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Revealing the functional role of sucrose nonfermenting-1-related protein kinase gene *SnRK2.10* in regulating starch degradation in banana (*Musa acuminata*)

Yunen Tu[1,2#], Zhao Zhu[1,2#], Peiguang Sun[2#], Min Zhang[3], Muhammad Moaaz Ali[2], Yueruxin Jin[2], Mulan Wu[4], Liying Wang[5], Lijuan Zhou[1], Juhua Liu[2], Zhiqiang Jin[2], Yuan Wang[6*] and Hongxia Miao[2*]

[1] *National State Key Laboratory of Tropical Crop Breeding, School of Tropical Agriculture and Forestry, Hainan University, Haikou 571100, China*
[2] *Institute of Tropical Bioscience and Biotechnology, Sanya Research Institute, Chinese Academy of Tropical Agricultural Sciences, 4 Xueyuan Road, Haikou 571101, China*
[3] *Institute of Pomology Science, Guizhou Academy of Agricultural Sciences, Jinzhu Street, Guiyang 550006, China*
[4] *College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China*
[5] *Agriculture College of Yanbian University, Yanbian University, Yanbian 133002, China*
[6] *Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*
[#] Authors contributed equally: Yunen Tu, Zhao Zhu, Peiguang Sun
[*] Correspondence: yuan.wang@genetics.ac.cn (Wang Y); miaohongxia@itbb.org.cn (Miao H)

Abstract

Starch degradation is a critical process influencing fruit quality and shelf life during postharvest ripening of banana. Sucrose nonfermenting-1-related protein kinase 2 (SnRK2) is a plant-specific Ser/Thr protein kinase family primarily involved in abscisic acid (ABA) signaling; however, its role in regulating fruit starch degradation remains largely unclear. In this study, 14 *MaSnRK2* genes were identified from the *Musa acuminata* genome and classified into three phylogenetic groups, all containing a conserved S_TKc catalytic domain. Evolutionary analysis indicated that the *MaSnRK2* family has undergone strong purifying selection, with six tandem duplication events contributing to its expansion. Promoter analysis revealed abundant *cis*-acting elements associated with development, hormone signaling, and stress responses. Among the identified members, *MaSnRK2.10* exhibited distinct functional differentiation and was markedly upregulated during fruit ripening, suggesting a specialized role in starch metabolism. Subcellular localization analysis showed that the MaSnRK2.10 protein is predominantly localized to the nucleus and cell membrane. Functional assays demonstrated that transient silencing of *MaSnRK2.10* increased total starch content, whereas transient overexpression reduced starch accumulation in banana fruit, confirming its involvement in the regulation of starch degradation. Furthermore, coexpression analysis and molecular docking predict that ERF, NAC1, NAC2, and NAC3 may bind to the *MaSnRK2.10* promoter, suggesting a complex transcriptional regulatory network. Collectively, this study systematically characterizes the genome-wide features of the *MaSnRK2* family and identifies *MaSnRK2.10* as a key regulator of starch degradation, providing valuable molecular insights and targets for improving banana fruit quality and shelf life.

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Introduction

Banana (*Musa* spp.) is a monocotyledonous herbaceous crop that originated in Southeast Asia and is currently cultivated in more than 135 countries across tropical and subtropical regions worldwide[1–3]. As one of the most important staple foods, bananas meet the dietary needs of over 400 million people, particularly in Africa[4]. Beyond its role as a fresh fruit, banana is widely consumed either raw or processed and serves as a functional ingredient in diverse food products[5].

Starch metabolism is a critical determinant of fruit quality. In starchy fruits such as banana[6–8], kiwifruit[9], and mango[10,11], starch degradation directly influences sweetness, texture, waxiness, and postharvest shelf life. In cereal crops, starch breakdown provides a sustained carbon supply during seed germination and early seedling establishment[12,13]. These observations underscore the physiological importance of starch catabolism in both reproductive and postharvest developmental contexts. Starch degradation is a highly coordinated and multistep biochemical process. Numerous enzymes participating in this pathway have been characterized, including glucan, water dikinase (GWD)[14,15], phosphoglucan water dikinase (PWD)[16], starch excess 4 (SEX4)[17], Like SEX4 isoforms 1 and 2 (LSF1/2)[8], starch phosphorylase (PHO)[6], α-amylases (AMY)[18], and β-amylase (BAM)[19]. Despite the identification of these core components, it remains unclear whether additional regulatory or catalytic proteins contribute to starch degradation in fresh fruit.

Sucrose nonfermenting-1-related protein kinase 2 (SnRK2) is a plant-specific serine/threonine protein kinase characterized by an N-terminal catalytic kinase domain and a C-terminal regulatory domain[20]. SnRK2 proteins are central regulators of plant growth, development, and abiotic stress responses, primarily through the abscisic acid (ABA) signaling pathway[20]. Based on phylogenetic classification, SnRK2 members are grouped into three subfamilies (I–III)[21]. Group I and II kinases are typically activated via ABA-independent mechanisms, whereas group III members (SnRK2.2/2.3/2.6) are strongly responsive to ABA and function as core components of ABA signaling[22]. In *Arabidopsis thaliana*, SnRK2.2 phosphorylates transcription factors (TFs) associated with ABI3 and VP1 (RAV), modulates the expression of abscisic acid-insensitive genes *ABI3*, *ABI4*, and *ABI5*, and thereby regulates seed development and dormancy[23]. In tomato, *SnRK2.3* enhances cytoplasmic glucose accumulation, phosphorylates the TF ABRE-binding factor 1.2 (AREB1.2), and regulates the expression of tonoplast sugar transporters TST1/2, ultimately affecting fruit sugar accumulation[24]. During strawberry ripening,

elevated ABA levels suppress *SnRK2.6* expression, relieving phosphorylation-mediated inhibition of the TF bHLH3, promoting the transcription of UDP-glucose: flavonol O-glucosyltransferase (UFGT), and accelerating fruit coloration[25]. In cassava, the MeSnRK2.3–MebHLH68 regulatory module links ABA signaling with starch biosynthesis[26]. Furthermore, several transcription factors—including MYBs, homeobox proteins (HBs), and AREB1—have been reported to regulate SnRK2 expression and participate in developmental and ripening processes[24,27–29]. For example, the R2R3-MYB transcription factor PsFLP positively regulates *SnRK2.3* expression to promote symmetric division of guard mother cells during stomatal development in *Pisum sativum*[27]. In apple, MdSnRK2.4 and MdSnRK2.9 phosphorylate MdHB1 and MdHB2, enhancing their protein stability and stimulating ethylene biosynthesis associated with fruit ripening and osmoregulation[28]. In banana, the MaMADS1-MaNAC083 transcriptional regulatory cascade regulates ethylene biosynthesis during banana fruit ripening[30]. The interaction between MuMADS1 and MaOFP1 affects banana ripening[31]. In both apple and tomato, the SnRK2.3–AREB1–TST1/2 cascade mediates sugar accumulation across the tonoplast[24]. Nevertheless, whether MYBs, HBs, AREB1, or other interacting factors regulate banana *SnRK2* genes and thereby influence fruit starch degradation remains to be elucidated.

In the present study, we identified 14 distinct *MaSnRK2* members in the banana A-genome database and systematically characterized their genome-wide features, evolutionary relationships, transcriptome profiles, and spatiotemporal expression patterns. Among them, the subcellular localization of the highly expressed MaSnRK2.10 protein was examined in *Nicotiana benthamiana* leaf cells. The functional significance of *MaSnRK2.10* during fruit ripening was further assessed through transient silencing and transient overexpression assays in banana fruit. Additionally, molecular docking analysis was employed to predict potential transcription factor partners of *MaSnRK2.10*. Collectively, these findings provide a theoretical framework for elucidating the role of SnRK2 genes in fruit starch degradation and offer valuable candidate genes for improving postharvest fruit quality and developing storage-tolerant banana cultivars through molecular breeding.

