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Proteases and protease inhibitors involved in plant stress response and acclimation

by

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Declaration

I, the undersigned, hereby declare that the thesis submitted herewith for the degree Philosophiae Doctor to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other university.

Anneke Prins

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Abstract

Proteases play a crucial role in plant defence mechanisms as well as acclimation to changing metabolic demands and environmental cues. Proteases regulate the development of a plant from germination through to senescence and plant death. In this thesis the role of proteases and their inhibitors in plant response to cold stress and CO₂ enrichment were investigated.

The activity and inhibition of cysteine proteases (CP), as well as degradation of their potential target proteins was investigated in transgenic tobacco plants expressing the rice cystatin, OC-I. Expression of OC-I caused a longer life span; delayed senescence; significant decrease in *in vitro* CP activity; a concurrent increase in protein content; and protection from chilling-induced decreases in photosynthesis. An initial proteomics study identified altered abundance of a cyclophilin, a histone, a peptidyl-prolyl cis-trans isomerase and two RuBisCO activase isoforms in OC-I expressing leaves. Immunogold labelling studies revealed that RuBisCO and OC-I is present in RuBisCO vesicular bodies (RVB) that appear to be important in RuBisCO degradation in leaves under optimal and stress conditions.



Plants need to respond quickly to changes in the environment that cause changes in the demand for photosynthesis. In this study the effect of CO₂ enrichment on photosynthesis-related genes and novel proteases and protease inhibitors regulated by CO₂ enrichment and/or development, was investigated. Maize plants grown to maturity with CO₂ enrichment showed significant changes in leaf chlorophyll and protein content, increased epidermal cell size, and decreased epidermal cell density. An increased stomatal index in leaves grown at high-CO₂ indicates that leaves adjust their stomatal densities through changes in epidermal cell numbers rather than stomatal numbers. Photosynthesis and carbohydrate metabolism were not significantly affected. Developmental stage affected over 3000 transcripts between leaf ranks 3 and 12, while 142 and 90 transcripts were modified by high CO₂ in the same leaf ranks respectively. Only 18 transcripts were affected by CO₂ enrichment exclusively. Particularly, two novel CO₂-modulated serine protease inhibitors modulated by both sugars and pro-oxidants, were identified. Growth with high CO₂ decreased oxidative damage to leaf proteins.



Abbreviations

ABA	-	abscissic acid
ACC	-	1-aminocyclopropane-1-carboxylate
ADP	-	adenosine diphosphate
AGPase	-	ADP glucose pyrophosphorylase
ANOVA	-	analysis of variance
Asp	-	asparagine
ATP	-	adenosine triphosphate
BBI	-	Bowman Birk Inhibitor
beta-lyc	-	lycopene beta-cyclase
BLAST	-	Basic Local Alignment Search Tool
BS	-	bundle sheath
BSA	-	bovine serum albumin
Bt	-	bacillus thuringiensis
°C	-	degree Celsius
C _a	-	ambient CO ₂ concentration
CA-1-P	-	2-carboxyarabinitol 1-phosphate
CatB	-	cathepsin B
CBF1	-	C promoter-binding factor 1
CE	-	carboxylation efficiency
CHAPS	-	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
CHCA	-	α-cyano-4-hydroxycinnamic acid
C _i	-	intercellular CO ₂ concentration
CIN	-	cytoplasmic invertase
CP	-	cysteine protease
CP4 EPSPS	-	5-Enol-pyruvylshikimate-3-phosphate synthase CP4
Ct	-	threshold cycle
CWIN	-	cell wall invertase
dATP	-	2'-deoxyadenosine 5'-triphosphate
dCTP	-	2'-deoxycytosine 5'-triphosphate
DEPC	-	diethyl pyrocarbonate
dGTP	-	2'-deoxyguanosine 5'-triphosphate
DMSO	-	dimethyl sulfoxide



