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Functional Plant Biology

Supplementary Material

Overexpression of *TaMPK3* enhances freezing tolerance by increasing the expression of *ICE-CBF-COR* related genes in the *Arabidopsis thaliana*

Rui Wang^A, Mengmeng Yu^A, Xin Zhao^A, Jingqiu Xia^A, Jing Cang^A, and Da Zhang^{A,*}

^ACollege of Life Science, Northeast Agricultural University, Harbin 150030, China.

*Correspondence to: Da Zhang College of Life Science, Northeast Agricultural University, Harbin 150030, China Email: zhangda@neau.edu.cn

Supplemental Information

Table S1. Primers lists used in this study

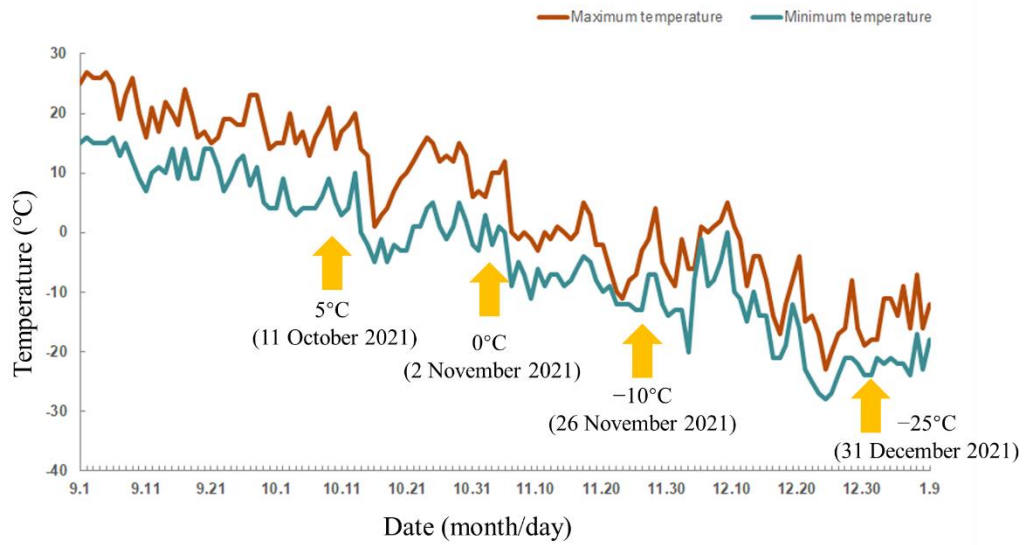
Primer name	Gene ID	Sequence (5'- 3')	Purpose
<i>TaACTIN-F</i>	TraesCS5B01G124100	CCTTAGTACCTTCCAACAGATGT	qRT-PCR
<i>TaACTIN-R</i>		CCAGACAACCTCGCAACTTAGA	
<i>TaMPK3-F</i>	TraesCS4D02G198600	CAGAGGATCACAGTTGAAGAGG	qRT-PCR
<i>TaMPK3-R</i>		TGCTCGAAGTCAAAGGAGAAG	
<i>TaMPK3-F</i>	TraesCS4D02G198600	ATGGACGGCGCTCCGGT	Gene clone
<i>TaMPK3-R</i>		CTAGTATCGGAAGTTGGGGTT	
<i>TaICE41-F</i>	TraesCS3A02G442200	ATGGAGAACTCGGCGGC	Gene clone
<i>TaICE41-R</i>		TCACGCAGGATACAGAGG	
<i>35S:TaMPK3-F</i>	TraesCS4D02G198600	CCTGCAGGCTGAGGCTTAATTAA+ATGGA CGGCGCTCCGGT	vector construction
<i>35S:TaMPK3-R</i>		TCCCGGGCTGAGGTTTAATTAA+CTAGTA TCGGAAGTTGGGGTT	
<i>TaMPK3-GFP-F</i>	TraesCS4D02G198600	GAGCTCGGTACCCGGGGATCC+ATGGACG GCGCTCCGGT	subcellular localization
<i>TaMPK3-GFP-R</i>		GGTGTGCGACTCTAGAGGATCC+GTATCGG AAGTTGGGGTT	
<i>BD-TaMPK3-F</i>	TraesCS4D02G198600	ATGGCCATGGAGGCCGAATTC+ATGGACG GCGCTCCGGT	Y2H
<i>BD-TaMPK3-R</i>		TCGACGGATCCCCGGAATTC+CTAGTATC GGAAGTTGGGGTT	
<i>AD-TaICE41-F</i>	TraesCS3A02G442200	GCCATGGAGGCCAGTGAATTC+ATGGAGA ACTCGGCGGC	Y2H
<i>AD-TaICE41-R</i>		ATGCCACCCGGGTGGAATTC +CTACATCGGTTCTGGAGACCG	
<i>TaMPK3-GFP^N-F</i>	TraesCS4D02G198600	ATGGCCATGGAGGCCGAATTC+ATGGACG GCGCTCCGGT	BiFC
<i>TaMPK3-GFP^N-R</i>		TCGACGGATCCCCGGAATTC+GTATCGG AAGTTGGGGTT	
<i>TaICE41-GFP^C-F</i>	TraesCS3A02G442200	GCCATGGAGGCCAGTGAATTC+ATGGAGA ACTCGGCGGC	BiFC
<i>TaICE41-GFP^C-R</i>		ATGCCACCCGGGTGGAATTC+CATCGC GTTCTGGAGACCG	
<i>AtACTIN2-F</i>	AT3G18780	TGTGCCAATCTACGAGGGTTT	qRT-PCR
<i>AtACTIN2-R</i>		TTCCCGCTCTGCTGTTGT	
<i>AtICE1-F</i>	AT3G26744	AGCTCCGTTGGAGTTGGAAG	qRT-PCR

