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Genome-wide identification and expression profiling of *TALE* gene family in banana under abiotic stresses and *Foc* 4 infection

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ABSTRACT

Three Amino Acid Loop Extension (TALE) is a member of the homeobox gene group. In addition to being crucial for growth and development, the TALE gene family is also critical for controlling how plants react to environmental stressors. Here, we used the banana genome database to extract 47 *MaTALE* genes. The evolutionary analysis grouped the sequences into seven subclades, named STM, KNOX-II, and BELL-I to BELL-V. Analysis of *MaTALE* promoters revealed that their *cis*-acting elements are associated primarily with hormone (especially ABA) and stress responsiveness. The STRING database further suggested that MaTALE proteins could work in tandem with other important TFs that play a significant role in plant growth. The expression analysis under drought, cold, and *Foc* 4 revealed their potential involvement in stress response. Exogenous ABA treatment significantly induced the expression of *MaTALE1/14/20* genes. The transient expression assay showed that MaTALE1 resides in the nucleus. Our data offer a valuable resource for developing integrated management strategies against abiotic (drought, cold) and biotic (*Foc* 4) stresses affecting bananas in Southern China.

Keywords: Banana | *Fusarium oxysporum* | *Foc 4* | Genome-wide | *MaTALE1* | PEG

1 | INTRODUCTION

The Three Amino Acid Loop Extension (TALE) superfamily comprises eukaryotic transcription factors critical for developmental regulation [1]. These proteins contain a highly conserved 63-amino acid homeodomain [2], distinguished by a three-amino acid loop insertion between helices 1 and 2 [3]. TALE genes encode proteins with KNOX and BELL domains that heterodimerize to regulate downstream targets [4]. In *Arabidopsis thaliana*, TALE factors are divided into BELL and KNOX subfamilies, whose specific interactions control developmental genes. Plant TALE proteins further feature KNOX1, KNOX2, ELK, and Homeobox KN domains [5]. The BELL subfamily is characterized by a conserved domain architecture, which usually includes SKY and BEL domains, a C-terminal Homeobox (HB) domain, and the VSLTLGL/ZIBEL domain.

As the founding member of the plant KNOX family, the maize homeobox gene *Knotted-1* (*Kn1*) has paved the way for characterizing KNOX functions in other species [4]. In line with that, the *Arabidopsis* Class I KNOX genes, including *SHOOTMERISTEMLESS* (*STM*), are critical for maintaining the shoot apical meristem (SAM). For instance, *stm* mutants fail to establish a functional SAM [6]. Beyond meristem regulation, Class I KNOX genes play diverse roles in plant development. They are known to repress differentiation and, in many contexts, inhibit secondary cell wall (SCW) formation. The synthesis of cotton fiber is regulated by *GhKNL1*, a class II member of the TALE family [7]. The regulation of flowers, xylem differentiation, and hormonal treatment may be influenced by the *Arabidopsis* KNOX Class I family genes (*STM, KNAT2, BREVIPEDICELLUS (BP)/KNAT1*, and *KNAT6*) [8]. Class I KNOX genes also exert inhibitory effects on secondary cell wall (SCW) production. The abundance of *KNOPE1* diminished progressively from primary to secondary growth and exhibited a negative correlation with lignin gene expression and lignin deposition, indicating that *KNOPE1* inhibited cell lignification in peach stems [9]. In contrast, Class II KNOX genes inhibit stem elongation and secondary cell wall (SCW) deposition, processes that are promoted by Class I genes. The *knat3/knat7* double mutants and the chimeric repressor of *KNAT3/KNAT7* exhibited analogous dwarfing phenotypes; however, *KNAT3* overexpression led to increased thickness of the interfascicular fiber secondary cell wall [10]. *KNAT3* binds to SCW-forming transcription factor *NST1/2* to control Ferulate 5-Hydroxylase (F5H) and enhance syringyl lignin formation [11]. *KNAT7* also activates the expression of IRXs in *Arabidopsis*, which positively regulates SCW biosynthesis [12]. Additionally, the KNOX genes regulate several target genes that are responsible for regulating hormone homeostasis. The expression of the ABA-responsive gene *ABI3* can be directly up-regulated by *KNAT3*. ABA treatment increased the expression and promoter activity of *MdKNOX19* in apples [4]. *MdKNOX19* directly binds to and upregulates *ABI5* to transmit ABA perception. These findings provide more evidence for a regulatory feedback loop involving KNOX and ABA signaling [4].

Drought stress is a detrimental abiotic stress that affects physiological and biochemical systems, reducing plant growth and yield [13]. The RNAi antisense lines of *MtKNOX3-like* in *Medicago truncatula* displayed compromised response to drought stress [14].

In another study, the expression of *GhBLH5-A05* in cotton was stimulated by drought stress. The *GhBLH5-A05* overexpression in both *Arabidopsis* and cotton enhanced drought tolerance, while its silencing led to greater sensitivity [15]. VIGS silencing of *GhKNOX4-AGh* and *GhKNOX22-D* genes affected cotton seedling growth and development under salt and drought [16]. Despite their distinctive role in stress biology, TALE genes have yet to be explored in banana.

Banana Fusarium wilt, commonly known as Panama disease, is a destructive condition caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*) [17,18]. Classified into races including *Foc* 1, *Foc* 2, and *Foc* 4 based on their host range, the pathogen poses a critical threat to banana cultivation. Specifically, races 1 and 4 jeopardize the sustainability and economic viability of banana crops worldwide [19]. Compounding these disease pressures, there are currently no effective chemical or agronomic controls for Fusarium wilt. Identifying Fusarium wilt in banana could be a key strategy to generate resilient lines.

Bananas (*Musa spp.*) are the most cultivated and globally traded fruits, mostly grown in tropical and subtropical countries [20]. As a significant dietary element, bananas are eaten raw as a dessert fruit or cooked, and they frequently provide a primary source of carbohydrates. In certain nations where bananas are a dietary staple, per capita consumption averages 0.5 kilograms per day and can exceed 1 kilogram in specific regions [20,21]. The vast tropical region in southern China provides ideal conditions for bananas, allowing for their cultivation and consumption on a large scale [22]. However, the production of banana is hampered by the *Foc* 4 pathogen, salinity, drought, and cold stress [23].

This study provides a comprehensive analysis of the TALE gene family in the banana genome. We performed computational characterization, including phylogeny, conserved motif identification, protein-protein interaction networks, and *cis*-regulatory element prediction. Furthermore, we examined *MaTALE* transcriptional responses to cold stress, drought stress, and *Foc* 4 infection. Our study reveals the critical role of *MaTALE* genes in regulating the banana plant's response to *Foc* 4, cold, and drought.

