Low-temperature-induced Regulatory Network Rewiring Via WRKY Regulators in Banana Peel

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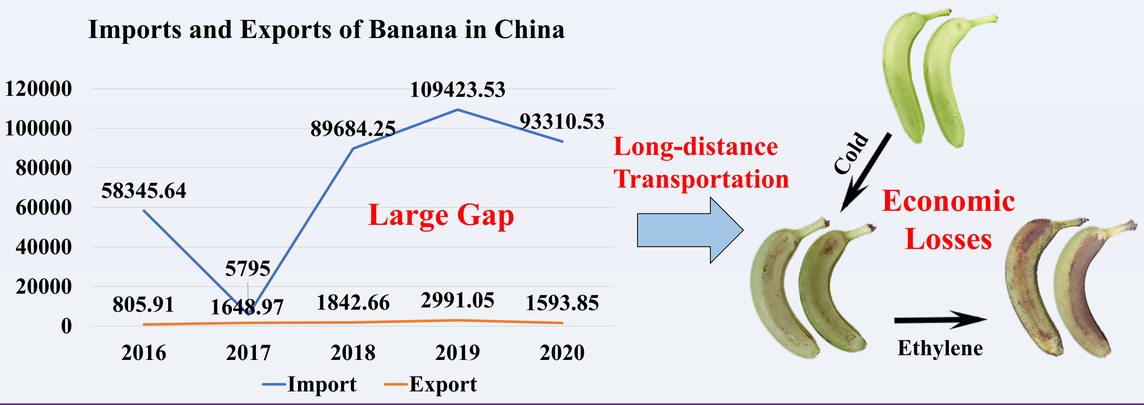
Abstract

Low-temperature storage is an effective means to extend the shelf life of fruits after harvest, but can lead to chilling injury in tropical fruits. It is still largely unknown how tropical fruits respond to low temperature compared to the cold response mechanisms of model plants. Banana (*Musa* spp.) fruits, as typical tropical fruits, are not cold tolerant, and lower temperatures can disrupt cellular compartmentalization and lead to severe browning. We observed Dynamic patterns of cold-induced transcripts are generally accompanied by concordant chromatin accessibility and histone modification changes. These upregulated genes were enriched in WRKY binding sites in their promoters and/or active enhancers, which could form enhancer-promoter interactions that appear to regulate critical browning pathways, including phospholipid degradation, oxidation, and cold tolerance. Our results illustrate the transcriptional reprogramming via WRKYs during banana peel browning at low temperature, and provide an extensive resource for studying gene regulation in tropical plants in response to cold stress, and potential targets for improving cold tolerance and shelf-life of tropical fruits.

Background



Banana is a popular fruit and also major food source for more than 4 million people in the world. Banana is mainly produced in Asia. In China, Fujian, Hainan, Guangxi, Yunnan and Guangdong are major areas of banana cultivation. In the last five years, imports of banana is much more than exports. These mean that a year-round supply of banana requires long-distance and long-time transportation across the cities or countries. Considering short shelf-life of banana, cold chain is an easy way to minimize metabolic activity. However, banana is sensitive to low temperature. Multiple chilling injury has emerged under low temperature, such as peel browning and pulp hardening. There injury symbols especially peel browning, seriously reduces banana quality, resulting in economic losses.