Materials and methods

Plant materials and treatments

The banana cultivar 'Cavendish' (*Musa acuminata*, AAA genotype) was cultivated in the Banana Germplasm Nursery (19° N, 110° E) at the Chinese Academy of Tropical Agricultural Sciences, Wenchang, Hainan Province, China. For spatial expression analysis, roots, leaves, and fruits were collected at 80 d after the emergence of the inflorescence from the pseudostem (DAF; days after flowering). For temporal expression analysis during fruit development and ripening, pulp samples were harvested at 0, 20, and 80 DAF, as well as at 0, 8, and 14 d postharvest (DPH). Each sampling point consisted of three biological replicates.

Genome-wide identification of *SnRK2* genes

The protein sequences of *Arabidopsis thaliana* AtSnRK2.1–AtSnRK2.10 (TAIR10) were used as query sequences to identify banana SnRK2 homologs via BLASTp (E-value ≤ 1×10^{-10}) against the banana A genome (DH Pahang, v2)[32]. Candidate proteins were further validated for conserved kinase domains using SMART and the NCBI Conserved Domain Database (CDD). After removing redundant sequences, 14 non-redundant *MaSnRK2* genes were identified and designated *MaSnRK2.1–MaSnRK2.14* according to their chromosomal positions. Physicochemical properties of the encoded proteins were predicted using ExPASy ProtParam[33]. Subcellular localization was predicted using Cell-PLoc 2.0 and WoLF PSORT[34].

Gene structure, conserved motifs, and phylogenetic analysis

Conserved motifs of SnRK2 proteins were identified using MEME Suite v5.5.0 with the following parameters: maximum motif number = 10 and motif width between 6 and 200 amino acids[35]. Exon–intron structures were visualized using TBtools based on genome annotation (GFF3) files[34]. Phylogenetic analysis was conducted using MEGA-X v10.1.1. A maximum likelihood (ML) tree was constructed under the JTT+G substitution model with 1,000 bootstrap replicates. The phylogenetic tree was further visualized and annotated using iTOL v6.

Selection pressure and collinearity analysis

Coding sequences (CDSs) of MaSnRK2 genes were extracted and compared with genome-wide collinear homologous genes using ParaAT2.0. Synonymous (Ks) and nonsynonymous (Ka) substitution rates, as well as Ka/Ks ratios, were calculated using the R package DoubleTrouble to evaluate selection pressure[36]. Intraspecific collinearity analysis was performed using MCScanX[37]. For interspecific synteny analysis, the banana A genome (DH Pahang v2) was used as a reference and compared with *A. thaliana* (TAIR10) and rice (Oryza sativa, MSUv7.0) genomes. The parameters were set as E-value ≤ 1×10^{-5} and match score ≥ 50[20].

Prediction of promoter *cis*-acting elements

The 2,000 bp upstream sequences from the translation start site of each *MaSnRK2* gene were retrieved as putative promoter regions. *Cis*-acting regulatory elements were predicted using PlantCARE. Plant-specific elements with E-value ≤ 1×10^{-4} were retained for further analysis. Distribution maps were generated using TBtools, and frequency heatmaps were visualized using the R package *ggplot2*[8].

Three-dimensional protein structure prediction

The three-dimensional structures of *MaSnRK2.1–MaSnRK2.14* monomeric proteins were predicted using AlphaFold 3[38]. Structural alignment, annotation of the S-TKc domain and activation loop, and root mean square deviation (RMSD) calculations were conducted using PyMOL v2.5.

Transcriptome sequencing and expression analysis

Total RNA was extracted using the RNAprep Pure Plant Kit (DP441; Tiangen Biochemical, Beijing, China), treated with DNase I, and assessed for integrity (RIN ≥ 7.5) using an Agilent 2100 Bioanalyzer. Libraries were prepared using the NEBNext Ultra RNA Library Prep Kit and sequenced on the Illumina NovaSeq 6000 platform (PE150). Each sample included three biological replicates and two technical replicates. Raw reads were quality-checked using FastQC v0.12.1. Adapters and low-quality reads (Q < 20) were removed using Trimmomatic v0.39. Clean reads were aligned to the banana A genome using HISAT2, and transcript assembly was performed with StringTie v2.2.1. Gene expression levels were calculated as fragments per

kilobase of exon model per million mapped fragments (FPKM). Differential expression analysis was conducted using DESeq2 with thresholds of $|\log_2 FC| \geq 1$ and FDR < 0.05. Heatmaps of the MaSnRK2 family across tissues and developmental stages were generated using pheatmap, and Z-score normalization was performed in TBtools. The transcriptome datasets have been deposited under accession numbers SRX3938704, SRX3938706, SRX3938707, SRX3938708, SRX3938709, SRX3938715, and SRX3938722 (BioProject: PRJNA432894).

RT-qPCR analysis

Gene expression of *MaSnRK2* members in different tissues and developmental stages was validated by quantitative real-time PCR (RT-qPCR) using SYBR Premix Ex Taq (TaKaRa, Shiga, Japan) on a Stratagene Mx3000P detection system (Stratagene, San Diego, CA, USA). Primer sequences are provided in Supplementary Table S1. *MaActin* (EF672732) and *MaUBQ2* (HQ853254) were used as internal reference genes. Relative expression levels were calculated using the $2^{-\Delta\Delta C_T}$ method[39]. Each reaction was performed with three biological replicates.

Subcellular localization of MaSnRK2.10

The full-length open reading frame (ORF) of *MaSnRK2.10* was cloned into the pART-CAM-EGFP vector to generate a *MaSnRK2.10*-GFP fusion construct. The recombinant plasmid and marker plasmid were transformed into *Agrobacterium tumefaciens* strain LBA4404. Agrobacterium-mediated transient expression was performed in *Nicotiana benthamiana* leaves[6]. After incubation at 25 °C for 48 h, GFP fluorescence was observed using a Nikon confocal laser scanning microscope (CLSM).

Transient overexpression and silencing of *MaSnRK2.10* in banana fruit

The ORF of *MaSnRK2.10* was inserted into the pCAMBIA3300 vector using *XbaI* and *KpnI* restriction enzymes for overexpression analysis. For gene silencing, the N-terminal S-TKc activation domain of MaSnRK2.10 was cloned into the pTRV2 vector using the same restriction sites. Constructs were introduced into *A. tumefaciens* strain GV3101. Banana fruit slices at 80 DAF were immersed in Agrobacterium suspension (OD_{600} = 0.6) and cultured on MS medium at 30 °C for 3 d[40]. I_2–KI staining was subsequently performed, and total starch, amylopectin, and amylose contents were quantified as previously described[40]. All experiments were conducted in triplicate.

Prediction of transcription factors and molecular docking analysis

Potential transcription factors (TFs) binding to the promoter region of *MaSnRK2.10* were predicted using PlantTFDB v5.0. Protein–DNA interaction models between identified TFs and *cis*-regulatory elements in the *MaSnRK2.10* promoter were simulated using AlphaFold 3[38]. Three-dimensional docking models were visualized in PyMOL v2.5.2 to infer potential regulatory mechanisms[41].

Statistical analysis

All statistical analyses were performed using R v4.4.2. For multiple comparisons, significance was evaluated using the least significant difference (LSD) test at $\alpha = 0.05$. Graphical representations were generated using appropriate R packages.

