

Tobacco Calmodulins Responsive to Tobacco Mosaic Virus-Induced Hypersensitive Reaction and Wounding

Hiromoto YAMAKAWA

A dissertation submitted to the Doctoral Program in Biological Sciences, the University of Tsukuba in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Science



January 2002

02304243

	page
Contents	- i
Abbreviations	- iv
Abstract	- 1
Introduction	- 3
Materials and Methods	- 7
Plant materials, synchronous HR induction by TMV, wounding	
and chemical treatments	- 7
Complementary DNA library screening	- 8
Reverse transcriptase-polymerase chain reaction (RT-PCR)	
and rapid amplification of cDNA ends (RACE)	- 9
Sequencing and sequence analyses	- 10
DNA gel blot analysis	- 10
RNA gel blot analysis	- 11
Production of recombinant CaM proteins in E. coli	- 11
Production and affinity purification of anti-(tobacco CaM) antibodies	- 12
Protein extraction and protein gel blot analysis	- 13
Measurement of proteasome activity	- 15
NAD ⁺ kinase assay	- 15
NO synthase assay	- 17
Calcineurin assay	- 18
Generation of transgenic tobacco plants	- 19
Results	- 21

	Cloning and sequence analysis of 13 tobacco CaM cDNAs	- 21
	Organ-specific expression of NtCaM genes	- 22
	Preparation of antibodies specific to individual type of tobacco CaMs	- 23
	Various expression profiles of tobacco CaM genes and predominant	
	accumulation of NtCaM13 protein during synchronous HR in	
	TMV-infected leaves	- 24
	Wound-induced expression of NtCaM1, 2, 3, 4, and 13 genes and	
	accumulation of NtCaM1 protein	- 25
	Response of NtCaM genes to treatment with SA and MeJA	- 26
	Enhanced accumulation of NtCaM proteins by the treatment with a	
	proteasome inhibitor	- 27
	NADK was activated by NtCaM1 and NtCaM3 but not by NtCaM13	- 28
	NOS was activated by NtCaM13 most effectively	- 29
	CaN was activated strongly by NtCaM3 and moderately by NtCaM1	
	and NtCaM13	- 29
	Expression of PR and wipk genes was affected in CaM transgenic plants	- 30
Dis	cussion	- 32
	Involvement of NtCaM13-type CaMs in HR signaling	- 32
	Involvement of NtCaM1-type CaMs in wound signaling	- 33
	Involvement of regulated degradation of NtCaM proteins	
	by proteasome in wound response	- 34
	Possible target proteins of NtCaMs	- 35

Potentiation of defense responses by the dynamic change

in CaM balance	- 39
References	- 40
Tables	- 51
Figures and Figure Legends	- 56
Acknowledgements	-108

Abbreviations

BSA bovine serum albumin

[Ca²⁺]_i concentration of cytosolic free Ca²⁺

CaM calmodulin

CaN calcineurin

EDTA ethylenediamine-N,N,N',N'-tetraacetic acid

EGTA ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid

FAD flavin adenine dinucleotide

HR hypersensitive reaction

JA jasmonic acid

MAP kinase mitogen-activated protein kinase

MeJA methyl jasmonate

4MU 4-methyl umbelliferone

4MUP 4-methyl umbelliferyl phosphate

NADK NAD⁺ kinase

NO nitric oxide

NOS nitric oxide synthase

PAGE polyacrylamide gel electrophoresis

PR pathogenesis-related

RACE rapid amplification of cDNA ends

ROS reactive oxygen species

RT-PCR reverse transcriptase-polymerase chain reaction

SA salicylic acid

SDS sodium dodecyl sulfate

TLC thin-layer chromatography

TMV tobacco mosaic virus

UTR untranslated region

WIPK wound-induced protein kinase