Characterization of Cold-induced Genes

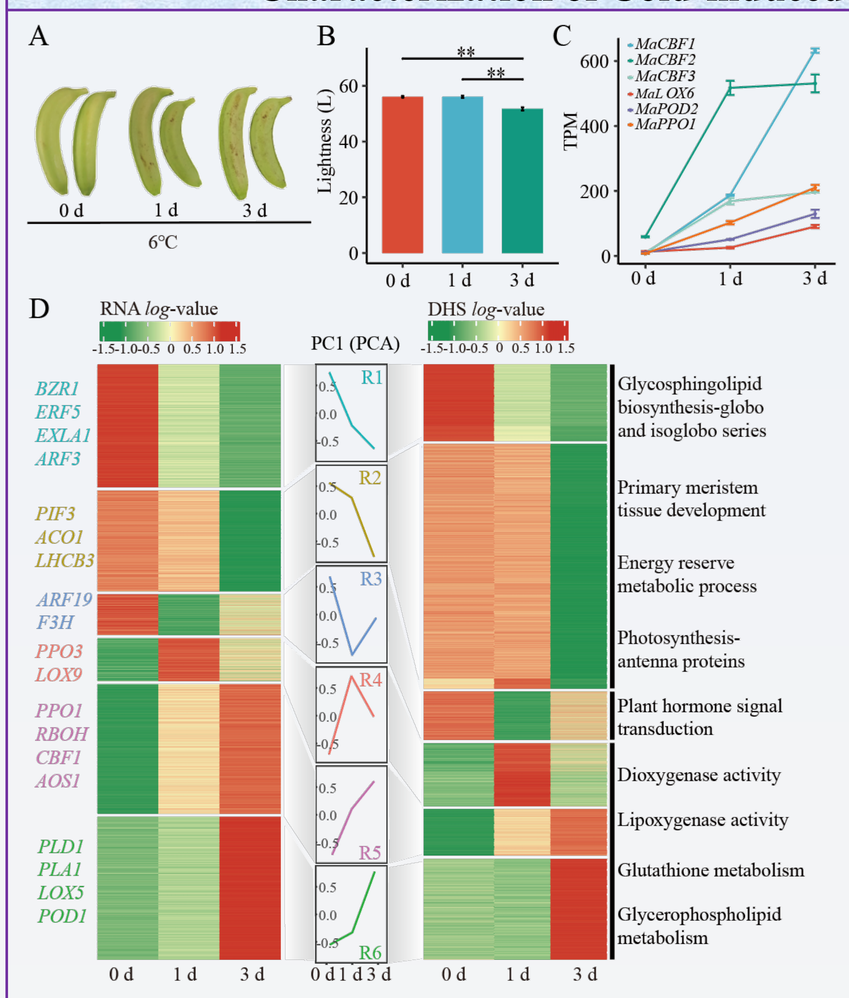


Fig. 1 Physiological changes and gene expression analysis of banana peels in response to low temperature. A, Dark spots appear on banana peels at low temperature. Fruits were kept at 6°C for 0 (as control), 1 or 3 days. **B,** Lightness of banana peels at low temperature after 0, 1 or 3 days at 6°C. **C,** Time course of gene expression for three *CBF*s, *LOX6*, *PPO1* and *POD2*, at low temperature. **D,** Heatmap of the RNA-seq and DHS datasets generated by *k*-means clustering analysis. PC1 represents a cluster of linear equation. RNA-seq and DNase-seq values were normalized. Representative genes are listed to the left. The patterns of PC1 are shown in the middle. Selected GO terms are listed to the right.

1. Low temperature slows down plant growth (R1 and R2) and strongly induces browning-related genes (BRGs) (R4, R5 and R6), including *MaPPO1*, *MaPLD1* and *MaLOX5*, which regulate critical browning pathways, including phospholipid degradation, oxidation.
2. The expression level changes of cold-respond genes in each clusters are along with the dynamics of chromatin accessibility.

