FUNCTIONAL GENOMICS OF THE PERIDERM: THE BIOSYNTHETIC GENE FHT, THE TRANSCRIPTIONAL REGULATOR STRIK AND THE TRANSCRIPTOME DECIPHERING

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Universitat de Girona

Doctoral thesis

Functional genomics of the periderm: the biosynthetic gene *FHT*, the transcriptional regulator *StRIK* and the transcriptome deciphering

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Doctorate program in Molecular Biology, Biomedicine and Health.

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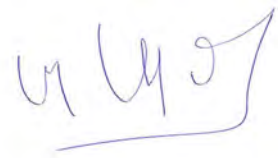

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The present thesis contain 50 supplementary material from chapters in printed and electronic format at the end of the document

This thesis is submitted in fulfilment of the requirements to obtain the doctoral degree from the Universitat de Girona

Universitat de Girona

Hereby, Dr. Mercè Figueras Vall·llosera, Prof. Dr. Marisa Molinas de Ferrer and Dr. Olga Serra Figueras, of the Universitat de Girona,

Certify:

That this doctoral thesis entitled "**Functional genomics of the periderm: the biosynthetic gene *FHT*, the transcriptional regulator *StRIK* and the transcriptome deciphering**.", that Pau Boher Genís has submitted to obtain the doctoral degree from the Universitat de Girona has been completed under their supervision, and meets the requirements to opt for the *International Doctor* mention.

In witness whereof and for such purposes as may arise, the following certification is signed:

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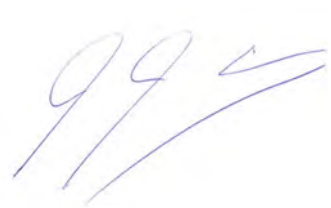

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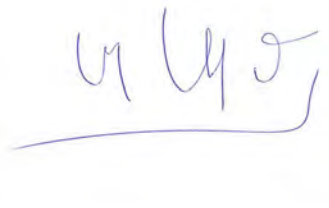

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Dedicatòria

"Cal saber viure en la incertesa sense perdre la passió."
Mark Levinson

La veritat és que no sabia quin era el millor moment per escriure aquesta part de la tesis, així que fidel al meu estil he acabat fent-ho a l'últim moment a corre-cuita la matinada abans d'enviar la tesi a imprimir, i amb companyia d'un bon got de vi. Espero complir els "estàndards de qualitat" i si em deixo algú no m'ho tingueu en compte ja em coneixeu.

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List of publications derived from this thesis

- Boher P, Serra O, Soler M, Molinas M, Figueras M. The potato suberin feruloyl transferase FHT which accumulates in the phellogen is induced by wounding and regulated by abscisic and salicylic acids. J Exp Bot. 2013; 64 (11):3225–3236.

- Boher P, Torrent X, Fernández S, Serra O, Soler M, Molinas M, Figueras M. The KH-domain RNA-binding protein StRIK from potato regulates stress- and transposable element-related genes in the periderm and affects flowering (*Unpubished manuscript*).

- Boher P, Serra O, Soler M, Molinas M, Figueras M. Comparative transcriptional profiling of cork and holm oak differentiating phellem tissues unveils molecular networks orchestrating phellem formation (*Unpubished manuscript*).

- Boher P, Sánchez A, Serra O, Soler M, Molinas M, Figueras M. Transcriptional characterization of a selected set of new cork candidate genes: mRNA abundance in phellem versus xylem tissue and effect of cork seasonal growth in their expression (*Unpubished manuscript*).

Table of abbreviations

General abbreviations

Abbreviation	Description
ABA	Abscisic acid
ABC	ATP-binding-cassette
ACP	Acyl carrier proteins
AGP	Arabinogalactan protein
APX	Ascorbate peroxidase
ATP	Adenosine triphosphate
BAHD	**B**EAT, benzyl alcohol acetyltransferase; **A**HCT, anthocyanin-O-hydroxycinnamoyltransferase; **H**CBT, anthranilate-N-hydroxycinnamoyl/benzoyltransferase; **D**AT, deacetylvindoline 4-O-acetyltransferase
BLAST	Basic Local Alignment Search Tool
BLASTN	protein (database)-nucleotide (query) BLAST
BLASTX	nucleotide (database)-nucleotide (query) BLAST
BLASTP	Protein(database)-protein (query) BLAST
bp	Base pair
BRs	Brassinosteroids
BSA	Bovine serum albumin
BWA-SW	Burrows-Wheeler Aligner, Smith-Waterman alignment
CASPL	CASPARIAN STRIP MEMBRANE DOMAIN PROTEINS-like
CAT	catalases
CAZy	Carbohydrate-Active Enzyme database
CDD	Conserved Domain Database
cDNA	complementary DNA
CE	Carbohydrate esterase
CHO	Carbohydrate
CK	Cytokinins
cm	Centimeter
cp	Cortical parenchyma
Ct	Threshold cycle
C-terminal	Carboxyl-terminal end
cv.	cultivar variant
CYPs	cytochrome P450 enzymes
d	days
DCAs	α,ω-dicarboxylic acids
DE	Differentially expressed
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E	Efficency (referred to primers)
EC	Enzyme code
EBI	European Bioinformatics Institute
EDTA	Ethylenediaminetetraacetic acid
en	Endodermis
ep	Epidermis
ER	Endoplasmic reticulum
EST	Expressed Sequence Tag
ex	exodermis
EXT	Extensins
F	ANOVA statistic
FAE	Fatty Acid Elongation complex
FARs	Fatty acyl reductases
FAS	Fatty Acid Synthetase complex
FDR	False Discovery Rate
FHT	fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase
FLA	fasciclin-like arabinogalatan proteins
FPKM	Fragments Per Kilobase Million
FT	Flowering time
fwd	Forward (referred to primers)
GA	Gibberellin

GC	Guanine and Cytosine content
GDP	Guanosine diphosphate
GFP	Green fluorescent protein
GH	Glycoside hydrolase
GO	Gene Ontology
GPATs	Glycerol-3-phosphate acyltransferases
GPX	Glutathione peroxidase
GT	Glycosyltransferase
GUS	β-glucuronidase gene
h	Hours
HCL	Hierarchical clustering
HD-ZIP	Homedomain-Leucine Zipper
IAA	Indole-3-acetic acid
IgG	Immunoglobulin G
INDEL	Insertion-deletion
JA	Jasmonic acid
kDa	kiloDalton
KEGG	Kyoto Encyclopedia of Genes and Genomes
KH	K homology (protein domain)
L.	Linnaeus
LAC	Laccase
LCFA	Long Chain Fatty Acid
LDH	Lactate dehydrogenase
LECRKs	Lectin receptors kinases
Log2FC	log2 fold-change
LRX	Leucine-rich repeat extensins
m	Months
M	Molar
MeJA	Methyl jasmonate
MEP	2-C-methyl-D-erythritol 4-phosphate
min	Minutes
miRNA	microRNA
ml	Milliliter
mm	Millimetre
mM	Millimolar
mm	Millimetre
mRNA	Messenger ribonucleic acid
MVA	Mevalonate
n	Number of replicates
nm	Nanometer
N-terminal	Amine-terminal end
N50	length of the longest contig such that all contigs of at least that length compose at least 50% of the bases of the assembly
NCBI	National Center for Biotechnology Information
NDP	Nucleoside diphosphate
ng	Nanogram
NGS	Next-Generation Sequencing
nM	Nanomolar
N-metabolism	Nitrogen metabolism
NMR	Nuclear magnetic resonance
nº	Number of items
nr	Non-redundant database
ns	not significant
nt	nucleotides
ºC	Degree Celsius
OPP	Oxidative pentose phosphate
ORF	Open Reading Frame
P	Pellet
P	Signficance value
PAL	Phenylalanine ammonia-lyase
PBS	Phosphate-buffered saline
PCA	Principal component analysis
PCD	Programmed cell death
Pc-G	Polycomb group
PCR	Polymerase chain reaction
pdm	Native periderm
PEP	phosphoenolpyruvate

PERKs	(PKR)-like ER kinase
pg	Phellogen
PGSC	Potato Genome Sequencing Consortium
PIN	PIN-FORMED proteins
pm	Phellem
PP	Prenyl diphosphate
PPi	Inorganic pyrophosphate
PPP	Pentose phosphate pathway
Pre-mRNA	Precursor mRNA
ProFHT::GUS-GFP	Construct of the *FHT* promoter fused to *GUS* and *GFP* reporter genes
PR-proteins	Pathogenesis-related proteins
Prx	Peroxidases
r	Pearson's correlation coefficient
Refseq	Reference Sequence Database
rev	Reverse (referred to primers)
RLKs	Receptor-like kinases
RNA	ribonucleic acid
RNAi	RNA interference
RNA-seq	RNA sequencing
ROS	Reactive oxygen species
rRNA	ribosomal RNA
RTA	Relative transcript abundance
RT-qPCR	Quantitative reverse-transcriptase PCR
S	Soluble
s	Seconds
SA	Salicylic acid
SAM	Shoot apical meristem
SAM cycle	S-Adenosyl methionine cycle
SCW	Secondary cell wall
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
siRNA	Small interfering RNA
SKS	Multicopper oxidase
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SPAD	Suberin (poly) aliphatic domain
SPPD	suberin a poly (phenolic) domain
SRA	Sequence Read Archive
sRNA	Small RNA
SSH	Suppression subtractive hybridization
ssp.	subspecie
t	t-student statistic
T6P	trehalose 6 phosphate
TAIR	The Arabidopsis Information Resource
TBST	TRIS-buffered saline-Tween
TC	Tentative consensus
TCA	Tricarboxylic acid
TE	Transposable elements
TE	Tracheary elements
TEM	Transmission Electron Microscopy
TF	Transcription factor
TRIS-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
UDP	Uridine diphosphate
UV	Ultraviolet
VLCFA	Very Long Chain Fatty Acid
WAKLs	Wall-Associated Kinase -Like
X-GlcA	5-bromo-4-chloro- 3-indolyl-β-d-glucuronic sodium salt
XTH	xyloglucan endotransglucosylase/hydrolases
xy	xylem
µg	microgram
µl	microlitre
µm	micrometre
ω-OHs	ω-hydroxy fatty acids

Gene abbreviations

Gene aliases	Description
AGO4	ARGONAUTE 4
4CL1	4-COUMARATE:COA LIGASE 1
A/N-INVB	ALKALINE/NEUTRAL INVERTASE B
AACT1	ACETOACETYL-COA THIOLASE 1
ABCB11/PGP11	P-GLYCOPROTEIN 11
ABCB21/PGP21	P-GLYCOPROTEIN 21
ABCB4	ATP BINDING CASSETTE SUBFAMILY B4
ABCG1	ATP-BINDING CASSETTE G1
ABCG20	ATP-BINDING CASSETTE G20
ABCG37/PDR9	PLEIOTROPIC DRUG RESISTANCE 9
ACCD	ACETYL-COA CARBOXYLASE CARBOXYL TRANSFERASE SUBUNIT BETA
ACO1	ACC OXIDASE 1
ACO4/EFE	ETHYLENE-FORMING ENZYME
ADT3	AROGENATE DEHYDRATASE 3
AFB2	AUXIN SIGNALING F-BOX 2
AGP14	ARABINOGALACTAN PROTEIN 14
AGP15	ARABINOGALACTAN PROTEIN 15
AGP18	ARABINOGALACTAN PROTEIN 18
AHK3	HISTIDINE KINASE 3
ALDH2B4	ALDEHYDE DEHYDROGENASE 2B4
ALDH3H1	ALDEHYDE DEHYDROGENASE 3H1
ALDH7B4	ALDEHYDE DEHYDROGENASE 7B4
ANAC087	ARABIDOPSIS NAC DOMAIN CONTAINING PROTEIN 87
AP1	APETALA1
ARF19	AUXIN RESPONSE FACTOR 19
ARF2	AUXIN RESPONSE FACTOR 2
ARF3	AUXIN RESPONSE FACTOR 3
ARF4	AUXIN RESPONSE FACTOR 4
ARF6	AUXIN RESPONSE FACTOR 6
ARF7	AUXIN RESPONSE FACTOR 7
ARF9	AUXIN RESPONSE FACTOR 9
ARIA	ARM REPEAT PROTEIN INTERACTING WITH ABF2
ARR1(B)	RESPONSE REGULATOR 1
ARR11(B)	RESPONSE REGULATOR 11
ARR12(B)	RESPONSE REGULATOR 1
ARR2(B)	RESPONSE REGULATOR 2
AS1	ASYMMETRIC LEAVES 1
AS2	ASYMMETRIC LEAVES 2
ASFT/RWP1/FHT	ALIPHATIC SUBERIN FERULOYL-TRANSFERASE, EDUCED LEVELS OF WALL-BOUND PHENOLICS 1
ASL1	ASYMMETRIC LEAVES 2-LIKE 1
ASN1	GLUTAMINE-DEPENDENT ASPARAGINE SYNTHASE 1
AT1G24430	HXXXD-type acyl-transferase family protein
AT2G23540	GDSL-like Lipase/Acylhydrolase superfamily protein
AT2G34930	disease resistance family protein / LRR family protein
AT2G34930	disease resistance family protein / LRR family protein
AT5G17680	disease resistance protein (TIR-NBS-LRR class), putative
AUD1	UDP-GLUCURONIC ACID DECARBOXYLASE 2
AXS2	UDP-D-APIOSE/UDP-D-XYLOSE SYNTHASE 2
BAM1	BARELY ANY MERISTEM 1
BOP2	BLADE ON PETIOLE2
BRI1	BRASSINOSTEROID INSENSITIVE 1
BRL3	BRI1-LIKE 3
BZIP11/GBF6	G-BOX BINDING FACTOR 6
C4H	CINNAMATE-4-HYDROXYLASE
CAD4	CINNAMYL ALCOHOL DEHYDROGENASE 4
CAD5	CINNAMYL ALCOHOL DEHYDROGENASE 5
CAD9	CINNAMYL ALCOHOL DEHYDROGENASE 9
CASPL1B-1	CASP-LIKE PROTEIN 1B1
CASPL1B-2	CASP-LIKE PROTEIN 1B2
CASPL1D-2	CASP-LIKE PROTEIN 1D2
CASPL1F-1	CASP-LIKE PROTEIN 1F1
CAT1	CATALASE 1

CAT1	CATALASE 1 (CAT1)
CAT2	CATALASE 2 (CAT2)
CBL9	CALCINEURIN B-LIKE PROTEIN 9 (CBL9)
CCoAOMT1	CAFFEOYL COENZYME A O-METHYLTRANSFERASE 1 (CCOAOMT1)
CCR-like3	NAD(P)-BINDING ROSSMANN-FOLD SUPERFAMILY PROTEIN
CCR-like5	NAD(P)-BINDING ROSSMANN-FOLD SUPERFAMILY PROTEIN
CesA2	CELLULOSE SYNTHASE A2 (CESA2)
CesA3	CONSTITUTIVE EXPRESSION OF VSP 1
CINV2	CYTOSOLIC INVERTASE 2 (CINV2)
CKX3	CYTOKININ OXIDASE 3 (CKX3)
CKX7	CYTOKININ OXIDASE 7 (CKX7)
CLF	CURLY LEAF (CLF)
CLV2	CLAVATA 2 (CLV2)
COB	COBRA (COB)
CPK11	CALCIUM-DEPENDENT PROTEIN KINASE 2 (CDPK2)
CRE1	WOODEN LEG (WOL)
CRY1	CRYPTOCHROME 1 (CRY1)
CSD1	COPPER/ZINC SUPEROXIDE DISMUTASE 1 (CSD1)
CSD3	COPPER/ZINC SUPEROXIDE DISMUTASE 3 (CSD3)
CslA9	ATCSLA09
CslD3	CELLULOSE SYNTHASE-LIKE D3 (CSLD3)
CYP714A	CYTOCHROME P450, FAMILY 714, SUBFAMILY A, POLYPEPTIDE 1 (CYP714A1)
CYP86A1	CYTOCHROME P450, FAMILY 86, SUBFAMILY A, POLYPEPTIDE 1 (CYP86A1)
CYP86B1	CYTOCHROME P450, FAMILY 86, SUBFAMILY B, POLYPEPTIDE 1 (CYP86B1)
DAHP	3-DEOXY-D-ARABINO-HEPTULOSONATE 7-PHOSPHATE SYNTHASE 1 (DHS1)
DIT2.1	DICARBOXYLATE TRANSPORT 2.1 (DIT2.1)
DRD1	DEFECTIVE IN RNA-DIRECTED DNA METHYLATION 1
DMS2	NUCLEAR RNA POLYMERASE D2A (NRPD2A)
DREB1A	DEHYDRATION RESPONSE ELEMENT B1A (DREB1A)
DWF1	DWARF 1 (DWF1)
EFS/SDG8	EARLY FLOWERING IN SHORT DAYS
ELF7	EARLY FLOWERING 7
ELF8	EARLY FLOWERING 8
ENO2	ENOLASE 2
ERF4	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 4 (ERF4)
ERF5	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 5 (ERF5)
ERF9	ERF DOMAIN PROTEIN 9 (ERF9)
ERS1	ETHYLENE RESPONSE SENSOR 1 (ERS1)
ETR2	ETHYLENE RESPONSE 2 (ETR2)
EXPA8	EXPANSIN A8 (EXPA8
FATB	FATTY ACYL-ACP THIOESTERASES B (FATB)
FER	FERONIA (FER)
FH8	FORMIN 8 (FH8)
FIE	FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)
FLC	FLOWERING LOCUS C (FLC)
GA2OX6	GIBBERELLIN 2-OXIDASE 6 (GA2OX6)
GER1	GDP-4-KETO-6-DEOXYMANNOSE-3,5-EPIMERASE-4-REDUCTASE 1 (GER1)
GID1B	GA INSENSITIVE DWARF1B
GID1C	GA INSENSITIVE DWARF1C
GLS1	GLUTAMATE SYNTHASE 1 (GLU1)
GLT1	PLASTIDIC GLC TRANSLOCATOR (PGLCT)
GLT1	NADH-DEPENDENT GLUTAMATE SYNTHASE 1 (GLT1)
GME1	GDP-D-MANNOSE 3',5'-EPIMERASE (GME)
GPAT6	GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE 6 (GPAT6)
GPAT8	GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE 8 (GPAT8)
GPT1	GLUCOSE 6-PHOSPHATE/PHOSPHATE TRANSLOCATOR 1 (GPT1)
GPTA5	LYCEROL-3-PHOSPHATE SN-2-ACYLTRANSFERASE 5
GSL10	GLUCAN SYNTHASE-LIKE 10
GSL3	GLUCAN SYNTHASE-LIKE 3
GSL5	GLUCAN SYNTHASE-LIKE 5
GSTF6	GLUTATHIONE S-TRANSFERASE 6
GSTU25	GLUTATHIONE S-TRANSFERASE TAU 25
GSTU7	GLUTATHIONE S-TRANSFERASE TAU 7
HD2C	HISTONE DEACETYLASE 2C
HD2C1	Histone deacetylation protein Rxt3-like
HAC1	HISTONE ACETYLTRANSFERASE OF THE CBP FAMILY 1
HAM3	HAIRY MERISTEM 3

HB-15/CNA	CORONA
HB-8	HOMEOBOX GENE 8
HCBT	N-HYDROXYCINNAMOYL/BENZOYLTRANSFERASE
HCT	HYDROXYCINNAMOYL-COA SHIKIMATE/QUINATE HYDROXYCINNAMOYL TRANSFERASE
HIRA	HOMOLOG OF HISTONE CHAPERONE HIRA
HMGR1	3-HYDROXY-3-METHYLGLUTARYL COA REDUCTASE 1
IAA16	INDOLEACETIC ACID-INDUCED PROTEIN 16
IAA26	INDOLE-3-ACETIC ACID INDUCIBLE 26
IAA27	INDOLE-3-ACETIC ACID INDUCIBLE 27
IAA9	INDOLE-3-ACETIC ACID INDUCIBLE 9
KAN1	KANADI 1
KASI	3-KETOACYL-ACYL CARRIER PROTEIN SYNTHASE I
KASIII	3-KETOACYL-ACYL CARRIER PROTEIN SYNTHASE III
KCS1	3-KETOACYL-COA SYNTHASE 1
KCS2/DAISY	3-KETOACYL-COA SYNTHASE 2
KCS20	3-KETOACYL-COA SYNTHASE 20
KNAT3	KNOTTED1-LIKE HOMEOBOX GENE 3
LAC12	LACCASE 12
LAC14	LACCASE 14
LAC3	LACCASE 3
LAC5	LACCASE 5
LACS9	LONG CHAIN ACYL-COA SYNTHETASE 9
LBD41	LOB DOMAIN-CONTAINING PROTEIN 41
LDH	Lactate/malate dehydrogenase family protein
LDL1/SWP1	LYSINE-SPECIFIC HISTONE DEMETHYLASE
LOX1	LIPOXYGENASE 1
LOX5	LIPOXYGENASE 5
LPD1 (E3)	LIPOAMIDE DEHYDROGENASE 1
LTA2 (E2)	PLASTID E2 SUBUNIT OF PYRUVATE DECARBOXYLASE
LUG	LEUNIG
M3/MAT3	METHIONINE ADENOSYLTRANSFERASE 3
MEE32	MATERNAL EFFECT EMBRYO ARREST 32
MFS	Major facilitator superfamily protein
MPDC2	GHMP kinase family protein
MS1	METHIONINE SYNTHESIS 1
MS2	METHIONINE SYNTHASE 2
MYB102	MYB-LIKE 102
MYB4	MYB DOMAIN PROTEIN 4
MYB52	MYB DOMAIN PROTEIN 52
MYB68	MYB DOMAIN PROTEIN 68
MYB93	MYB DOMAIN PROTEIN 93
MYC2/JAI1	MYC2, JASMONATE INSENSITIVE 1
NAC038	NAC DOMAIN CONTAINING PROTEIN 38
NAC058	NAC DOMAIN CONTAINING PROTEIN 58
NAC1	NAC DOMAIN CONTAINING PROTEIN 1
NADP-ME4	NADP-MALIC ENZYME 4
NAP1	NAP1-RELATED PROTEIN 1
NCED1	CAROTENOID CLEAVAGE DIOXYGENASE 1
NEK6	NIMA (NEVER IN MITOSIS, GENE A)-RELATED 6
OMT1	O-METHYLTRANSFERASE 1
OST1	OPEN STOMATA 1
PAL1	PHE AMMONIA LYASE 1
PAL2	PHENYLALANINE AMMONIA-LYASE 2
PAL3	PHENYLALANINE AMMONIA-LYASE 3
PAL4	PHENYLALANINE AMMONIA-LYASE 4
PDC	Thiamine pyrophosphate dependent pyruvate decarboxylase family protein
PDC2	PYRUVATE DECARBOXYLASE-2
PDH (E1α)	PYRUVATE DEHYDROGENASE E1 ALPHA
PDH (E1β)	PYRUVATE DEHYDROGENASE E1 BETA
PFK3	PHOSPHOFRUCTOKINASE 3
PFK5	PHOSPHOFRUCTOKINASE 5
PFK7	PHOSPHOFRUCTOKINASE 7
PFP-α	Phosphofructokinase family protein
PKL	PICKLE
PIE	PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1
PIN3	PIN-FORMED 3

PIN-LIKES 3	Auxin efflux carrier family protein
PK	Pyruvate kinase family protein
PKp1	PLASTIDIAL PYRUVATE KINASE 1
PMT5	POLYOL/MONOSACCHARIDE TRANSPORTER 5
PP2CA	PROTEIN PHOSPHATASE 2CA
PPT1	PHOSPHATE/PHOSPHOENOLPYRUVATE TRANSLOCATOR
Prx11	Peroxidase superfamily protein
Prx20	Peroxidase superfamily protein
Prx52	Peroxidase superfamily protein
Prx73/RHS19	Peroxidase superfamily protein, ROOT HAIR SPECIFIC 19
RAP2.12	RELATED TO AP2 12
RAP2.2	RELATED TO AP2 2
RAP2.3/EBP	RELATED TO AP2 3, ETHYLENE-RESPONSIVE ELEMENT BINDING PROTEIN
RAP2.4	RELATED TO AP2 4
REV	REVOLUTA
RIK	RS2-INTERACTING KH PROTEIN
SAD6	STEAROYL-ACYL CARRIER PROTEIN Δ9-DESATURASE6
SAM1	S-ADENOSYLMETHIONINE SYNTHETASE 1
SAM2	S-ADENOSYLMETHIONINE SYNTHETASE 2
SAP18	SIN3 ASSOCIATED POLYPEPTIDE P18
SCR	SCARECROW
SEF1	SERRATED LEAVES AND EARLY FLOWERING
SMT1	STEROL METHYLTRANSFERASE 1
SOC1	SUPPRESSOR OF OVEREXPRESSION OF CO 1
SP1L5	SPIRAL1-LIKE5
STM	SHOOT MERISTEMLESS
STP14	SUGAR TRANSPORT PROTEIN 14
SUC3/SUT2	SUCROSE TRANSPORTER 2
SUC4/SUT4	SUCROSE TRANSPORTER 4
SUD3	UDP-XYL synthase 6 (UXS6)
SUS3	SUCROSE SYNTHASE 3
SUS4	SUCROSE SYNTHASE 4
SVP	SHORT VEGETATIVE PHASE
SWN1	SWINGER
THE1	THESEUS1
TIR1	TRANSPORT INHIBITOR RESPONSE 1
TPS6	TREHALOSE -6-PHOSPHATASE SYNTHASE S6
TPS9	TREHALOSE -6-PHOSPHATASE SYNTHASE S9
UGD3	UDP-GLUCOSE DEHYDROGENASE 3
UGE5	UDP-D-GLUCOSE/UDP-D-GALACTOSE 4-EPIMERASE 5
ULT1	ULTRAPETALA1
VIP3	VERNALIZATION INDEPENDENCE 3
VIP4	VERNALIZATION INDEPENDENCE 4
VRN2	REDUCED VERNALIZATION RESPONSE 2
WOX4	WUSCHEL RELATED HOMEOBOX 4
WRI1	WRINKLED 1
WRKY43	WRKY DNA-BINDING PROTEIN 43
WRKY56	WRKY DNA-BINDING PROTEIN 56
WRKY65	WRKY DNA-BINDING PROTEIN 65
XTH22	XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 22
XTH23/XTR6	XYLOGLUCAN ENDOTRANSGLYCOSYLASE 6
XTH27/EXGT-A3	ENDOXYLOGLUCAN TRANSFERASE A3
XTH28	XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 28
XTH30	XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 28
XTH5	XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 5
XTH9	XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 9

List of figures

1. Scientific background

3. Results

Supplemental data

List of tables

3. Results

Supplemental data

(●) Supplementary materials that for space reasons are not included in the printed version of this thesis can be found at the end of the document in electronic format (CD-ROM attached).

Table of contents

Summary

The periderm is a complex structure essential to protect plant secondary body and wounded tissues against water loss, solar irradiation and pathogens. This barrier function is achieved by the phellem (cork) layer, the external multilayered tissue of the periderm formed by dead cells made impervious by suberin deposition into cell walls. Despite the critical role of phellem for plants survival in the terrestrial environment, the knowledge of the molecular processes involved in its formation is yet very limited.

During the past decade, several transcriptomic and proteomics studies as well as reverse genetic approaches have been performed to unravel the molecular genetics of periderm formation and suberin synthesis. Among these studies, the potato FHT (fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase) gene was revealed as an essential player for the sealing properties of potato tuber periderm and suberin synthesis. Notwithstanding, most of the above mentioned strategies were mainly focused to identify the molecular basis of suberin synthesis, remaining elusive other key aspects of phellem biogenesis such as primary metabolism, meristem regulation or phytohormone signaling.