2 | RESULTS

2.1 | Genome-wide characterization of MaTALE genes

We identified putative MaTALE genes via a BLAST search of the banana genome using *Arabidopsis* TALE sequences as queries. After validating these candidates with HMMER and removing duplicates, we retained 47 unique genes, which we named MaTALE1–MaTALE47 according to their genomic order (Table 1).

2.2 | Chromosomal localization of MaTALE genes

The *MaTALE* genes were mapped to 11 chromosomes, with an uneven distribution (Figure 1). Chromosome 4 had the highest density (13 genes; *MaTALE12–24*), followed by chromosome 10 (*5* genes*; MaTALE40–45*). Chromosomes 1, 2, and 5 each had 4 genes, while chromosome 8 had only one (*MaTALE37*).

2.3 | Evolutionary and conserved domain analysis of TALE proteins

A phylogenetic tree was constructed from TALE protein sequences of banana, *Arabidopsis*, wax gourd, and cucumber using MEGA 6.0. Within this tree, the STM subfamily harbored the highest number of TALE genes, with KNOX-II being the next most abundant (Figure 2A). The BELL clade was divided into five subfamilies (BELL-I to BELL-V), with BELL-I and BELL-V containing the most genes and BELL-II, -III, and -IV containing fewer. Analysis of conserved domains indicated that KNAT group proteins feature KNOX1, KNOX2, ELK, and HOX domains, while BEL group proteins contain POX and HOX domains (Figure 2B). Homology models of the 3D structures further highlighted distinct structural differences between the KNAT and BEL groups (Figure 2C).

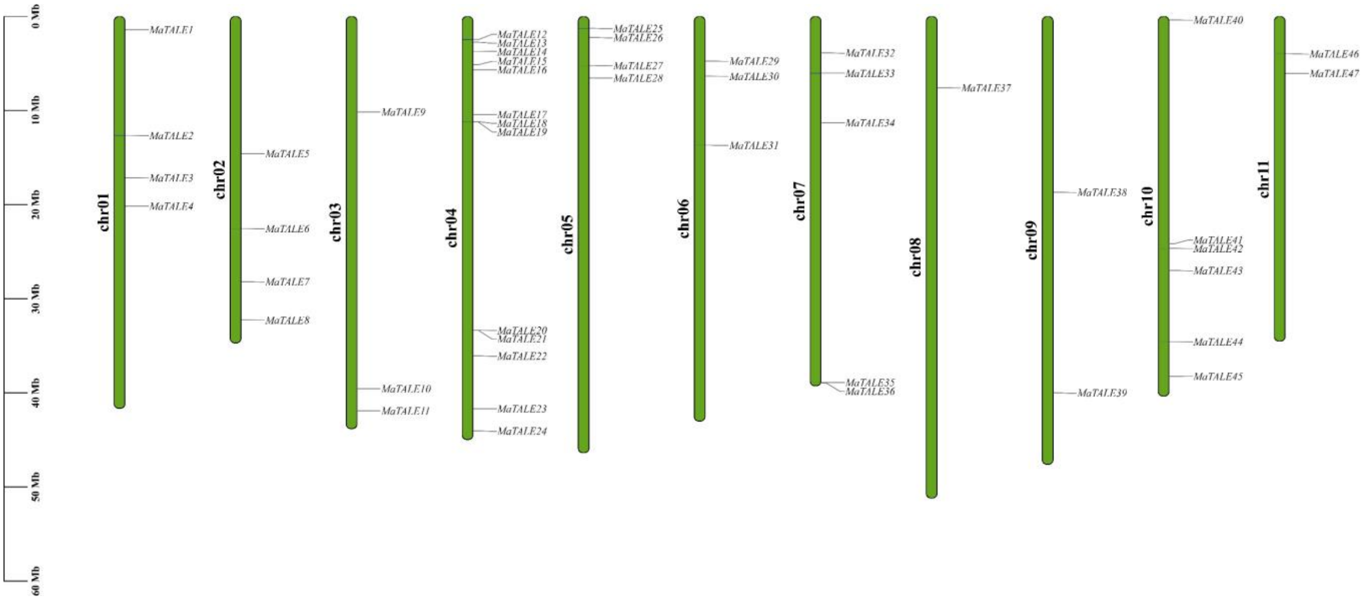

Figure 1 | Chromosomal localization of MaTALE genes. Chromosome numbers are indicated at the top of each bar. This schematic was generated using TBtools-II.

