

Supplementary Materials: Molecular Characterization of MaCCS, a Novel Copper Chaperone Gene Involved in Abiotic and Hormonal Stress Responses in *Musa acuminata* cv. Tianbaojiao

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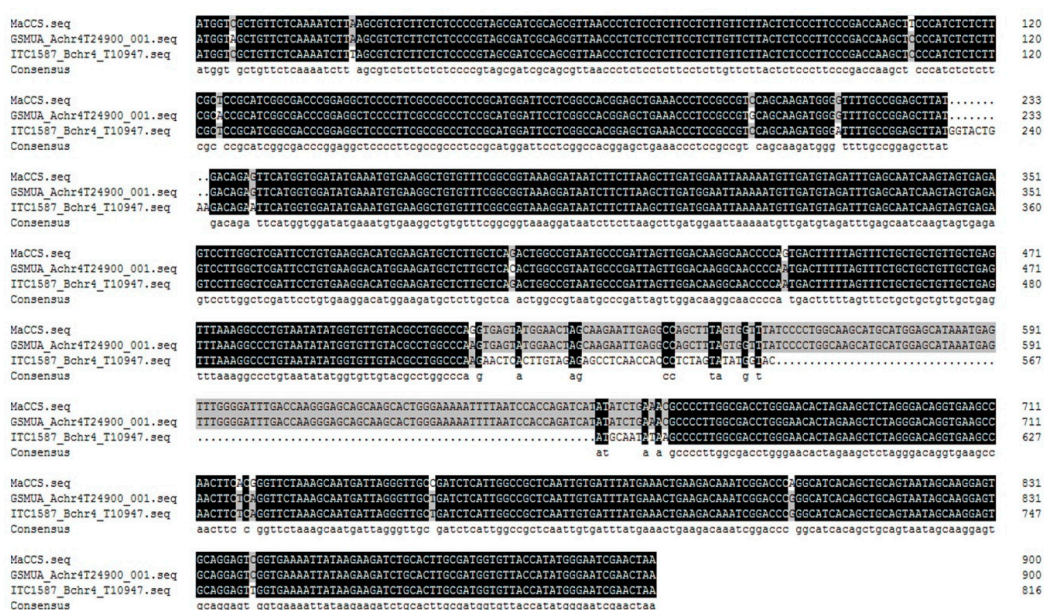


Figure S1. Sequence alignments of CCs from Tianbaojiao and two wild bananas. Nucleotides identical in all sequences are indicated with a black background, and nucleotides conserved only between two sequences are indicated with a gray background.