To obtain more profound insights on the role of *FHT* we produced a polyclonal FHT antibody and transgenic potato plants bearing the *FHT* promoter fused to reporter genes. Our work provides evidences that *FHT* is expressed in cells undergoing suberization and that is induced by wounding and abscisic acid, whereas salicylic acid represses FHT. Singularly, the FHT accumulation in the periderm is restricted to the phellogen derivative cells with phellem identity and the protein levels are maintained for several months after harvest, suggesting a possible mechanism for the maintenance of phellem apoplastic barrier once tuber growth ceases. The cytosolic localization of FHT is surprising considering the hydrophobic nature of its substrates.

To contribute to the better understanding of periderm regulation we performed a first approach to the function of the potato K homology (KH)-domain RNA-binding protein *RIK* (*RS2-INTERACTING KH PROTEIN)*. Transcript profile of *StRIK* gene indicates that is ubiquitously expressed and induced by wounding in tubers. Transcriptome analysis of *StRIK* downregulated transgenic lines suggests that it is involved in the regulation of stress-inducible genes and transposable element-related genes in the

periderm. In addition, *StRIK* silenced lines frequently showed floral transition in agreement with additional roles in other plant tissues.

To widen the knowledge of phellem formation, we performed a global transcriptome analysis by RNA-seq of the outer bark of the cork oak and holm oak, yielding a final assembly of 16,865 contigs. Functional analyses of these sequences allowed us to obtain a comprehensive view of the biochemical and regulatory pathways induced during phellem formation. The comparison between cork oak and holm oak bark transcriptomes highlights genes upregulated in cork oak involved in cell wall formation, cell growth, primary metabolism and suberin accumulation. Similarly, genes related with meristem maintenance, auxin transport and ethylene metabolism and signaling are also more induced in cork oak. On the other hand, ABA signaling, biotic and abiotic stress responses and regulation are the main functional categories upregulated in holm oak.

To better understand the function of some of the candidate genes identified we further analysed their expression patterns. Genes involved in the biosynthesis of fatty acid, isoprenoids, suberin and some NAC and R2R3-MYB transcription factors are upregulated in cork versus wood, whereas they show similar transcript accumulation of genes related to meristem and phytohormones. The transcript profiles in cork during the growing season evidence that genes related with cell wall and suberin metabolism, oxidative stress, ethylene and gibberellin receptors show the highest expression in June, when cork growth rate is supposed to be maximal. On the other hand, most of the genes involved in the regulation of meristem development, including an auxin transporter and a cytokinin receptor, are induced in April, when phellogen activity initiates.

As a whole this work provides a better understanding of the periderm biology. The clarification of FHT roles in suberized tissues, the effects of *StRIK* silencing in tuber periderm transcriptome and the molecular networks unveiled by the transcriptomics approaches in the outer bark of cork and holm oak, provide valuable information for future functional studies in phellem tissue that, ultimately, might be useful for breeding programs addressed to improve the skin quality of potatoes and commercial cork production.

Resum

La peridermis és una estructura complexa, essencial per la protecció dels òrgans vegetals madurs (secundaris) i els teixits cicatricials contra la perduda d'aigua, la radiació solar i els patògens. Aquesta funció barrera de la peridermis es dóna gràcies al fel·lema (súber), el teixit pluriestratificat de cèl·lules mortes amb parets suberificades situat a la seva part externa. Tot i que el fel·lema té un paper crític per la supervivència de les plantes terrestres, el coneixement sobre les bases moleculars que controlen la seva formació és avui dia encara molt limitat.

Durant l'última dècada s'han realitzat varis estudis a nivell del transcriptoma i el proteoma així com diverses aproximacions de genètica inversa per tal de desxifrar els mecanismes genètics responsables de la formació de la peridermis i la síntesis de suberina. D'entre aquests estudis, el gen FHT (de l'anglès, *fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase*) va ser caracteritzat per tenir un paper clau en la funció barrera de la peridermis del tubercle de patata i la síntesis de suberina. No obstant això, la majoria d'aquestes aproximacions estaven centrades principalment en la identificació de les bases moleculars de la síntesis de suberina, deixant sense resoldre altres aspectes claus de la biogènesis del fel·lema com ara el metabolisme primari, la regulació del meristema i la senyalització hormonal.

Per tal d'aprofundir en el coneixement de la funció de FHT, vam produir un anticòs policlonal i plantes transgèniques transformades amb la construcció del promotor de *FHT* fusionat a dos gens delators. El nostre treball evidencia que *FHT* s'expressa en cèl·lules sotmeses a processos de suberificació, i que *FHT* s'indueix per ferida i àcid abscísic, mentre que és reprimit per àcid salicílic. De forma singular, l'acumulació de FHT es troba restringida a les cèl·lules derivades del fel·logen amb identitat de fel·lema i els nivells de la proteïna es mantenen durant varis mesos després de la recol·lecció dels tubercles, el que suggereix un mecanisme per mantenir la funció barrera una vegada el tubercle deixa de créixer. La localització citosòlica de FHT és sorprenent considerant el caràcter hidrofòbic dels seus substrats.

Per tal de contribuir a una millor comprensió de la regulació de la periderma es va realitzar una primera aproximació a la funció de la proteïna d'unió a ARN amb domini KH, *StRIK (RS2-INTERACTING KH PROTEIN)*. El perfil d'expressió indica que el gen s'expressa de forma ubiqua i és induït per ferida en tubercles. L'anàlisi del

transcriptoma de les línies transgèniques silenciades pel gen *StRIK* suggereix que està involucrat en la regulació de gens induïbles per estrès i gens relacionats amb la transposició al fel·lema. A més, les línies *StRIK* silenciades eventualment mostren transició floral, indicant rols addicionals de *StRIK* en altres teixits vegetals.

Per tal d'ampliar els coneixements sobre la formació del fel·lema, vam realitzar una anàlisi global del transcriptoma de l'escorça externa de l'alzina surera (*Quercus suber*) i l'alzina (*Quercus ilex*), generant un total de 16,865 *contigs*. L'anàlisi funcional d'aquestes seqüències ens ha permès obtenir un visió més completa de les vies bioquímiques i reguladores induïdes durant la formació del fel·lema. La comparativa entre els transcriptomes de l'alzina surera i l'alzina mostra que els gens més expressats en l'alzina surera estan relacionats amb la formació de la paret cel·lular, el creixement cel·lular, el metabolisme primari i l'acumulació de suberina. De forma semblant, els gens relacionats amb el manteniment del meristema, el transport d'auxines i el metabolisme i senyalització de l'etilè també estan més induïts a l'alzina surera. Per altre banda, la senyalització de l'àcid abscísic, la resposta a estrès biòtic i abiòtic i la regulació en general, són les principal categories funcionals que estan més induïdes a l'alzina.

Amb la finalitat de contribuir a un millor coneixement d'alguns dels gens identificats vam estudiar amb més detall el seu patró d'expressió. Gens relacionats amb la síntesis d'àcids grassos, isoprenoides, suberina i alguns factors de transcripció de la família NAC i R2R3-MYB estan més induïts en el fel·lema de l'alzina surera en comparació al xilema, mentre que els gens de meristema i hormones presenten nivells de transcrit semblants. Els perfils d'expressió durant el creixement estacional del fel·lema evidencien que els gens de paret cel·lular, suberina, estrès oxidatiu i els receptors d'etilè i gibberel·lines presenten el seu màxim d'expressió al Juny, el mes on hi ha la taxa màxima de creixement del fel·lema. Per altra banda, la majoria de gens relacionats amb el desenvolupament de meristema, inclòs un transportador d'auxines i un receptor de citoquinines estan més induïts a l'Abril, quan s'inicia l'activitat del fel·logen.

En conjunt aquest treball proporciona un millor coneixement de la biologia de la peridermis. La major comprensió del paper de FHT en els teixits suberitzats, els efectes del silenciament de *StRIK* en el transcriptoma de la periderma dels tubercles i les xarxes moleculars descobertes mitjançant les aproximacions transcriptòmiques en l'escorça

externa de l'alzina surera i l'alzina proporcionen informació valuosa per futurs estudis funcionals en el fel·lema que, en última instància, podria ser útil per a programes de millora genètica adreçats a millorar la qualitat de la pell del tubercle de patata i la producció del suro.

Resumen

La peridermis es una estructura compleja, esencial para la protección de los órganos vegetales maduros (secundarios) y los tejidos cicatriciales contra la pérdida de agua, la radiación solar i los patógenos. Esta función barrera de la peridermis se da gracias al felema (súber), el tejido pluriestratificado de células muertas y con paredes suberificadas que se encuentra en su parte externa. A pesar de que el felema tiene un papel crítico para la supervivencia de las plantas terrestres, el conocimiento sobre las bases moleculares que controlan su formación es hoy en día aún muy limitado.

Durante la última década se han realizado varios estudios a nivel de transcriptoma y del proteoma así como diversas aproximaciones de genética reversa con el fin de descifrar los mecanismos genéticos responsables de la formación de la peridermis y la síntesis de suberina. De entre estos estudios, el gen *FHT* (del inglés, *fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase*) fue caracterizado por tener un papel clave en la función barrera de la peridermis del tubérculo de patata y la síntesis de suberina. No obstante, la mayoría de estas aproximaciones estaban centradas principalmente en la identificación de las bases moleculares de la suberina, dejando sin resolver otros aspectos claves de la biogénesis del felema como el metabolismo primario, la regulación del meristemo y la señalización hormonal.

Para mejorar el conocimiento de la función de FHT, producimos un anticuerpo policlonal y plantas transgénicas transformadas con la construcción del promotor de *FHT* fusionado a dos genes delatores. Nuestro trabajo evidencia que *FHT* se expresa en células sometidas a procesos de suberificación, i que *FHT* es inducido por herida y ácido abscísico, mientras que es reprimido por ácido salicílico. De forma particular, la acumulación de FHT se encuentra restringida en las células del felógeno con identidad de felema y los niveles de la proteína se mantienen durante varios meses después de la recolección de los tubérculos, lo que sugiere un mecanismo para el mantenimiento de la función barrera una vez el tubérculo deja de crecer. La localización citosólica de FHT es sorprendente ya que los sustratos de esta enzima son hidrofóbicos.

Para contribuir a una mayor comprensión de la regulación de la peridermis realizamos una primera aproximación a la función de la proteína de unión a ARN con un dominio KH, *StRIK (RS2-INTERACTING KH PROTEIN)*. El perfil de expresión indica que el gen se expresa de forma ubicua i es inducida por herida en tubérculos. El análisis del

transcriptoma de las líneas transgénicas silenciadas por el gen *St*RIK sugiere que está involucrado en la regulación de genes inducibles por estrés i genes relacionados con la transposición en el felema. Además, las líneas *StRIK* silenciadas eventualmente muestran transición floral, indicando roles adicionales de *StRIK* en otros tejidos vegetales.

Con la finalidad de ampliar los conocimientos sobre la formación del felema, realizamos un análisis global del transcriptoma de la corteza externa del alcornoque (*Quercus suber*) y la encina (*Quercus ilex*), generando un total de 16,865 *contigs*. El análisis funcional de estas secuencias nos ha permitido obtener una visión más completa de las vías bioquímicas y reguladoras inducidas durante la formación del felema. La comparativa entre los transcriptomas de alcornoque y encina muestra una mayor expresión en el alcornoque de genes relacionados con la formación de la pared celular, el crecimiento celular, el metabolismo primario y la acumulación de suberina. De forma similar, los genes relacionados con el mantenimiento del meristemo, el transporte de auxinas y el metabolismo y señalización del etileno estan más inducidos en el alcornoque. Por el contrario, la señalización mediada por ácido abscísico, la respuesta a estrés biótico y abiótico y la regulación en general, son las principales categorías funcionales que se encontraban más inducidas en la encina.

Con el fin de contribuir a un mejor conocimiento de alguno de los genes identificados estudiamos con más detalle su patrón de expresión. Genes relacionados con la síntesis de ácidos grasos, isoprenoides, y algunos factores de transcripción de la familia NAC y R2R3-MYB estan más inducidos en el felema del alcornoque en comparación con el xilema, mientras que presentaban niveles de acumulación de transcrito parecidos para los genes de meristemo y hormonas. Los perfiles de expresión durante el crecimiento estacional del felema evidenciaron que los genes de pared celular, suberina, estrés oxidativo, y los receptores de etileno y giberelinas presentan su máximo de expresión en Junio, el mes en que se produce un mayor crecimiento del felema. En cambio, la mayoría de los genes relacionados con el desarrollo del meristemo, incluidos un transportador de auxinas y un receptor de citoquininas estan más inducidos en Abril, cuando se inicia la actividad del felógeno.

En conjunto este trabajo proporciona un mejor conocimiento de la biología de la peridermis. La mayor comprensión del papel de FHT en los tejidos suberificados, los

efectos del silenciamiento de *StRIK* en el transcriptoma de la peridermis de los tubérculos y las redes moleculares descubiertas mediante las aproximaciones transcriptómicas en el alcornoque y la encina, proporcionan una información valiosa para futuros estudios funcionales en el felema que, en última instancia, podría ser de utilidad para programas de mejora genética dirigidos a mejorar la calidad de la piel del tubérculo de patata y la producción del corcho.

Scientific background

1. Scientific background

1.1. Periderm: a plant barrier developed in secondary organs and wounded tissues

The evolutionary transition of plants from water to land occurred about 450 million years ago. When plants conquered the terrestrial environment they were faced with competing demands so several structural innovations were developed to adapt their existence in land. Among them, essential adaptations were the formation of supportive tissues (collenchyma and sclerenchyma) which allows plants to stand up against gravity, the development of conducting systems (xylem and phloem) to effectively transport fluids, and the evolution of anchorage and absorption organs for water and nutrients uptake from soil (root). Additionally, effective evolutionary strategies were developed in plant aerial parts to cope with the ever changing water potential gradient between the plant body and the surrounding environment to prevent an uncontrolled loss of water that may cause lethal dehydration. Therefore the need of waterproofing barriers for plants survival to prevent desiccation is self-evident (Lendzian, 2006). In all primary aerial surfaces of ferns and seed plants this is achieved by the cuticle, a lipophilic and non-cellular layer which seals the epidermis of aerial organs such fruits, leaves, primary stems and flowers. In addition, the vital gas exchange of CO_2 and O_2, essential for photosynthesis and respiration, and the controlled water evaporation, is facilitated by stomata which concertedly evolved with cuticles (Edwards *et al.*, 1998). Nevertheless, either during the secondary growth of stem or upon wounding, the complex formed by the epidermis, cuticle and stomata become damaged or destroyed and is never regenerated but it is replaced by a completely new structure which is the periderm.

The periderm has a secondary origin because it arises from a new lateral meristem usually formed within the outer part of cortex named the phellogen or cork cambium. The phellogen generates the multilayered phellem (cork) tissue centrifugally and the phelloderm centripetally (Lendzian, 2006). The cork cell layer is the responsible of the periderm barrier function because cork cells walls are made impervious to moisture and gases by suberin depositions. The phelloderm is a tissue which resembles the parenchymatous cells from the cortex or phloem and their cells are the inner derivatives of phellogen. The complex formed by the phellem, phellogen and phelloderm constitutes the periderm (Fig. 1.1). Lenticels are aerenchymatous areas interspersed in

the phellem used as the new paths for the gas exchange between the inside of plant secondary body and the atmosphere. Hence, plants similarly cope with the problems associated with vital gas exchange during either the primary or secondary growth by the cuticle-epidermis-stomata or the phellem-lenticels complexes respectively.

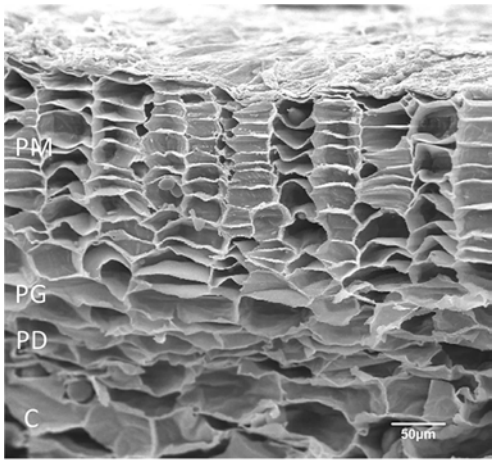

Fig. 1.1. Structure of the periderm of potato tuber. Scanning electron microscope image extracted from Serra (2008) showing the layers of the periderm: phellem (PM), phellogen (PG) and phelloderm (PD), and cortical parenchyma (C). Bar = 50 µm.

1.1.1. *Periderm occurrence*

Periderm formation is a common biological process occurring in roots and stems of woody angiosperms and gymnosperms and tubers. Periderm might also occur in the oldest parts of some eudicots herbs and in some monocots. Periderm is also generated in the exposed surfaces of the abscission zones of plants, and in the development of protective coats close to necrosed or wounded tissues in response to mechanical wounding or parasite attack forming a special periderm, denominated wound periderm or wound cork. Another type of periderm is found in the surface of fruits such as apples and pears, which develop the so called russeting phenotype which results from the replacement of the outer layers of the fruit partially or entirely over its surface by a periderm (Evert, 2006).

1.1.2. *Periderm development*

The phellogen can develop from different cell types such as living epidermal, collenchyma or parenchyma cells that eventually dedifferentiate becoming meristematic and undergo periclinal divisions. The first periclinal division resulted in two cells; the inner is the phelloderm which usually does not divide anymore. The outer cell undergoes a second periclinal division resulting in two cells, the outer differentiates into the phellem (cork) and the inner cell constitutes the phellogen initial which continues to divide by periclinal division but occasionally also performs anticlinal divisions in a

manner that the circumference layer of phellem is continuously increased (Fahn, 1967). Because phellogen development is asymmetrical, the phellem growth usually is much higher than the phelloderm, hence is formed by more layers of cells. The number of phellem cells produced in each season is variable among different species.

The phellem cells are characterized by having a prismatic shape, suberized walls and compactly arranged in radial rows lacking intercellular spaces. The recently divided phellem cells after mitoses have thin primary walls which enable cells to expand. However, during maturation, a thick layer of suberin is deposited on the walls which finally end with the death of the cells. In this process, the protoplast of the phellem cells is lost and the cell lumen becomes filled with air or pigmented substances (Fahn, 1967). Two common types of phellem cells are those which are hollow, thin-walled and somewhat widened radially, and those which are thick-walled and radially flattened (Evert, 2006).

With the continuation of secondary thickening the first periderms are replaced by more internal periderms. Then, every later-formed periderm develops deeper in the cortex or primary phloem or even deeper within the secondary phloem if secondary thickening persevered. Considering the ontogeny of the first-formed periderm two types of formation of subsequent periderms can be distinguished. When the first phellogen is formed in inner layers the additional periderms usually form a complete cylinder (e.g. *Vitis*), while in plants where it is formed in outer layers of cortex or in the epidermis, the additional periderms develop displaying a scale or shell shape (e.g. *Pinus*) (Fahn, 1967). Moreover, at some point the outer layers of periderms are sloughed off so the thickness of the remaining periderms on a plant is almost constant. However in some plants such *Quercus suber* and *Aristolochia* thick cork layers can be accumulated on the stems surface (Fahn, 1967).

The cadence of subsequent periderm formation is very variable among species. While for certain genera the additional periderms develop in the first year of growth of the stem, in species such apple and pear trees it might last up to eight years of stem growth until subsequent periderms are developed. In a few species of *Populus* and *Prunus* the first phellogen keeps active for twenty or thirty years, and in *Quercus suber* and some other species the first phellogen is active throughout the plant lifespan or for many years and displays seasonal differences in the type of phellem cells produced which appeared

as annual growth rings of phellem (Fahn, 1967). At the start of the growing season the phellogen is highly active and thin-walled phellem cells are produced, while by the end of the season cell divisions become infrequent and thick-walled phellem cells are formed.

1.1.3. *Control of periderm initiation and cambial and phellogen activities relationship*

Numerous factors seem to be involved in the regulation of the origin and development of phellogen. Inducing factors affecting phellogen initiation include the exposure of inner tissues to air, increased sap pressure, photoperiod, thermoperiod, tissue water potential, oxygen pressure, low light intensity and several growth regulating substances (Waisel, 1995). Interestingly, Borger and Kozlowski (1972) showed that when leaves of green ash were excised, immediately after their emergence, the seedling failed to develop a periderm indicating that initiation of phellogen depended on foliar products. Moreover, the formation of periderm in the stems of woody plants is delayed in comparison to that of the secondary vascular tissues. Investigations performed by Whitmore (1962) reported a positive correlation between xylem:bark ratios and initiation of phellogen. Accordingly it was suggested that the internal mechanical pressure exerted in the bark by the growing secondary xylem might be a primary cause for phellogen initiation. In addition, while the annual rhythm of phellogen activity was found positively correlated with the rhythm of cambium in four oaks species (*Quercus boissieri*, *Q. ithaburensis*, *Q. calliprinos* and *Q. suber*) (Ephrat, 1971), studies performed in other species such *Robinia* or *Acacia* showed that the activities of phellogen and cambium were not interdependent (Arzee *et al.*, 1970). Despite both meristems contribute to the radial growth, the average rate of phellogen activity is much slower than the rate of cambium activity (Waisel, 1995).

1.1.4. *Morphology of rythidome, a succession of periderms and internal dead tissues*

Rythidome (outer bark) formation occurs by the successive development of periderms deeper in the living tissues of plant axis due to the continuation of secondary thickening. All tissues exterior to the most recent periderm become sealed off from water and nutrients supply and subsequently die (Howard, 1977). Hence, dead tissues are accumulated on the periphery and constitute the rythidome or outer bark, which

increases its thickness by further addition of cork layers with enclosed cavities of dry cortical and phloem tissues (Fahn, 1967). Then, the term *rythidome* (outer bark) included all tissues external to the innermost phellogen and should be distinguished from the term *bark* which refers to all tissues external to the vascular cambium. The living tissues between the rythidome and the vascular cambium form the *inner bark* which is composed of functional secondary phloem and fibers. When the first periderm is formed close to the stem surface, a small amount of primary tissues is cut off and therefore no rythidome is considered to be formed (Fig. 1.2A). In plants in which the first periderm is formed deeper in the stem axis, thicker layers of tissues connected to cork remain on the stem surface and therefore exhibit rythidome morphology (Fig. 1.2B) (Fahn, 1967).

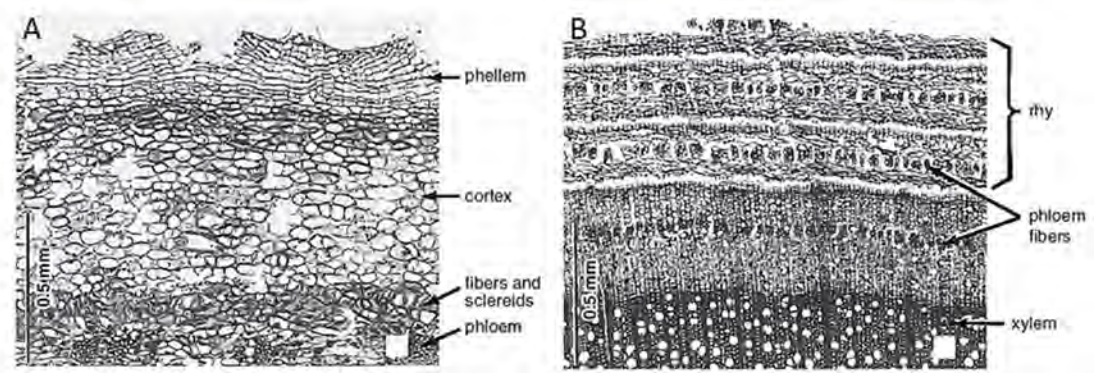

Fig. 1.2. Detail of periderm and rythidome in transverse sections of stems extracted from Evert (2006). (A) Phellem with cracks from *Talauma*. (B) Rythidome (rhy) from *Lonicera tatarica* with layers of periderm alternate with layers derived from secondary phloem and phloem fibers.

1.1.5. *Closing layer and wound periderm formation*

Wounding induces metabolic and related cytological responses that direct, under favorable conditions, the fulfillment of the wound closure (Bostock and Stermer, 1989). The ultrastructural changes evident in cells bordering the injured cell layer indicate intense transcriptional, translational and secretory activities (Evert, 2006). The sequence of events that occurs during wound healing in the stems of gymnosperms and woody angiosperms is similar to that exhibited by a wounded potato tuber, probably the most extensively studied object in this regard (Schreiber *et al.*, 2005). In the wound response, periderm formation is preceded by the sealing of the newly exposed surface by an impervious layer of cells named the closing or boundary layer. Closing layer originates from cells present at the time of wounding and it is formed immediately below the dead

cells at the surface of the wound. A first obvious response is the deposition of callose at plasmodesmata on the boundary cells walls next to the damaged cells, thus sealing symplasmic connections at this interface. Primary walls and middle lamella become lignified followed by suberization of the walls through the deposition of suberin lamella over the inner surfaces of walls. The ligno-suberized closing layer provides impervious barrier to water and pathogen invasion of the inward living tissues and facilitates the optimal conditions for wound periderm formation. Then, periclinal divisions take place beneath the closing layer to initiate the wound phellogen. Newly developed cork cells are distinguished from the closing layer by their radial alignment within the wound periderm (Fig. 1.3). Development of wound periderm is important for the storage life of potatoes and other horticultural products or when plant explants are cut for propagation (Evert, 2006). Wound periderm formation is notably influenced by environmental factors such temperature and humidity (Artschwager, 1923; Artschwager and Starrett, 1931).

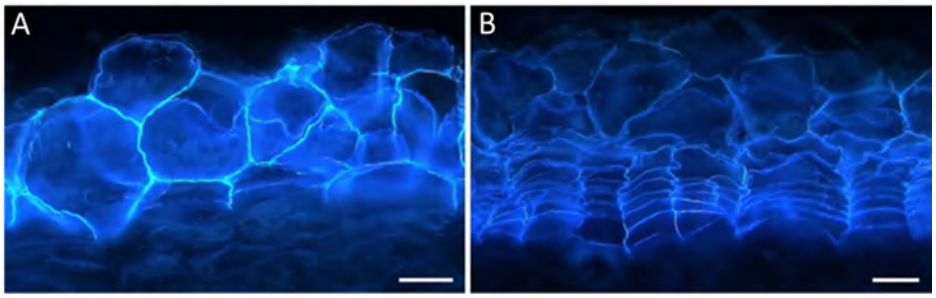

Fig. 1.3. Detail of wounded-tuber closing layer after 5 days (d) of wound-healing and after 56 d of wound-healing, extracted from Neubauer *et al.* (2012). (A) The 5 d time point includes only the closing layer formation while (B) 56 d also include the accumulation of layers of phellem cells (files of rectangular cells). Sections observed under UV (A, B) showing the suberin autofluorescence. Bar = 5 µm.

1.1.6. *Lenticels*

Lenticels are differentiated structures of the periderm that are characterized by a loose arrangement of cells that facilitate gaseous exchange and thus, provide a pronounced permeability to water, oxygen, and carbon dioxide (Lendzian, 2006). Lenticels develop beneath the stomata on the plant axes concurrently with the first periderm (Waisel, 1995). Then, a periclinal dividing meristem, the lenticel phellogen, is stablished. Outwardly the lenticel phellogen produces the complementary or filling cells and once these cells have increased in number, they push towards the epidermis until it is fractured (Waisel, 1995). The main differences of the complementary tissue compared

to the phellem adjacent cells are the presence of intercellular spaces, the thinner walls and the more radially elongated cell shape. Lenticels usually are formed by the nonsuberized complementary tissue alternated with compact closing layers of suberized cells. An experiment in *Betula* periderms showed that lenticels permeance to water and oxygen vary along the seasons suggesting that structural changes in the closing layer and filling tissue of lenticels are somehow regulated to modulate vital gas exchange (Lendzian, 2006). An example of seasonal development in lenticels is the Norway spruce in which the maximum permeability of lenticels coincides with the full active period of wood production (Kartusch and Rosner, 2003).