Results

Genome-wide identification and structural characterization of MaSnRK2 family members

All SnRK2 protein sequences were retrieved from the banana A genome database[32]. Fourteen SnRK2 members were identified and designated MaSnRK2.1–MaSnRK2.14 according to their chromosomal positions (Fig. 1a). Phylogenetic analysis classified the 14 MaSnRK2 proteins into three groups (I–III). Group I comprised MaSnRK2.1–MaSnRK2.6; Group II included MaSnRK2.8, MaSnRK2.10, and MaSnRK2.13; and Group III contained MaSnRK2.7, MaSnRK2.9, MaSnRK2.11, MaSnRK2.12, and MaSnRK2.14 (Fig. 1a). Conserved motif analysis revealed that Motifs 1–7, and 10 were shared across all groups, whereas Motifs 8 and 9 were absent in Group I members (Fig. 1b). Gene structure analysis showed that all *MaSnRK2* genes contained nine coding sequences (CDSs) interrupted by eight introns, displaying highly conserved exon–intron organization (Fig. 1c). Domain analysis confirmed that each protein harbors a conserved serine/threonine protein kinase catalytic domain (S_TKc, cd14662) with comparable amino acid lengths (Fig. 1d). Three-dimensional structural prediction demonstrated that the S_TKc domain forms canonical secondary structural elements, including α-helices and β-sheets (Fig. 1e), which are essential for maintaining protein structural stability.

Physicochemical properties of the MaSnRK2 proteins are summarized in Supplementary Table S2. Amino acid lengths ranged from 328 aa (MaSnRK2.12) to 371 aa (MaSnRK2.9). Molecular weights varied between 37.78 kDa (MaSnRK2.12) and 42.61 kDa (MaSnRK2.9), and theoretical isoelectric points ranged from 4.77 (MaSnRK2.4) to 8.07 (MaSnRK2.12). All proteins were predicted to be hydrophilic (GRAVY < 0). Subcellular localization prediction indicated nuclear localization for all members (Supplementary Table S2), suggesting their potential involvement in nuclear regulatory processes.

Evolutionary analysis of the *SnRK2* gene family

Phylogenetic analysis incorporating SnRK2 proteins from banana, *Arabidopsis thaliana*, and rice (*Oryza sativa*) revealed three major clades (A–C), with protein names and accession numbers listed in Supplementary Table S3. Notably, *MaSnRK2.3* clustered with rice *OsSnRK3* in Group C. The majority of SnRK2 proteins from all three species were distributed within Groups A and B (Fig. 2a). Ka/Ks ratio analysis demonstrated that all SnRK2 orthologous gene pairs across the three species exhibited Ka/Ks < 1 (Fig. 2b), indicating strong purifying selection during evolutionary divergence. The conserved clustering patterns of Groups A and B further suggest functional conservation and shared evolutionary origin. Chromosomal mapping showed that *MaSnRK2* genes are unevenly distributed across seven chromosomes (Fig. 2c). Specifically, *MaSnRK2.2*, *MaSnRK2.4*, and *MaSnRK2.1* are located on chromosomes 1, 2, and 3, respectively; *MaSnRK2.5* and *MaSnRK2.6* are clustered on chromosome 4; *MaSnRK2.3* and *MaSnRK2.10* reside on chromosome 5; and four genes (*MaSnRK2.7*, *MaSnRK2.12*, *MaSnRK2.13*, and *MaSnRK2.14*) are located on chromosome 6. Additionally, *MaSnRK2.9* is located on chromosome 10, while *MaSnRK2.8* and *MaSnRK2.11* are positioned on chromosome 11 (Fig. 2c). The clustered chromosomal distribution suggests that certain *MaSnRK2* members may have originated through local duplication events.

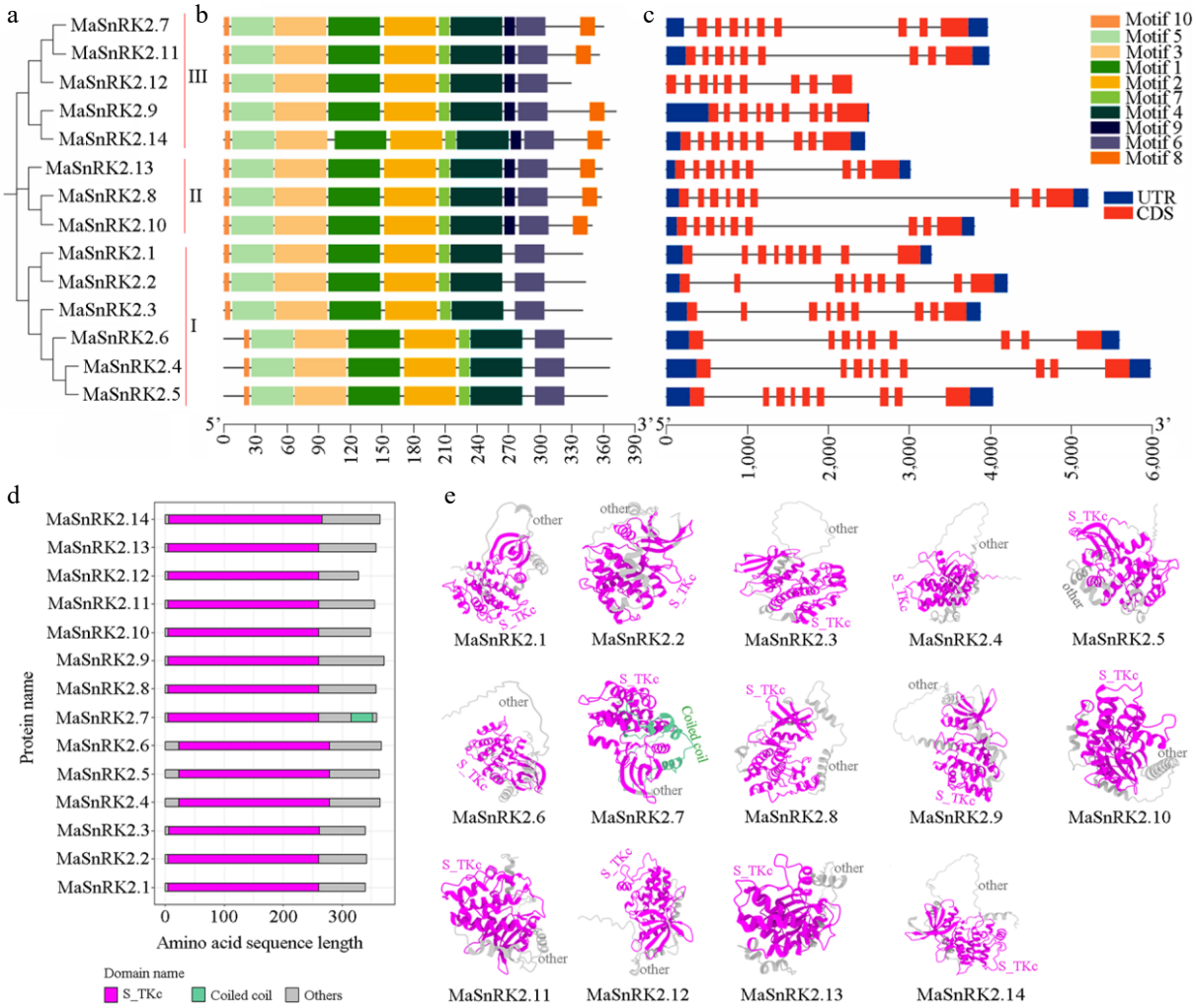

Fig. 1 Bioinformatics analyzes of *MaSnRK2* genes family. (a) Phylogenetic evolution of the MaSnRK2 protein family. (b) Motif analysis of the *MaSnRK2* gene family. (c) Gene structure analysis of the *MaSnRK2* gene family. (d) Protein domain analysis of MaSnRK2 family members. (e) Three-dimensional (3D) structure prediction of MaSnRK2 proteins. Purple represents a conserved serine/threonine protein kinases catalytic domain (S_TKc, cd14662). Green represents a coiled coil domain. Gray represents the other domain.