DNA	-	deoxyribonucleic acid
DNase	-	deoxyribonuclease
dNTP	-	deoxyribonucleotide triphosphate
dTTP	-	2'-deoxythymidine 5'-triphosphate
DREB1A	-	dehydration response element B1A (
DTT	-	dithiotreitol
E	-	efficiency
E1	-	uibiquitin-activating enzyme
E2	-	ubiquitin-conjugating enzyme
E3	-	ubiquitin ligase
E64	-	<i>trans</i> -epoxysuccinyl-L-leucylamido(4-guanidino)butane
EDTA	-	ethylenediaminetetraacetic acid
ER	-	endoplasmic reticulum
FACE	-	free-air CO ₂ enrichment
G3P	-	glyceraldehyde 3-phosphate
GA	-	gibberellic acid
Gin	-	glucose insensitive
GPCR	-	G-protein coupled receptor
gus	-	β-glucuronidase
h	-	hour(s)
HB	-	hemoglobin
HSP	-	heat shock protein
HXK	-	hexokinase
Incw4	-	cell wall invertase 4
IPM	-	integrated pest management
JA	-	jasmonic acid
J _{max}	-	CO ₂ saturated rate of photosynthesis
kDa	-	kilodalton
KV	-	KDEL vesicles
Lhc	-	light harvesting complex
LSU	-	large subunit
M	-	mesophyll
MAP	-	mitogen-activated protein
ME	-	malic enzyme



min	-	minute(s)
MOPS	-	3-(N-Morpholino)propanesulfonic acid
MS	-	mass spectrometry
MW	-	molecular weight
NAD	-	nicotinamide adenine dinucleotide
NADP	-	nicotinamide adenine dinucleotide phosphate
NCBI	-	National Center for Biotechnology Information
NR	-	nitrogen reductase
OC-I	-	oryzacystatin I
OCE	-	OC-I expressing tobacco
PAGE	-	polyacrylamide gel electrophoresis
PAL	-	phosphoammonia lyase
PARP	-	poly (ADP-ribose) polymerase
PBS	-	phosphate buffered saline
PCR	-	polymerase chain reaction
PCD	-	programmed cell death
PEP	-	phosphoenolpyruvate
PEPC	-	phosphoenolpyruvate carboxylase
PGA	-	phosphoglycerate
Pi	-	inorganic phosphate
pI	-	isoelectric point
PMSF	-	phenylmethylsulphonyl fluoride
ppm	-	parts per million
PR	-	pathogenesis-related
psy	-	phytoene synthase
qPCR	-	quantitative realtime PCR
RACE	-	rapid amplification of cDNA ends
RbcL	-	ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit
Rbcs	-	ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit
RGS1	-	regulator of G-protein signalling1
RMA	-	Robust Multichip Average
RNA	-	ribonucleic acid
RNAse	-	ribonuclease
ROS	-	reactive oxygen species



Rpm	-	revolutions per minute
RQ	-	relative quantity
RT	-	reverse transcriptase
RuBisCO	-	ribulose-1, 5-bisphosphate carboxylase/oxygenase
RuBP	-	ribulose-1, 5-bisphosphate
RVB	-	RuBisCO vesicular body
s	-	second(s)
SAG	-	senescence-associated gene
SD	-	standard deviation
SDS	-	sodium dodecyl sulphate
SE	-	standard error
SELDI-TOF MS-		surface-enhanced laser desorption ionization - time of flight mass spectrometry
SA	-	salicylic acid
serpin	-	serine protease inhibitor
SnRK	-	SNF-1 related kinase
SPP	-	sucrose phosphate phosphatase
SPS	-	sucrose phosphate synthase
SSU	-	small subunit
SUS	-	sucrose synthase
SuSy	-	sucrose synthase
SUT	-	sucrose transporter
TAE	-	Tris-Acetic acid-EDTA
TBS	-	Tris-buffered saline
TCA	-	trichloroacetic acid
TFA	-	trifluoroacetic acid
TP	-	triose phosphate
T-6-P	-	trehalose-6-phosphate
U	-	units
UV	-	ultraviolet
UDP	-	uridine 5'-diphosphate
UDPG	-	uridine 5'-diphosphoglucose
V	-	volt
VIN	-	vacuolar invertase



- VPE - vacuolar processing enzyme
- v/v - volume per volume
- WIP - wound-induced protein



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