<i>AtICE1-R</i>		GCTGCACGTTTCTGGAACAG	
<i>AtCBF1-F</i>	AT4G25490	GCTTTTCAAGATGAGACGTGTG	qRT-PCR
<i>AtCBF1-R</i>		TAAATAGCTTCCACCATCGTCT	
<i>AtCBF2-F</i>	AT4G25470	TGACGTGTCCTTATGGAGCTA	qRT-PCR
<i>AtCBF2-R</i>		CTGCACTCAAAAACATTTGCA	
<i>AtCBF3-F</i>	AT4G25480	CGTTTCAGGATGAGATGTGTGA	qRT-PCR
<i>AtCBF3-R</i>		CTCATCGTGCATATAAAACGCA	
<i>AtCOR15A-F</i>	AT2G42540	GCTTCAGATTTTCGTGACGGATAAAAC	qRT-PCR
<i>AtCOR15A-R</i>		GCAAAAACATTAAGAATGTGACGGTG	
<i>AtCOR47-F</i>	AT1G20440	CAGTGTCGGAGAGTGTGGTG	qRT-PCR
<i>AtCOR47-R</i>		ACAGCTGGTGAATCCTCTGC	
<i>AtRD29A-F</i>	AT5G52310	GCCGAGAACTTCAGATTGG	qRT-PCR
<i>AtRD29A-R</i>		CCATTCCTCCTCCTCCTTTC	
<i>AtKINI-F</i>	AT5G15960	TGGAGCTGGAGCACAACA	qRT-PCR
<i>AtKINI-R</i>		GACCCGAATCGCTACTTGTTC	

Table S2. Cis-elements analysis of *TaMPK3* promoter region

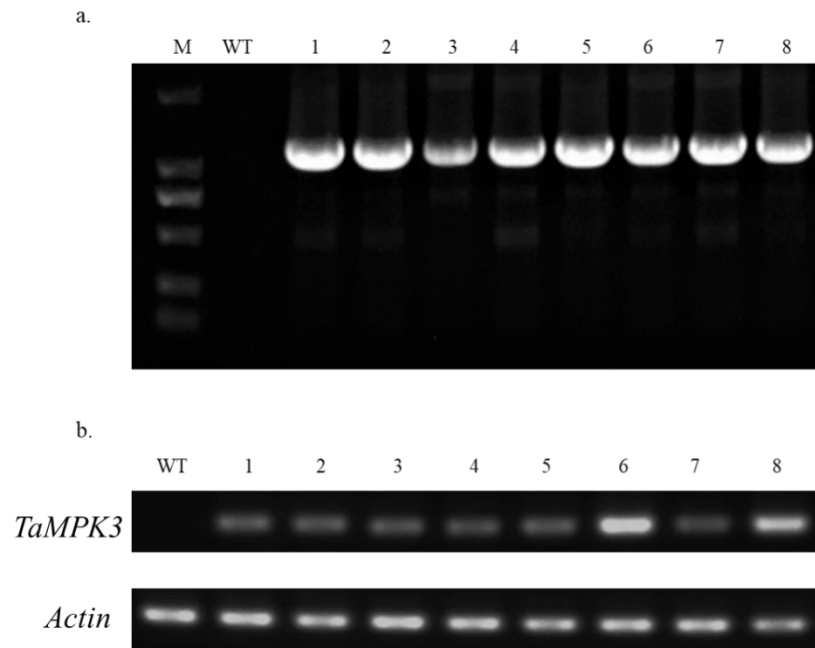
Category	Element	Description	Number
Stress	DRE	cis-acting element of dehydration reaction	2
	TC-rich repeats	cis-acting element involved in defense and stress responsiveness	1
Light	G-box	cis-acting regulatory element involved in light responsiveness	1
	Sp1	light responsive element	1
	GT1-motif	light responsive element	1
	TCT-motif	part of a light responsive element	1
Regulation	TATA-box	core promoter element around -30 of transcription start	18
	CAAT-box	common cis-acting element in promoter and enhancer regions	15
	A-box	cis-acting regulatory element	5
	CCAAT-box	MYBHv1 binding site	2
	Circadian	cis-acting regulatory element involved in circadian control	2
	ARE	cis-acting regulatory element essential for the anaerobic induction	1
Hormone	CGTCA-motif	cis-acting regulatory element involved in the MeJA-responsiveness	3
	TGACG-motif	cis-acting regulatory element involved in the MeJA-responsiveness	3
	ABRE	cis-acting element involved in the abscisic acid responsiveness	1

Supplementary Figure S1



Supplementary Figure S1: Temperature change trend of Dn1 from sowing to sampling. The red line represents the highest temperature of the day, and the blue line represents the lowest temperature of the day.

Supplementary Figure S2



Supplementary Figure S2: Homozygous detection of T_3 transgenic *Arabidopsis thaliana*
a. DNA level detection of *TaMPK3* overexpression plants; b. Semi-quantitative RT-PCR detection of *TaMPK3* overexpression plants