Cis-acting element analysis of MaSnRK2 promoters

Promoter analysis revealed that all *MaSnRK2* genes contain canonical core promoter elements, including the CAAT-box and TATA-box. Numerous hormone-responsive and stress-related *cis*-elements were also identified (Fig. 3). Abscisic acid-responsive elements (ABREs) were present in the promoters of *MaSnRK2.1*, *MaSnRK2.2*, *MaSnRK2.4*, *MaSnRK2.5*, *MaSnRK2.9*, *MaSnRK2.11*, and *MaSnRK2.12*. Methyl jasmonate-responsive elements (TGACG motif and CGTCA motif) were detected in the majority of promoters. Additional elements associated with auxin (AuxRR-core, TGA-element), salicylic acid (TCA-element, SARE), gibberellin (TATC-box), and defense/stress response (TC-rich repeats) were also identified. Moreover, most *MaSnRK2* promoters contained multiple light-responsive elements (Fig. 3). Collectively, these results indicate that *MaSnRK2* gene expression may be finely regulated by diverse hormonal signals and environmental cues, supporting their potential roles in banana growth, development, and stress adaptation.

Collinearity analysis of the *MaSnRK2* gene family

Genome-wide synteny analysis revealed several collinear gene pairs among *MaSnRK2* members (Fig. 4a). *MaSnRK2.9* exhibited collinearity with both *MaSnRK2.12* and *MaSnRK2.14*. Similarly, *MaSnRK2.10* showed syntenic relationships with *MaSnRK2.8* and *MaSnRK2.13*, while *MaSnRK2.4* formed collinear pairs with *MaSnRK2.5* and *MaSnRK2.6*. These findings suggest that segmental duplication events contributed to the expansion of the *MaSnRK2* gene family and that duplicated genes retained substantial sequence and structural similarity. Cross-species collinearity analysis between banana, *A. thaliana*, and rice detected no conserved syntenic pairs involving SnRK2 genes (Fig. 4b), indicating that the SnRK2 family may have undergone lineage-specific expansion following species divergence or experienced structural genomic rearrangements that disrupted collinearity conservation.

Expression patterns of the *MaSnRK2* gene family

To investigate potential functional roles, expression profiles of *MaSnRK2* genes were analyzed across multiple tissues (roots, stems, leaves, and fruits) and developmental stages (0 and 20 DAF; 0, 8, and 14 DPH). Transcriptome analysis revealed that *MaSnRK2.1*, *MaSnRK2.3*, *MaSnRK2.8*, and *MaSnRK2.10* were highly expressed in fruit compared with other tissues (Fig. 5a). During fruit ripening, *MaSnRK2.1*, *MaSnRK2.8*, and *MaSnRK2.10* were significantly

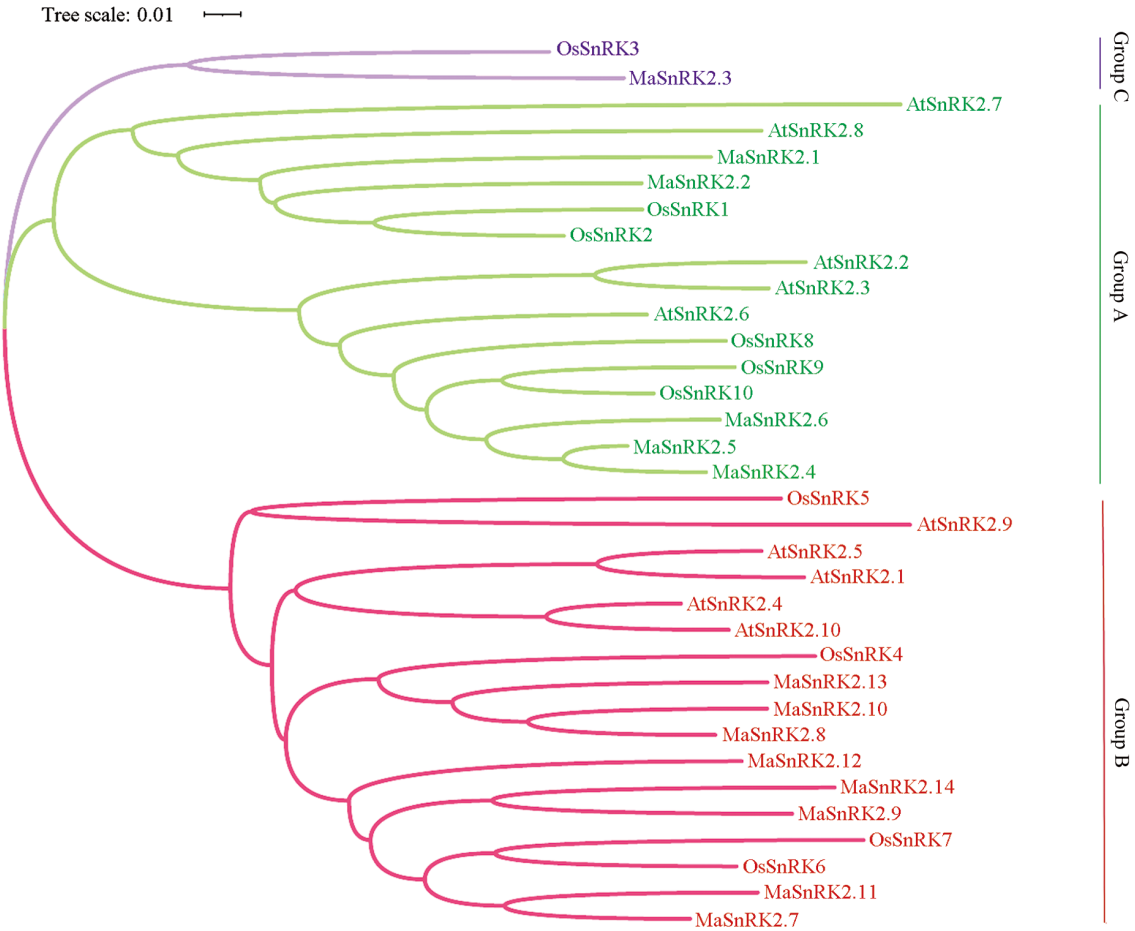

Fig. 2 Phylogenetic, Ka/Ks ratio, and chromosomal distribution of SnRK2 proteins from banana, *A. thaliana*, and rice species. The phylogenetic tree constructed by MEGA X v.10.1.1 software. (a) The SnRK2s were divided into three groups including group I, II, and III based on banana MaSnRK2s and other plants SnRK2 amino acid sequences. The MaSnRK2s, OsSnRK2s, and AtSnRK2s represent SnRK2s from *Musa acuminata*, *Oryza sativa*, and *Arabidopsis thaliana*, respectively. (b) Boxplot with scatter points of Ka/Ks values by species.

upregulated at early postharvest stages (0 and 8 DPH), with *MaSnRK2.10* showing the highest expression level (Fig. 5b, Supplementary Table S4). RT-qPCR validation confirmed that *MaSnRK2.10* expression patterns were consistent with transcriptomic data across tissues and developmental stages (Fig. 5c, d), supporting the reliability of the transcriptome data and suggesting a potential role for *MaSnRK2.10* in early postharvest fruit physiology (Supplementary Tables S5, S6).