Although lenticels are usual components of stems and roots periderms (Fig. 1.4), in some cases they occur on the surface of leaves on certain species (the so-called 'cork warts'), and also on the surface of some fruits such apples or pears (Evert, 2006). Lenticels occurrence is an agronomic important trait for tuber storage and quality and for the production of cork stoppers obtained from the bark of *Quercus suber*.

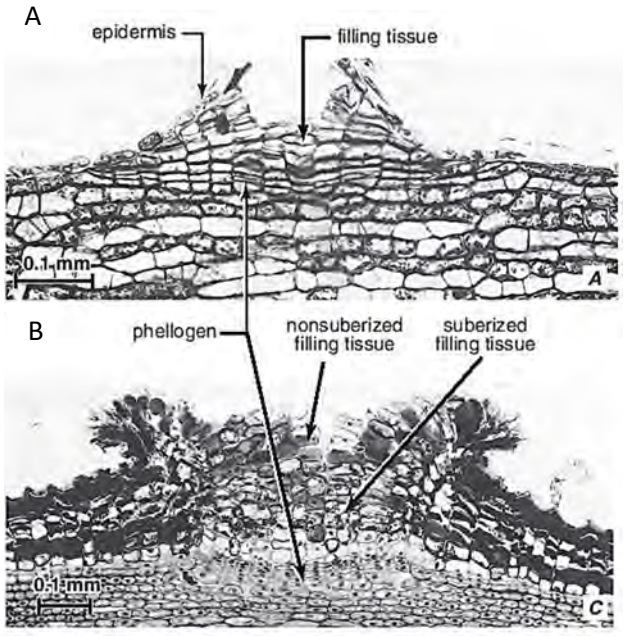

Fig. 1.4. Detail of lenticels in transverse sections of stems, extracted from Evert (2006). (A) Young lenticel of avocado (*Persea americana*) with no closing layer present. (B) Lenticel of elderberry (*Sambucus canadensis*) with compact layer of suberized cells interior to loosely structured nonsuberized filling tissue.

1.2. Plant models for periderm studies

Despite periderm formation is a common feature of all higher plants, most of the knowledge about periderm and suberin biosynthesis has been mainly obtained by studies in cork oak tree and potato plant. The bark of the cork oak tree (*Quercus suber L.*) and the skin of potato (*Solanum tuberosum*) tubers are the paradigms of suberized

tissues because their phellem cells synthesize large amounts of suberin which can be isolated from adjacent tissues for their analysis (Fig. 1.5).

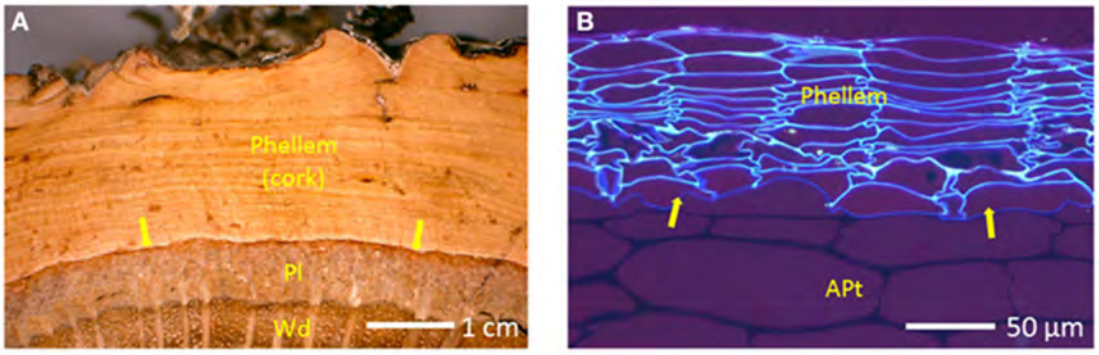

Fig. 1.5. Detail of the bark of cork oak (*Quercus suber)* tree and potato (*Solanum tuberosum*) tuber periderm, the principal models for periderm studies, extracted form Graça (2015). (A) Outer tissues of *Q. suber* trunk, displaying the phellem (*the cork*), the phellogen (lateral meristem, arrows) and the phelloderm (not seen); *Pl*, phloem; *Wd*, xylem (wood). (B) Outer tissue of potato tuber, displaying the phellem (suberized tissues), phellogen (arrows) and phelloderm (not seen); *Apt*, amylaceous parenchyma tissue.

Cork used as a commercial material for bottle cork or isolating boards comes from the cork oak trees which are native from the Mediterranean region. Cork consists of thin-walled cells, dead at maturity and with a hollow lumens filled with air. The features that give commercial value to cork are its imperviousness to gases and liquids and its strength, elasticity and lightness (Fahn, 1967). The chemical composition of the cork tree bark has been widely analysed (Silva *et al.*, 2005). On average it contains 63% of suberin and 15% of waxes and tannins (Pereira, 1988). Cork oak tree has a phellogen that is formed by a continuous layer which encircles the stems and branches, and produces an external layer of cork tissue. In the first year of growth, the first phellogen arises in the cell layer just beneath the epidermis (Graça and Pereira, 2004). The first cork obtained from the cork oak tree has little commercial value. When the cork tree is about twenty years old, the first periderm is debarked and a new phellogen arises from the cortex just few millimeters below the site where the first periderm was formed. Cork produced by the new formed phellogen grows very rapidly, and after nine years it can be stripped off to obtain commercial cork (Costa *et al.*, 2001). This process can be repeated about every nine-year until the tree is about 150 years old. After successive strippings, the new phellogen arises from nonconducting phloem tissue. Essentially, cork oak is the canonical model for cork because each year it can produce about 60 layers of almost pure cork cells with high amounts of suberin and forms a raw material with industrial value. However molecular functional studies using cork oak tree are

impaired due its low efficiency transformation and its long regeneration time (Álvarez-Fernández and Ordás, 2012).

Potato (*Solanum tuberosum*) tubers are underground storage organs covered by the skin (periderm) that protects the inner flesh from dehydration and pathogens. The potato tuber periderm has been widely studied because it is a crop of great agronomic importance (Schmidt and Schönherr, 1982; Vogt *et al.*, 1983; Lulai and Freeman, 2001; Sabba and Lulai, 2002). Potato tubers are an outstanding model for periderm functional studies because transgenic potatoes can be produced in a reasonable time and enough amounts of periderm can be obtained for ultrastructural, chemical and physiological studies (Serra *et al.*, 2009*a*,*b*, 2010*b*). Moreover, the release of the potato genome (Potato Genome Sequencing Consortium; Xu *et al.*, 2011) has further promoted the use of this plant for molecular and functional genetic studies. Aliphatic suberin has been widely analysed in native potato periderm (Kolattukudy and Agrawal, 1974; Graça and Pereira, 2000; Schreiber *et al.*, 2005) but potato plant has also been used as a well-defined model for suberized plant tissues in general and many studies have described the formation of wound-induced periderm in potato tubers (Schreiber *et al.*, 2005). Another important agronomic aspect is the process of periderm maturation during the storage period of tubers. In this process the periderm becomes resistant to excoriation and the periderm water permeability decreases due to the deposition of suberin and waxes in the cell wall during the first days of storage (Schreiber *et al.*, 2005). The understanding of the molecular processes involved in tuber periderm is crucial for a better knowledge of this protective tissue and to improve the storage life of tubers.

1.2.1. *Molecular studies for the identification of candidate genes of phellem tissue in the cork oak bark and potato tuber skin models*

Despite cork is a difficult tissue for molecular studies due to cork cells are dead at maturity and because of its phenolic composition, proteomics and transcriptomics approaches have been conducted to identify relevant genes for native and wound-induced periderm formation in the potato tuber and the bark of cork tree (Soler *et al.*, 2007; Barel and Ginzberg, 2008; Chaves *et al.*, 2009; Ginzberg *et al.*, 2009; Ricardo *et al.*, 2011; Teixeira *et al.*, 2014).

The study of the genomics of cork cells from the cork tree performed in our laboratory (Soler *et al.*, 2007) was the first global study to the molecular biology of periderm and

suberin. Soler and coworkers by means of a Suppression Subtractive Hybridization (SSH) obtained a library of ESTs preferentially induced in cork (compared to the proliferative mass of somatic embryo) which was spotted on a microarray to compare the cork (a suberin-producing tissue) and wood (non-suberin-producing tissue) transcriptomes. The list of genes identified was composed mainly of predicted enzymes involved in the synthesis, transport and polymerization of suberin, some stress proteins and some regulatory proteins. Among the regulatory proteins there was NAC, MYB and WRKY transcription factors and a homolog to the maize RS2-INTERACTING KH PROTEIN (RIK). Moreover, based on this list the same authors analysed for a selection of genes the seasonal variation in their mRNA abundances (Soler *et al.*, 2008). On the other hand, a work published later by Ricardo *et al.* (2011) identified by a proteomic analysis of young shoots and 8-year-old branches 99 proteins involved in cork formation. The proteins identified were related to the carbohydrate metabolism probably linked to cell wall reorganization, protein stability, regulation and many defense proteins, but any protein involved in the lipid components of suberin were identified (Ricardo *et al.*, 2011). Recently, the first application of high-throughput RNA sequencing in cork tissue was performed to compare the gene expression in high-quality cork and bad-quality cork to identify candidate genes for the production of cork of superior quality (Teixeira *et al.*, 2014). As a whole, it was found that genes involved in regulatory processes such as those involved in DNA synthesis, RNA processing, proteolysis or transcription factors involved in abiotic stress response were more induced in bad-quality cork while good-quality displayed an stronger induction of heat-shock proteins and suberin related genes. In addition, also recently the small RNA fractions of leaves and differentiating phellem in cork oak were deep sequenced in order to identify specific microRNAs (miRNAs) involved in cork formation (Chaves *et al*., 2014).

Considering the complexity of *Quercus suber* cork, few studies has been addressed to understand the biochemical pathways involved in its biogenesis compared to the periderm model of potato tuber skin. The study of potato tuber periderm provides a simpler system and therefore has been more used as a model for the study of suberization. By wounding potato tubers a rapid formation of uniform suberin layers can be obtained. Overall, potato tuber has allowed the identification of genes or enzymes related to suberin biosynthesis and deposition (Espelie *et al.*, 1986; Stark *et al.*,

1994; Bernards and Razem, 2001; Razem and Bernards, 2003). Identically, to the proteins identified in *Q. suber* stems (Ricardo *et al.*, 2011), previous works which analysed the proteins in suberizing potato tuber tissues (Barel and Ginzberg, 2008; Chaves *et al.*, 2009) also highlighted the high involvement in phellem of proteins related to biotic and abiotic stress indicating the strong requirements for cell protection against the stressful events occurring in active phellem such as oxidative stress. In particular, Barel and Ginzberg (2008) identified proteins differentially accumulated in potato tuber skin compared to tuber flesh. The skin proteome was characterized by proteins involved in cell proliferation, one-carbon metabolism, the oxidative respiratory chain and some caffeoyl-CoA O-methyltransferase and peroxidases candidates for suberization process. However, the major functional category was stress-related proteins (63%). Proteins from potato tuber slices were analysed in six different time points during the wound-healing process as well as proteins present in native periderm by Chaves *et al.* (2009). Tuber slicing triggered differential induction of proteins that led to changes in metabolism, activation of plant defense responses and secondary metabolism involved in cell-wall reinforcement (Chaves *et al.*, 2009). Moreover, this work also highlights the existence of a succession of proteomics events along the reconstruction of wound-periderm and showed also that the molecular processes of native and wound-periderm formation are not fully equivalent. Another work was performed to analyze the transcript profiles of heat-stressed potato tubers based on a microarray experiment (Ginzberg *et al.*, 2009). The heat-enhanced development and the accumulation of suberized skin-cells were along with a major induction of response to stress genes (33%) and cell proliferation and development (28%), which was considered a protective mechanism against heat by forming a thicker periderm. Thereafter, Soler *et al.* (2011) performed the first transcriptomic approach addressing the regular development of periderm tissue in potato. An SSH library was performed to identify genes upregulated in potato periderm by using cDNA of the underlying parenchyma flesh to subtract common genes. This work presented a comprehensive list of 108 genes involved in suberin and wax biosynthesis, stress and defense, regulation, cell wall and others. In agreement with the previous works, the most abundant category was stress and defense (25.6%) and the second one regulation (24.4%). Suberin and wax biosynthesis was represented by 11.6% of the genes. The relative contribution to the functional categories of the genes identified in this work differ from the cork oak periderm SSH library published before (Soler *et al.*, 2007). While genes involved in regulation, stress and

defense were richer in the potato SSH library, fewer genes related to secondary metabolism including suberin were found in comparison with the SSH library from cork oak. It was suggested that a possible explanation could be the better insulation of living phellem cells in cork oak compared to potato from environmental stress factors because cork oak can develop a thicker layer of cork (60–100 cell layers) compared to the 6-10 cork cells in potato, and the protection conferred by the cork rings of previous years.

1.3. Suberin: an apoplastic biopolymer

The occurrence of suberin is very variable in plants: it is found in both internal and external tissues in all plant organs, and its formation is induced by abiotic and biotic environmental stimulus stress stimulus. The suberin is found in the native periderm of shoots, roots and tubers; the wound-healing periderm and the root exo- and endodermal cell walls (Schreiber *et al.*, 2005). The composition, structure, deposition pattern and functions of suberin have been reviewed by several authors (Kolattukudy, 2001; Bernards, 2002; Franke and Schreiber, 2007; Pollard *et al.*, 2008; Ranathunge *et al.*, 2011; Beisson *et al.*, 2012). Suberin cell wall depositions create a hydrophobic barrier against uncontrolled water and/or solute diffusion through the apoplast and restricts pathogen invasion during plant development in different tissues. Basically, suberin can be deposited into plant cell walls wherever and whenever it is necessary to form a boundary to separate plant tissues from surrounding environment or different tissues inside the plant body (Kolattukudy, 2001).

Suberin is a glycerol-based polyester composed of two major domains, a poly (aliphatic) domain (SPAD) and a poly (phenolic) domain (SPPD), which is deposited between the primary cell wall and the plasma membrane. Suberin deposition usually occurs as a distinct lamella that covers the inner surface of the original primary cellulose wall. The suberin lamella has a layered appearance under the transmission electron microscope (TEM) because it consists of alternating electron-dense layers and electron-lucent layers oriented parallel to the cell wall (Thomson *et al.*, 1995) (Fig. 1.6). Another important component are the suberin-associated waxes which are embedded in the SPAD domain and also contribute significantly to the barrier function (Kolattukudy, 2001; Bernards, 2002; Franke and Schreiber, 2007; Pollard *et al.*, 2008). The SPAD is

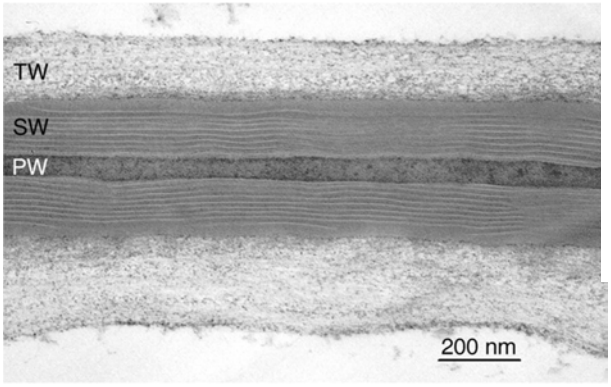

Fig. 1.6. TEM micrographs of suberin lamella from cork cell walls of potato tuber periderms, extracted from (Serra *et al.*, 2009).The polysaccharide primary wall (PW) and tertiary wall (TW) as well as the suberized secondary wall (SW) formed by the typical suberin lamella are indicated.

of primary importance for suberin function as a barrier of water and it consist of mainly ω-hydroxy fatty acids, α,ω-dicarboxylic acids, mid-chain oxygenated fatty acids, fatty acids and primary alcohols (Kolattukudy, 2001; Pollard *et al.*, 2008). Glycerol is esterified to ω-hydroxy and α,ω-dicarboxylic fatty acids to form the suberin matrix polymer (Moire *et al.*, 1999; Graça and Pereira, 2000; Graça and Santos, 2006). The SPPD probably plays a major role in pathogen defense and its components principally consist of hydroxycinnamates such as ferulic acid (Bernards *et al.*, 1995; Kolattukudy, 2001; Bernards, 2002). An scheme of the overall biochemical reactions for SPAD and SPDD precursors was proposed by Bernards (2002) which showed that at the most basic level SPAD and SPDD components derive from products of carbohydrate metabolism such pyruvate, phosphoenolpyruvate and the erytrose-4-phosphate. A branching point occurs between the synthesis of fatty acid and the shikimate pathways. Fatty acid synthesis yields the 16:0 and 18:00 precursors to all aliphatic monomers and the shikimate pathway yields the phenylalanine amino acid which is the precursor for almost all phenylpropanoids required for the aromatic domain (Bernards, 2002). However, the characterization of the whole suberin macromolecular assembly has been hampered because to date it has not been possible to obtain unaltered intact fraction of suberin aliphatic and aromatic domains. Then models of the chemical and macromolecular structure of suberin have been deduced based on the chemical analysis of partial depolymerization and the ^{13}C NMR spectroscopy of suberizing tissues (Stark *et al.*, 1994; Bernards *et al.*, 1995; Graça and Pereira, 2000; Ranathunge *et al.*, 2011). Despite the macromolecular structure of suberin is unclear yet, several models have been proposed (Kolattukudy, 2001; Bernards, 2002; Graça and Santos, 2007; Franke and Schreiber, 2007) in which the monomeric building blocks contain long-chain and very long-chain α,ω-dicarboxylic acids esterified to glycerol at both ends forming the

core of the suberin macromolecule. In this model suberin polymer might form a two- and three-dimensional network by forming ester linkages to additional α,ω-dicarboxylic acids and ω-hydroxy-acids leading to the formation of the insoluble polymer (Franke and Schreiber, 2007). The poly (aliphatic) domain was proposed to be a glycerol-based lipid-phenolic heteropolymer composed of fatty acids and derivatives, and phenolics composed mostly by esterified acid ferulic. Thus, it has been proposed that the lamellar structure of suberin observed in TEM corresponds to the aliphatic constituents (translucent lamellae) and the aromatic constituents (dense lamellae) (Schmutz et al., 1993, 1996; Graça and Santos, 2007). The poly (phenolic) domain is covalently linked to carbohydrates of the primary cell wall and has been shown to be a lignin like domain (Fig. 1.7). However, the fact that the down-regulation of ferulic acid in the aliphatic suberin did not affect the lamellar appearance of suberin (Serra *et al.*, 2010*b*) contradicts the model which proposed key roles of ferulic acid in the lamellar structure of suberin (See for review Serra *et al.*, 2010*a*) denoting that the structural basis of suberin lamella is not understood.

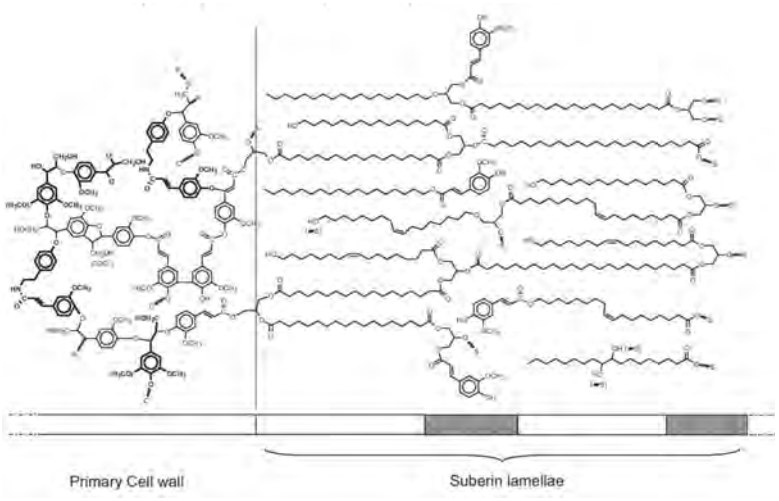

Fig. 1.7. Proposed model of the assembly of compounds in a macromolecular structure of suberin, extracted from Bernards (2002). The poly (phenolic) domain (SPDD) is displayed restricted in the primary cell wall. The poly (aliphatic) domain is glycerol-based polyester. In this model aliphatic zones are the translucent bands and the phenolics the opaque bands of the suberin lamellae observed under TEM.

Suberin deposition needs the synthesis of aliphatic, phenolic and glycerol monomers, followed by their transport to the cell wall where they are polymerized to form the suberin macromolecular structure. As mentioned above, the aliphatic suberin synthesis begins with the synthesis of fatty acids that occurs in plastids by the Fatty Acid Synthetase (FAS) complex. The fatty acids obtained, are then transported in the endoplasmic reticulum and there they are modified by specific suberin proteins.

Last years, some of the key genetic actors responsible of the biosynthesis and regulation of suberin, mostly related to the aliphatic polyester domain, have been unveiled (See for a review, Ranathunge *et al.*, 2011; Vishwanath *et al.*, 2015). The most significant advances have been made using analytical and molecular tools developed mostly for *Arabidopsis thaliana* and *Solanum tuberosum* (potato). Briefly, fatty acid elongation is performed by β-ketoacyl-CoA synthases (*KCS2/DAISY*, *KCS20*, *StKCS6*) (Franke *et al.*, 2009; Lee *et al.*, 2009; Serra *et al.*, 2009*a*) from the Fatty Acid Elongation (FAE) complex forming the Very Long Chain Fatty acids (VLCFAs); fatty acid reduction to primary alcohols and α,ω-diols are produced by the fatty acyl reductases (*FAR1*, *FAR4*, *FAR5*) (Domergue *et al.*, 2010; Vishwanath *et al.*, 2013); fatty acid oxidation is performed by the cytochrome P450 enzymes (*CYP86A1/HORST*, *CYP86B1/RALPH*, *StCYP86A33*) (Li *et al.*, 2007; Höfer *et al.*, 2008; Compagnon *et al.*, 2009; Serra *et al.*, 2009*b*) forming ω-hydroxy fatty acids (ω-OHs); and the esterification of ω-OHs and DCAs to the glycerol-3-phosphate molecules (G3P) by the enzymes glycerol-3-phosphate acyltransferases (*GPAT5*, *GPAT7*) (Beisson *et al.*, 2007; Yang *et al.*, 2012*b*) which produces the sn-2 monoacylglycerols. Further oxidation of ω-OHs to DCA may be catalyzed by the same cytochromes P450s that form the ω-OHs or additional ones. The activation of fatty acids to fatty acyl-CoAs is catalyzed by long-chain acyl-CoA synthetases (LACSs). The molecular mechanisms by which the suberin building blocks are transported intracellularly are unclear. However, members of the ATP-binding-cassette (ABC) transporters (*StABCG1*, *ABCG2*, *ABCG6*, *ABCG20*) have been shown to be involved in the transport of suberin through the plasma membrane to the apoplast (Landgraf *et al.*, 2014; Yadav *et al.*, 2014). Genes involved in the polymerization of the suberin blocks have not been characterized yet. However, it is expected that polyester synthases may be able to extend sn-2 monoacylglycerols with other suberin monomers to synthesize the insoluble suberin polyester. The main aromatic monomer present in the aliphatic suberin polyester is ferulic acid derived from the phenylpropanoids. Activated ferulic acid (feruloyl-CoA) is then linked to ω-OHs and primary alcohols by the action of BAHD-type acyltransferases (*ASFT/HHT*, *FHT*, *FACT*, *PtFHT1*) to produce alkyl ferulates (Gou *et al.*, 2009; Molina *et al.*, 2009; Serra *et al.*, 2010*b*; Kosma *et al.*, 2012; Cheng *et al.*, 2013) (Fig. 1.8). Regarding suberin regulation putative regulators include members of the WRKY, NAC and MYB transcription factors which showed preferentially expression in tissues undergoing suberization and frequently showed co-expression patterns with suberin-related genes. Moreover

members of these transcription factors families are involved in processes related to suberin, such as the synthesis of phenylpropanoids, fatty acid elongation, secondary wall modification (Ranathunge *et al.*, 2011). However, to date only the *MYB41* (Fig. 1.8) and *StNAC103* have been functionally characterized for their role in the control of suberin deposition (Kosma *et al.*, 2014; Verdaguer *et al.*, 2016).

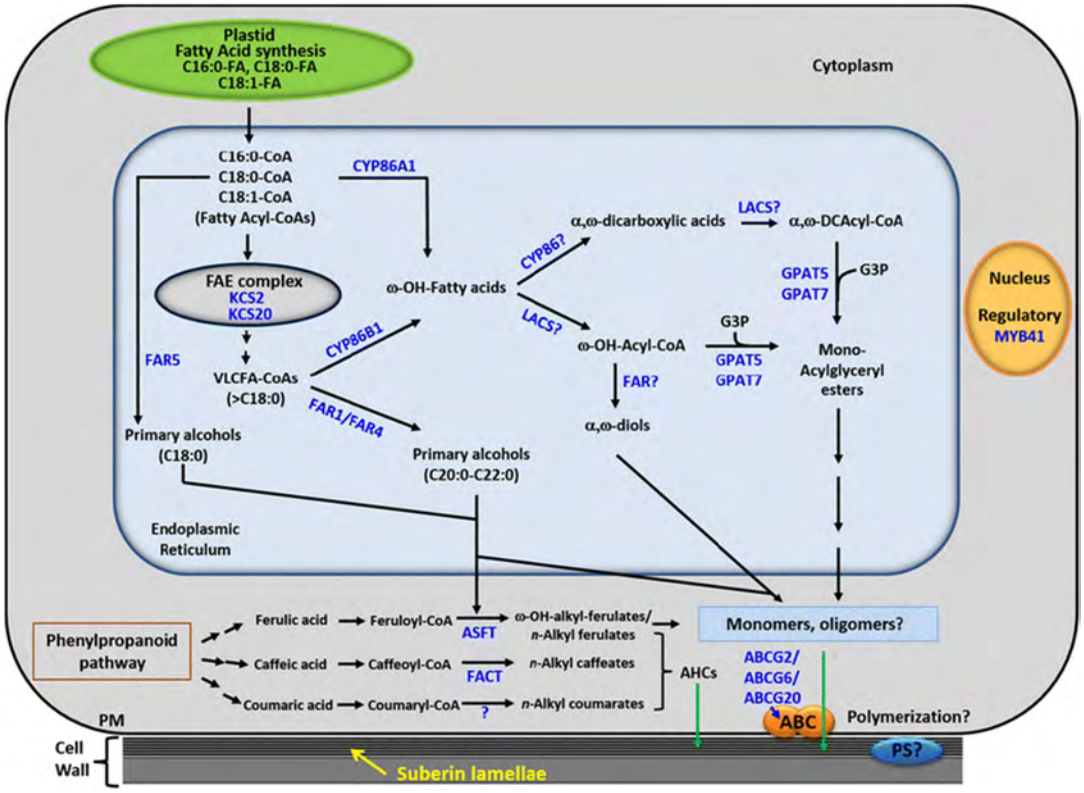

Fig. 1.8. Overview of the biochemical pathways involved in suberin synthesis and export to the cell wall, extracted from Vishwanath *et al.* (2015). In blue are highlighted specific protein names identified in *Arabidopsis thaliana*.