The Importance of The WRKY Family in Response to Low Temperature

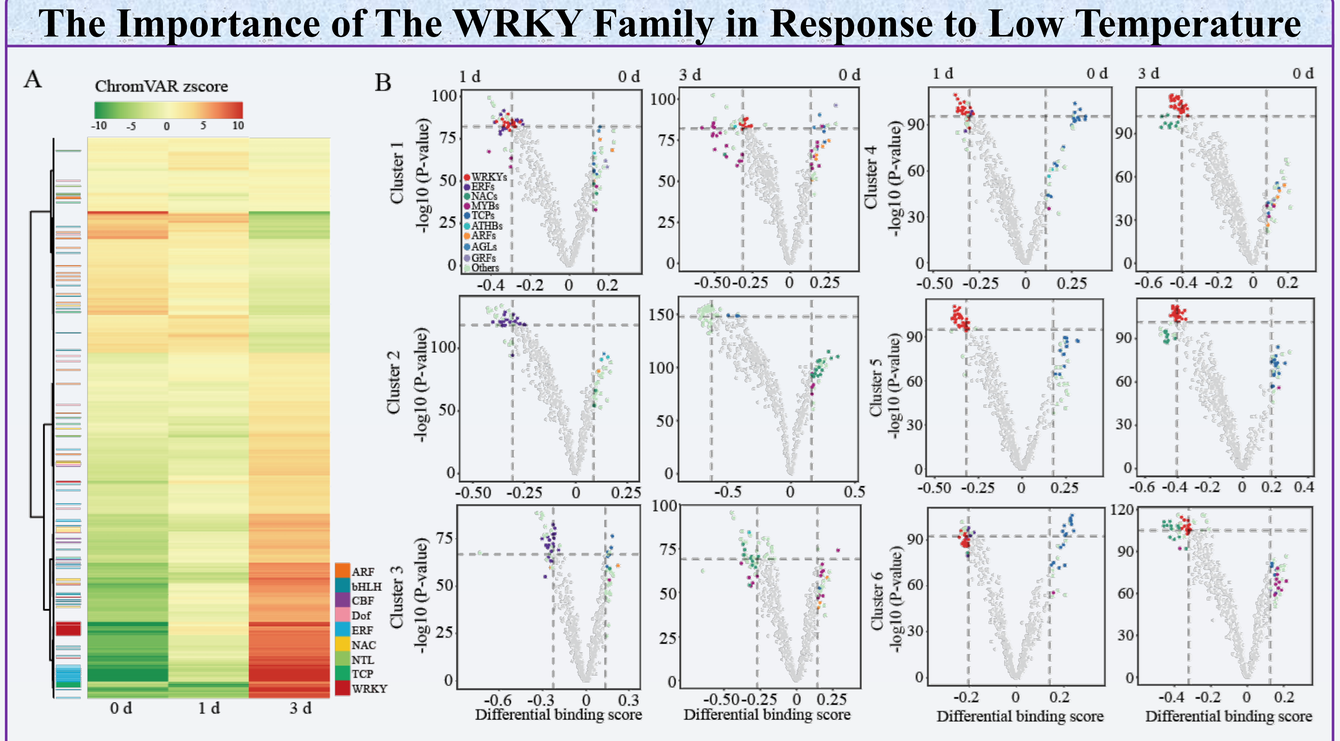


Fig. 3 Footprint analysis of *cis*-regulatory elements. A, Identification of TF activity. Heatmap showing motif activity, as determined. TF families are shown to the left. The heatmap represents the Z-score of motif activity, based on DNase-seq results. **B,** Footprint analysis for each cluster in Fig. 1D.

1. The enrichment for the binding sites of TFs like WRKYs, NACs, CBFs, TCPs and ERFs after 3 days in the cold.
2. WRKY regulator family plays a core role in response to low temperature and is strongly associated with banana peel browning.