Subcellular localization and functional analysis of MaSnRK2.10 in starch metabolism

To determine subcellular localization, the ORF of *MaSnRK2.10* was fused to GFP and transiently expressed in *Nicotiana benthamiana* leaves. Confocal microscopy showed that the *MaSnRK2.10*-GFP fusion protein predominantly localized to the nucleus and plasma membrane, whereas free GFP displayed diffuse cellular distribution (Fig. 6a). Functional analysis was performed using transient silencing and overexpression assays in banana fruit discs. Silencing of *MaSnRK2.10* resulted in darker I_2–KI staining relative to controls (Fig. 6b), indicating reduced starch degradation. Transcript analysis confirmed significant downregulation of *MaSnRK2.10* expression (Fig. 6c). Quantitative measurements showed increases in total starch (17.94%), amylopectin (14.80%), and amylose (3.14%), compared with the empty vector control (Fig. 6d). Conversely, transient overexpression of *MaSnRK2.10* altered I_2–KI staining intensity (Fig. 6e) and significantly increased transcript levels (Fig. 6f). However, carbohydrate contents did not show a uniform decrease; total starch, amylopectin, and amylose exhibited changes of approximately 14%, 8.53%, and 5.39%, respectively (Fig. 6g, Supplementary Table S7). These results indicate that *MaSnRK2.10* modulates starch metabolism rather than acting as a simple modulator of starch degradation.

Prediction of upstream transcriptional regulators of MaSnRK2.10

Potential transcription factors (TFs) interacting with the *MaSnRK2.10* promoter were predicted (Fig. 7a). Expression profiling revealed that *NAC1*, *NAC2*, *NAC3*, and ERF transcription factors were significantly upregulated during fruit ripening (Fig. 7b, Supplementary Table S8). Binding prediction analysis demonstrated high interaction scores between these TFs and the *MaSnRK2.10* promoter (Fig. 7c). Molecular docking simulations further characterized potential protein–DNA interactions. Specifically, Lys156 of NAC1 was predicted to interact with DG4 and DT5 bases within the promoter sequence 'cttgttcctcacgcctcct', forming OP2–N2/H2 interactions with bond lengths of 10.85 Å and 10.04 Å, respectively (Fig. 7e). Arg155 of NAC2 was predicted to bind DT11 in the sequence 'tgcttccttgttccacg' via O3′–NH2 interaction (9.46 Å) (Fig. 7f). DG16 within

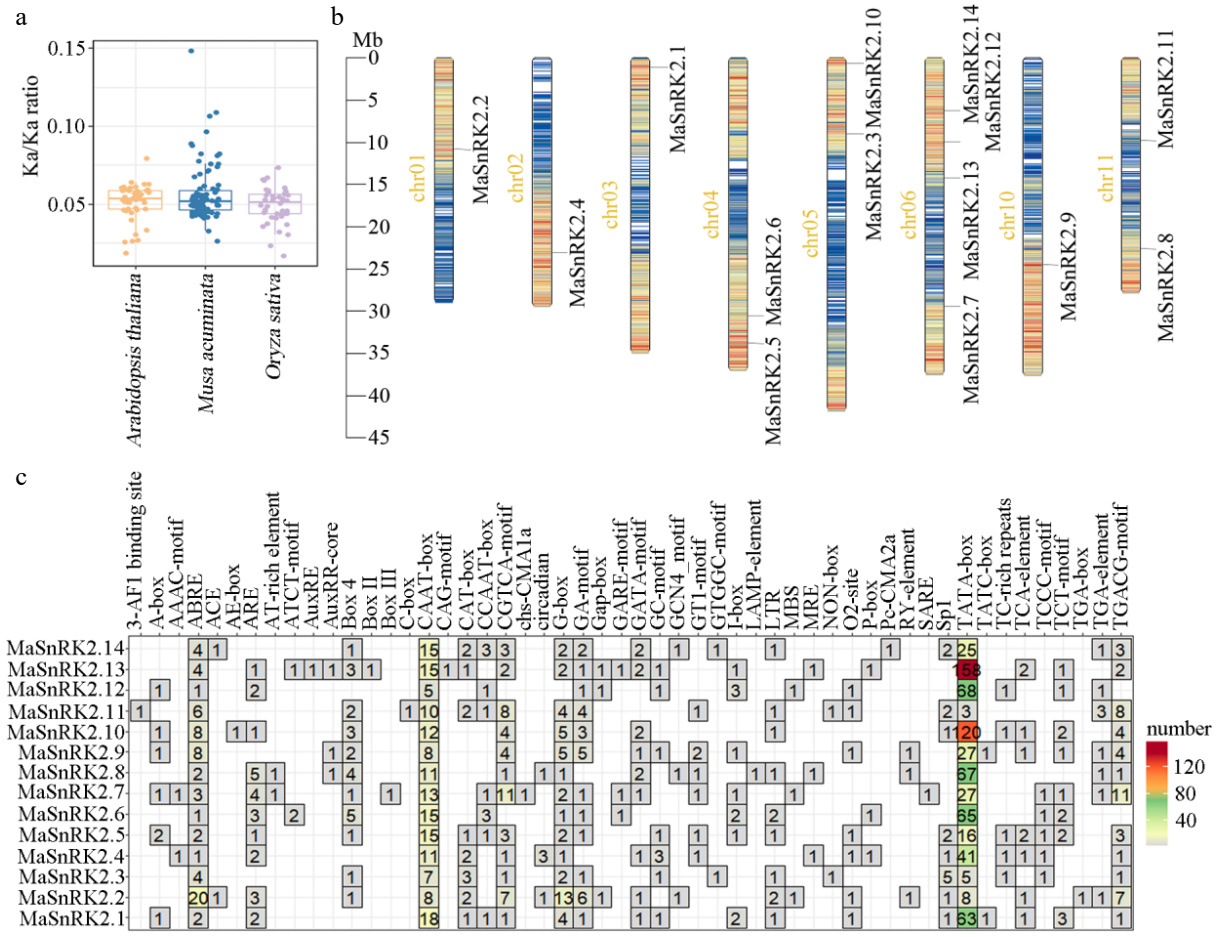

Fig. 3 *Cis*-acting elements analysis of *MaSnRK2* family. (a) Chromosomal localization of 14 *MaSnRK2* gene family members. Different colors represent different gene densities on the chromosome. Specifically, green, blue, and orange represent low, medium, and high gene density, respectively. (b) Promoter *cis*-acting elements of *MaSnRK2* genes; the number represents the number of *cis*-acting elements in each *MaSnRK2* promoter.

'tcctcacgcct' interacted with Arg90 via OP1–NE (7.61 Å) (Fig. 7g). ERF was predicted to bind DC3 and DG4 bases in 'gccgccgttgccg' through OP1–NH2 (Arg75) and O6–NH2 (Arg44) interactions, respectively (11.10 Å) (Fig. 7d, Supplementary Table S9). These findings identify candidate transcription factors potentially involved in regulating *MaSnRK2.10* expression and provide a theoretical framework for future experimental validation of transcriptional regulatory networks governing starch degradation in banana fruit.

Discussion

Starch degradation is a central metabolic process governing postharvest quality formation and shelf life in banana[6], mango[11], kiwifruit[9], peach[42], and apple[18]. Numerous genes and transcription factors (TFs) involved in fruit starch degradation have been identified, including *AMY1*[18], *BAM9b*[43], *PHO1*[6], *LSF1*[8], *MYB10.1*[42], *MYB16L*[44], *MYB44*[45], *ERF17– bHLH149*[18], *BEL1*[46], *GTL1a*[47], *APETALA2* (AP2a)[48], *Dof3*[49], *NAC029*[50], *WRKY32*[51], and *C2H2*[52].