1.3.1. *The potato FHT gene, a key BAHD acyltransferase for suberin synthesis and the sealing properties of the periderm*

A previous transcriptomic approach of the cork oak (*Quercus suber*) phellem performed in our laboratory highlighted a putative involvement of a BAHD family member in the suberin synthesis which was annotated as N-hydroxycinnamoyl/benzoyltransferase-like (HCBT) (Soler *et al.*, 2007). Additionally, the transcripts levels of *HCBT* showed very similar patterns to that of previously characterized suberin genes (*GPAT5* and *CYP86A1*) during the cork seasonal growth (Soler *et al.*, 2008). Then its potato ortholog was identified and was renamed as FHT (fatty ω-hydroxyacid/fatty alcoholhydroxycinnamoyl transferase) based on the *in vitro* activity of the protein (Serra

et al., 2010*b*). The *FHT*-RNAi plants showed reduction in alkyl ferulates, and ferulic acid and ω-hydroxyacids levels in the suberin-associated waxes and the suberin polymer, respectively. The *FHT*-RNAi periderm also showed more 'liquid-like' aliphatic chains and abundant aromatic constituents; the resulting tissues showed attenuated cooperative hydroxyfatty acid motions, resistance to transesterification, and a mechanically compromised periderm layer (Serra et al., 2010b; Serra et al., 2014). The skin of the *FHT* RNAi tubers appeared duller and showed intense grid-like splitting and cracking (Fig. 1.9). This macroscopic appearance agrees with the SEM cross-sectional micrographs showing more phellem cell layers and less regular cell organization in the silenced lines. Surprisingly *FHT* downregulation did not affect to the typical suberin lamellar structure although the periderm permeability was strongly increased and the maturation of tuber periderm was prevented. Altogether these results suggested that the *FHT* function is important for the sealing properties and maturation of the periderm in potato tubers by the synthesis of alkyl ferulates. In agreement with above, *ASFT/HHT* mutants, the putative ortholog in Arabidopsis (At5g41040), showed a decrease in suberin ferulate esters (Gou *et al.*, 2009; Molina *et al.*, 2009).

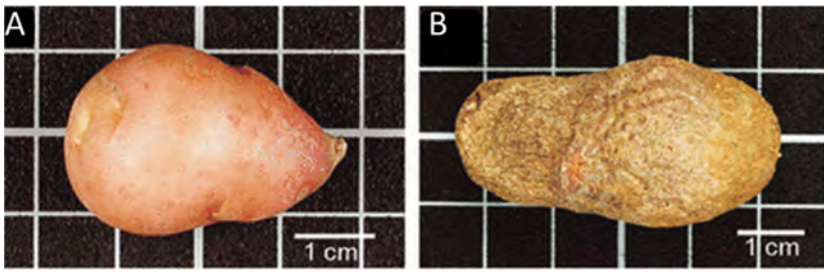

Fig. 1.9. Periderm morphology of 21-day-stored tubers of (A) wild-type and (B) transgenic *FHT* RNAi potato (*Solanum tuberosum*) plants. Extracted from Serra *et al*. 2010b.

1.4. Molecular control of periderm initiation, growth and differentiation

Secondary (lateral) meristems (vascular cambium and phellogen) contain the pluripotent stem cells that allow the radial growth of woody species throughout their life cycle (Miyashima *et al.*, 2013). The vascular cambium gives rise to the secondary phloem (outwardly) and the secondary xylem (inwardly). Analogously, the phellogen meristem gives rise to the phellem (outwardly) and the parenchymatous phelloderm (inwardly). Little is known about most processes involved in the initiation, maintenance and differentiation of the phellogen compared to the vascular cambium which has been much studied. However, the similitude between the asymmetrical and radial patterning

of wood/phloem and phellem/phelloderm tissues suggests that both processes might share common regulators.

Phellem formation is the result of the balance between meristem (phellogen) maintenance and the commitment of the new divided cells to differentiation. Because this balance will determine the number of newly formed phellem cells each growing season, it must be tightly controlled by genetic and hormonal factors as it has been reviewed for the cambial meristem (Milhinhos and Miguel, 2013; Sorce *et al.*, 2013; Ursache *et al.*, 2013; Ye and Zhong, 2015) and modulated by environmental cues (Schrader *et al.*, 2004*a*; Druart *et al.*, 2007).

1.4.1. *Genetic regulators*

Several studies of genes associated to vascular cambium have revealed conservation between the molecular mechanisms which control the proliferation and maintenance of the shoot apical meristem (SAM) and the vascular cambium. For instance, homologous genes to key regulatory proteins of the SAM such as WUSCHEL (WUS) together with the signaling peptide CLV3 and the receptor kinase CLV1, the transcription factors SHOOTMERISTEMLESS (STM), KNOX proteins (KNAT1 and KNAT6) and AINTEGUMENTA (ANT) (Ha *et al.*, 2010) have been found highly expressed in the vascular cambium cells (Schrader *et al.*, 2004*b*). Later, functional analysis in poplar of *PttSTM/ARBORKNOX1* (*ARK1*, the ortholog to Arabidopsis *STM* gene) and *PttKNOX1/ARBORKNOX2* (*ARK2*, the ortholog to Arabidopsis *KNAT1* gene), demonstrated their role in the regulation of cambium activity and xylem differentiation (Groover *et al.*, 2006*a*; Du *et al.*, 2009). A functional study of *ANT* in Arabidopsis roots and poplar stems demonstrated its role in secondary organ thickening mediated by cytokinins (Randall *et al.*, 2015). In Arabidopsis it has been shown that the TDIF-TDR-WOX4 signaling module constitutes a peptide-receptor-transcription factor signaling pathway controlling the maintenance and proliferation of vascular meristem during secondary growth (Hirakawa *et al.*, 2010), which is equivalent to the WUS/CLV feedback loop pathway that keep the stem cell population in the SAM. It has been seen that the onset of vascular tissue differentiation from cambium cells also involves the interaction between the class III HD-ZIP (*ATHB-8*, *PHV*, *CNA*, *PHB*, *REV*) and KANADI (*KAN1-4*) genes playing important roles in regulating vascular patterning, organ polarity, polar auxin transport and xylem and phloem differentiation (Emery *et*

al., 2003; Ilegems *et al.*, 2010*b*). Moreover, members of the NAC and MYB transcription factors families are known to act as top-level and second-level master regulators to activate a battery of downstream transcription factors that regulate the coordinated expression of secondary wall biosynthetic genes during the differentiation of woody tissues (Zhong and Ye, 2014).

In addition, the expression control of key meristem genes in the SAM such as WUS or KNOX genes has been shown to be dependent on the activities of chromatin remodeling factors. For instance the ATP-dependent SWI/SNF2 enzyme SPLAYED (SYD) and the three component CAF1 complex control WUS expression, and the polycomb group (PcG) proteins FERTILISATION INDEPENDENT ENDOSPERM (FIE) and CURLY LEAF (CLF) restrict KNOX gene expression (Nieuwland *et al.*, 2009). Therefore epigenetics is expected to be also an important factor in the regulation of lateral meristems.

1.4.2. *Hormonal regulation*

It has also been demonstrated that plant hormones play important roles in the regulation of vascular cambium activities. For instance, the auxin gradients, which are maximal in the cambial zone (Uggla *et al.*, 1996), are suggested to be essential for cambial proliferation because decreased auxin levels have been shown to reduce cell divisions in the xylem (Tuominen *et al.*, 1997; Nilsson *et al.*, 2008). Moreover, the downregulation of auxin efflux carriers (*PIN3* and *PIN4*) decrease auxin polar flow and inhibit cambium activity (Zhong and Ye, 2001). The importance of auxin-mediated signaling in the vascular development was also demonstrated by the overexpression of *PttIAA3* repressor in transgenic poplar which resulted in a reduction in cell divisions of the vascular cambium (Nilsson *et al.*, 2008). Cytokinins (CKs) hormones stimulate cell divisions (Miller *et al.*, 1955) and have been shown to play important roles in the maintenance and proliferation of cambial cells. Several cytokinin receptors (e.g. *HK3*, *WOL/CRE1*) are highly expressed in the vascular cambium zones and the reduction of cytokinin levels in engineered transgenic poplar trees reduced the number of cambial cells and stem diameter (Nieminen *et al.*, 2008). Similar conclusions were reported in studies of transgenic Arabidopsis plants with impaired cytokinin biosynthesis (Matsumoto-Kitano *et al.*, 2008). Several works have showed that ethylene can stimulate cambial growth in herbaceous and trees species (Biro *et al.*, 1980; Little and

Pharis, 1995). Consistent with this Love *et al.* (2009) developed a system by using transgenic ethylene-overproducers and ethylene insensitive poplar trees which demonstrates that endogenous ethylene stimulates cambium activity and increases wood formation. Furthermore it was demonstrated that xylem differentiation in Zinnia cell culture system is strictly dependent on ethylene synthesis (Pesquet and Tuominen, 2011). Brassinosteroids (BRs) have been suggested to be involved in the regulation of xylem development (Turner *et al.*, 2007). In particular BRs induced programmed cell death (PCD) and secondary cell wall (SCW) deposition during tracheary elements (TE) differentiation in Zinnia system (Fukuda, 1997; Yamamoto *et al.*, 1997) and regulate the expression of HD-Zip III genes during vascular differentiation (Ohashi-Ito *et al.*, 2002). However, BRs have been also shown to promote cell divisions in the procambial cells (Caño-Delgado *et al.*, 2004). Concerning gibberellins, both the bioactive hormone and the expression of genes of the signal transduction pathway are concentrated predominately in the expansion zone of developing xylem in poplar (Israelsson *et al.*, 2005), indicating a role of gibberellin during the expansion of secondary xylem cells. Moreover, overexpression of the gibberellin receptor *PttGID1* results in increased wood formation in transgenic poplar (Mauriat and Moritz, 2009). Further evidence of the gibberellins role in xylem cell expansion was demonstrated by overexpression of a key enzyme regulating gibberellin biosynthesis (Eriksson *et al.*, 2000). Besides the hormonal promoting signals, abscisic acid (ABA) probably is a repressor of cambium development and secondary growth in woody species through a hypothesized negative interaction with auxin (Little and Wareing, 1981) under suboptimal conditions with regards to water availability (Berta *et al.*, 2010). Moreover, it is likely the existence of hormone crosstalk between some of the above mentioned hormones to coordinate their signaling pathways that converge to common targets to regulate cambial activities as has been shown in other developmental processes such as the cytokinin and ethylene involvement in the modulation of auxin transport in lateral root formation (Negi *et al.*, 2008; Marhavý *et al.*, 2014).

However, to date little progress has been made to underscore the genetic and hormonal factors which are behind the control of the phellogen activity. Only very recently, a role for the Populus *SHR-like* gene, *PtSHR2B*, has been proposed in the phellogen activity through cytokinin homeostasis based on the functional studies of transgenic lines in hybrid aspen overexpressing *PtSHR2* (Miguel *et al.*, 2015). Using a well-defined

wound-healing model system in potato, Lulai *et al.*, (2016) demonstrated a role for cytokinins and auxins in wound periderm development. Despite the existence of overlapping genetic networks controlling apical and cambial meristems has been reported (Schrader *et al.*, 2004*b*; Groover *et al.*, 2006*a*), there is little evidence so far to support that this genetic conservation is extended to the phellogen as well, even though it may be expected.

1.4.3. *Mechanical stress*

Another factor that has been hypothesized to influence meristem growth is the mechanical signals. It has been shown that the overall tensions generated in growing tissues can modulate local expansion rates by influencing cell wall and microtubule organization (Murray *et al.*, 2012). In the same line, a very recent work demonstrated the role of mechanicals signals in the expression of the key meristematic identity gene *SHOOT MERISTEMLESS (STM)* in Arabidopsis (Landrein *et al.*, 2015).

1.4.4. *Environmental inputs*

Seasonal changes in the growth conditions have been also shown to influence cambium activity in Arabidopsis. For instance treatments that stop or delay flowering also lead to an enhanced secondary growth (Chaffey *et al.*, 2002). Moreover, the late flowering double mutant *soc1 ful1* in Arabidopsis shows enhanced wood development (Melzer *et al.*, 2008) denoting a link between flowering time and secondary growth. Furthermore, Sibout *et al.,*(2008) observed a tight correlation between flower development and secondary growth comparing different recombinant inbred lines (RIL) in Arabidopsis accessions because the major Quantiative Trait Loci (QTL) identified for xylem-to-phloem ratio and flowering time coincided, and was linked to *FLOWERING LOCUS C (FLC)*.

Objectives

2. Objectives

The main goal of the present work is to gain deeper insights into the molecular mechanisms that give rise to the phellem formation by studying the promoter and the protein of the previously characterized *FHT* gene, the effects of *RIK* silencing in the periderm and also by identifying new candidate genes by transcriptomic approaches. To address these challenges, we used molecular techniques both *in planta* and *in vitro* and bioinformatics tools as well. The following are the specific objectives:

1. Understand the suberization process in roots and tubers by defining the spatiotemporal expression patterns of the central suberin biosynthetic gene *FHT* (Chapter I).

- **1.1.** Clarify the activation of the *FHT* promoter and the accumulation of the FHT protein in potato native tissues.
- **1.2.** Reveal the activation of the *FHT* promoter and the accumulation of the FHT protein during tuber development and maturation.
- **1.3.** Determine the *FHT* promoter induction and FHT protein accumulation by wound-healing in above and underground potato organs.
- **1.4.** Uncover the regulation of the FHT protein accumulation by the hormones abscisic acid, salicylic acid and jasmonic acid.
- **1.5.** Establish the subcellular localization of the FHT enzyme.

2. Obtain insights on the role of the gene *StRIK* in the periderm (Chapter II)

- **2.1.** Analyze the *RIK* transcript accumulation in potato native and wounded tissues.
- **2.2.** Analyze the transcript accumulation of *RIK* cork oak and holm oak ortholog in cork bark and during the growing season.
- **2.3.** Predict by *in silico* analyses of the promoter region and the co-expression network of *RIK* its putative function, partners and factors regulating the expression.
- **2.4.** Confirm the predicted nuclear subcellular location of *StRIK*.
- **2.5.** Study the effects of *StRIK* downregulation on the potato plant development and especially in the periderm transcriptome.

3. Expand the understanding of the phellem transcriptome and highlight new candidate genes for phellem formation in cork oak and holm oak using RNA-seq (Chapter III).

- **3.1.** Generate a *de novo* assembly of phellem transcriptome.
- **3.2.** Perform the functional annotation of the contigs obtained and classify them in relevant functional categories according to public databases and literature.
- **3.3.** Identify differential expressed genes between cork oak and holm oak phellem libraries.
- **3.4.** Highlight the biological processes differentially regulated between cork oak and holm oak phellem libraries by analyzing the enrichment of functional categories.
- **3.5.** Provide new candidate genes for phellem formation based on their expression pattern, their functional assignment and literature.
- **3.6.** Search suberin candidate genes by *in silico* co-expression analysis.
- **3.7.** Identify *in silico* sequence variants (SNPs and INDELs) of contigs.

4. Obtain a better insight to the function of some phellem candidate genes identified in the RNA-seq analysis by studying their transcriptional pattern in cork oak tissues (Chapter IV).

- **4.1.** Compare the transcript accumulation of the candidate genes between cork and wood tissues.
- **4.2.** Determine the transcript profile of the candidate genes along the cork growing season.

Results

3. Results

The results are presented and discussed in four different chapters structured as scientific articles:

- **Chapter I:** The potato suberin feruloyl transferase FHT which accumulates in the phellogen is induced by wounding and regulated by abscisic and salicylic acids.

  The results from this chapter have been published in Journal of Experimental Botany as Boher *et al*. (2013).

- **Chapter II:** The KH-domain RNA-binding protein StRIK from potato regulates stress- and transposable element-related genes in the periderm and affects flowering.

- **Chapter III:** Comparative transcriptional profiling of cork and holm oak phellem tissues unveils molecular networks orchestrating phellem formation.

- **Chapter IV:** Transcriptional characterization of a selected set of new cork candidate genes: mRNA abundance in phellem versus xylem tissue and effect of cork seasonal growth in their expression.

Chapter I

The potato suberin feruloyl transferase FHT which accumulates in the phellogen is induced by wounding and regulated by abscisic and salicylic acids

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ABSTRACT

The present study provides new insights on the role of the potato (*Solanum tuberosum*) suberin feruloyl transferase FHT in native and wound tissues, leading to conclusions about hitherto unknown properties of the phellogen. In agreement with the enzymatic role of FHT, it is shown that its transcriptional activation and protein accumulation are specific to tissues that undergo suberization such as the root boundary layers of the exodermis and the endodermis, along with the tuber periderm. Remarkably, *FHT* expression and protein accumulation within the periderm is restricted to the phellogen derivative cells with phellem identity. FHT levels in the periderm are at their peak near harvest during periderm maturation, with the phellogen becoming meristematically inactive and declining thereafter. However, periderm FHT levels remain high for several months after harvest, suggesting that the inactive phellogen retains the capacity to synthesize ferulate esters. Tissue wounding induces *FHT* expression and the protein accumulates from the first stages of the healing process onwards. *FHT* is up-regulated by abscisic acid and down-regulated by salicylic acid, emphasizing the complex regulation of suberin synthesis and wound healing. These findings open up new prospects important for the clarification of the suberization process and yield important information with regard to the skin quality of potatoes.

Key words: ABA, BAHD suberin feruloyl transferases, cell wall suberization, FHT promoter, phellem, phellogen, potato periderm, suberin, wound-healing periderm.

INTRODUCTION

The potato enzyme FHT (fatty ω-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase) and the respective *Arabidopsis* orthologue ASFT/RWP1/AtHHT (At5g41040) have previously been characterized both *in vitro* and *in planta* (Gou *et al.*, 2009; Molina *et al.*, 2009; Serra *et al.*, 2010b). Categorized as acyltransferases of the BAHD family capable of undertaking the *in vitro* catalytic transfer of ferulic acid from feruloyl-CoA to ω-hydroxyfatty acids and fatty alcohols, both enzyme orthologues are responsible for supplying monomers to suberin (reviewed by Liu, 2010; Serra *et al.*, 2010a). Suberin consists of a complex cell wall polymer which is used by land plants to regulate the apoplastic transport of water (see, among others, Bernards, 2002; Ranathunge *et al.*, 2011; Beisson *et al.*, 2012), being composed of an aliphatic domain cross-linked with a lignin-like aromatic domain that is fixed to the primary cell wall. The aliphatic domain is made up of a glycerol-based fatty acid-derived polyester which forms a matrix in which soluble lipids or waxes are embedded.

Ferulate esters are structural yet minor components of the aliphatic suberin, and are also constituents of the waxes embedded within the suberin (Schreiber *et al.*, 2005; Graça, 2010). Suberin (bound) and wax (soluble) alkyl ferulates play crucial roles with regard to the apoplastic barrier. Potatoes deficient in FHT are characterized by a large reduction of ferulate in both suberin and waxes, displaying a periderm that is a 14-fold more permeable to water compared with wild-type potatoes (Serra *et al.*, 2010b). The above statement is in agreement with an increased permeability of the suberized tissues of *Arabidopsis rwp1* mutants (Gou *et al.*, 2009). On the other hand, potato tubers deficient in FHT present a rough skin similar to that of russet potato varieties, and are unable to complete the periderm maturation process correctly and consequently remain prone to suffer from skinning injury over a long period after harvest (Serra *et al.*, 2010b).

The periderm consists of the dermal structure that replaces the plant epidermis of secondary (mature) organs and tubers (Peterson and Barker, 1979). It comprises three tissues: the phellem, the phellogen or mother layer, and the phelloderm. The phellem or cork layer is composed of 6–12 layers of dead cells with suberized walls that prevent water loss and act as an effective barrier to plant pathogens. The phelloderm connects the periderm to storage tissues (tuber flesh) and consists of one or a few layers of cells

with cellulosic walls which can hardly be distinguished from the cortical parenchyma. The phellogen functions as a meristem given that consecutive new layers of phellem are produced as the outer layers are sloughed off during tuber growth. While the phellogen continues to be physiologically active, its cell walls remain thin and prone to fracture, leading to potato skinning. Nonetheless, when tuber growth ceases by vine killing or harvest, the periderm enters a maturation period during which the phellogen becomes meristematically inactive, with cell walls thickening and becoming resistant to excoriation (Lulai and Freeman, 2001), while at the same time the adjacent phellem cells complete their full suberin and wax load (Schreiber *et al.*, 2005). Once mature, no new phellem cell layers are added nor are further changes observed in the periderm (Sabba and Lulai, 2005; Lendzian, 2006). However, very little is known about changes in phellogen cells during periderm maturation except for the modifications in cell wall composition studied by Sabba and Lulai (2005) and Neubauer *et al.* (2013).

Potatoes react to skinning or other types of injury by forming a wound periderm beneath the wound surface (Morris *et al.*, 1989). Native and wound periderms are similar in structure and composition, and follow analogous maturation processes (Lulai and Freeman, 2001), although the wound periderm is more permeable to water and is proportionally enriched by wax alkyl ferulates (Schreiber *et al.*, 2005). The wound healing ability that includes suberin deposition at the wound site is essential to extend the storage life of potatoes. Abscisic acid (ABA) is a potent phytoregulator that reduces evapotranspiration and hastens the wound-associated deposition of suberin (Soliday *et al.*, 1978; Lulai *et al.*, 2008), in contrast to ethylene which is not required for wound suberization (Lulai and Suttle, 2004, 2009). Furthermore, jasmonic acid (JA) is rapidly induced by wounding, but neither JA treatment nor inhibition of JA accumulation have any effect on suberin deposition (Lulai *et al.*, 2011). Clarifying the effects of plant hormones in wound-associated suberization may contribute further to better understand the healing processes and might help to improve the quality and storage life of potatoes. Notwithstanding the crucial role played by FHT with regard to the water barrier function coupled to the external appearance of the tuber periderm, an in-depth study of the role of FHT as regards suberized tissues is still awaited. The present work was designed to provide experimental evidence for *FHT* promoter activity and protein accumulation in the native periderm together with other constitutively suberized tissues, as well as to widen FHT studies into the wound induced suberization process. For these

reasons a polyclonal antibody was produced and potato plants stably transformed with a *FHT* promoter::GUS–GFP (β-glucuronidase–green fluorescent protein) construct were obtained. FHT temporal and spatial profiles in normal and mechanically injured tissues are reported. The results show that *FHT* is specifically expressed in cells undergoing suberization and that it is induced by wounding and regulated by ABA and salicylic acid (SA). Information is presented on FHT accumulation in the periderm, providing a new significant insight with reference to phellogen cells once tuber growth ceases, which might be useful to improve potato storage.

RESULTS

FHT localization in the native periderm and root tissues

In order to verify the FHT expression profile and test the FHT polyclonal antibody, protein extracts derived from potato tissues were analysed by western blot (Fig. 1). A band with an electrophoretic mobility corresponding to 55 kDa, in accordance with that predicted for the FHT protein, was only present in the periderm and root tissues which contain suberized tissues. This band was absent in stem, leaf, and tuber flesh (tuber parenchyma) which correspond to unsuberized tissues and also in the controls incubated with the pre-immune serum (data not shown). These results are in agreement with the *FHT* transcript profile carried out by northern blot analysis (Serra *et al.*, 2010b) and validate the use of the FHT antiserum in further studies. The tuber periderm and the root tissues were analysed at a histological level to determine in which precise cells the *FHT* promoter is active and the protein accumulates. Plants of *S. tuberosum* ssp. *andigena*, chosen because tuberization can be induced by photoperiod, were stably transformed with a construct carrying the *FHT* promoter region (2541 bp upstream of the translation initiation codon) fused to the GUS and GFP coding regions. Potato tubers cut in half

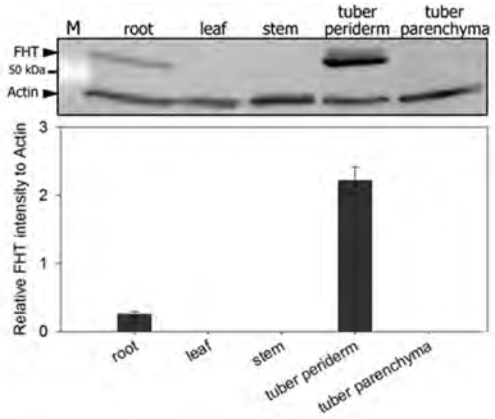

Fig. 1. **FHT protein profile of potato tissues**. Protein extracts derived from root, leaf, stem, tuber periderm, and tuber parenchyma separated by SDS–PAGE and analysed by western blot using antiserum against FHT. Actin was used as the internal control. The 50 kDa molecular mass marker is indicated to the left of the panel. Relative FHT accumulation with respect to actin is quantified for each lane. Relative intensity values are means ±SD of two independent biological replicates.

and stained for GUS activity showed the blue marker specifically at the region of the periderm that covers the tuber surface (Fig. 2A, arrowheads), while it was found to be absent from the apical bud region which had not yet developed a periderm (Fig. 2A, arrow). The thin sections used for microscopy analysis allowed the distinction between the suberized phellem, made up of dead cells, and the adjacent non-suberized layers, the phellogen and phelloderm, by means of suberin autofluorescence (Fig. 2B). GUS activity was particularly localized beneath of the phellem innermost cell layer and concentrated in a single layer of live cells corresponding to the phellogen (Fig. 2B, C). The immunolocalization of FHT was performed using a secondary antibody conjugated to Alexa Fluor 488 as its green fluorescence contrasts with the faint dark-yellow autofluorescence emitted by suberin under blue excitation. In the immunostained periderm sections, the green fluorescence showed no overlap with the suberin autofluorescence and was restricted to a single cell layer of live cells corresponding to the phellogen (Fig. 2D–G). The antiserum and the FHT affinity-purified antibodies were both used in these experiments to rule out a possible cross-reactivity. No green fluorescence was observed in the negative controls performed with the pre-immune serum nor using only the primary or secondary antibodies; in the same way, green

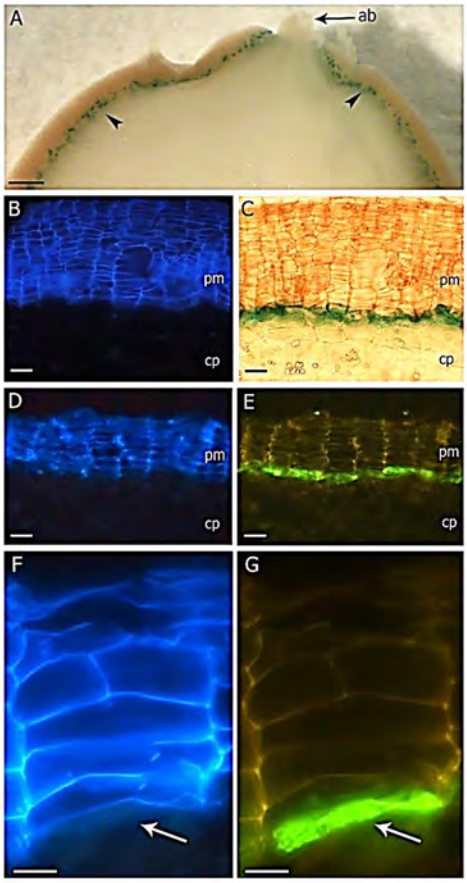

Fig. 2. FHT expression in native tuber periderm of potato. (A–C) GUS activity directed by the FHT promoter in transgenic tubers. (A) An *in vitro* cultured tuber cut in half and showing GUS staining specific to the periderm located beneath the phellem (arrowheads). No signal was detected in the apical bud region (arrow). (B) Cryosection of the GUS-stained periderm showing the suberin autofluorescence of the phellem and (C) the GUS blue marker located in a single cell layer beneath the phellem. (D–G) FHT immunolocalization using the Alexa Fluor 488-labelled FHT purified antibody. Sections observed under UV (D, F) showing the suberin autofluorescence and under blue excitation (E, G) showing the green fluorescence of labelled FHT antibody located in the phellogen cell layer (white arrow). Scale bars=500 µm (A), 50 µm (B–E), and 20 µm (F, G). cp, cortical parenchyma; pm, phellem.

fluorescence was absent in tubers of FHT silenced lines (data not shown). Upon inspection of the periderm in some cork-warts that form spontaneously in stems of *in vitro* cultured potato plants, GUS activity restricted within the phellogen cell layer was confirmed (Supplementary Fig. S1). Thus, the FHT transcriptional and translational activity of the native periderm is specific to the phellogen cells. On the other hand, root tissue was examined using primary roots of *in vitro* cultured plants carrying the *ProFHT::GUS-GFP* construct. In roots stained for GUS activity, the blue marker was restricted to the exodermis, located beneath the epidermis, as well as the endodermis, located between the cortex and the stele (Fig. 3). In root cross-sections, GUS staining overlapped with the autofluorescence signal (Fig. 3A, B). Whole-mount roots observed under bright field and confocal microscopy exhibited GUS activity, and GFP fluorescence localized in these suberized cell layers (Fig. 3C–E).