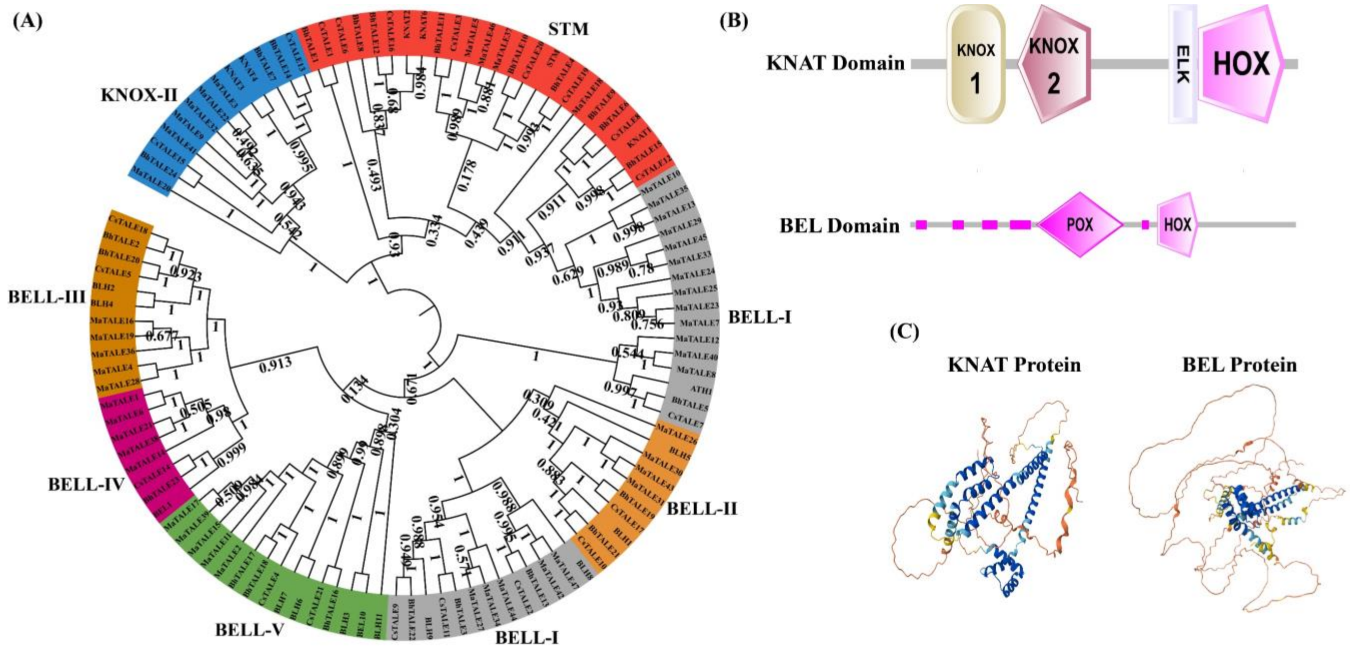

Figure 2 | (A) Analysis of the MaTALE gene family using phylogenetic tools. A phylogenetic tree was constructed based on the amino acid sequences of proteins from four species: *Arabidopsis* (19 proteins), banana (47 proteins), cucumber (21 proteins), and wax gourd (24 proteins). The analysis was performed using the maximum likelihood method. All MaTALE were categorized into seven categories, and the resulting phylogenetic tree was presented by employing ITOL. (B) Conserved domain analysis of MaTALE proteins. (C) 3D structure of the KNAT and BEL protein.

2.4 | Conserved motif analysis of MaTALE proteins

To assess the functional diversity of MaTALE proteins, we identified conserved motifs using the MEME suite. Ten unique motifs were discovered (Figure 3). Six family members (MaTALE1, 8, 12, 16, 19, 20) contained the broadest catalog of key domains, including KNOX1 (motif 2), KNOX2 (motif 3), ELK (motif 6), Homeobox_KN (motif 8), and POX (motif 10). In contrast, a more restricted pattern was seen in other proteins, including MaTALE3, 13, and 15, which contained only KNOX1, KNOX2, and Homeobox_KN, while MaTALE6 featured just ELK and Homeobox_KN. Overall, the Homeobox_KN and POX domains were the most prevalent among all MaTALE proteins (Figure 3).

2.5 | Gene ontology (GO) analysis of *MaTALE* genes

Gene Ontology (GO) analysis was performed to predict the functional roles of MaTALE genes in banana growth. By examining MaTALE protein sequences, we identified dominant GO terms associated with key developmental processes, including floral meristem specification, shoot formation, xylem development, hormone response, regulation of secondary cell wall (SCW) metabolism, and the vegetative-to-reproductive phase transition (Figure 4). Terms linked to molecular functions, such as the regulation of transcription, were also identified.

2.6 | Interactive protein analysis

To elucidate the functional context of MaTALE proteins, we constructed a protein-protein interaction (PPI) network using the STRING database with MaTALE1 as the reference. This analysis identified a cluster of key interactors, including NAC073, NAC012, MYB63, MYB75, MYB46, OFPs, and BELL-family BLH proteins (Figure 5A). Enrichment analysis of this PPI network further indicated that MaTALE1 and its partners are predominantly associated with secondary cell wall (SCW) biogenesis (Figure 5B).

2.7 | *Cis*-acting elements of *MaTALE* genes in banana

To investigate the probable regulatory mechanisms of the MaTALE gene family, we identified the *cis*-acting elements associated with each member (Figure 5C). These elements are implicated in plant hormone signaling, development, stress responses, and light perception. Analysis of the 2,000-bp promoter sequences revealed multiple *cis*-acting elements. These included stress-responsive elements (LTR, MBS, ARE), transcriptional regulatory elements (MYB and W-box), and components associated with plant hormone signaling (P-box, TGA-element, ABRE). The predominant motif is the *cis*-acting element associated with ABA reactivity, comprising 30% of the analyzed hormone response motifs. MeJA reactivity-related cis-acting components of the TGACG motif made about 13%. Additionally, we discovered that the TCA element in response to SA occurred 29 times and accounted for 10% of the 19 *MaTALE* gene promoters. Drought stress was associated with the MBS factor, accounting for 12% (Figure 5C). These results imply that these hormones and drought stress may influence *MaTALE* gene transcription.

2.8 | Expression analysis of *MaTALE* genes under cold, drought, and pathogen stress

The presence of stress and hormone-responsive *cis*-elements in the promoter region of *MaTALE* genes led us to examine their expression under drought, cold, and pathogen (*Foc* 4) stress (Figure 6). The expression analysis revealed a varied expression pattern under drought stress. For instance, the *MaTALE1* displayed a high expression pattern in control (Con-1-3) and drought (D-1-3) treated banana leaf samples (Figure 6A).

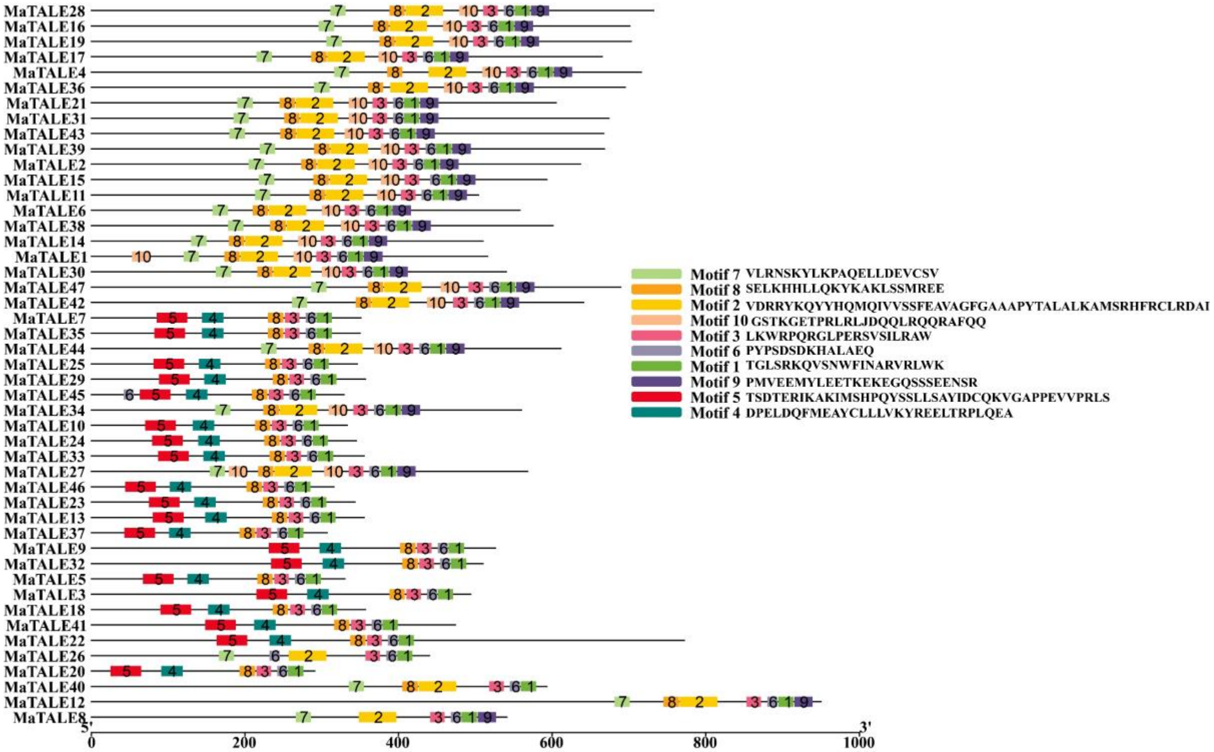

Figure 3 | Identification of conserved protein motifs in the MaTALE family. Analysis was performed on MaTALE protein sequences using the MEME web server.