Sucrose nonfermenting-1-related protein kinase 2 (SnRK2) is a plant-specific serine/threonine protein kinase that plays pivotal roles in plant growth, development, abiotic stress responses, and abscisic acid (ABA) signaling[53–55]. However, the genome-wide characteristics and functional roles of *MaSnRK2* genes in banana have not previously been systematically investigated. In the present study, 14 *MaSnRK2* genes (*MaSnRK2.1–MaSnRK2.14*) were identified in the banana A genome (Fig. 1). All encoded proteins contained a conserved S_TKc catalytic domain and displayed highly conserved motif composition and exon–intron organization. This structural conservation is consistent with reports in *Arabidopsis thaliana*[22], rice (*Oryza sativa*)[56], and cassava (*Manihot esculenta*)[26], indicating that SnRK2 proteins are evolutionarily conserved across monocots and dicots. Interestingly, the predicted coiled-coil domain in MaSnRK2.7 is unique among banana SnRK2 family members. Coiled-coil motifs typically serve as platforms for protein dimerization or interaction with binding partners. Although such domains are rarely annotated in SnRK2s, their presence in MaSnRK2.7 suggests a potential regulatory mechanism involving conformational changes or protein complex assembly, possibly linked to developmental or stress signaling. This structural divergence warrants further investigation into its functional significance.

Phylogenetic analysis categorized banana SnRK2 proteins into three subgroups (I–III), consistent with classifications reported in rice, *A. thaliana*, and cassava[22,26,56]. Members of Group I exhibited close evolutionary relationships with rice orthologs OsSnRK1 and OsSnRK2, whereas Groups II and III clustered with OsSnRK4, OsSnRK6, OsSnRK7, and OsSnRK3 (Fig. 2a). Interestingly, this phylogenetic classification (Fig. 1a; Subgroup I–III) did not fully align with the grouping based on gene structure or expression patterns (Fig. 2; Group A–C). Such discrepancies are not uncommon in multigene families, often reflecting functional differentiation following gene duplication events[35]. Thus, presenting both classifications provides

Fig. 4 Intergenomic and intragenomic synteny relationship of the SnRK2 family members among banana (*Musa acuminata*), Arabidopsis (*Arabidopsis thaliana*), and rice (*Oryza sativa*). (a) Intergenomic synteny relationship of the MaSnRK2 members in the banana genome. Green lines indicate intrachromosomal collinearity between MaSnRK2.4 and MaSnRK2.5 or MaSnRK2.6. Red lines indicate intrachromosomal collinearity between MaSnRK2.10 and MaSnRK2.8 or MaSnRK2.13. Purple lines indicated intrachromosomal collinearity between MaSnRK2.9 and MaSnRK2.12 or MaSnRK2.14. (b) Collinear SnRK2 family gene pairs between rice and banana, and between banana and *A. thaliana*.

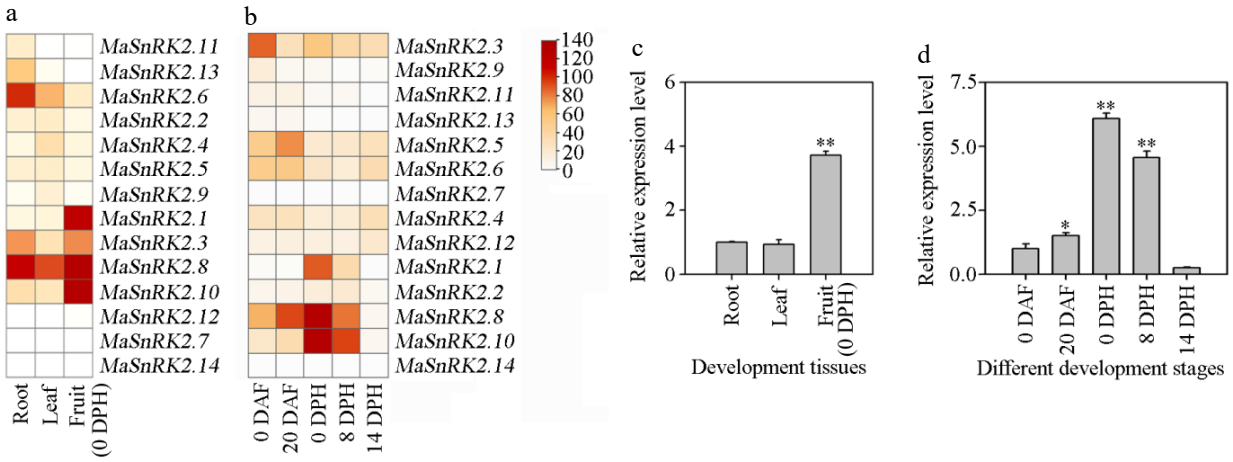

Fig. 5 Expression profiles of *MaSnRK2* family genes in the different organs and different developmental stages by banana transcriptome and qRT-PCR. (a), (b) Expression of *MaSnRK2s* in different organs and during different stages of banana fruit development and ripening. The heat map with clustering was created based on the FPKM value of the *MaSnRK2s*. Differences in gene expression changes are shown in color in the red-yellow scale. (c) Expression of *MaSnRK2.10* in different tissues. (Note: the expression data were detected by real-time fluorescence quantitative PCR [RT-QPCR]). (d) Expression of *MaSnRK2.10* at different stages of fruit development and ripening. Data are presented as means ± standard deviations, $n = 3$ biological replicates.

complementary views of the evolutionary and functional diversification of banana SnRK2s. Ka/Ks ratios below 1.0 for all *MaSnRK2* homologous pairs indicate that the gene family has undergone strong purifying selection during evolution (Fig. 3a), suggesting functional constraint and conservation. Gene duplication represents a primary mechanism driving gene family expansion[57]. The clustered chromosomal distribution and intragenomic collinearity observed among *MaSnRK2.9* and *MaSnRK2.12/2.14*, *MaSnRK2.10*