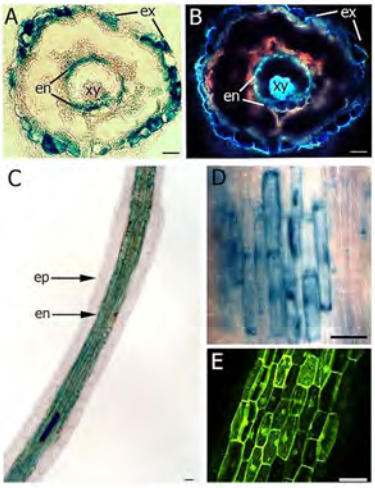

Fig. 3. *FHT* expression in root tissues of potato. GUS and GFP expression driven by the *FHT* promoter is restricted to the exodermis and endodermis. (A and B) Root cross-section under bright field (A) and UV excitation (B). In the endodermis and exodermis, the GUS signal overlaps with the suberin autofluorescence. (C–E) Whole mounts showing GUS activity localized (C) in the endodermal and (D) in the exodermal cells. (E) Confocal microscope image showing GFP accumulation in exodermal cells. Scale bars=25 μm (A, B), 50 μm (C, D, E). ex, exodermis; en, endodermis; ep, epidermis; xy, xylem vessels.

FHT expression throughout tuber development, maturation, and storage

Developing tubers of *ProFHT*::*GUS-GFP* plants were collected and stained for GUS activity at a number of main developmental stages according to Kloosterman *et al.* (2008): stolon tip, stolon swelling, tuber initiation, and early, middle, and late tuber growth stages. The blue marker begins to become visible through the skin when the developing tubers reach the stage of early tuber growth (Fig. 4A). The blue colour is first detected at the tuber basal end region and progressively extends upwards to cover the entire tuber surface (Fig. 4A, B). Lenticels showed up as deep blue dots indicative of an intense GUS activity (Fig. 4B) in agreement with a greater fluorescence intensity of FHT (Fig. 4C, D). These observations are in accordance with the periderm developmental gradient and confirm an intense activity in the lenticular phellogen of

growing tubers. Furthermore, periderm samples obtained at different time points throughout the maturation and ageing process of tubers (up to 10 months of storage at 4 ºC) were analysed by western blot; as shown in Fig. 5, the level of FHT was greater in samples which were obtained near to harvest, coinciding with the periderm maturation period, while it decreased thereafter. However, the FHT level still remained high after 4 months of storage, and FHT was even detected after 10 months of storage. It is noteworthy that one tuber stained for GUS after a 7 month storage period at 4 ºC displayed a faint blue surface colour in contrast to an intense blue colour of the lenticels (Supplementary Fig. S2); however, two other tubers kept in the same conditions showed no visible GUS signals.

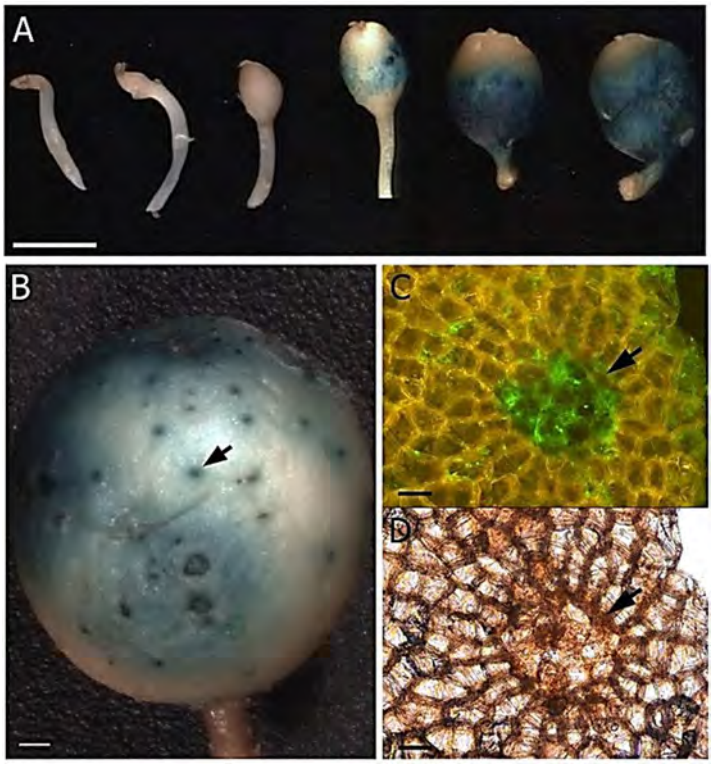

Fig. 4. *FHT* induction in developing tubers of potato. (A and B) GUS signal observed through the surface of tubers in *ProFHT::GUS-GFP* potato plants. (C and D) FHT immunolocalization in a lenticel. (A) Tubers grown in soil sampled at the stolon tip, stolon swelling, tuber initiation, and early, middle, and late tuber growth stages. The GUS staining starts to become visible at the basal end when tubers enter the growth stage and the signal progressively covers the whole tuber surface. (B) Tuber in a late growth stage showing lenticels as dark blue dots (arrow). (C and D) Detail of a lenticel stained for FHT under blue light excitation (C) and under bright light (D). Scale bars=5 mm (A), 1 mm (B), 50 µm (C, D).

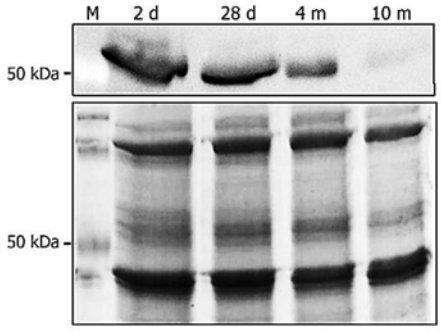

Fig. 5. FHT levels in the potato periderm during tuber maturation and ageing (storage). Western blot analysis (upper panel) shows that a higher level of FHT is observed close to the harvest period and thereafter decreases, although it is still detected after 10 months of storage at 4 ºC. SDS–polyacrylamide gel stained with Coomassie Brilliant Blue (lower panel) showing that equal total protein amounts were loaded in each lane. d, days; m, months.

Temporal and spatial FHT pattern in healing tissues

In order to elucidate the participation of FHT in the healing process, its expression in mechanically injured tissues was investigated. Fully expanded leaflets of plants bearing the *ProFHT*::*GUS–GFP* construct were injured with a 'dog brush' and left to heal. In wounded leaflets the FHT level peaks after 72 h and decrease subsequently by a half at 96 h following injury (Fig. 6A). When leaflets were examined for GUS activity 48 h after wounding, the blue marker appeared to be restricted to the scar tissues at the margin of wounds (Fig. 6B–D), corresponding to the suberin autofluorescence area (data not shown). Young (primary) stems were superficially injured with a scalpel and left to heal. In wounded stems 48 h after injury the GUS blue colour also appeared confined to the site of damage (Fig. 6E), being more intense at the wounded margins yet also detectable in the central areas in which only the epidermis was eroded. In tubers, the healing process was examined in single cuts or in excised parenchyma discs at 0, 24, 48, and 72 h, and 6 d after injury. A certain amount of FHT was detected 24 h after injury and levels increased as the healing process progressed (Fig. 7A). Compared with 24 h after injury, the amount of FHT relative to actin was increased by 9- to 10-fold after the sixth day. Tubers with single cuts were used to examine the *FHT*

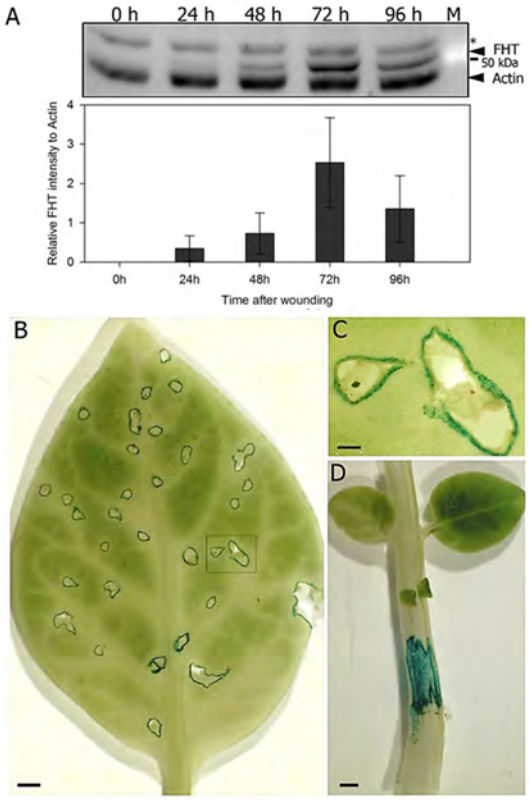

Fig. 6. FHT in wound-healing leaflets and stems of potato. (A) The upper panel shows the FHT protein profile in mechanically injured leaflets monitored by western blot using actin as a loading control. The 50 kDa molecular marker is shown to the right. The asterisk indicates an extra band not corresponding to the molecular weight of FHT or actin. The lower panel shows the FHT accumulation relative to actin as quantified for each lane (values are means ±SD of three independent biological replicates). (B) Injured leaflet stained for GUS activity 48 h after wounding. (C) Detail of wound lesions in B. (D) Injured stem stained for GUS activity 48 h after wounding. Scale bars=1 mm (B, D), 200 µm (C).

transcriptional activity 48 h after wounding. In these tubers, the entire severed surface showed a very intense GUS signal (Fig. 7B, arrows) which connects to the wounded edges, with the GUS signal being distinct in the intact periderm covering the undamaged surface (Fig. 7B, arrowheads). Microscopic examination revealed that the GUS staining localized within the live parenchyma cells closest to the injured surface (1–2 cells from the wounded surface) (Fig. 7C, D) as seen by the green fluorescent signal in FHT immunostained tissue sections (Fig. 7E, F). Some of these parenchyma cells were not yet suberized although they showed signs of amyloplast depletion.

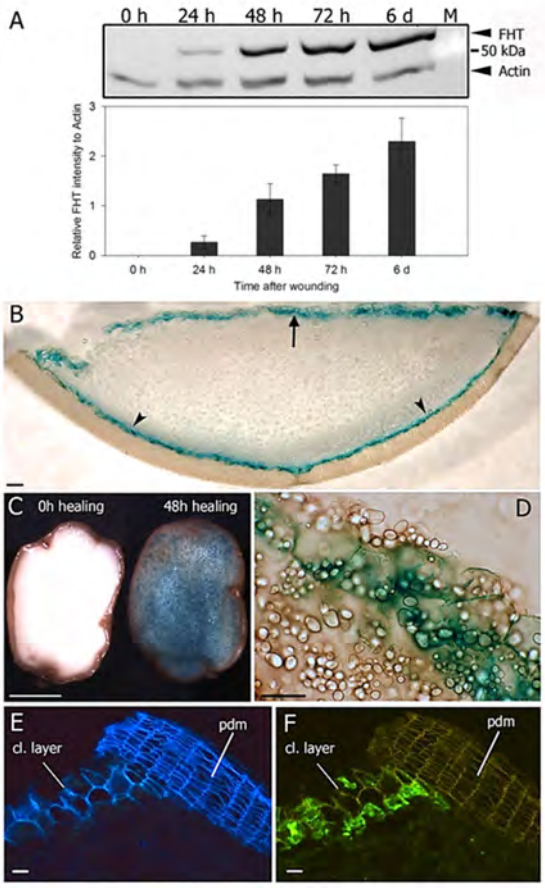

Fig. 7. FHT in wound-healing tubers of potato. (A) The upper panel shows the FHT protein profile in healing potato discs monitored by western blot using actin as a loading control. The lower panel shows FHT accumulation relative to actin as quantified for each lane (values are means ±SD of three independent biological replicates). FHT accumulation is observed 24 h after injury and increases progressively up to the sixth day. (B) Section of a transgenic tuber 48 h after injury showing GUS activity localized on the wound surface (arrow) and also in the native periderm (arrowheads). (C) A tuber cut in half stained for GUS activity at 0 h and 48 h after wounding. (D) Thin section of the wound showing FHT promoter activity localized in the live parenchyma cells closest to the wound surface. (E and F) Cryosection of the wound obtained 72 h after injury showing the contact zone between the wound and the native periderm. Observed under (E) UV excitation to show the suberin autofluorescence and (F) under blue light excitation to show the green fluorescence of the FHT. Scale bars=100 µm (B), 5 mm (C), 50 µm (D–F). cl. layer, wound closing layer; pdm; native periderm.

Phytohormones and FHT induction in healing tissues

In order to better understand the role of ABA in wound induced suberization and to discern possible effects of JA and SA, FHT accumulation was examined in potato tuber discs treated with 0.1 mM hormone solutions for 1 h and afterwards left to heal. Upon examination 24 h and 48 h after wounding, the ratio between the intensity of the FHT and actin bands was greater in the ABA-treated discs than in the non-treated discs where the FHT band was barely visible (Fig. 8A). Thus, ABA treatment enhances the induction of FHT in healing tissues of potato. Examination at the same time periods

revealed that discs treated with JA showed no effects on FHT accumulation in comparison with the controls (Fig. 8B). In contrast, in the SA-treated discs, FHT protein expression was not detected at 24 h following wounding and the intensity of the band 48 h after wounding was lower compared with that of the control discs (Fig. 8C), thus pointing to a regulatory effect of SA in wound-induced suberization which is antagonistic to that of ABA.

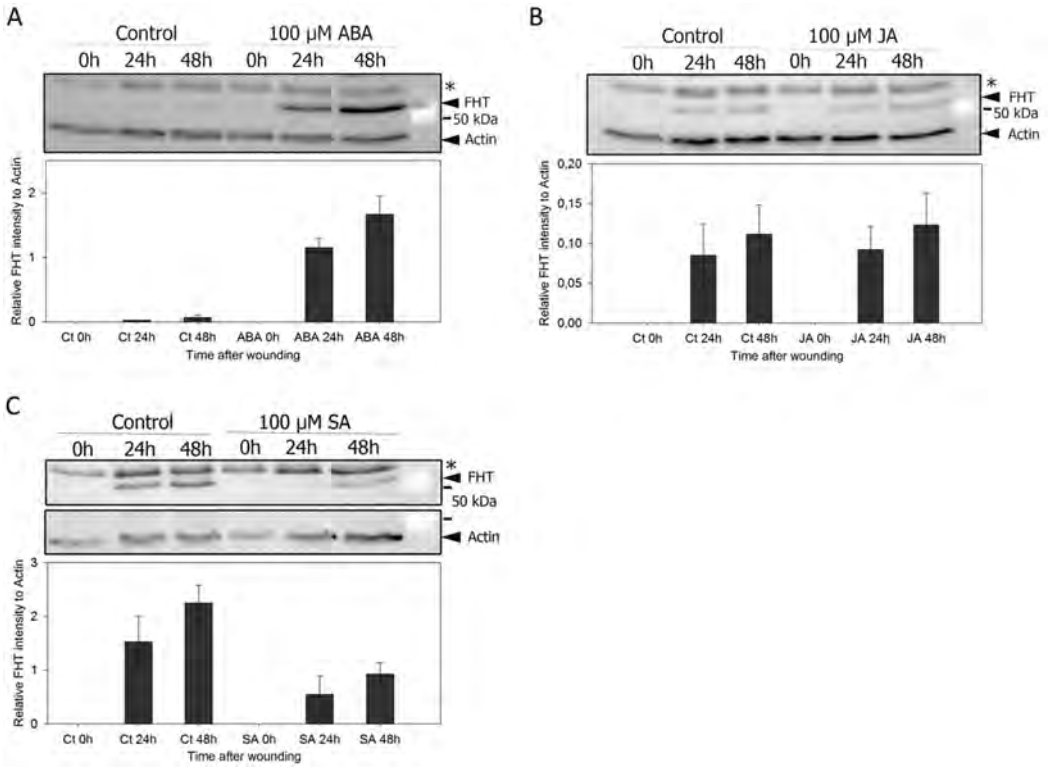

Fig. 8. ABA and SA but not JA modify FHT expression in healing potato discs. Protein extracts were analysed by western blot (upper panels) with FHT antiserum. Actin was used as a loading control. The lower panels show FHT accumulation relative to actin as quantified for each lane (values are means ±SD of three independent biological replicates). (A) FHT induction by ABA was monitored in wound-healing potato tuber discs. ABA treatment enhances FHT accumulation during the wound-healing process (t-test, $P < 0.01$). (B) No significant differences between JA treatment and the control treatment with regard to FHT protein accumulation were detected. (C) FHT protein accumulation is reduced in SA-treated discs compared with the control treatment (t-test, $P < 0.05$). The molecular marker is shown to the right. Asterisks mark extra bands that do not correspond to the expected molecular weights of the proteins analyzed.

Subcellular localization of FHT

Sequence analysis of FHT using TMHMM (Krogh *et al.* 2001), SignalP (Petersen *et al.*, 2011), and the WolfPSORT (Horton *et al.*, 2007) programs to predict the subcellular localization anticipated no transmembrane helices and no signal peptide; therefore, they forecast a cytosolic localization of the protein. The experimental evidence for the FHT subcellular localization was obtained by ultracentrifugation of the protein homogenates

from native and wounded periderm as well as root tissue. The protein extracts were separated into supernatant and pellet fractions expected to contain soluble (cytosolic) and microsomal proteins, respectively. These fractions were analysed by western blot using antibodies against FHT, a cytosolic protein marker (the UDP-glucose pyrophosphorylase, UGPase) protein, and a microsomal protein marker calreticulin (Fig. 9). The calreticulin antibody reacted only with the pellet fractions, confirming that microsomal proteins are localized in the pellet. Conversely, the UGPase antibody reacted with the supernatant, although a faint reaction also appeared in the pellet of the tuber-wound periderm. The FHT protein behaved in a similar manner to UGPase, a result consistent with a cytosolic localization in accordance with the *in silico* predictions.

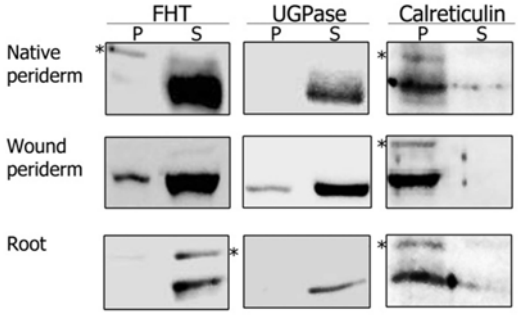

Fig. 9. FHT immunodetection in the subcellular fractions derived from suberized tissues. Protein fractions of native and wound periderm as well as root tissues were obtained by ultracentrifugation and analysed by western blot. In addition to the FHT antiserum, UGPase and calreticulin antibodies were also used as cytosolic and microsomal markers, respectively. S, soluble (cytosolic) fraction; P, pellet (microsomal fraction). The asterisks mark non-specific bands.

DISCUSSION

FHT is accumulated in the phellogen

FHT encodes a potato feruloyl transferase involved in suberin and wax biosynthesis that is necessary for periderm integrity (Serra *et al.*, 2010b). FHT silenced tubers display a defective skin, lose large amounts of water, and remain prone to excoriation (skinning) for a long period after harvest (Serra *et al.*, 2010b). Here it is demonstrated that *FHT* is specifically expressed and that the protein accumulates in the phellogen cell layer (Fig. 2). No FHT protein—or only extremely faint traces—was observed in the innermost layers of the phellem. Thus, *FHT* becomes active in phellogen cells before suberin deposition starts or at least before it can be detected. It is remarkable that *ASFT*, the *FHT Arabidopsis* orthologue, is the only gene among seven other suberin reporter genes that is expressed much earlier than the start of suberin deposition in endodermal cells (Naseer *et al.*, 2012). Also worth mentioning is the fact that the aromatic suberin is laid down in the cell wall well in advance of the aliphatic suberin (Lulai and Corsini, 1998).

The early accumulation of ferulate may be a critical aspect for the coupling of the aromatic and aliphatic suberin domains, considering that ferulate esters are able to form covalent bonds with cell wall polysaccharides and polyphenolics while leaving the aliphatic chain ready for esterification (Liu, 2010). On the other hand, the maximum FHT accumulation in the periderm occurs during progression of the periderm maturation (Fig. 5), a complex physiological process that usually takes place at harvest and in which the phellogen becomes meristematically inactive (Lulai and Freeman, 2001), while at the same time the phellem completes its full suberin and wax load (Schreiber *et al.*, 2005). The mature periderm maintains the FHT levels although with a decreasing trend (Fig. 5). This sustained FHT presence suggests a continuous function of this protein in phellogen cells of the mature periderm which remain meristematically inactive. Such a function may be related to the maintenance of the integrity of the apoplastic barrier: a pool of FHT kept at a basal level may rapidly provide new ferulate esters if eventually the phellogen receives the appropriate stimuli to undergo phellem differentiation. Such a mechanism may be effective with regard to microfissures or small cracks that could promote water loss and the entry of microorganisms. Lenticels are special areas of the periderm which are crucial to regulate gas exchange. They form early in developing tubers by periclinal divisions of cells beneath the stomata, giving rise to a particular phellogen which produces a type of suberized tissue that is permeable to water and gases (complementary tissue). The phellogen then extends from lenticels to build up a complete layer of native periderm (Adams, 1975; Tyner *et al.*, 1997). The preponderance of the FHT transcriptional activity and protein accumulation in lenticels (Figs. 4, 5) agree with an intense activity of the lenticular phellogen in developing tubers. Moreover, the regulation of gas exchange by lenticels is based on the long-term structural changes which involve phellogen activity and suberin biosynthesis, namely the formation of a closing layer of highly suberized and dense cells to restrict gas exchange, or the enlargement of the lenticular area by proliferation to increase gas exchange (Lendzian, 2006). Environmental factors such as temperature and humidity have been related to the proliferation of the lenticular phellogen during tuber storage (Adams, 1975). Lenticel disorders in fresh market potatoes have been related to suberin deposition in lenticels (Makani, 2010).

FHT in the root boundary layers

FHT and its *Arabidopsis* orthologue ASFT (Molina *et al.*, 2009) are specifically expressed in root exodermal and endodermal cells where suberization occurs, although not in other cells (Fig. 3). Together the endodermis and exodermis are effective water and ion barriers while both possess Casparian strips and develop suberin lamellae (Enstone *et al.*, 2003). The strips develop earlier than lamellae and are important to prevent the apoplastic bypass of salts into the stele (Chen *et al.*, 2011). In addition, both the exodermis and endodermis are variable barriers that develop closer to or further from the root tip depending on abiotic stress (Enstone *et al.*, 2003) or pathogens (Thomas *et al.*, 2007). Moreover, the rate of suberization (Hose *et al.*, 2001) and the proportion between aliphatic and aromatic monomers in the root suberin (Zimmerman *et al.*, 2000) also depend on stress factors such as drought, anoxia, or salinity. In agreement with this, some genes involved in root suberin deposition are expressed under salt, osmotic treatment, or drought (Franke *et al.*, 2009; Lee *et al.*, 2009; Domergue *et al.*, 2010). In addition, suberin mutants, such as *GPAT5*, *esb1*, and the *FHT* ortholog *AtHHT/rwp* show modified sensitivities to salt stress (Beisson *et al.*, 2007; Baxter *et al.*, 2009; Gou *et al.*, 2009). Therefore, the contribution of *FHT* with regard to the regulation of root suberin deposition under stress cues such as anoxia, drought, or biotic stress could be surmised, taking into account the predicted *cis*-regulatory elements of the *FHT* promoter (Supplementary Table S1).

FHT is induced by injury

Tissues react to injury by forming a suberized and lignified closing layer which in most tissues is followed by active cell division that gives rise to a new phellogen and thereafter a wound periderm. In potato, leaves are characterized by the formation of a closing layer which is adjacent to the wounded margin and lacks cell division (Bloch, 1941), while tubers develop a wound periderm as has been widely documented (see, among others, Morris *et al.*, 1989; Sabba and Lulai, 2002). In leaves, FHT protein accumulation peaks after the third day following wounding when the formation of the closing layer is completed (Fig. 6A). In tubers, FHT accumulates early but keeps increasing at least up to the sixth day after injury (Fig. 7A) when the formation of the wound periderm is almost completed. These observations prove a rapid and massive induction of FHT during the healing process concomitant with suberin deposition. It has been shown that deposition of the aromatic suberin precedes that of the aliphatic suberin

(Yang and Bernards, 2006). In mechanically injured potato leaves, the gene encoding phenylalanine ammonia lyase (*PAL*), an enzyme that operates at the very early steps of the phenylpropanoid biosynthesis, peaks 2 h after wounding and returns to its original level ~6 h afterwards (Joos and Halborck, 1992). In wounded potato tubers, suberization-associated anionic peroxidases appear after day 2 post-wounding and gradually increase until day 8 (Chaves *et al.*, 2009). In leaves of *Arabidopsis*, the *DAISY* transcript which encodes a fatty acid elongase peaks 1 h after wounding (Franke *et al.*, 2009), while transcripts encoding fatty acid reductases (*FAR*) peak 48 h after injury (Domergue *et al.*, 2010).