Enhancers Paly an Important Role in Low Temperature

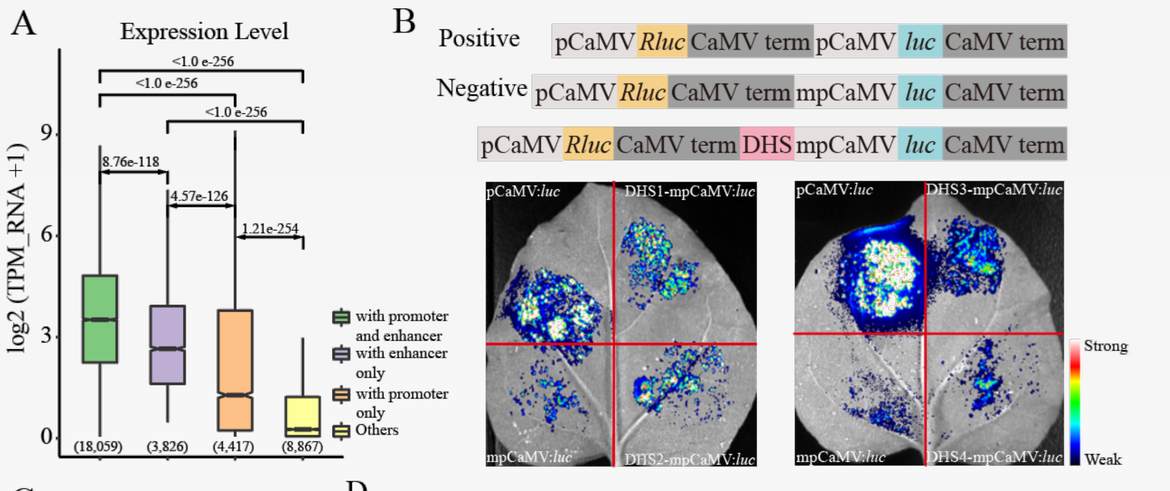


Fig. 2 Analysis of candidate enhancers in response to low temperature. A, Boxplots of gene expression for genes with only promoter, with only enhancer, with both, or with neither. The number of genes for each category is given in parentheses. **B,** Schematic diagrams for the reporter constructs of the dual-luciferase assay (top) and results of the assay in *Nicotiana benthamiana* leaves showing that candidate enhancers increase *LUC* transcription levels (bottom). **C,** Number of candidate enhancers in response to low temperature. Venn diagram shows the number of time-specific, common and cold-induced enhancers. **D,** Integrated Genome Viewer browser window of normalized read coverage for RNA-seq, DNase-seq data and putative loops for representative genes at 0, 1 and 3 days at 6°C.

1. the expression levels of genes with promoters and candidate enhancers are significantly higher than those with promoters or candidate enhancers alone.
2. Low temperature induced a large number of enhancers, which played an crucial role in increasing expression level of cold-respond genes, some of which are related in phospholipid degradation and oxidation.