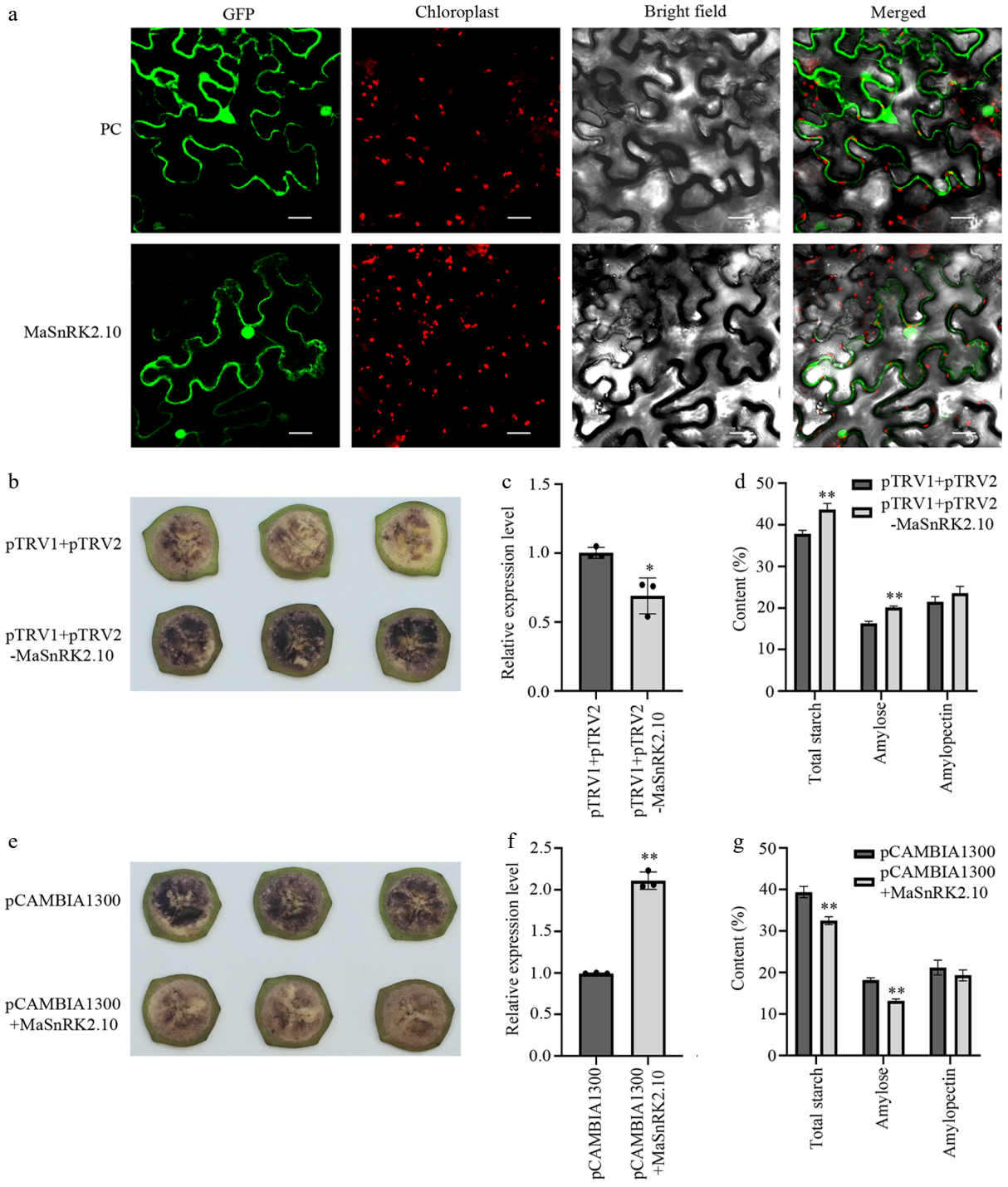

Fig. 6 Co-localization of MaSnRK2.10 in *Nicotiana benthamiana* and its functional analysis in banana (*M. acuminata*) fruit. (a) Co-localization of MaSnRK2.10. The green fluorescent protein (GFP) fluorescence is represented by green, whereas red fluorescence indicates chloroplast red fluorescent proteins. A composite image was created by merging the GFP and RFP fluorescence images, represented under 'merge'. Bar = 10 μm. (b) Iodine (I_2-KI) staining of banana fruit discs showing reduced starch degradation following transient suppression of *MaSnRK2.10*. (c) Relative expression levels of *MaSnRK2.10* in banana fruit discs after gene silencing, as determined by quantitative reverse transcriptase PCR (RT-qPCR). (d) Quantification of total starch, amylose, and amylopectin contents in banana fruit discs following *MaSnRK2.10* suppression. (e) Iodine (I_2-KI) staining of banana fruit discs showing increased starch degradation following transient overexpression of *MaSnRK2.10*. (f) Relative expression levels of *MaSnRK2.10* in banana fruit discs after gene overexpression, as determined by RT-qPCR. (g) Quantification of total starch, amylose, and amylopectin contents in banana fruit discs following *MaSnRK2.10* transient overexpression. Data represent the mean ± standard error (SE) of three independent biological replicates. Statistically significant differences between samples were determined by Analysis of Variance (ANOVA) (* $p < 0.05$; ** $p < 0.01$).

and *MaSnRK2.8/2.13*, and *MaSnRK2.4* and *MaSnRK2.5/2.6* suggest that segmental or tandem duplication events contributed to family expansion (Fig. 4a). The absence of conserved collinear pairs between banana and either *A. thaliana* or rice (Fig. 4b) implies lineage-specific expansion and divergence following species differentiation.

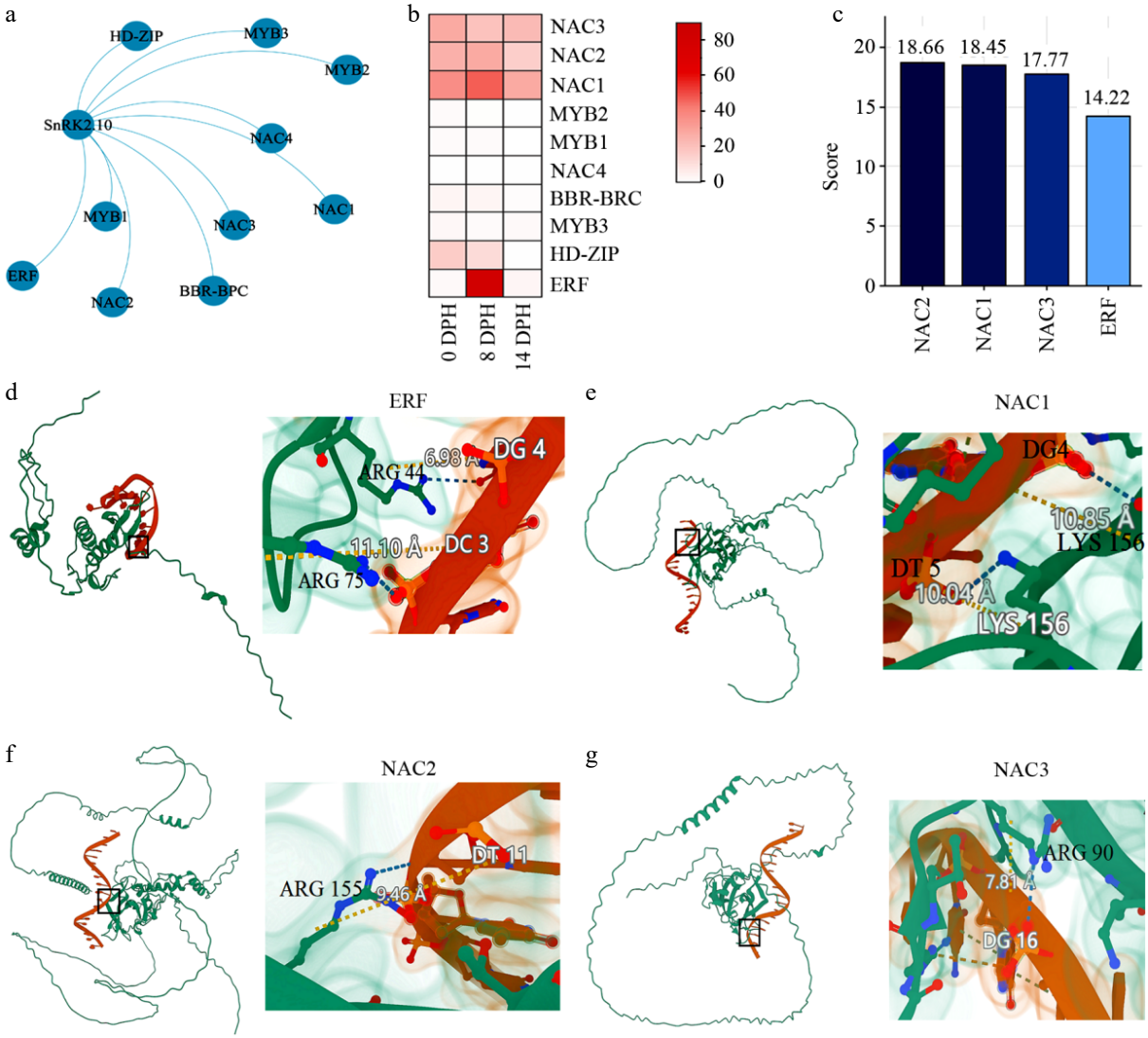

Fig. 7 Prediction and validation of transcriptional regulators targeting the *MaSnRK2.10* promoter in banana (*Musa acuminata*). (a) Predicted transcriptional regulatory network involving transcription factors targeting the *MaSnRK2.10* promoter. (b) Expression profiles of candidate transcription factors (*NAC1*, *NAC2*, *NAC3*, and *ERF*) associated with the *MaSnRK2.10* regulatory network across different ripening stages of banana fruit. (c) Predicted binding scores of NAC1, NAC2, NAC3, and ERF to the *MaSnRK2.10* promoter based on *in-silico* analysis. (d)–(g) Molecular docking simulations showing the predicted binding interactions between the *MaSnRK2.10* promoter and transcription factors (d) ERF, (e) NAC1, (f), NAC2, and (g) NAC3, supporting their potential regulatory roles in starch biosynthesis.