FHT is regulated by ABA and SA

Injury and pathogen attack activate JA, ethylene, ABA, and SA production, and these signals are transduced to a number of genes which are essential for plant protection (Bruxelles and Roberts, 2001). Moreover, interactions among these pathways allow for antagonistic and synergistic effects (Atkinson and Urwin, 2012). Suberin and lignin deposition are involved in most defense reactions (Thomas *et al.*, 2007). FHT is induced by wounding (Figs 6, 7) and responds to ABA and SA treatments (Fig. 8), presenting predicted *cis*-regulatory motifs for biotic and abiotic stress as well as ABA, JA, and SA responsiveness (Supplementary Table S1). A positive effect of ABA with regard to the induction of suberin genes and suberin deposition has been documented in potato (Soliday *et al.*, 1978; Roberts and Kolattukudy, 1989; Lulai *et al.*, 2008), *Arabidopsis* (Lee *et al.*, 2009), and tomato (Leide *et al.*, 2011). Moreover, Suttle *et al.* (2013) showed that endogenous ABA concentrations in potato tubers decrease after injury and reach a minimum after 24 h; nonetheless, the concentration then increases from the third to the seventh day in a pattern parallel to that of FHT (Fig. 7A). Furthermore, Lulai *et al.* (2008) reported that endogenous ABA concentrations increase after tuber harvest and then decrease during tuber storage, displaying an age-dependent pattern also similar to that of FHT (Fig. 5). According to Kumar *et al.* (2010), treatment with ABA partly restores the healing ability of older tubers by enhancing the accumulation of suberin aromatics. These authors also demonstrated that the age-induced loss of the healing ability is partly due to a reduced capacity to accumulate ABA and modulate the production of suberin aromatics through PAL. A similar modulation might also be contemplated through FHT. On the other hand, injury of potato tubers triggers a rapid increase (by 5-fold) of the basal JA content which peaks 4–6 h after wounding and

thereafter returns to basal levels, a pattern compatible with a role in the early wound response (Koda and Kikuta, 1994). However, Lulai *et al.* (2011) showed no effect of JA treatment or inhibition of JA accumulation on suberin biosynthesis in the wound closing layer, in agreement with the lack of an enhancing or inhibiting effect of JA with regard to FHT induction (Fig. 8B). In contrast, Ozeretskovskaya *et al.* (2009) reported a positive effect of exogenous JA in reference to periderm proliferation, but this finding opposes the more general view that one of the functions of the wound-induced JA is related to the inhibition of growth by mitotic suppression (Zhang *et al.*, 2008). Concerning SA, its role in wound responses has so far not been elucidated (Vlot *et al.*, 2009). Previous experiments using potato discs have to date been unable to detect any effect of exogenous SA in connection with the healing process (Ozeretskovskaya *et al.*, 2009). However, SA impedes FHT induction after injury (Fig. 8C), acting in an antagonistic manner with respect to ABA. The antagonistic interaction among the ABA and SA signalling pathways has already been reported in several stress and defense responses (Jiang *et al.*, 2010; Sánchez-Vallet *et al.*, 2012).

FHT is located in the cytosol

Most factors that contribute to the transport and polymerization of suberin monomers are still unknown and the subcellular organization of the enzymes of the suberin biosynthesis pathway remains unclear (Pollard *et al.*, 2008; Beisson *et al.*, 2012). The endoplasmic reticulum (ER) has been reported as the location of some suberin, cutin, and wax enzymes, such as CER4/FAR3, CYP86A1/Horst KCS, KCR, and LACS (Rowland *et al.*, 2006; Höfer *et al.*, 2008; Joubès *et al.*, 2008; Beaudoin *et al.*, 2009; Weng *et al.*, 2009). Thus, the ER is supposed to be the place where reduction, hydroxylation, and elongation of the very long fatty acid chains occur. It is noteworthy that FAR proteins 1, 4, and 5 provide the fatty alcohols required for FACT, a feruloyl transferase closely related to FHT (Kosma *et al.*, 2012). However, subcellular fractionation indicates that FHT is absent from the ER but present in the cytosol. Moreover, two cutin BAHD acyltransferases also localize in the cytoplasm, and one of them, DCF (defective in cutin ferulate), has a homologous enzyme activity to that of FHT (Panikashvili *et al.*, 2009; Rautengarten *et al.*, 2012). The cytosolic localization of these enzymes is intriguing when the hydrophobic nature of their substrates is considered. In fact, Pascal *et al.* (2012) hypothesized an interaction with microsomal

proteins as a requirement for CER2, which is annotated as BADH acyltransferase and is localized only in the cytosol.

MATERIALS AND METHODS

Plant material

Potato plants (*Solanum tuberosum*) subspecies *tuberosum* (cv. Désirée) and *andigena* were propagated as described by Serra *et al.* (2010b). For the *andigena* plants, tuber induction was performed in soil when plants reached the 14-leaf stage by setting short-day conditions (8 h light/16 h dark) and *in vitro* as described by Dobránszki (2001). The commercial potato cv. Kennebec used for the wound healing and hormone experiments was purchased from a local supermarket.

Phytohormone treatments

Potato discs (3 mm thick and 13 mm in diameter) were obtained by cutting cylinders of parenchyma tissue excised from tubers with a cork borer. Hormone stock solutions were prepared at 0.1 M ABA (Sigma, A-1049) in dimethylsulphoxide (Lulai *et al.*, 2008), 0.1 M JA (Sigma, J-2500), and 0.25 M SA (Sigma, S-7401) in ethanol. ABA, JA, and SA assays were performed on freshly cut discs at a final concentration of 0.1 mM diluted with milliQ water. Discs were placed in the hormone solutions (≤30 discs/100 ml of solution) and incubated at room temperature for 1 h on a rotatory shaker (50 cycles min^{-1}) to achieve uniform hormone permeation. After treatment, discs were removed from the solution and allowed to wound heal at room temperature in saturated humidity and dark conditions. As a control, the same protocol was applied to potato discs in treatments without phytohormones and with the respective dimethylsulphoxide or ethanol volumes. Control and treated discs were collected and frozen in liquid nitrogen for analysis.

Generation of ProFHT::GUS-GFP transgenic potatoes

The promoter of *FHT* was obtained by Genome Walker (Clontech) and using the *Solanum phureja* genome (http://solanaceae.plantbiology.msu.edu/pgsc_download.shtml; Potato Genome Sequencing Consortium, 2011). A fragment consisting of 2541 bp upstream of the initial ATG codon (KC695749) was amplified with the forward primer 5′-GCACGAAGTTTCCAAGCATT-3′ and the

reverse primer 5′-TTCTCAAATTAAAAATCCTGTTT-3′. This sequence was cloned into the GATEWAY entry vector pENTR/D-TOPO (Invitrogen) and transferred into the GATEWAY destination vector pKGWFS7 (Karimi *et al.*, 2002) by LR reaction (Invitrogen). Potato leaves were infected with *Agrobacterium tumefaciens* strain GV2260 and stably transformed with the *ProFHT*::*GUS-GFP* recombinant plasmid according to Banerjee *et al.* (2006). Kanamycin-resistant plants were regenerated and grown *in vitro* until tuber development.

FHT polyclonal antiserum and western analysis

The FHT protein was purified as described by Serra *et al.* (2010b) and the polyclonal antibody was obtained from the Antibody Production Service of the CSIC (Barcelona). Following standard protocols, two rabbits were respectively immunized with 1 mg of purified FHT. To obtain reactivity of the antibody against both the native and non-native proteins, each injection contained both the native and the heat-denatured antigen (1:1). Dot-blot and western blot assays confirmed that an antiserum dilution of 1:10 000 was able to detect 1 ng of the native protein and 100 ng of the denatured protein. The antiserum was purified as follows: a membrane containing 100 μg of purified FHT was incubated with 100 mM glycine at pH 2.5 for 10 min to remove poorly bound proteins, blocked with 5% skimmed milk powder in TRIS-buffered saline–Tween (TBST) for 45 min, followed by overnight incubation with 10 ml of the antiserum, and subsequently washed thoroughly with TBST buffer. Purified antibodies were eluted with 100 mM glycine (pH 2.5) and then neutralized with TRIS-HCl (pH 8) until a pH of 7 was reached. Soluble proteins were extracted from tissues with a buffer containing 56 mM $NaCO_3$, 56 mM dithiothreitol (DTT), 2% SDS, 12% sucrose, and 2 mM EDTA in a ratio of 1 ml per 0.5 g of fresh tissue. Protein concentrations were determined using the Bradford assay. Extracts were resolved in either 10% or 12% acrylamide SDS–polyacrylamide gels and blotted onto nitrocellulose membranes (Millipore) using 40 μg of total protein. The membranes were blocked and then probed overnight at 4 ºC against a 1:10 000 dilution of crude rabbit anti-FHT serum and a 1:4000 dilution of mouse anti-actin (Agrisera) used as a loading control. Primary antibodies were detected by means of secondary antibodies against rabbit (Nordic Immunology) and mouse (Calbiochem), respectively, which were conjugated to a peroxidase. Peroxidase activity was detected by chemiluminiscence (Millipore) and images of the blots were used for quantification

via densitometry (Flurochem SP, AlphaInnotech). Band quantification was performed by Quantity One Software (Bio-rad).

Detection of FHT promoter activity

Plant tissues were immersed in an ice-chilled 90% acetone (v/v) bath and incubated for 20 min on ice, after which they were rinsed with water. Tissues were infiltrated with 1 mM 5-bromo-4-chloro- 3-indolyl-β-d-glucuronic sodium salt 3·H2O (X-GlcA, Duchefa), 50 mM sodium phosphate buffer (pH 7), 1 mM potassium ferrocyanide, 1 mM potassium ferricyanide, 10 mM EDTA, and 0.05% (v/v) Triton X-100 for 20 min under vacuum, incubated at 37°C for a maximum of 48 h, and then cleared with 70% (v/v) ethanol. Stained tissues were washed 2–3 times with phosphate-buffered saline (PBS) and cryoprotected through a series of 0.1, 0.5, and 1 M sucrose in PBS solution in order to carry out sectioning in a Cryocut 1800 (Reichert-Jung) cryotome. Observations were made using a Nikon SMZ-1000 stereomicroscope and an Olympus Vannox microscope, and micrographs were obtained using a set of Infinity X, Deltapix, and Nikon digital cameras. Transgenic roots were observed using a Nikon CS1 90i Eclipse confocal laser-scanning microscope. For the visualization of GFP, fluorescent samples were excited at 488 nm with an argon ion laser and emission was monitored at 500–530 nm; images were obtained using the EZ-C1 software.

Immunohistochemical detection of FHT

Tissues fixed by vacuum infiltration for 90 min in 4% paraformaldehyde (w/v) in PBS were subsequently washed twice with PBS and twice with distilled water. Waxes were removed through an ethanol– xylol series (Sauer *et al.*, 2006) and cryosectioning was performed. Dried sections were incubated in PBS for 10 min, blocked with 2% bovine serum albumin (BSA) solution in PBS for 30 min, and then labelled by incubation with the purified FHT antibody diluted 1:50 in 2% BSA at 4 ºC overnight, followed by a secondary goat antirabbit IgG Alexa Fluor 488 (Invitrogen) diluted 1:500 in 2% BSA. Whole-mount tissues were treated according to Sauer *et al.* (2006) and then incubated with the purified FHT antibody diluted 1:25 for 240 min at 37 ºC, followed by incubation with an Alexa Fluor 488- (Invitrogen) labelled secondary antibody diluted 1:500 for 180 min at 37 ºC. Fluorescence images were observed with an epifluorescence LEICA DMR-XA microscope and images were taken with a Jenoptik ProgRes C14 digital camera.

Subcellular fragmentation assay

Plant material was ground in liquid nitrogen, and protein extraction and subcellular fractionation were performed as described by Rautengarten *et al.* (2012). The extracted proteins in the supernatant and pellet fractions were analysed via western blot as described above. Blots were probed with rabbit anti-UGPase (Agrisera) at a 1:3000 dilution, rabbit anti-calreticulin (Abcam) at a 1:1000 dilution, and crude rabbit anti-FHT at a 1:10 000 dilution at 4 ºC overnight. After three consecutive washing steps, the membranes were incubated for 1 h at room temperature with a goat anti-rabbit antibody (Nordic Immunology) conjugated to peroxidase 1:40 000 dilution. Peroxidase activity was detected by chemiluminiscence as described above (Millipore).

Chapter II

The KH-domain RNA-binding protein StRIK from potato regulates stress- and transposable element-related genes in the periderm and affects flowering

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ABSTRACT

Genetic factors involved in the regulation of periderm development are basically unknown. In a previous transcriptomic approach in the outer bark of cork oak we identified a K homology (KH)-domain RNA-binding protein, *RIK* (*RS2-INTERACTING KH PROTEIN*), as upregulated in the phellem (in comparison to xylem). In the present work we have performed molecular and *in silico* analyses of the potato *StRIK* and the Arabidopsis *RIK* genes to obtain more insights to their function. By RT-qPCR we determined a *StRIK* widespread expression in potato plant tissues including periderm and upon wounding in potato tuber discs. Phenotypic observations of *RIK* downregulated transgenic lines revealed in a preliminary experiment that *StRIK* silencing induces floral transition at the shoot apical meristem. The transcriptome analyses of the tuber periderm of *StRIK* defective lines revealed differential expression of genes related to RNA processing, stress and DNA transposition, in agreement with the *in silico* co-expression network of Arabidopsis homolog *RIK* gene, which is enriched in functions related to regulation, splicing, gene silencing, development and stress. These results are in line with the nuclear localization of the RIK protein. Considering the overall results, we suggest that potato *StRIK* might play roles in the post-transcriptional regulation of pre-mRNAs but also in the chromatin regulation which might be involved in the control of transposable elements in response to stress and in diverse plant developmental processes such as flowering or periderm formation.

Chapter III

Comparative transcriptional profiling of cork and holm oak phellem tissues unveils molecular networks orchestrating phellem formation

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ABSTRACT

The phellem (cork) is the covering tissue in plant mature (secondary) organs and healing tissues but despite its importance for plant survival, the molecular mechanisms underlying its formation are far from being fully understood. To gain new insights the phellem transcriptome was profiled in two different model systems: the outer bark of the cork tree (*Quercus suber* L.) from which commercial cork is obtained and that of holm oak tree (*Quercus ilex* L.) which consists of a rythidome. Both transcriptomes were sequenced, functionally analysed and the genes identified were catalogued in the following categories: carbohydrate, amino acid, acyl-lipid and isoprenoid metabolisms, cell wall, transcription factors, phytohormone and meristem-related genes and integrators of environmental cues. Cork oak and holm oak transcriptomes reflected similitudes in their complexity and were classified similarly in the main biological processes. However, many genes showed differential expression between both oaks (16.4%). In comparison, cork oak was enriched in cell wall synthesis and loosening, and in suberin-related secondary pathways synchronously with the primary metabolic pathways that supply the precursors. Stem cell identity genes, auxin polar transport and ethylene synthesis and perception were also more induced in cork oak. In contrast, genes associated to ABA signaling, to biotic and abiotic stress responses, and to regulatory processes such as chromatin remodeling, regulation of transcription and protein synthesis were enriched in holm oak. A few genes involved in the vernalization response were also more induced in holm oak. Globally this study provides a comprehensive view of the whole phellem transcriptome and identifies candidate genes for phellem that may explain the different specificities of the phellem tissue in both *Quercus* species.

Chapter IV

Transcriptional characterization of a selected set of new cork candidate genes: mRNA abundance in phellem versus xylem tissue and effect of cork seasonal growth in their expression.

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ABSTRACT

The biogenesis of cork (phellem) secondary tissue involves the sequential stages of meristem initiation and proliferation, cell expansion, massive suberin deposition and the entry to an irreversible program of cell death. Here, based on the transcriptomic analysis on cork tissue performed in Chapter III, we selected a set of candidate genes of the cellular processes occurring in cork formation. From this selection we compared for 26 genes the mRNA abundances in cork to a non-suberin-producing secondary tissue such as wood (xylem) to identify genes more specifically required for cork formation and suberin synthesis. The mRNA abundance along the cork growing season was also analysed for 21 genes and statistically evaluated to identify common trends by building gene clusters. Genes involved in *de novo* fatty acid, isoprenoids and suberin biosynthesis, and NACs and R2R3-MYBs transcription factors candidates for suberin regulation, were preferentially expressed in cork. In comparison, meristem- and phytohormone-related genes showed fewer differences between cork and wood tissues. The transcript profiles along the cork growing season showed significant seasonality for most of the genes analysed and displayed two major trends. On the one hand, cell wall and suberin metabolisms, oxidative stress tolerance genes, and ethylene and gibberellin receptors showed the highest expression in June when cork differentiation is supposed to be at maximum rate. On the other hand, most of the genes involved in the regulation of meristem development, including an auxin transporter and a cytokinin receptor, were grouped in the same cluster which peaked in April, when phellogen activity initiates. Our study provides a first approach to the function of some new cork candidate genes by identifying structural and regulatory genes preferentially expressed in cork, indicating certain similarities in the regulation of wood- and cork-forming tissues

Conclusions

4. Conclusions

From objective 1: Understand the suberization process in roots and tubers by defining the spatiotemporal expression patterns of the central suberin biosynthetic gene FHT (Chapter I).

1. The *FHT* expression is restricted to the native suberized tissues (root exo- and endodermis and the tuber periderm) and at subcellular level the protein is located in the cytosol.
2. In the tuber periderm, the *FHT* gene turns on in the phellogen derivatives cells before suberin depositions can be detected, suggesting FHT as an early marker of the suberization process.
3. FHT protein levels are retained in the periderm for several months after harvest suggesting a mechanism for the maintenance of phellem apoplastic barrier integrity once tuber growth ceases.
4. *FHT* gene is induced by wounding and abscisic acid whereas is repressed by salycilic acid.

From objective 2: Obtain insights on the role of *StRIK* gene in the regulation of periderm formation (Chapter II)

5. *StRIK* transcript is expressed in suberized and unsuberized plant tissues, hence indicating that the role of this gene is not specific of phellem and suberized tissues.
6. The nuclear localization of StRIK supports its putative role on the processing of pre-mRNAs predicted by *in silico* analyses.
7. Silencing of *StRIK* did not cause obvious effects in periderm structure but frequently induced floral transition at the shoot apical meristem.
8. Downregulation of *StRIK* in the periderm affects the expression of genes involved in the stress response, DNA transposition and RNA processing.

From objective 3: Expand the understanding of the phellem transcriptome and highlight new candidate genes for phellem formation in cork oak and holm oak using RNA-seq (Chapter III).

9. The *de novo* assembly of phellem transcriptome yielded 16,865 contigs which were annotated with Gene ontologies, KEGG pathways, MapMan 'bins', conserved protein domains and against several sequence databases. Using Arabidopsis available public databases and literature, a set of 3,209 contigs were classified in the following relevant functions for the phellem: carbohydrate metabolism, amino acid metabolism, acyl-lipid metabolism, isoprenoids metabolism, cell wall proteins, transcription factors families, phytohormone-related genes, meristem regulation and the pathways involved in the integration of environmental cues.
10. The genes upregulated in cork oak were enriched in cell wall synthesis and loosening, suberin-related secondary pathways as well as sucrose metabolism, glycolysis, *de novo* synthesis of fatty acids, phenylalanine and methionine metabolism; suggesting that primary metabolic pathways are coordinately expressed to supply the molecular building blocks for phellem cell growth and suberin deposition.
11. The genes involved in meristem maintenance and proliferation and auxin transporters were also upregulated in cork oak suggesting molecular mechanisms that contribute to the major development of cork oak phellem.
12. The genes upregulated in holm oak were enriched in ABA signaling, biotic and abiotic stress responses, and to regulatory processes such as chromatin remodeling, regulation of transcription and protein synthesis. Thus, it suggests that phellem cells in holm oak are subjected to more stress during their development and the major induction of ribosomes or chromatin related genes might contribute to maintain cellular homeostasis and the correct gene regulation.
13. Genes known to be involved in the integration of environmental cues and in the epigenetic regulation were differentially regulated indicating that these genes might be involved in the transcriptional and developmental differences between the phellem of cork oak and holm oak trees.

14. Several structural and regulatory genes showed *in silico* co-expression with suberin genes. Among them stand out the members of the class III peroxidases and CASP-like proteins, which have not been yet related with the suberization process, and several NAC, R2R3-MYB and WRKY transcription factors.
15. We have identified *in silico* potential SNPs and INDELs which, upon their experimental validation, could be used as molecular markers for genetic population studies or to identify potential biomarkers for cork production by detecting specific variants of phellem candidate genes in cork oak and holm oak genotypes.

From objective 4: Obtain a better insight to the function of some phellem candidate genes identified in the RNA-seq experiment by studying their transcriptional pattern in cork oak tissues (Chapter IV).

16. Genes involved in fatty acid, isoprenoids and suberin biosynthesis, and some NAC and R2R3-MYB transcription factors are upregulated in cork compared to wood, which reinforced their expected role in cork formation.
17. Most of the meristem- and phytohormonal-related genes analysed showed similar transcript accumulation in cork and wood, suggesting similarities in the regulation of both secondary tissues.
18. Genes involved in lipid, cell wall and suberin metabolism as well as a cell death inhibitor and ethylene and gibberellins receptors showed their highest expression in June, when cork growth is supposed to be maximum.
19. Several genes involved in meristem regulation, including an auxin transporter and a cytokinin receptor, were grouped in the same cluster with an expression peak in April when phellogen activity initiates.

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Annex

Supplemental data for Chapter I

The potato suberin feruloyl transferase FHT which accumulates in the phellogen is induced by wounding and regulated by abscisic and salicylic acids

This supplemental data include *FHT* promoter driven GUS expression analyses in stem cork wards and in freshly-harvested and stored potato tubers, and the description of the putative *cis*-elements identified *in silico* in *FHT* promoter.

Supplementary figures

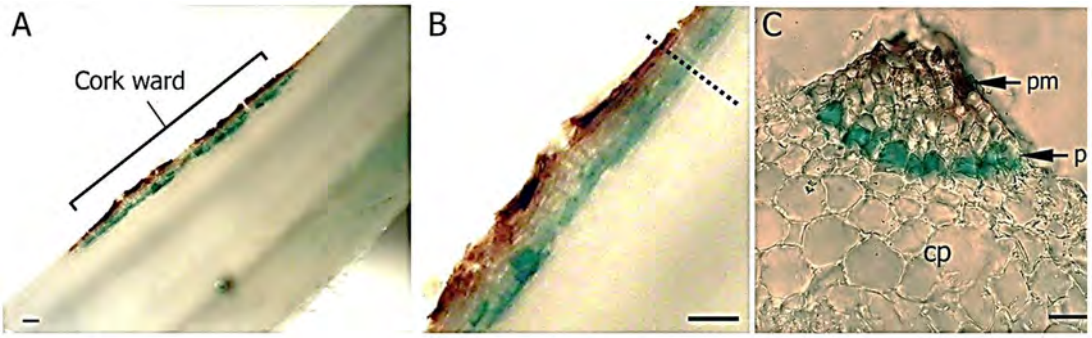

Fig. S1. GUS activity driven by *FHT* promoter in the stem of *in vitro* cultured ssp. *Andigena* plants. (A) Whole-mount showing a cork ward. (B) Detail of A. (C) Cryotome cross-section of a cork wards. Dotted line in B indicates the cross section orientation. Scale bars = 100 µm (A, B), 50 µm (C). Pg, phellogen; pm, phellem; cp, cortical parenchyma.

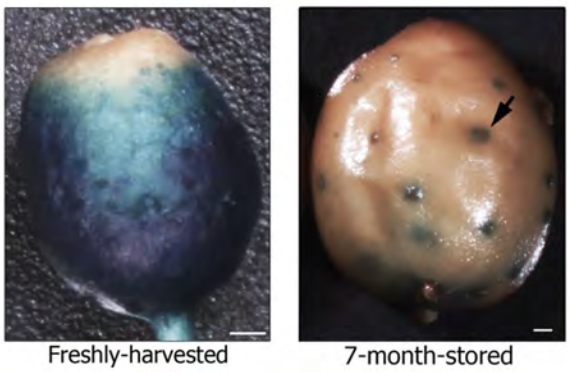

Fig. S2. A freshly-harvested and a 7-month-stored tuber stained for GUS activity. GUS expression is still detected mainly in the lenticels of tuber periderm after 7 months storage (arrow). Scale bars = 1 mm.

Supplementary tables

Table S1. Putative *cis*-elements found in *FHT* promoter**.** The putative *FHT* promoter region (2541 bp upstream of the translation initiation) was examined using PLACE (Higo et al., 1999) and PlantCare (Lescot et al., 2002) databases. In agreement with the *FHT* promoter activity and protein profile in potato, the analysis reveals *cis*-regulatory elements specific of tuberous root (e.g. SPBF binding site); root (e.g. rolD; SORLIP 1); motifs involved in phenylpropanoids (e.g. E box element recognized by R2R3-MYB, BHLH factors that control light and tissue-specific activation of phenylpropanoids genes); motifs related to water stress and inducible by ABA (e.g. MYB and MYC binding sites, CAT-box, SORLIP1 and WRKY); motifs related to wounding (e.g. W-box responsive to methyl jasmonate); and motifs related to biotic stress and salicylic acid responsiveness (W-box, GT-1, elicitor-responsive, Salicylic-induced W-box and salicylic responsive TCA-elements).