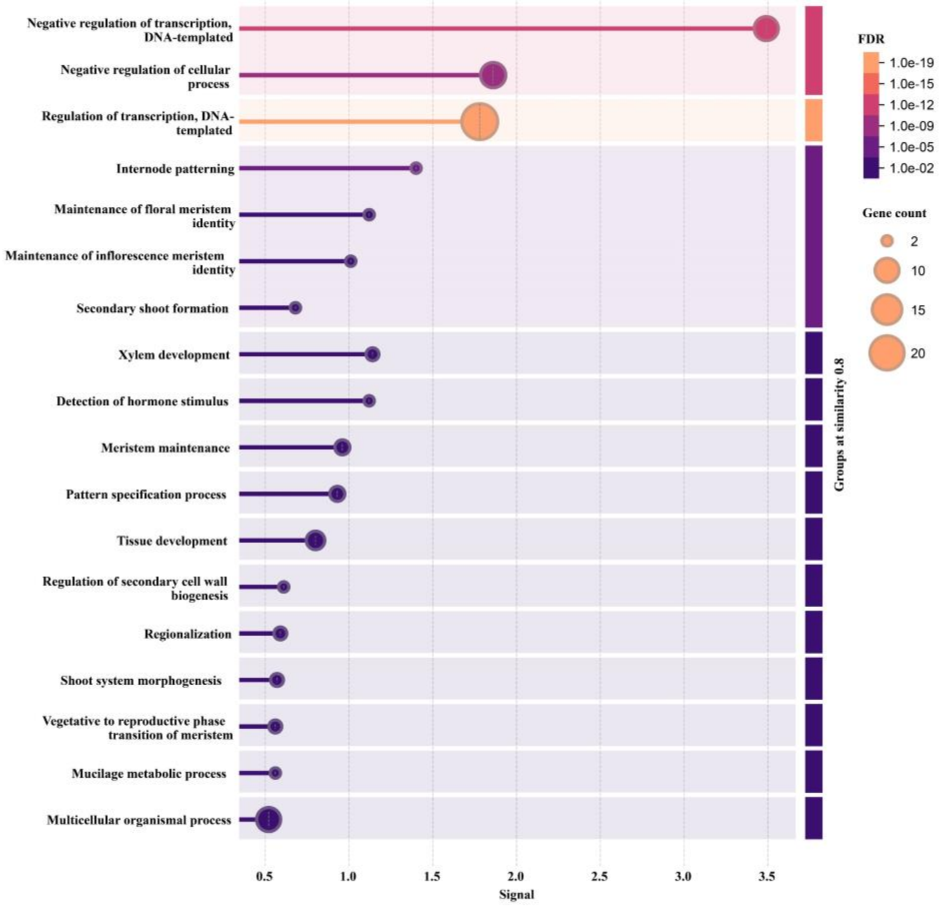

Figure 4 | Functional prediction of MaTALE genes by GO enrichment. Enriched terms are categorized and shown according to biological process, molecular function, and cellular component.

Table 1 | Genomic information of *MaTALE* genes in banana.

Locus	Name	Chr. No	Subfamily	Subcellular
Macma4_01_g02090	*MaTALE1*	1	BELL	Nucleus
Macma4_01_g17530	*MaTALE2*	1	BELL	Nucleus
Macma4_01_g21010	*MaTALE3*	1	KNOX	Nucleus
Macma4_01_g22010	*MaTALE4*	1	BELL	Nucleus
Macma4_02_g02590	*MaTALE5*	2	KNOX	Nucleus
Macma4_02_g07790	*MaTALE6*	2	BELL	Nucleus
Macma4_02_g16610	*MaTALE7*	2	KNOX	Nucleus
Macma4_02_g22910	*MaTALE8*	2	BELL	Nucleus
Macma4_03_g13540	*MaTALE9*	3	KNOX	Nucleus
Macma4_03_g28940	*MaTALE10*	3	KNOX	Nucleus
Macma4_03_g32070	*MaTALE11*	3	BELL	Nucleus
Macma4_04_g02880	*MaTALE12*	4	BELL	Nucleus
Macma4_04_g03350	*MaTALE13*	4	KNOX	Nucleus
Macma4_04_g04730	*MaTALE14*	4	BELL	Nucleus
Macma4_04_g06800	*MaTALE15*	4	BELL	Nucleus
Macma4_04_g07650	*MaTALE16*	4	BELL	Nucleus
Macma4_04_g14150	*MaTALE17*	4	BELL	Nucleus
Macma4_04_g15170	*MaTALE18*	4	KNOX	Nucleus
Macma4_04_g15230	*MaTALE19*	4	BELL	Nucleus
Macma4_04_g25350	*MaTALE20*	4	KNOX	Nucleus
Macma4_04_g25370	*MaTALE21*	4	BELL	Nucleus
Macma4_04_g28960	*MaTALE22*	4	KNOX	Nucleus
Macma4_04_g37060	*MaTALE23*	4	KNOX	Nucleus
Macma4_04_g41060	*MaTALE24*	4	KNOX	Nucleus
Macma4_05_g01930	*MaTALE25*	5	KNOX	Nucleus
Macma4_05_g03060	*MaTALE26*	5	BELL	Nucleus
Macma4_05_g07130	*MaTALE27*	5	BELL	Nucleus
Macma4_05_g08960	*MaTALE28*	5	BELL	Nucleus
Macma4_06_g06460	*MaTALE29*	6	KNOX	Nucleus
Macma4_06_g08990	*MaTALE30*	6	BELL	Nucleus
Macma4_06_g19850	*MaTALE31*	6	BELL	Nucleus
Macma4_07_g05320	*MaTALE32*	7	KNOX	Nucleus
Macma4_07_g08150	*MaTALE33*	7	KNOX	Nucleus
Macma4_07_g15090	*MaTALE34*	7	BELL	Nucleus
Macma4_07_g30110	*MaTALE35*	7	KNOX	Nucleus
Macma4_07_g30220	*MaTALE36*	7	BELL	Nucleus
Macma4_08_g10240	*MaTALE37*	8	KNOX	Nucleus
Macma4_09_g19870	*MaTALE38*	9	BELL	Nucleus
Macma4_09_g23490	*MaTALE39*	9	BELL	Nucleus
Macma4_10_g00320	*MaTALE40*	10	BELL	Nucleus
Macma4_10_g10820	*MaTALE41*	10	KNOX	Nucleus
Macma4_10_g11460	*MaTALE42*	10	BELL	Nucleus
Macma4_10_g14320	*MaTALE43*	10	BELL	Nucleus
Macma4_10_g26520	*MaTALE44*	10	BELL	Nucleus
Macma4_10_g32340	*MaTALE45*	10	KNOX	Nucleus
Macma4_11_g05020	*MaTALE46*	11	KNOX	Nucleus
Macma4_11_g07780	*MaTALE47*	11	BELL	Nucleus