Promoter analysis revealed that *MaSnRK2* genes contain multiple *cis*-acting regulatory elements associated with phytohormone responses, stress signaling, and developmental regulation. In maize, a naturally occurring 20 bp deletion in the *ZmSnRK2.10* promoter reduces transcript abundance and increases shoot Na^+ accumulation under salt stress[58], underscoring the functional importance of promoter architecture. Similarly, in *A. thaliana*, double mutants of *snrk2.2 snrk2.3* exhibit reduced expression of ABA-responsive genes containing ABRE motifs, leading to altered seed germination and root growth[59]. The promoter of *MaSnRK2.10* is enriched with hormone-responsive elements, including ABRE, AuxRE, and GARE motifs, as well as stress-related elements (MBS and LTR) and development-associated elements (O2-site and MRE). Such enrichment suggests that *MaSnRK2.10* may integrate hormonal and developmental signals during fruit ripening. The presence of conserved TATA-box and CAAT-box elements further confirms canonical promoter structure, consistent with *SnRK2* promoters in cassava, pepper, *A. thaliana*, and rice[26,60,61].

The *SnRK2* genes are implicated in starch biosynthesis, seed germination, and fruit development across species. For example, *MeSnRK2.3* is highly expressed in cassava storage roots[26], and pepper *CaSnRK2* genes exhibit strong expression in developing fruit tissues and under abiotic stress[60]. In strawberry, SnRK2.6 expression is ABA-inducible and negatively correlated with ripening progression[25]. In tomato, ABA acts as a core hormone regulating fruit ripening. Through the dual mechanisms of transcription and post-translational mediated by SnRK2s, it collaboratively regulates the activities of ACO and ACS in the ethylene synthesis pathway, thereby precisely initiating and advancing the fruit ripening process[62]. In banana, *MaSnRK2.1*, *MaSnRK2.8*, and particularly *MaSnRK2.10* were significantly upregulated during early ripening stages (Fig. 5). Among them, *MaSnRK2.10* exhibited the highest expression level, suggesting a central regulatory role. Subcellular localization analysis demonstrated that the MaSnRK2.10 protein localizes predominantly to the nucleus and plasma membrane, consistent with previous findings for TaSnRK2.8 in wheat[63] and

ZmSnRK2 (ZmSAPK8) in maize[64]. Functional assays further supported its role in starch metabolism. Silencing of *MaSnRK2.10* led to increased total starch, amylose, and amylopectin accumulation, accompanied by darker I_2–KI staining (Fig. 6b), indicating reduced starch degradation. Conversely, transient overexpression of *MaSnRK2.10* decreased starch components in banana fruit. These results provide direct functional evidence for the role of *MaSnRK2.10* in regulating starch degradation during banana ripening. This regulatory role aligns with findings in cassava, where overexpression of *MeSnRK2.3* enhanced starch accumulation and growth[26], highlighting conserved yet context-dependent functions of SnRK2 proteins in carbohydrate metabolism. Mechanistically, we propose that MaSnRK2.10 may directly phosphorylate rate-limiting enzymes (e.g., AMY, BAM) or act upstream of transcription factors (e.g., bZIP, NAC), regulating starch catabolic genes. Future kinase assays and phosphor-site mutagenesis are needed to identify its bona fide targets.

Transcriptional regulation is critical for coordinating starch degradation during banana ripening. For instance, *MaMADS36* directly activates MaBAM9b expression to enhance starch degradation[65], whereas MaMYB3 represses multiple starch-degradation-related genes (*GWD1*, *SEX4*, *BAM7–8*, *AMY2B*, *AMY3*, *AMY3A*, *AMY3C*, *MEX1*, and *pGlcT2-1*) and delays ripening[66]. MaMYB44 and MaMYB73 form a complex that suppresses *EXPA15* and *BAM3*, influencing fruit firmness and starch metabolism[45]. In the present study, four candidate TFs—*NAC1*, *NAC2*, *NAC3*, and *ERF*—were predicted to bind the *MaSnRK2.10* promoter. Their expression patterns were significantly correlated with *MaSnRK2.10* during early ripening. Molecular docking simulations supported the potential for direct protein–DNA interactions, suggesting that these TFs may regulate *MaSnRK2.10* transcription. Although these findings are based on computational modeling and co-expression analysis, and require experimental validation (e.g., EMSA or ChIP assays), they provide a plausible regulatory framework linking upstream TF activity with SnRK2-mediated starch degradation. Further experiments will be conducted—such as yeast one-hybrid and EMSA assays—to further verify these predicted interactions.

Most studies have focused on transcriptional or enzymatic mechanisms. In contrast, our study identifies MaSnRK2.10, a Ser/Thr protein kinase, as a new player associated with starch degradation. This finding is conceptually significant because it highlights a potential post-translational regulatory layer—reversible protein phosphorylation—into the starch catabolic pathway. Given that SnRK2 kinases respond to ABA and stress signals, MaSnRK2.10 may integrate ripening-related or environmental cues into starch metabolism. While its precise targets remain unknown, our work opens a new direction for investigating how starch breakdown is fine-tuned at the protein level, complementing existing transcriptional models. Future studies should explore whether MaSnRK2.10 phosphorylates known starch-degrading enzymes (e.g., MaPHO1, MaBAM9b) or acts independently.

Conclusions

This study provides a comprehensive genome-wide characterization of the *MaSnRK2* gene family in banana and reveals its functional relevance to fruit starch degradation. Fourteen *MaSnRK2* genes were identified and classified into three conserved phylogenetic groups, exhibiting highly conserved gene structures, protein motifs, and evolutionary patterns under strong purifying selection, with tandem duplication contributing to family expansion. Promoter analysis indicated that *MaSnRK2* genes, particularly *MaSnRK2.10*, harbor diverse *cis*-acting elements associated with phytohormone signaling, stress responses, and developmental regulation, suggesting complex transcriptional control. Expression profiling demonstrated that *MaSnRK2.10* is specifically and highly expressed during early fruit ripening, and subcellular localization showed that the MaSnRK2.10 protein is distributed in the nucleus and cell membrane. Functional assays further confirmed that *MaSnRK2.10* significantly regulates starch degradation, as transient silencing increased starch, amylose, and amylopectin contents, whereas transient overexpression reduced their accumulation in banana fruit. In addition, several candidate transcription factors were predicted to regulate *MaSnRK2.10* expression, implying a potential regulatory network underlying starch metabolism during ripening. Together, these findings identify *MaSnRK2.10* as a key regulator of starch degradation and provide a solid theoretical basis for future molecular improvement of starch metabolism and postharvest quality in banana.

Author contributions

The authors confirm their contributions to the paper as follows: study conception and design, draft manuscript preparation: Miao H, Wang Y; data collection: Tu Y, Zhu Z, Sun P, Jin Z, Liu J; analysis and interpretation of results: Miao H, Zhu Z, Sun P, Zhang M, Jin Y, Wu M, Wang L, Zhou L; resources: Miao H, Jin Z, Liu J; manuscript writing and revising: Miao H, Tu Y, Ali MM. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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