Group	Motif name	Sequence	Motif nº	Function
Stress				
	WBOXNTCHN48	CTGACY	1	"W box". Elicitor-responsive element
	WBOXNTERF3	TGACY	7	"W box". Response to wounding
	OSE1ROOTNODULE	AAAGAT	5	Organ-specific elements (OSE) in infected cells of root nodules
	OSE2ROOTNODULE	CTCTT	4	Organ-specific elements (OSE) in infected cells of root nodules
	PREATPRODH	ACTCAT	1	Pro- or hypoosmolarity-responsive element
	RAV1AAT	CAACA	6	RAV1 binding site, cold responsiveness
	MYB1AT	WAACCA	6	Element involved in dehydration-responsiveness
	MYBCORE	CNGTTR	2	Element involved in response to water stress
	GT1GMSCAM4	GAAAAA	10	Pathogenesis and salt-induced element
	CURECORECR	GTAC	12	Copper-response element
	CCAATBOX1	CCAAT	4	HSE (Heat shock element)
	ANAERO1CONSENSUS	AAACAAA	5	Response to anaerobiosi
	ARE	TGGTTT	2	Cis-acting regulatory element essential for the anaerobic induction
	HSE	AGAAAATTCG	2	Cis-acting element involved in heat stress responsiveness
	TC-rich repeats	ATTTTCTTCA	4	Cis-acting element involved in defense and stress responsiveness
	ACGTATERD1	ACGT	6	Involved in etiolation-induced, dehydration
Hormones				
ABA	WBBOXPCWRKY1	TTTGACY	1	WRKY proteins binding site, responsive to ABA
	CAT-box	GCCACT	1	Element involved in meristem expression and ABA responsiveness
	MYB2AT	TAACTG	2	Element involved in response to water stress and ABA
	MYB2CONSENSUSAT	YAACKG	2	MYB recognition site involved in dehydration and ABA response

	MYCATERD1	CATGTG	1	MYC binding site involved in response to dehydration and ABA
	MYCATRD22	CACATG	1	MYC binding site involved in response to dehydration and ABA
	MYCCONSENSUSAT	CANNTG	12	MYC binding site involved in response to dehydration and ABA
	SORLIP1AT	GCCAC	4	Light-inducible, root-specific and ABA responsive element
Methyl Jasmonate	CGTCA-motif	CGTCA	1	Cis-acting regulatory element involved in the MeJA-responsiveness
	TGACG-motif	TGACG	1	Cis-acting regulatory element involved in the MeJA-responsiveness
Salicylic acid	TCA-element	GAGAAGAATA	1	Cis-acting element involved in salicylic acid responsiveness
	WBOXATNPR1	TTGAC	6	"W-box"; binding site for SA-induced WRKY transcription factor
Ethylene	ERELEE4	AWTTCAAA	1	Ethylene responsive element
Auxin	ASF1MOTIFCAMV	TGACG	1	Response to auxin and salicylic acid
	ARFAT	TGTCTC	1	Auxin response factor binding site
	TGA-element	AACGAC	1	Auxin-responsive element
	AuxRR-core	GGTCCAT	2	Cis-acting regulatory element involved in Auxin responsiveness
	CATATGGMSAUR	CATATG	4	Element involved in auxin responsiveness
Gibberellin	GARE2OSREP1	TAACGTA	1	Gibberellin-responsive element (GARE)
	GAREAT	TAACAAR	2	GARE (GA-responsive element)
	MYBGAHV	TAACAAA	2	Central element of gibberellin (GA) response complex (GARC)
	PYRIMIDINEBOXOSRAMY1A	CCTTTT	3	Gibberellin-respons cis-element of GARE and pyrimidine box
	TATCCACHVAL21	TATCCAC	1	Conserved cis-acting response complex (GARC)
	PYRIMIDINEBOXHVEPB1	TTTTTTCC	1	"Pyrimidine box". Required for GA induction
Tissue specific				
	ROOTMOTIFTAPOX1	ATATT	25	Root specific element from rolD gene
	SP8BFIBSP8BIB	TACTATT	1	Tuberous root specific elment
	RHERPATEXPA7	KCACGW	1	Right part of RHEs (Root Hair-specific cis-Elements)
	EBOXBNNAPA	CANNTG	12	RRE element. Tissue-specific activation of phenylpropanoids biosynthesis genes
	RYREPEATBNNAPA	CATGCA	3	Required for seed specific expression
	POLLEN1LELAT52	AGAAA	15	Element required for pollen specific expression
	AACACOREOSGLUB1	AACAAAC	2	Endosperm-specific elment

	BOXIINTPATPB	ATAGAA	2	Conserved elment in promoters of plastids genes.
	CACTFTPPCA1	YACT	38	Element related to mesophyll expression
	TAAAGSTKST1	TAAAG	10	Dof1 protein controlling guard cell-specific gene expression
Others	AMYBOX1	TAACARA	2	Conserved sequence in alpha-amylase gene promoter
	ARR1AT	NGATT	23	ARR1-binding element, response element
	BIHD1OS	TGTCA	9	Binding site of BELL homeodomain transcription factor
	CAATBOX1	CAAT	41	CAAT promoter consensus sequence
	CAREOSREP1	CAACTC	3	Element found in promoter region of a cystein proteinase gene
	CARGCW8GAT	CWWWWWWWWG	6	Binding site for AGAMOUS-like 15
	CIACADIANLELHC	CAANNNNATC	2	Element involved in circadian expression
	CPBCSPOR	TATTAG	2	Cytokinin-enhanced protein binding in vitro
	DOFCOREZM	AAAG	49	Core site for binding of Dof proteins
	EECCRCAH1	GANTTNC	2	Consensus motif for enhancer elements, EE-1 and EE-2
	GATABOX	GATA	19	Element required for high level, light regulated, and tissue specific expression
	GCN4OSGLUB1	TGAGTCA	1	Specific site for a basic leucine zipper transcription factor
	GT1CONSENSUS	GRWAAW	33	Consensus GT-1 binding site in many light-regulated genes
	GT1CORE	GGTTAA	3	Binding site for nuclear protein factor GT-1, light-dependent response element
	GTGANTG10	GTGA	11	Element found in late pollen gene g10
	HDZIP2ATATHB2	TAATMATTA	1	Binding site for homeobox gene (ATHB-2). Shade avoidance response
	HEXMOTIFTAH3H4	ACGTCA	1	"Hexamer motif" found in promoter of wheat histone genes H3 and H4
	IBOXCORE	GATAA	7	Conserved sequence upstream of light-regulated genes
	INRNTPSADB	YTCANTYY	7	"Inr (initiator)" elements found gene promoter without TATA boxes
	LECPLEACS2	TAAAATAT	2	Core element in LeCp binding cis-element
	MARTBOX	TTWTWTTWTT	4	"T-Box", motif found in SAR (scaffold attachment region)
	MYBPZM	CCWACC	1	Core of consensus myb homolog binding site
	MYBST1	GGATA	2	Core motif of MybSt1 (a potato MYB homolog) binding site
	NAPINMOTIFBN	TACACAT	1	Sequence of napin promoter
	NTBBF1ARROLB	ACTTTA	2	Dof protein binding site
	POLASIG1	AATAAA	14	poly A signal
	POLASIG2	AATTAAA	5	poly A signal
	POLASIG3	AATAAT	5	poly A signal

RBCSCONSENSUS	AATCCAA	1	rbcS general consensus sequence
REALPHALGLHCB21	AACCAA	3	Required for phytochrome regulation
RYREPEATGMGY2	CATGCAT	1	RY repeat motif
RYREPEATLEGUMINBOX	CATGCAY	2	RY repeat found in seed-storage protein genes
S1FBOXSORPS1L21	ATGGTA	3	Found in genes encoding plastid ribosomal protein S1 and L21
SEF1MOTIF	ATATTTAWW	1	SEF1 (soybean embryo factor 1)
SEF3MOTIFGM	AACCCA	2	SEF3 binding site (soybean embryo factor 3)
SEF4MOTIFGM7S	RTTTTTR	13	SEF4 binding site (soybean embryo factor 4)
Skn-1_motif	GTCAT	9	Cis-acting regulatory element required for endosperm expression
SORLIP2AT	GGGCC	1	Sequences over-represented in light-induced promoters
SORLREP3AT	TGTATATAT	2	Sequences over-represented in light-induced promoters
SURECOREATSULTR11	GAGAC	1	Core of sulfur-responsive element (SURE)
TATABOX2	TATAAAT	2	TATA box
TATABOX4	TATATAA	2	TATA box
TATABOX5	TTATTT	19	TATA box
TATAPVTRNALEU	TTTATATA	1	TATA-like sequence
TATCCAOSAMY	TATCCA	2	Element found in alpha-amylase promoters
TATCCAYMOTIFOSRAMY 3D	TATCCAY	1	RAmy3D alpha-amylase gene promoter
TBOXATGAPB	ACTTTG	5	Tbox. Reduce light-activation gene transcription
TRANSINITDICOTS	AMNAUGGC	1	Context sequence of translational initiation codon in dicots
TRANSINITMONOCOTS	RMNAUGGC	1	Context sequence of translational initiation codon in monocots
WBOXHVISO1	TGACT	7	SUSIBA2 bind to W-box element
WRKY71OS	TGAC	17	A core of TGAC-containing W-box
5UTR Py-rich stretch	TTTCTTCTCT	1	Cis-acting element conferring high transcription levels
AAAC-motif	CAACAAAAACCT	1	Light responsive element
AAGAA-motif	gGTAAAGAAA	1	
ACE	CTAACGTATT	1	Cis-acting element involved in light responsiveness
AE-box	AGAAACAA	1	Light responsive element
Box 4	ATTAAT	2	Module involved in light responsiveness
Box I	TTTCAAA	2	Light responsive element
ATCT-motif	AATCTAATCT	1	Module involved in light responsiveness
chs-Unit 1 m1	ACCTACCACAC	1	Module involved in light responsiveness
GT1-motif	GGTTAAT	4	Light responsive element
MNF1	GTGCCC(A/T)(A/T)	1	Light responsive element

O2-site	GATGATATGG	2	Cis-acting regulatory element involved in zein metabolism regulation
TATA-box	TTTTA	144	Core promoter element around -30 of transcription start
Unnamed__4	CTCC	6	
Unnamed__6	taTAAATATct	1	
Unnamed__8	CATTTTTGT	1	
TCT-motif	TCTTAC	3	Part of a light responsive element
LAMP-element	CCAAAACCA	1	Part of a light responsive element
TGACGTVMAMY	TGACGT	1	Required for high level expression
TATABOXOSPAL	TATTTAA	1	TATA box found in phenylalanine ammonia-lyase gene promoter

Supplementary references

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Supplemental data for Chapter II

The KH-domain RNA-binding protein StRIK from potato regulates stress- and transposable element-related genes in the periderm and affects flowering

This supplemental data includes pictograph representations of *RIK* gene expression patterns, the identification of the tentative consensus (TC) sequences which do not overlap with the *StRIK* gene model isolated by Soler (2008), StRIK protein alignments, the sequence used to generate the *StRIK*-RNAi construct, the screening of *StRIK*-RNAi silenced transgenic lines, photographs of *StRIK*-RNAi plants forming fully developed flowers and the number of small RNA loci identified. It also includes several tables containing the protein motifs identified in the StRIK protein sequence, a BLASTN analysis to identify off-targets of *StRIK*-RNAi fragment and the primers used for the RNA-seq validation by RT-qPCR. The table headings of the supplemental data presented in electronic format attached to the end of this thesis are also included.

Supplementary figures *(starts in next page)*

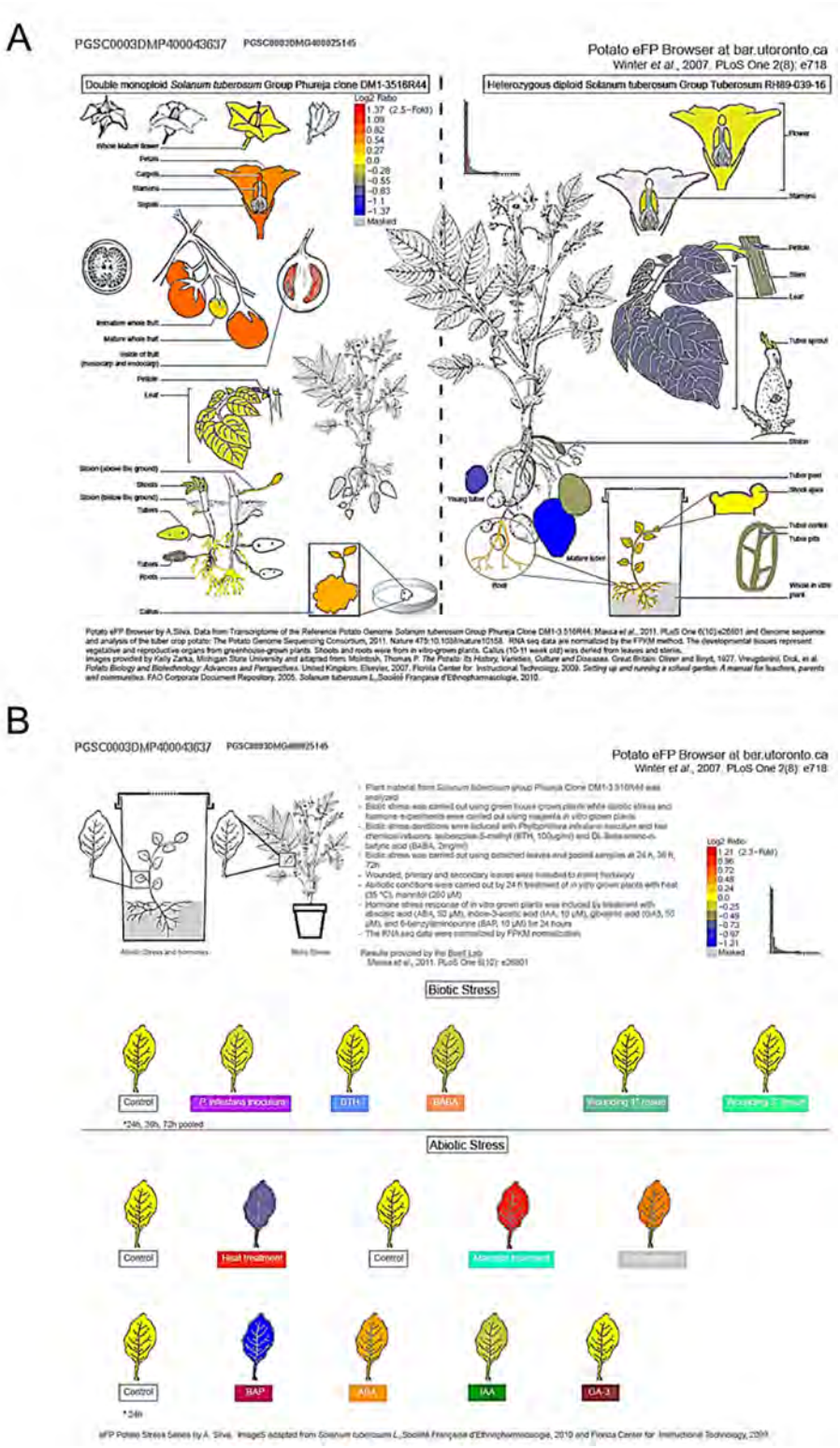

Fig. S1. Pictograph representations of the *StRIK* expression profile in potato tissues and organs (A) and in plants treated with different hormones and stresses (B) by eFP Browser database.

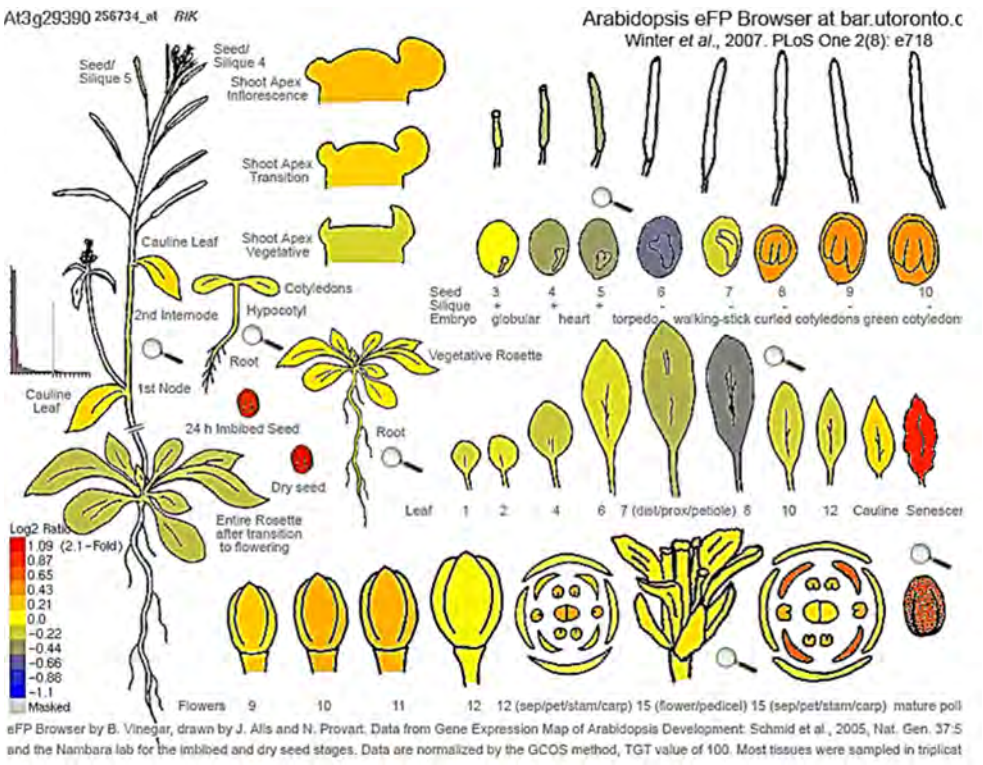

Fig. S2. Pictograph representations of the *RIK* expression profile in Arabidopsis by eFP Browser database.

TC207045

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TC203962

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Fig. S3. Identification of novel transcribed regions of *StRIK* gene based on Tentative Consensus (TC) from TIGR database. Four different TCs showing only partial overlapped regions with *StRIK* gene (TC225105, TC203962, TC207945 and DV625908) were aligned to the genomic region of the *StRIK* gene. In grey are highlighted the exons of the *StRIK* coding sequence. Underlined regions correspond to the TCs sequences and in yellow is highlighted the regions which differed from TCs and exons (novel transcribed regions). In blue the primers used for all TC amplifications are highlighted.

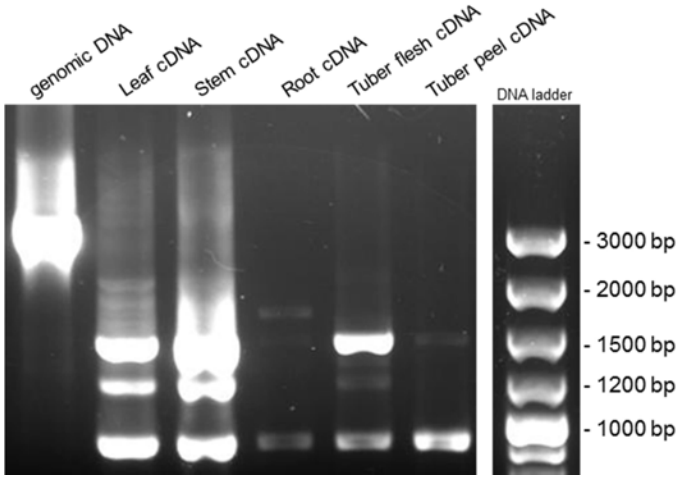


Fig. S4. Detection by PCR analyses of the novel transcribed regions comprised between exon 7 and 11 of *StRIK* gene in genomic DNA and in potato leaf, stem, root, tuber flesh and tuber peel tissues cDNA libraries. The expected size of the amplicons are the following: genomic DNA sequence, 3501 bp; full-length sequence obtained by Soler (2008), 903 bp; TC225105, 1172 bp; DV625908, 1364 bp; TC203962, 1568 bp. In Fig. S3 the primers used for PCR amplifications are shown in blue.

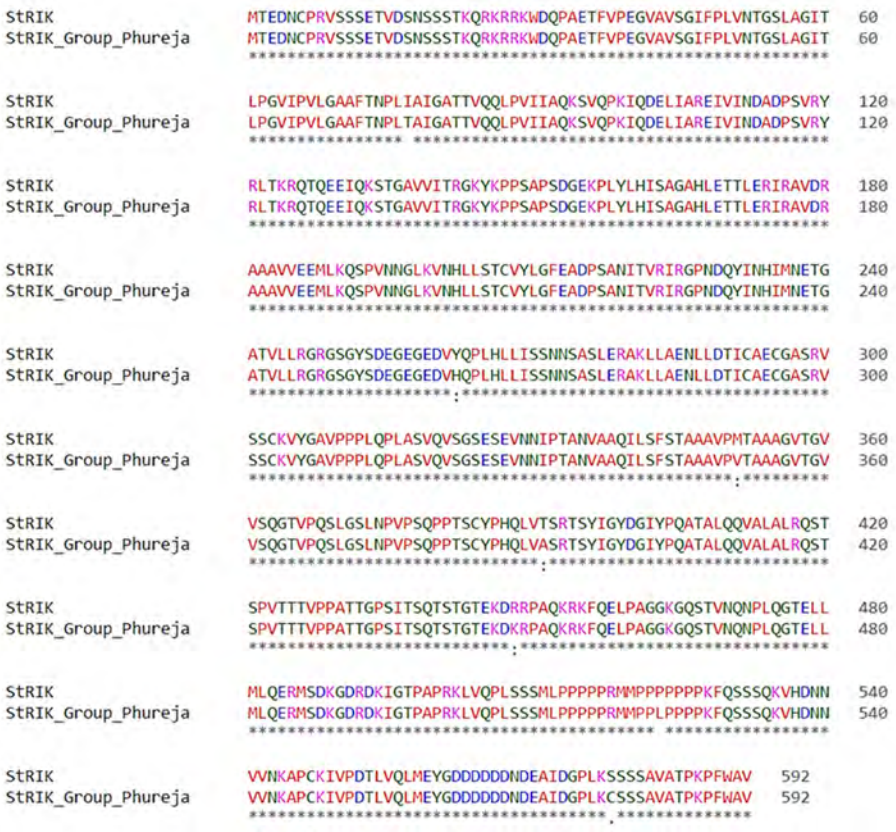
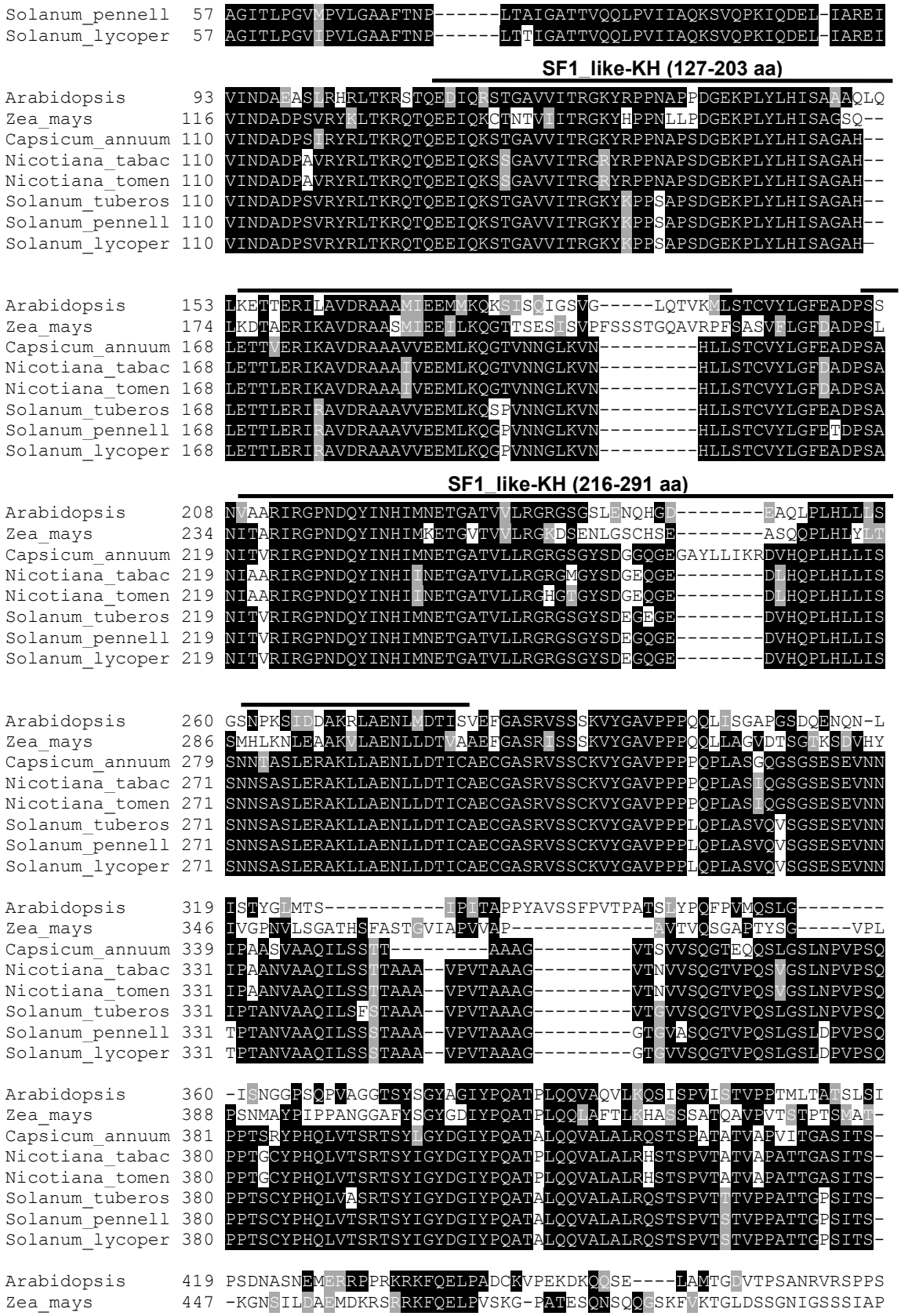


Fig. S5. Amino acid sequence alignment between the sequence obtained of StRIK from *S. tuberosum* Group Tuberosum (Soler, 2008) and from *S. tuberosum* Group Phureja (PGSC0003DMP400043638). The identical amino acid residues are indicated with an asterisk (*), different residues but with strongly similar properties with a period (.) and the residues with weakly similar properties with a colon (:).


SF1_like-KH (127-203 aa)
SF1_like-KH (216-291 aa)

```
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Nicotiana_tabac 439 -QTSIGSEKDKRPAQKRKFQELPAAGKSQVTVNQVCAKSP--------------------
Nicotiana_tomen 439 -QTSIGSEKDKRPAQKRKFQELPAAGKSQVTVNQVCAKSP--------------------
Solanum_tuberos 439 -QTSTGTEKDKRPAQKRKFQELPAGGKGQSTVNQNPLQGTELLMLQE-------------
Solanum_pennell 439 -QTSTGSEKDKRPAQKRKFQELPAVGKGQATVNQNPLQAMELLMLQE-------------
Solanum_lycoper 439 -QTSTGSEKDKRPAQKRKFQELPAGGKGQATVNQNPLQATELLMLQE-------------

                                                  Proline-rich region (498-527 aa)
Arabidopsis     475 PRSV------MPPP----------PPKTIAPPPSKTMSPPSSKSMLPPPPRSKTMSPLSS
Zea_mays        505 PKKVHPGSNGMLPQEEADMPSHLSISTKMLPPPLKSMLPLPPRSMPPPPPKSM-------
Capsicum_annuum 486 ------YFSFMVPMQRYHAPSGF-----GILSNVNMLI----------------------
Nicotiana_tabac     ------------------------------------------------------------
Nicotiana_tomen     ------------------------------------------------------------
Solanum_tuberos 485 ------------RMSDKGDRDKI-----GTPAPRKLVQPLSSSMLPPPPPRMM-------
Solanum_pennell 485 ------------RISEKGDTDKI-----GIPTHRKLVQPLSSSMLPPPPPRMM-------
Solanum_lycoper 485 ------------RISEKGDTDKI-----GIPTPRKLVQPLSSSMLPPPPPRMM-------

                    ___________
Arabidopsis     519 KSMLPPPPRFTLTTQRSRLQDNHISVKKPNPVPDTLIKLMEYGDDEDDDDDPDEP---LT
Zea_mays        558 -PPPP--PKFPSDEFLSRNENKFFPLKEPTAPPRSFDAISVLPSERRPREPKEEKN---K
Capsicum_annuum     ------------------------------------------------------------
Nicotiana_tabac     ------------------------------------------------------------
Nicotiana_tomen     ------------------------------------------------------------
Solanum_tuberos 521 -PPLPPPPKFQSSSQKVHDNNVVNKAPC-KIVPDTLVQLMEYGDDDD-DDNDEAIDGPLK
Solanum_pennell 521 -PPPPPPPKFQSSSQKVHDNNVVDKAPS-KIVPDTLVQLMEYGDDDD-DDNDEAIDGPLK
Solanum_lycoper 521 -PPPPPPPKFQSSSQKVHDNNMVNKAPS-KIVPDTLVQLMEYGDDDDDDDNDEAIDGPLK

Arabidopsis     576 TRS------------
Zea_mays        612 R-HTCV---------
Capsicum_annuum     ---------------
Nicotiana_tabac     ---------------
Nicotiana_tomen     ---------------
Solanum_tuberos 578 CSSSAVATPKPFWAV
Solanum_pennell 578 SSSSAVATPKPFWAV
Solanum_lycoper 579 SSSSAVATPKPFWAV
```

Fig. S6. Amino acid alignment of the potato RIK (*StRIK*) with the most similar RIK proteins of different species and the two orthologs of maize and Arabidopsis. Amino acid alignment for the Arabidopsis (AAY24687.1), *Zea mays* (AAY24682.1), *Capsicum annuum* (XP_016559014.1), *Nicotiana tabacum* (XP_016444079.1), *Nicotiana tomentosiformis* (XP_009595397.1), *Solanum tuberosum* Group Phureja (PGSC0003DMP400043638), *Solanum lycopersicum* (XP_004233384.1), *Solanum pennellii* (XP_015065578.1) is shown. The two predicted SF1_like-KH conserved protein domains and the proline-rich region are indicated by bold lines. The amino acids that are identical are shaded in black and the ones that are similar in grey.