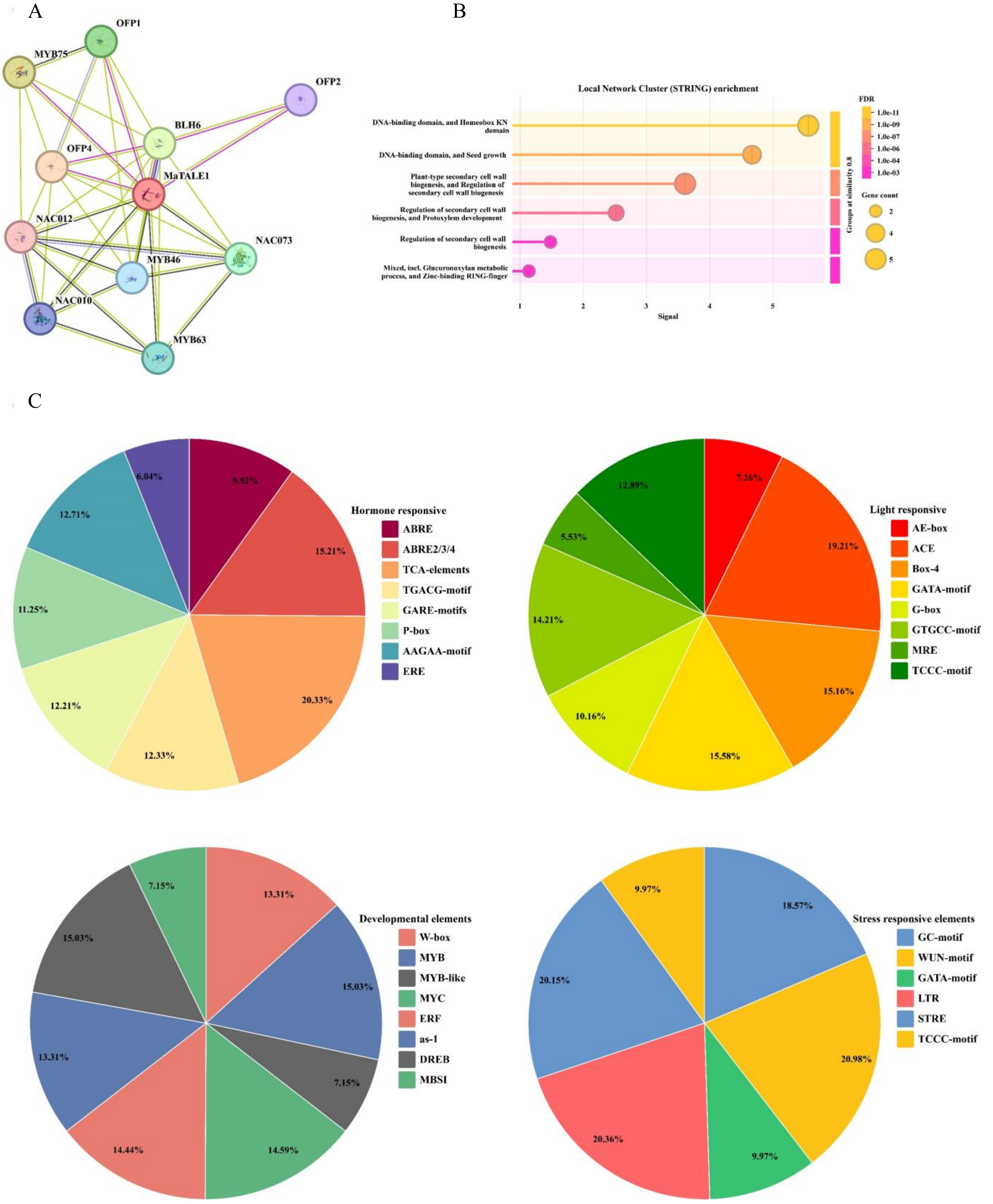

Figure 5 | (A) The predictive functional associates of MaTALE proteins. The MaTALE1 served as a reference in the String online tool for functional predictive analysis. (B) Local cluster analysis of MaTALE proteins together with their interactive partners. (C) MaTALE genes' cis-acting components in the banana genome database. The data depicted hormone-responsive (HR), stress response (stress responsive), and developmental elements (DE).