Network Analysis of Banana Peel Browning With Core WRKYs

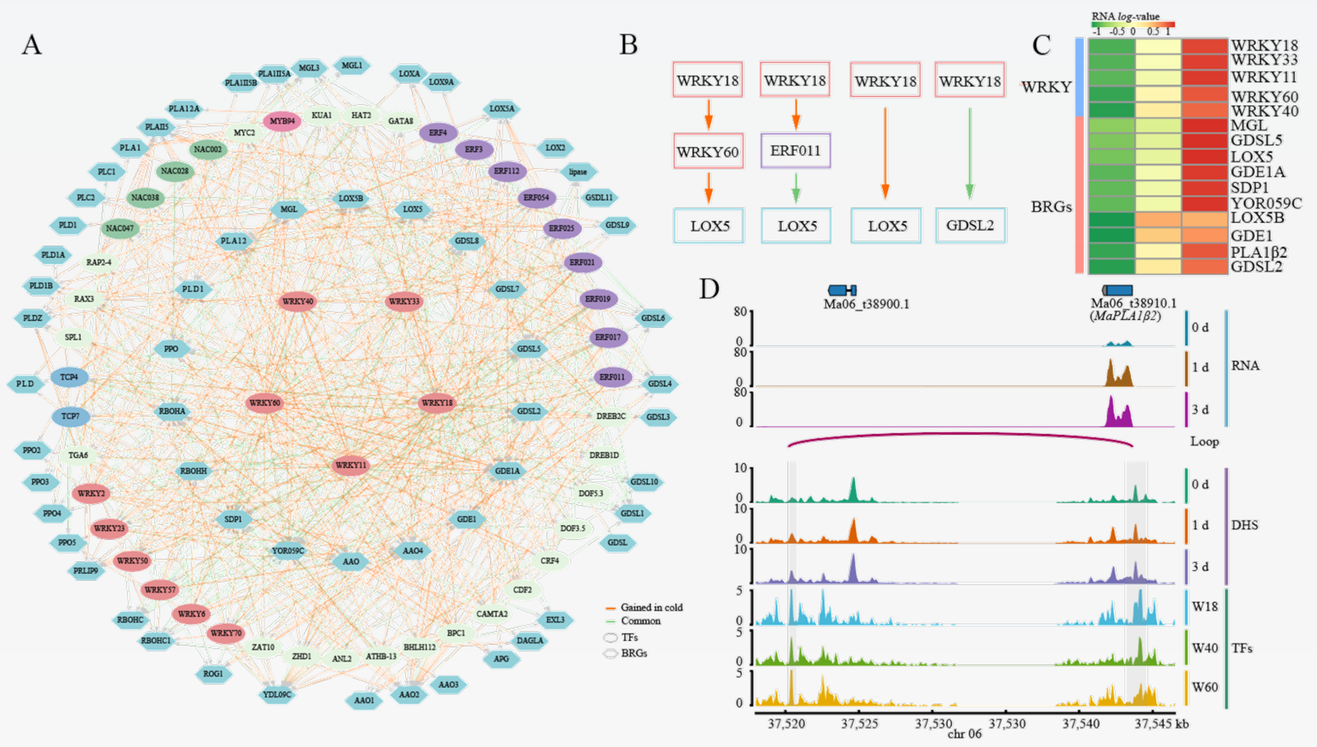
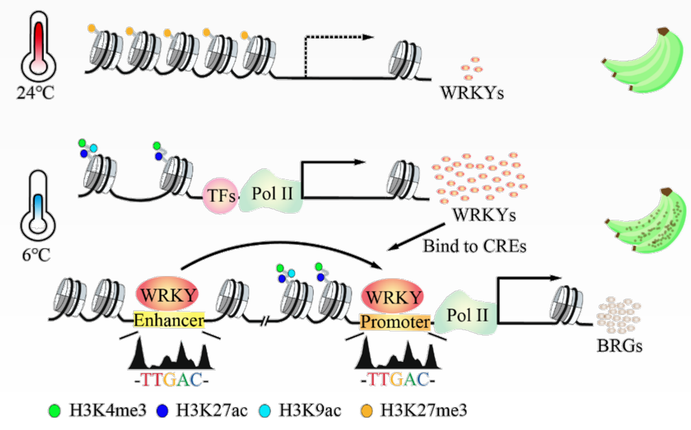


Fig. 4 Dynamics of browning-related interaction networks. A, Rewiring of browning-related networks by five WRKY TFs at low temperature. Dynamic TF binding is represented by orange (regulation gain) and green (common regulation of the control and cold treatment) edges, at low temperature. Ellipses, TFs; hexagons, BRGs, based on binding data generated by DAP-seq. **B,** Example of TF interaction pattern in browning-related interaction networks. **C,** Heatmap of expression levels of the five WRKY genes and their direct BRG targets. **D,** Integrated Genome Viewer window of normalized read coverage of RNA-seq, DNase-seq, TF binding (DAP-seq) and loops for representative genes.

1. Low-temperature-induced regulatory network rewiring via WRKY regulators in banana peel.
2. BRGs are directly or indirectly regulated by the five WRKYs at low temperature, along with increased expression level.

An Model of Banana Peel Browning at Low Temperature



Cold-induced genes are frequently associated with accessible chromatin and enrichment of active histone marks along their promoters. Among these genes, WRKYs target to the promoter region and also to enhancers of BRGs, likely forming a chromatin loop that further increase the expression of BRGs