025**, *47*, e70023.
39. Xing, L.; Zhu, M.; Luan, M.; Zhang, M.; Jin, L.; Liu, Y.; Zou, J.; Wang, L.; Xu, M. MiR169q and NUCLEAR FACTOR YA8 enhance salt tolerance by activating PEROXIDASE1 expression in response to ROS. *Plant Physiol.* **2022**, *188*, 608–623.
40. Wang, J.; Mao, L.; Li, Y.; Lu, K.; Qu, C.; Tang, Z.; Li, J.; Liu, L. Natural variation in BnaA9.NF-YA7 contributes to drought tolerance in *Brassica napus* L. *Nat. Commun.* **2024**, *15*, 2082.
41. Chen, H.; Wang, T.; He, X.; Cai, X.; Lin, R.; Liang, J.; Wu, J.; King, G.; Wang, X. BRAD V3.0: An upgraded Brassicaceae database. *Nucleic Acids Res.* **2022**, *50*, D1432–D1441.
42. Finn, R.D.; Clements, J.; Eddy, S.R. HMMER web server: Interactive sequence similarity searching. *Nucleic Acids Res.* **2011**, *39*, W29–W37.
43. Lamesch, P.; Berardini, T.Z.; Li, D.; Swarbreck, D.; Wilks, C.; Sasidharan, R.; Muller, R.; Dreher, K.; Alexander, D.L.; Garcia-Hernandez, M.; et al. The Arabidopsis Information Resource (TAIR): Improved gene annotation and new tools. *Nucleic Acids Res.* **2012**, *40*, D1202–D1210.
44. Mistry, J.; Chuguransky, S.; Williams, L.; Qureshi, M.; Salazar, G.A.; Sonnhammer, E.; Tosatto, S.; Paladin, L.; Raj, S.; Richardson, L.J.; et al. Pfam: The protein families database in 2021. *Nucleic Acids Res.* **2021**, *49*, D412–D419.
45. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* **2020**, *13*, 1194–1202.
46. Wilkins, M.R.; Gasteiger, E.; Bairoch, A.; Sanchez, J.C.; Williams, K.L.; Appel, R.D.; Hochstrasser, D.F. Protein identification and analysis tools in the ExPASy server. *Methods Mol. Biol.* **1999**, *112*, 531–552.
47. Bailey, T.L.; Johnson, J.; Grant, C.E.; Noble, W.S. The MEME suite. *Nucleic Acids Res.* **2015**, *43*, W39–W49.
48. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780.
49. Yang, S.; De Angelis, D. Maximum likelihood. *Methods Mol. Biol.* **2013**, *930*, 581–595.
50. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874.
51. Lescot, M.; Dehais, P.; Thijs, G.; Marchal, K.; Moreau, Y.; Van de Peer, Y.; Rouze, P.; Rombauts, S. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* **2002**, *30*, 325–327.
52. Giorgi, F.M.; Ceraolo, C.; Mercatelli, D. The R language: An engine for bioinformatics and data science. *Life* **2022**, *12*, 648.
53. Ashburner, M.; Ball, C.A.; Blake, J.A.; Botstein, D.; Butler, H.; Cherry, J.M.; Davis, A.P.; Dolinski, K.; Dwight, S.S.; Eppig, J.T.; et al. Gene ontology: Tool for the unification of biology. *Gene Ontol. Consortium. Nat. Genet.* **2000**, *25*, 25–29.
54. Kanehisa, M.; Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **2000**, *28*, 27–30.

55. Bao, Y.; Bai, X.; Bu, C.; Chen, H.; Chen, H.; Chen, K.; Chen, M.; Chen, M.; Chen, M.; Chen, P.; et al. Database Resources of the National Genomics Data Center, China National Center for Bioinformation in 2025. *Nucleic Acids Res.* **2025**, *53*, D30–D44. <https://doi.org/10.1093/nar/gkae978>. PMID: 39530327.
56. Zhang, S.; Chen, X.; Jin, E.; Wang, A.; Chen, T.; Zhang, X.; Zhu, J.; Dong, L.; Sun, Y.; Yu, C.; et al. The GSA Family in 2025: A Broadened Sharing Platform for Multi-Omics and Multimodal Data. *Genom. Proteom. Bioinform.* **2025**, *23*, qzaf072. <https://doi.org/10.1093/gpbjnl/qzaf072>. PMID: 40857552.

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