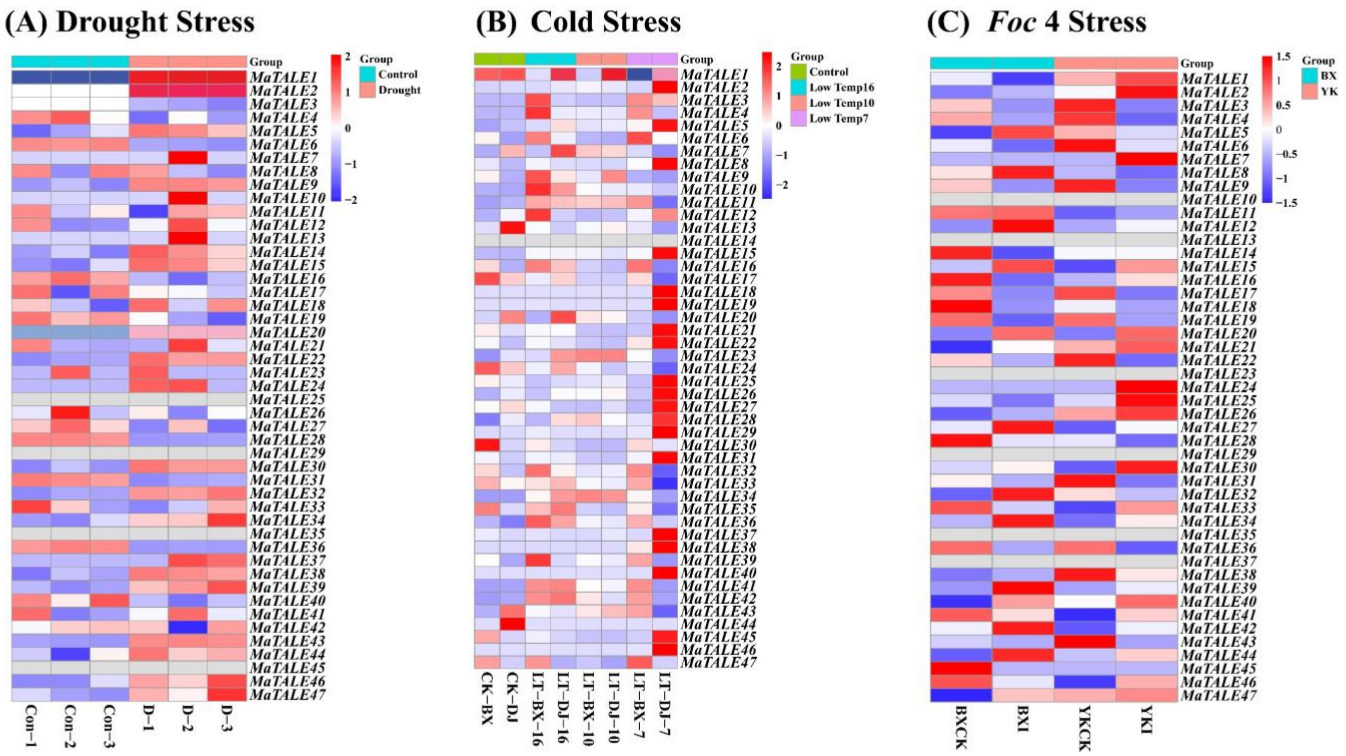

Figure 6 | Heatmap expression analysis of MaTALE genes under various stresses. (A) Analysis of the MaTALE gene expression in response to drought stress. (B) Expression analysis of MaTALE genes in response to low temperature (LT) in banana line BX (Baxijiao) and DJ (Dajiao) at 16, 10, and 7℃, showing log2(fold change) values with the scale limit [+ 2, −2]. The heatmap was drawn with Tbtools. The red color displays high expression, whereas the blue color shows low expression.

Similarly, the expression of *MaTALE4* is upregulated in Con-1-3 and in D-1-3 in a consistent pattern. Several genes, such as the *MaTALE3* gene induced in Con-1-3 but not in D-1-3. The *MaTALE30, MaTALE31, MaTALE32,* and *MaTALE43* showed dominant expression in the Con-1-3 and in the D-1-3 (Figure 6A). Cold stress is an economically significant abiotic stress in the banana production industry. To understand the role of *MaTALE* genes in regulating banana response to cold stress, we analyzed the expression of these genes in cold-sensitive (BX) and cold-tolerant (DJ) cultivars under 16℃, 10℃, and 7℃. Similar to that of drought stress, the *MaTALE1* genes showed high expression in control (CK) samples of the BX and DJ cultivars. At 16℃, no significant difference was observed between BX and DJ; however, the expression of *MaTALE1* sharply reduced in BX compared to DJ at 10℃ and 7℃ (Figure 6B). The *MaTALE2/3* displayed downregulated expression across the samples. A large number of genes were recorded for insignificant expression difference in BX and DJ at all the given temperatures. The *MaTALE23 was* induced significantly at 10℃ and 7℃ in DJ than in BX, whereas the *MaTALE34* displayed consistent high expression (Figure 6B).

The expression of the *MaALE* gene family was further examined in the BX and YK lines subjected to the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* race 4 (Foc *4*) causes *Fusarium* wilt disease (Figure 6C). The widely cultivated BX variety has good agronomic traits but is susceptible to *Foc* 4, whereas the YK cultivar exhibits strong resistance. The expression of the *MaTALE1* gene was strongly induced in the resistant YK line inoculated with *Foc* 4 (YKI) but suppressed in the inoculated susceptible BX line (BXI) (Figure 6C). The *MaTALE3/4/6/14/19/20/21/28* displayed high expression across the samples (BXCK, BXI, YKCK, and YKI). In contrast, *MaTALE9, MaTALE32*, and *MaTALE42* were specifically upregulated in both YKCK and YKI samples compared to BXI. Separately, a distinct cluster of *MaTALE* genes showed no significant differential expression across any samples (Figure 6C).

2.9 | Expression analysis under ABA treatment and functional characterization of *MaTALE1*

Since *MaTALE* gene expression varied under drought, cold, and *Foc* 4 stress, we selected four candidate genes to analyze their response to ABA using qRT-PCR. Among these, *MaTALE1* expression was sharply induced by 0.5 mM ABA treatment, showing increased levels at 6, 12, and 16 hours. Expression peaked at 16 hours with a 10-fold increase relative to the control (0 h) (Figure 7A). Similarly, the expression of *MaTALE14* and *MaTALE20* was induced at all measured time points compared to the control (0 h). In contrast, *MaTALE36* expression declined sharply at 6, 12, and 16 hours, reaching a minimum of 0.5-fold its initial level by 16 hours (Figure 7A). To experimentally verify our in-silico predictions for MaTALE protein localization, we conducted a transient expression assay. Consistent with its predicted nuclear localization, MaTALE1 was confirmed to reside in the nucleus (Figure 7B).

3 | DISCUSSION

TALE transcription factors control essential processes, including plant growth, meristem maintenance, and organ morphogenesis. These proteins are characterized by the presence of KNOX, ELK,