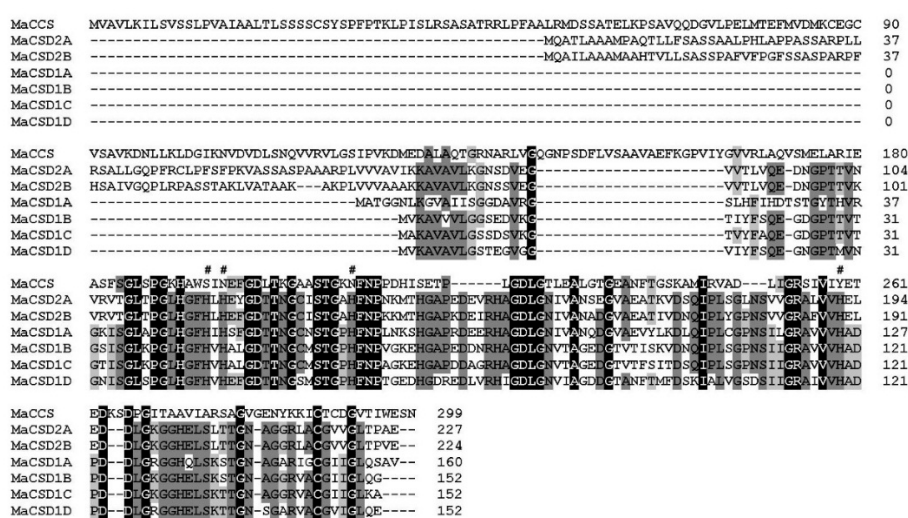


Figure S2. Multiple sequence alignment of the deduced MaCCS protein and Cu/ZnSOD (MaCSD) proteins. Identical conserved residues are shown with a black background, while similar residues are indicated with a gray background. Gaps (-) have been introduced to optimize the alignment. A hash sign (#) indicates the copper-binding ligands (histidine residues) of Cu/ZnSODs. Amino acid sequences of the MaCSDs are downloaded from the NCBI database with the following accession numbers: MaCSD2A (AFV46365), MaCSD2B (AIF32069), MaCSD1A (AFC17494), MaCSD1B (AGC13157), MaCSD1C (AGC13158) and MaCSD1D (AGC85069).

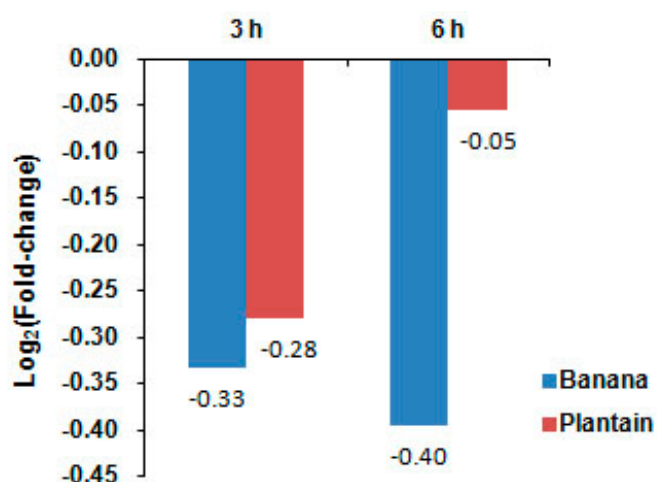


Figure S3. Expression levels of the CCS gene (genome locus ID: GSMUA_Achr4G24900_001) in response to cold treatment (10 °C) according to the transcriptomics data. The expressions of CCS are presented using fold change compared to control (0 h). Log₂ (fold-change value) is shown at the top of bar.

Table S1. Oligonucleotide primers used in this study.

Primer Name	Primer Sequence (5'–3')	Tm/°C	Extension Time/s	Purpose
CCS-ORFF	GCAAGTAATAAAAGAGTGAATCGGAG	54	55	ORF/gDNA amplification
CCS-ORFR	CTTACAGCAGGTCAGTGTGG			
CCS-5R1 5P	CACTAAAGCTGGCCTCAATTCTTGC CGACTGGAGCACGAGGACACTGA	58	35	1st round of 5'RACE
CCS-5R2 5NP	GCCAAGGACTCTCACTACTTGATTGC GGACACTGACATGGACTGAAGGAGTA	60	30	2nd round of 5'RACE
CCS-3F1 AUAP	GAGCAATCAAGTAGTGAGAGTCCTTG GGCCACGCGTCGACTAGTAC	58	50	1st round of 3'RACE
CCS-3F2 AUAP	CTAGCAAGAATTGAGGCCAGCTTTAG GGCCACGCGTCGACTAGTAC	60	40	2nd round of 3'RACE
CCS-ProF	GAACATGAGCATCCTGATAAAAGTG	55	120	Promoter amplification
CCS-ProR	AAGAGGAGAGGGTTAACGCTG			
CCS-SubF	CATGCCATGGATGGTCGCTGTCTCAAATC *	51	54	Subcellular localization
CCS-SubR	GGACTAGTGTTCCGATTCCCATATGGTAAC *			
CAC-QF	AACTCCTATGTTGCTCGCTTATG	57	30	qRT-PCR
CAC-QR	GGCTACTACTTCGGTTCCTTAC			
CCS-QF	GAAGATGCTCTTGCTCAGACTG	57	30	qRT-PCR
CCS-QR	GGCTAGTCCATACTCACCTGG			

* Restriction enzyme sites are in bold.