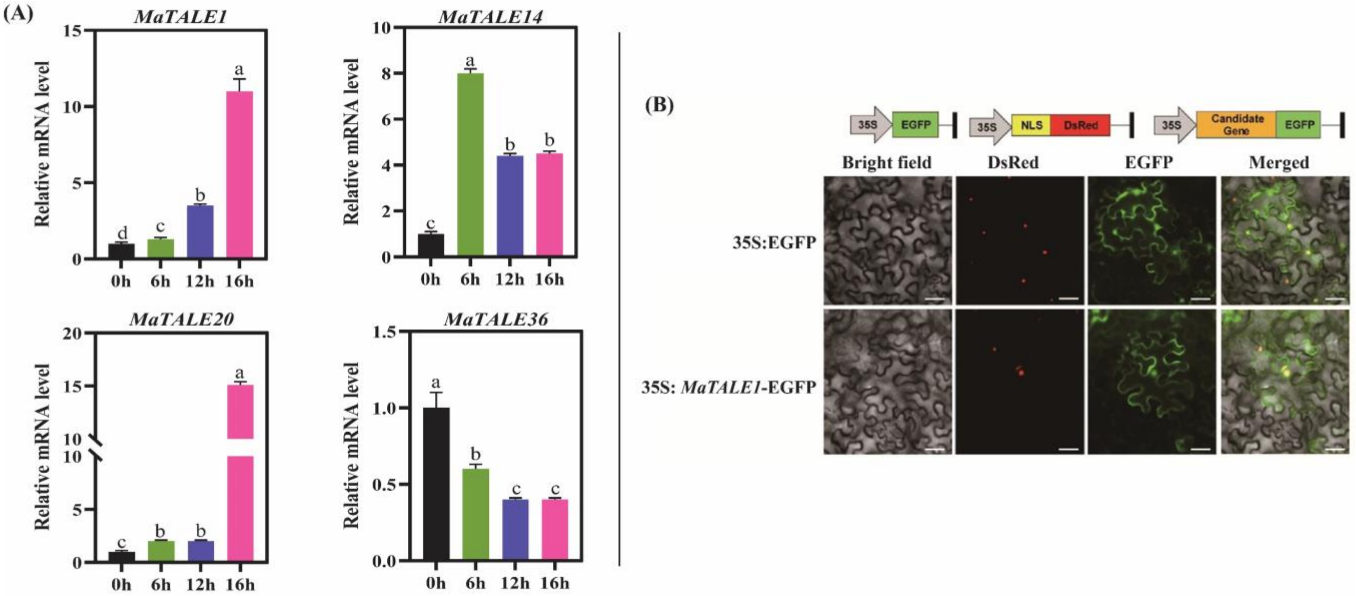

Figure 7 | (A) Analysis of MaTALE gene expression in response to ABA. (B) Subcellular localization of MaTALE1 was determined by transiently expressing a 35S: MaTALE1-EGFP fusion construct in Nicotiana benthamiana. The data illustrate MaTALE gene expression at CK (0h), as well as at 6 h, 12 h, and 16 h intervals. The histogram bars represent expression levels, whereas the error bars depict means ± SEM.

and Homeobox domains. Functionally, the Homeobox domain is responsible for DNA binding, whereas the ELK domain, featuring an amphipathic helix, enables interactions with other proteins [8,24]. The regulatory functions of the *TALE* gene family in banana remain poorly characterized.

3.1 | MaTALE genes are widely distributed in the banana genome

The early 20th century saw a surge in research on TALE [4,25], a eukaryotic gene family that is widely distributed. This study presents a comprehensive bioinformatic analysis of 47 MaTALE genes identified in the banana genome. These genes are distributed across all eleven chromosomes (Figure 1) and are predicted to encode nuclear-localized proteins (Table 1). To investigate their evolutionary relationships, we constructed an unrooted phylogenetic tree of the encoded proteins. Unlike previous studies [24,26], we classified these proteins into seven separate categories (Figure 2A). While the BELL genes are further divided into five categories, the KNOX genes have been separated into two groups (Figure 2A). Our classification results are also supported by the ML-phylogenetic tree built with the best model and the annotations of the SMART database [27,28]. Each subfamily is characterized by distinct domain architectures. Gene structure analysis further revealed that every *MaTALE* gene contains at least one intron, and members within the same subfamily exhibit a high degree of genomic conservation. Protein motif analysis and annotation revealed that members of the same class have identical protein motifs (Figure 3), which is in line with previous studies on the poplar *TALE* family [29].

Gene transcription is controlled by the *cis*-elements selective binding of transcription factors at the gene promoter region. Numerous *cis*-elements linked to hormonal responses and abiotic stress were identified in the *MaTALE* promoter sequence, including methyl jasmonate, abscisic acid, and gibberellin (Figure 5C), corroborating other studies [5,30]. The findings indicated a conservative component within the *MaTALE* gene promoter. ABRE has been connected in studies to high salt stress, drought, and ABA induction in plants [31]. Additionally, ARE, MBS, and LTR are all components that are associated with stress. Involvement of the *MaTALE* gene family in banana abiotic stress was found to be significant. Predictions of gene functions and analysis of protein-protein networks point to the MaTALE family as a critical regulator of SCW. Consistent with the earlier research, MaTALE1 interacts with several proteins found in floral organs, as shown by protein-protein network analysis [32].

3.2 | MaTALE genes are crucial stress regulators

Several plant species have shown that members of the TALE gene family can react to environmental stresses [4]. The transcriptome analysis and cloning of eleven KNOX genes in *Camellia japonica* L. showed that these genes significantly affect drought and salinity tolerance [33]. Hormonal treatments and stressful environmental factors, such as drought, ABA, MeJA, and SA, can affect the 19 KNOX genes in *Dendrobium huoshanense*, according to studies [34]. The GA pathway gene *PagGA20ox1* may be influenced by Poplar's *PagKNat2/6b*, which in turn mediates drought responses [35]. The promoters of the TALE gene family in soybean contain stress-responsive *cis*-elements, suggesting that salt and drought may cause alterations in the expression of *GmTALE* [36]. There is evidence that certain TALE genes have a role in stress adaptation in cotton, as they are upregulated in response to abiotic stimuli. In *Arabidopsis*, the homologous gene *KNAT7* is linked to the *ZmTALE1*, *ZmTALE37*, and *ZmTALE38* genes in maize [37]. Reportedly, this gene has the ability to control lignin synthesis in *Arabidopsis*, which in turn influences the development of vascular tissue [11]. According to research, plants' ability to withstand drought is greatly influenced by the process of lignin production [38]. In addition, the *ZmTALE24* gene, which is thought to be similar to *Arabidopsis's KNAT1*, controls gibberellin activity and, by extension, vascular tissue development through its interactions with DELLA, which are negative components of the gibberellin signaling pathway [3]. The leaves of *Toona sinensis* exhibited increased salt sensitivity and enhanced tolerance to osmotic stress following the transient expression of the *TsBLH4* and *TsKNOX6* genes [39]. The repression of the *GhKNOX4-AGh* and *GhKNOX22-D* genes in cotton seedlings exposed to salt and drought had a notable impact on their growth and development [16]. By controlling stomata opening and oxidative stressors, *GhKNOX4-A* and *GhKNOX22-D* might play a role in drought response [16]. Concerning the reported studies, we observed varied expression trends of *MaTALE* genes following drought stress (Figure 6A and 7). For instance, the *MaTALE1 was* sharply induced following drought stress. The expression of *MaTALE1 was* sharply enhanced following ABA treatment (Figure 7A). The enrichment of a high number of ABA-responsive *cis*-elements in the upstream region of *MaTALE1* genes and high expression under ABA could potentially fine-tune the banana response to drought and *Foc* 4 stress. A key defense response regulated by ABA is stomatal closure, which creates a physical barrier to hinder pathogen entry [40]. Additionally, ABA enhances resistance against root invasive pathogens [41]. In wheat subjected to *Puccinia striiformis* f. sp. *tritici* (Pst) and *Blumeria graminis* f. sp. *tritici* (Bgt), TALE gene expression was time-dependent. A group of genes, including *TaTALE4-5B, TaTALE17-5A, TaTALE17-4B, TaTALE9-4A*, and *TaTALE21-7B* were upregulated specifically at 24 hours post-inoculation. In contrast, *TaTALE17-4D* and *TaTALE21-7D* exhibited a more prolonged response, remaining elevated at 48 hours [42]. Enhanced cold tolerance in *CmBLH2*-overexpressing lines was associated with increased antioxidant enzyme activity, leading to reduced ROS accumulation and oxidative damage. The high expression of *MaTALE1* under ABA (Figure 7A) and *Foc* 4 (Figure 6C) stress provides clues for its possible role against the devastating pathogen. This contrasted with RNAi lines, which displayed a diminished reactive oxygen species scavenging capacity [43]. In our research, while the expression analysis provides a controlled and direct functional readout for different stresses, it may not fully replicate the complex regulatory networks and physiological context of a whole plant. Through transgenic approaches (overexpression/knockdown) or, more relevantly, through CRISPR-Cas9 genome editing in banana, which represents a promising strategy for both functional characterization and potential direct application in crop improvement.

4 | CONCLUSION

The banana genome contains 47 *MaTALE* genes, which have been divided into 7 groups based on their evolutionary relationships. They are important regulators of the trade-off between growth and immunity because they contain stress-responsive *cis*-acting elements and hormones. The microarray analysis suggested that *MaTALE* genes are crucial in regulating banana response to drought, cold, and *Foc* 4 stress. High expression of *MaTALE1* under drought, cold, and *Foc* stress and ABA hormone was instrumental. Considering *MaTALE1* significant role, it may be an effective tool for functional research to produce banana lines that are resistant to multiple abiotic/biotic stresses.

5 | MATERIALS AND METHODS

5.1 | Identification and isolation of *MaTALE* genes from the Banana genome

We used the BLAST algorithm in Ensembl Plants to identify TALE family sequences by searching the banana genomes against *Arabidopsis* TALE proteins, applying an e-value cutoff of 1e-10. Putative TALE sequences were further characterized by analyzing conserved domains with the CDD and Pfam databases and by predicting subcellular localization using the Plant-mPLoc server (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/#).

5.2 | Physical location and synteny of *MaTALE* genes

Using TBtools [44], we determined the chromosomal distribution of MaTALE genes by extracting their gff3 annotations from the banana genome database and mapping them to chromosomes.

5.3 | Phylogenetic analysis of *MaTALE* proteins

A phylogenetic tree was built from TALE amino acid sequences (banana, cucumber, wax gourd, *Arabidopsis*). Initial sequence alignment used Clustal-Omega [45]. Phylogenetic relationships were then estimated using Maximum Likelihood (ML) analysis with 1000 bootstrap replicates on the IQ-TREE web server, and the final tree was visualized in iTOL v5.

5.4 | Conserved motif analysis of MaTALE proteins

We identified conserved motifs in MaTALE proteins using MEME (http://meme-suite.org/index.html) with the following parameters: a zero-or-one occurrence per sequence (ZOPS) model, discovery of up to 10 motifs, and motif widths ranging from 6 to 100 residues. The resulting motifs were visualized using TBtools [44].

5.5 | Interactive protein partners

Protein-protein interaction (PPI) networks of MaTALE proteins were predicted using STRING (v11.5). *Arabidopsis* was used as the reference, with parameters set to a minimum confidence score of 0.7, a maximum of 5 first-shell interactors, and 10 second-shell interactors. Cytoscape (v3.8.2) was employed for network visualization.

5.6 | Gene Ontology analysis of *MaTALE* genes

We analyzed MaTALE protein sequences using Blast2GO with default settings for Gene Ontology (GO) annotation. Consistent with [7], we categorized results into the three primary GO domains: cellular component, molecular function, and biological process.

5.7 | Promoter analysis of *MaTALE* genes

Using PlantCARE, we identified all predicted cis-regulatory elements within the 1.5-kb promoter regions upstream of the start codon (ATG) for each MaTALE gene. The identified elements were categorized by function, with a focus on growth, hormone response, and stress-related motifs.

5.8 | Prediction of 3D protein structures

The full protein sequences of MaTALE proteins were used for homology modeling via Phyre2 [46]. The resulting models underwent energy minimization on the YASARA Energy Minimization server, followed by the exclusion of explicit solvent in Accelrys Discovery Studio v4.1. Final structures were rendered using PyMOL [47].

5.9 | RNA extraction and expression profiling

Total RNA was isolated from banana leaf tissues with the RNAprep

Pure Plant Kit (Tiangen, Beijing, China). This RNA was then reverse-transcribed into cDNA using the HiScript II Q RT SuperMix (Vazyme, Nanjing, China). Gene expression was determined by quantitative PCR (qPCR) using SYBR Green PCR Master Mix (TransGen, Beijing, China). Relative expression levels of target genes were calculated using the $2^{\wedge-\Delta\Delta CT}$ method, normalized to the reference gene *Actin*. The primers used are listed in Supplementary Table S1.

5.10 | Subcellular localization of MaTALE1

The MaTALE1 coding sequence (CDS) was cloned into two vectors for functional analysis: the stable expression vector pCAMBIA1302, which carries a β-glucuronidase (GUS) reporter, and the transient expression vector pGreen35S::GFP for subcellular localization via green fluorescent protein (GFP) in *Nicotiana benthamiana*. The recombinant pGreen35S::GFP plasmids were transfected into *Agrobacterium tumefaciens* strain GV3301 and then used to transform *N. benthamiana* leaves. These transformed tissues were cultured in the dark at 28°C for 48 hours on 1/2MS plates. GFP fluorescence was subsequently imaged using a ZEISS Axio Image D2 fluorescence microscope.

5.11 | Statistical analysis

Statistical analyses were performed in SPSS. Data are expressed as mean ±SD. Significant differences ($p < 0.05$) were determined by one-way ANOVA with Tukey's post hoc test, denoted by lowercase letters above bars.

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AUTHOR CONTRIBUTIONS

Conceptualization: R.S., H.S., and H.C. | Methodology: H.S., H.C., and S.G. | Investigation: S.G., and H.S. | Visualization: H.C and S.G. | Funding acquisition: R.S., and S.G. | Project administration: R.S., and S.G. | Supervision: R.S. | Writing – original draft: R.S., H.C., and S.G. | Writing – review and editing: H.S. The authors confirm their contributions to the paper as follows.

DATA AVAILABILITY STATEMENT

All the data generated or analyzed during this study are included in this published article and its supplementary information file at https://trendsacademics.com/tpb/index.php/ojs/article/view/9/9

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the author(s) used ChatGPT 3.5 in order to improve readability and language. After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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