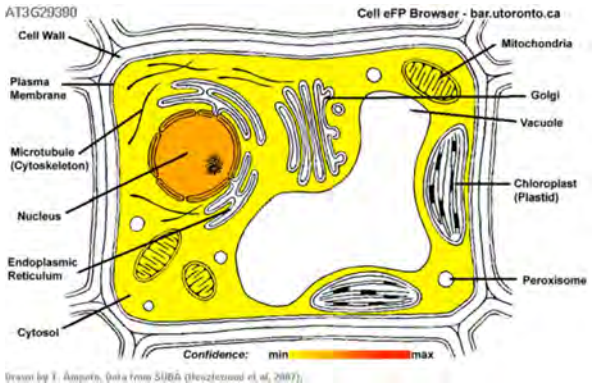

Fig. S7. Representation of the Arabidopsis RIK gene predicted subcellular location by SUBA3 database.

StRIK Group Phureja (PGSC0003DMG400025145)

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GTCTCAAGATTTCAATAAACTCTTGCTTGGTTTGTCTATTAGTGATGACCTACCTATCTTCATTACCCCTTTTTTTCTTGCATTTCTTGTTA
CTAAGTGGAAGTGTGAAAATCCATTAGGTCCAAGTAACATAGTAGAACTTCTTTTACTTTTCATTTTTATCTTCCACTTCTTATTTTTGTAT
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ATCTCAGATACATTAGTCCAGCTAATGGAATATGGGGATGACGACGACGATGATAATGATGAAGCAATTGACGGACCTTTGAAATGTAGCTC
AAGTGCGGTAGCAACTCCAAAGCCATTCTGGGCTGTTTAA

Fig. S8. Genomic *StRIK* sequence. In red characters is shown the sequence used to generate the *StRIK*-RNAi construct. The 12 exons of *StRIK* shadowed in grey. The start initiation codon and the STOP codon are highlighted in green.

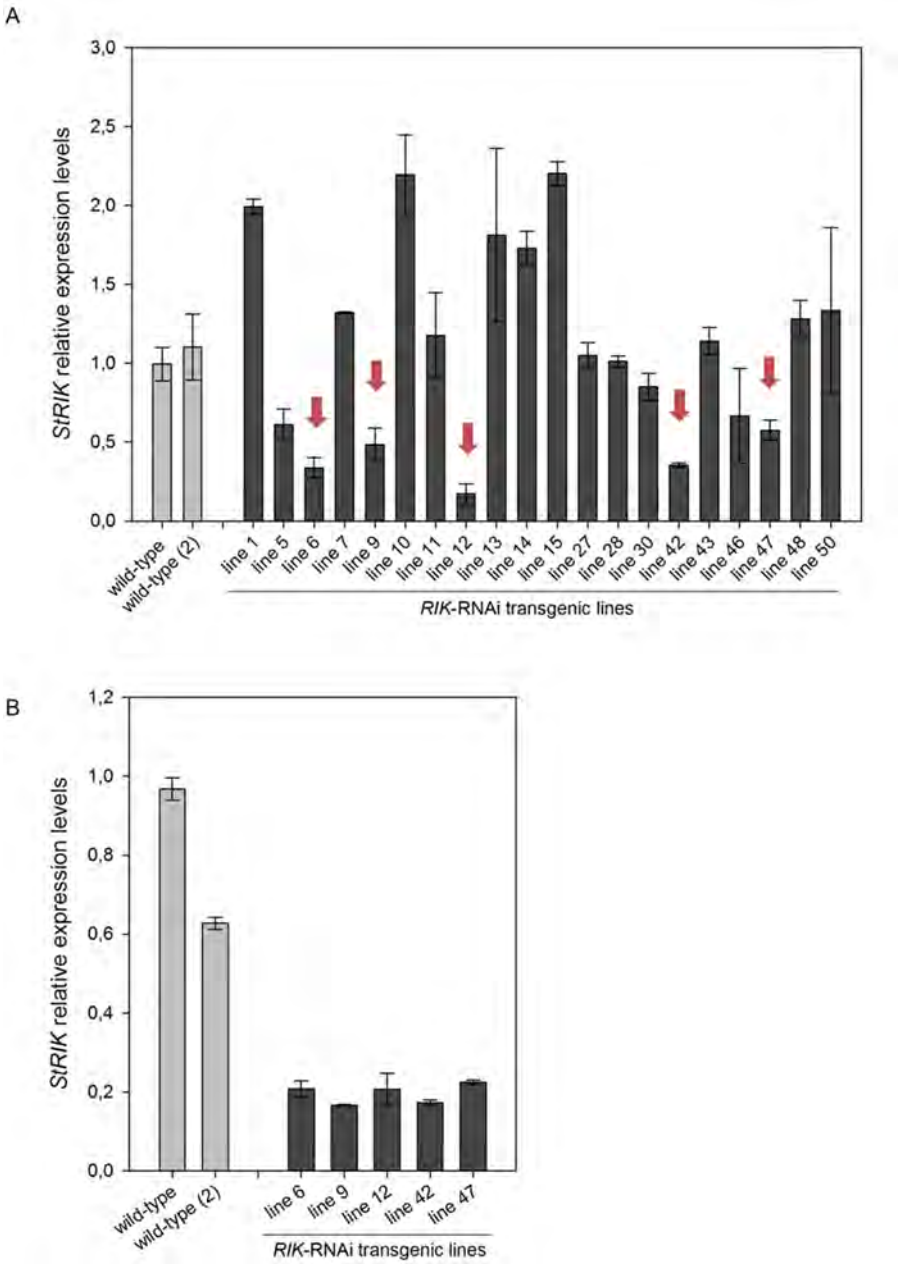

Fig. S9. Screening of *StRIK*-RNAi silenced transgenic lines by RT-qPCR in potato (A) leaves and (B) phellem tissues from two consecutive plantations

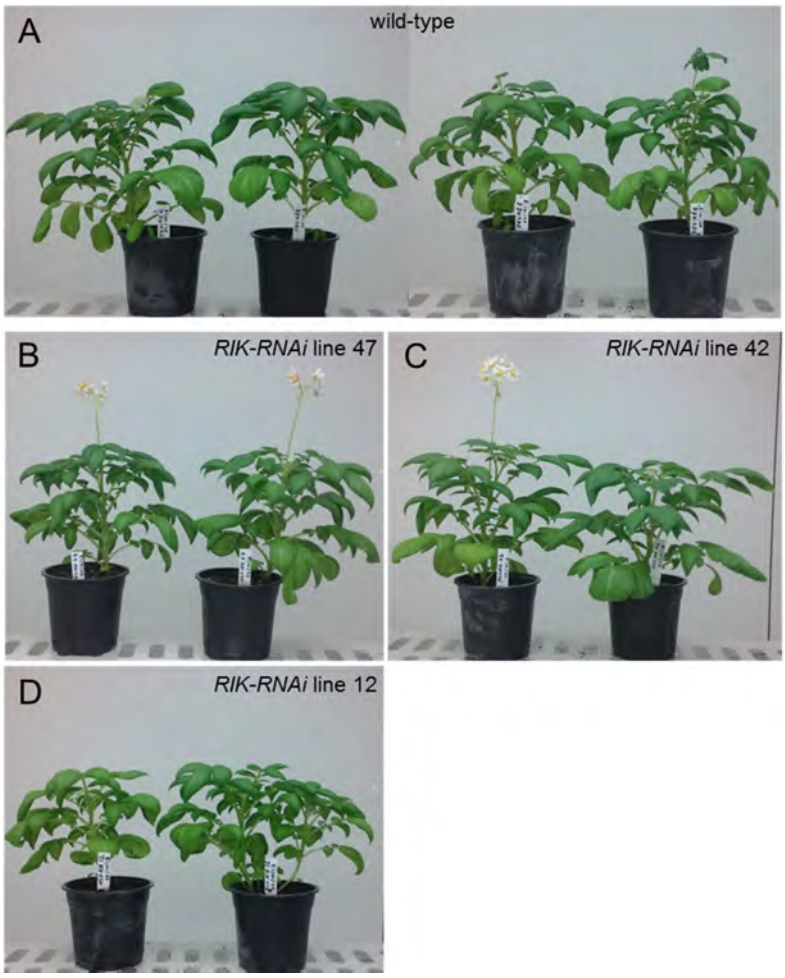

Fig. S10. *StRIK* silenced lines forming fully developed flowers.

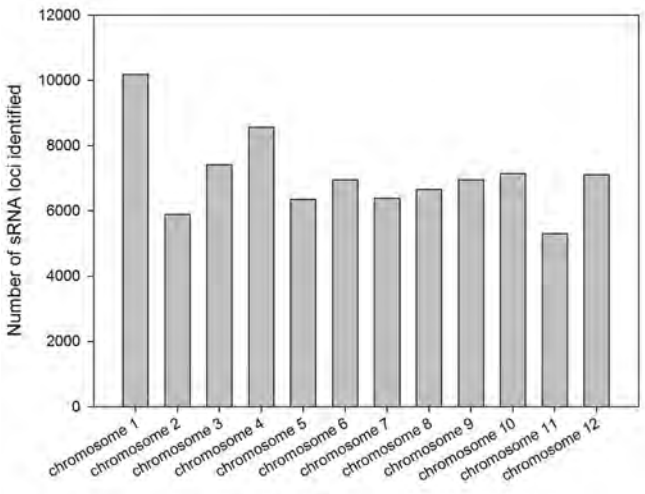

Fig. S11. Total number of identified small RNAs loci for each chromosome of the potato genome.

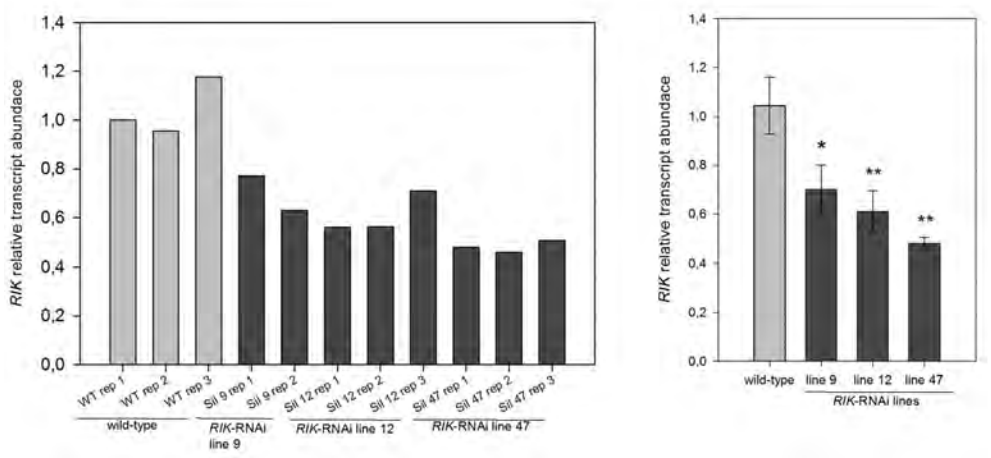

Fig. S12. RT-qPCR re-confirmation of the *StRIK* silencing in isolated phellem from *StRIK*-RNAi independent lines and biological replicates prior to high-throughput RNA sequencing (t test; *, P < 0.05; **, P < 0.01).

Supplementary tables

Table S1. Tissue and condition gene expression FPKM levels of the *StRIK* gene based on the RNA-seq data extracted from The potato Genome Sequencing Consortium (PGSC) (Xu et al., 2011).

Gen	Gene ID	S1 [RH* Flower]	S2 [RH Leaf]	S3 [RH Petiole]	S4 [RH Shoot Apex]	S5 [RH Stem]	S6 [RH Stolon]	S7 [RH Young Tuber]	S8 [RH Mature Tuber]	S9 [RH Root]	S10 [RH Stamen]	S11 [RH Water-Stressed Leaf]	S12 [RH Tuber Pith]	S13 [RH Tuber Peel]	S14 [RH Whole in vitro Plant]	S15 [RH Tuber Sprout]	S16 [RH Tuber Cortex]
RIK	PGSC0003DMT400064730	30	20	37	27	23	34	21	12	43	40	43	23	22	42	39	24

*RH refers to the heterozygous diploid *S. tuberosum* Group Tuberosum cultivar, RH89-039-16 (RH), one of the two potato species sequenced by the PGSC

Table S2. List of motifs identified in StRIK protein sequence by ScanProsite (http://prosite.expasy.org/scanprosite/)

motif	start	end	motif sequence
PS00006|CK2_PHOSPHO_SITE	10	13	SssE
PS00001|ASN_GLYCOSYLATION	18	21	NSSS
PS00005|PKC_PHOSPHO_SITE	21	23	StK
PS00008|MYRISTYL	41	46	GVavSG
PS00008|MYRISTYL	54	59	GSlaGI
PS00008|MYRISTYL	69	74	GAafTN
PS00005|PKC_PHOSPHO_SITE	117	119	SvR
PS00005|PKC_PHOSPHO_SITE	123	125	TkR
PS00004|CAMP_PHOSPHO_SITE	124	127	KRqT
PS00006|CK2_PHOSPHO_SITE	127	130	TqeE
PS00006|CK2_PHOSPHO_SITE	152	155	SdgE
PS00006|CK2_PHOSPHO_SITE	170	173	TtlE
PS00008|MYRISTYL	197	202	GLkvNH
PS00001|ASN_GLYCOSYLATION	219	222	NITV
PS00005|PKC_PHOSPHO_SITE	221	223	TvR
PS00001|ASN_GLYCOSYLATION	237	240	NETG
PS00008|MYRISTYL	249	254	GSgySD
PS00001|ASN_GLYCOSYLATION	272	275	NNSA
PS00005|PKC_PHOSPHO_SITE	302	304	ScK
PS00006|CK2_PHOSPHO_SITE	322	325	SgsE
PS00006|CK2_PHOSPHO_SITE	324	327	SesE
PS00006|CK2_PHOSPHO_SITE	442	445	TgtE
PS00005|PKC_PHOSPHO_SITE	444	446	TeK
PS00006|CK2_PHOSPHO_SITE	444	447	TekD
PS00005|PKC_PHOSPHO_SITE	487	489	SdK
PS50099|PRO_RICH	498	527	PaprklvqplsssmlpppppprmmpplpppP
PS00005|PKC_PHOSPHO_SITE	533	535	SqK
PS00005|PKC_PHOSPHO_SITE	585	587	TpK

Table S6. Potato transcripts from PGSC database most homologous to the *StRIK*-RNAi fragment used for the silencing obtained by a BLASTN analysis.

Accession	Description	Length	E value	Coverage	Identity
PGSC0003DMT400064729	Protein RIK	246	1E-137	100.00	100.00
PGSC0003DMT400064730	Protein RIK	246	1E-137	100.00	100.00
PGSC0003DMT400064731	Protein RIK	227	1E-126	92.28	100.00
PGSC0003DMT400055483	Conserved gene of unknown function	18	0.23	7.32	100.00
PGSC0003DMT400014640	EMB1865	18	0.23	8.94	95.45
PGSC0003DMT400014644	EMB1865	18	0.23	8.94	95.45
PGSC0003DMT400014643	EMB1865	18	0.23	8.94	95.45
PGSC0003DMT400030423	Extensin	17	0.92	6.91	100.00
PGSC0003DMT400042918	ATP binding protein	17	0.92	6.91	100.00
PGSC0003DMT400073845	Dnajc14 protein	17	0.92	6.91	100.00

Table S8. List of primers used for St*RIK* transcript profile study and for the RNA-seq results validation, with their specific parameters after analyzing them with NetPrimer software tool.

Gene	Sequence (5'→3')	nt	Tm (ºC)	GC%	ΔG (kcal/mol)	*3' end stabillity* (kcal/mol)	*Self Dimer* (kcal/mol)	*Hairpin* (kcal/mol)	*Cross dimer*
RIK protein	fwd: ACGAAGCAAAGGAAACGGAGA	21	61.5	47.6	-37.45	-7.82	-	-	-
	rev: AGCAGCACCCAAGACTGGAA	19	56.0	47.3	-33.17	-9.64	-7.21	-2.91	
transposase	fwd: ATGAGAAGCCCGATTGGATG	20	59,7	50.0	-35.13	-8.07	-	-	-4.54 (3')
	rev: GATGAGTCCCTGGTGCCTTT	20	58,7	55.0	-34.34	-8.56	-	-	
Dehydration responsive element binding protein	fwd: TGATTCTGCTTCCTGGGCTAC	21	59,4	52.3	-35.63	-7.04	-	-	-
	rev: TCGTCCTTTCCTTCGTCTTTC	21	58.6	47.6	-35.51	-7.06	-	-	
RNase H family protein	fwd: TGAACCACTTGGAGGGATGAA	21	60,0	47.6	-35.08	-6.95	-	-	-7.97 (3')
	rev: CAGTGAGCGGAAAACCAAAA	20	58,7	45.0	-34.95	-7.79	-	-	
Retrotransposon protein	fwd: TGGCTCATCACGAAAAGAACA	22	59,0	42.8	-36.51	-6.82	-	-	-6.02 (3')
	rev: AAAGGCTCGACTTACACACCAC	22	58,6	50.0	-35.7	-7.71	-6.76	-	
K+ channel inward rectifying	fwd: GCAAGATACGCCAACAAATCCT	22	61,1	45.4	-38.11	-7.72	-4.05 (3')	-	-4.42 (3')
	rev: CGTCCACCACTTTCACTTTCATT	23	60,8	43.4	-37.55	-6.95	-	-	

The following additional supplemental data of Chapter II can be found in electronic format (CD-ROM) attached to the end of this thesis:

Table S3. Co-expression network of *RIK* gene in Arabidopsis: List of 300 most co-expressed genes.

Table S4. Gene ontology enrichment of the 300 genes most co-expressed with Arabidopsis *RIK* gene

Table S5. List of the *cis*-regulatory elements identified in the promoter region of *StRIK* 3000 bp upstream to the start initiation codon.

Table S7. List of all differentially regulated genes between *StRIK*-RNAi and wild type phellem identified by RNA-seq.

Supplemental data for Chapter III

Comparative transcriptional profiling of cork and holm oak phellem tissues unveils molecular networks orchestrating phellem formation

This supplemental data includes MA plot, GO enrichments, KEGG pathways classifications, and MapMan pathways diagrams of the set of genes differentially regulated between the phellem of cork oak and holm oak. It also includes a summary of the bioinformatics analyses results of the reads obtained by 454 sequencing, a selection of the genes identified *in silico* showing strong co-expression with suberin genes and a selection of candidate genes for phellem formation, representative of the biochemical and regulatory pathways discussed in Chapter III. The tables headings of the supplemental data presented in electronic format attached to the end of this thesis are also included.

Supplementary figures

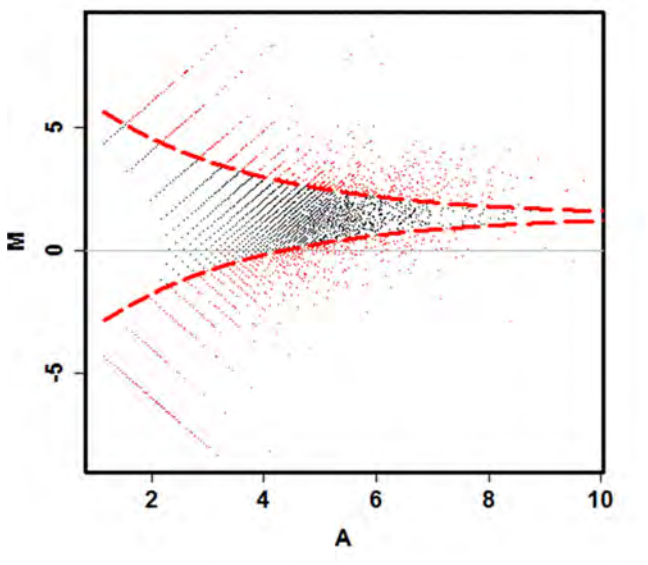

Fig. S1. The MA plot shows the $\log_2$ fold change (M) and the $\log_2$ expression (A). The red points correspond to genes showing significant differences (q-value < 0.05).

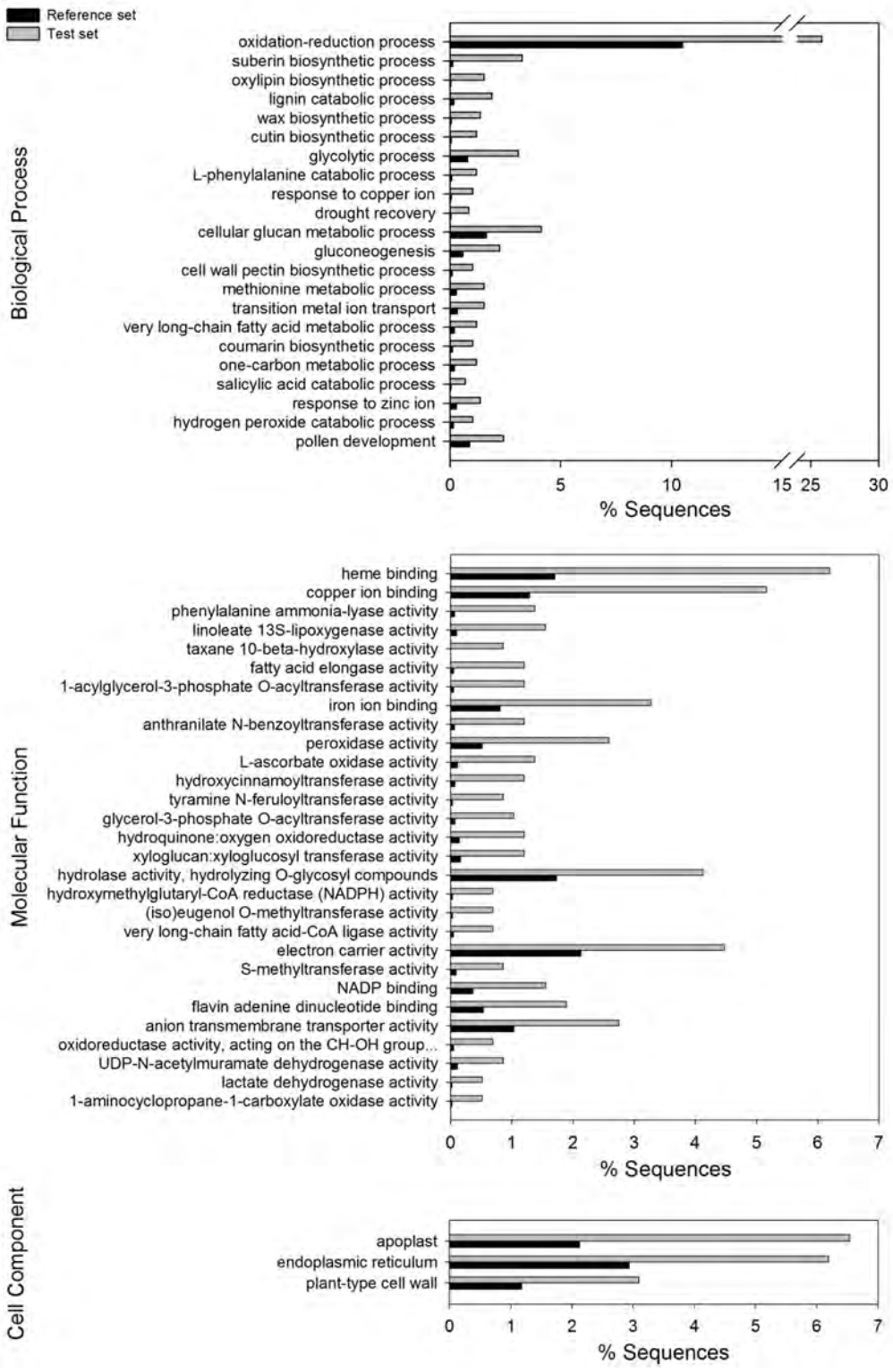

Fig. S2. GO enrichment of the set of the contigs upregulated in cork oak. The percentage of the most specific gene ontologies of biological processes, molecular functions and cell components are shown for the test set (cork oak upregulated contigs) and the reference set (all contigs).

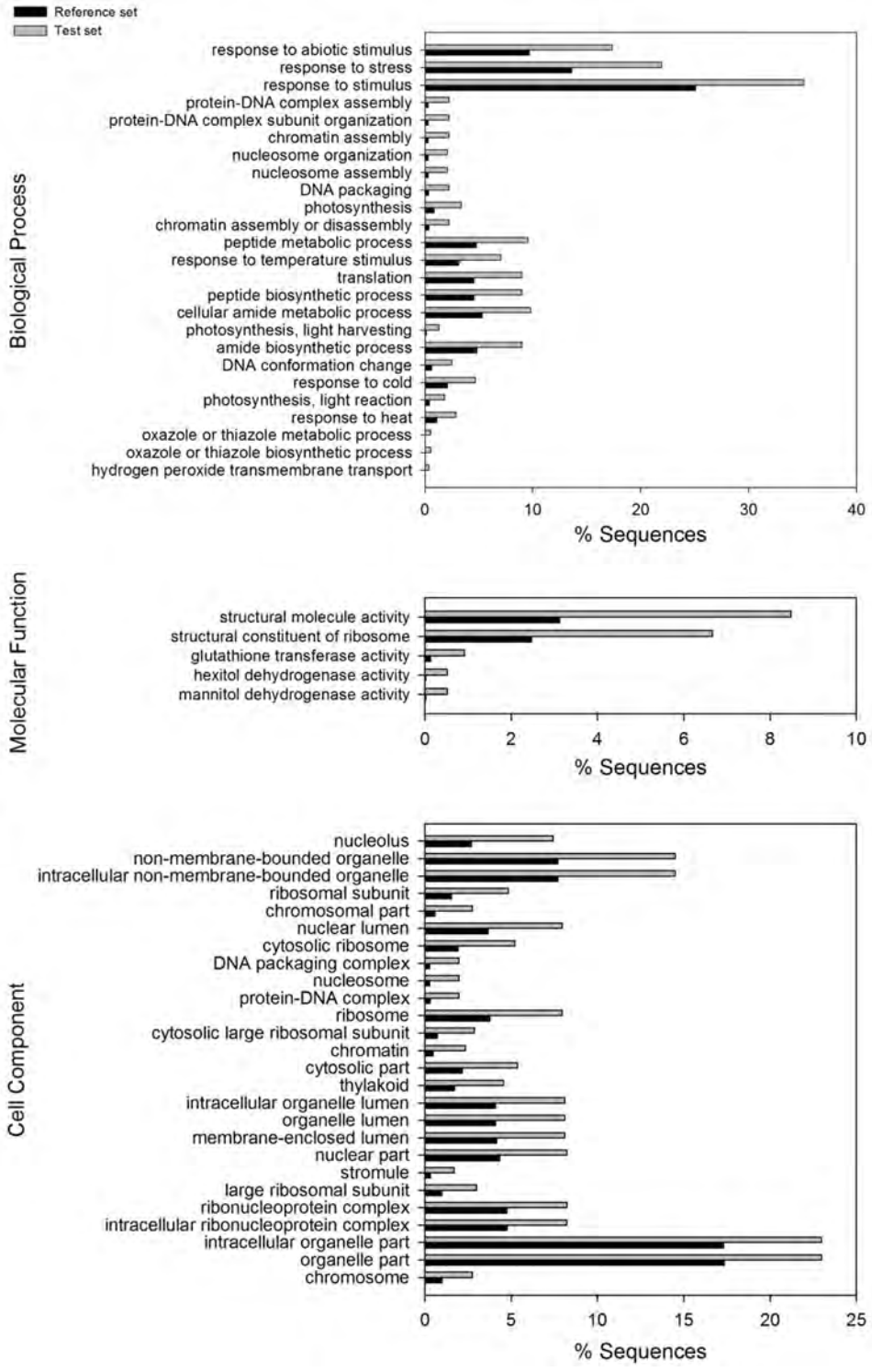

Fig. S3. GO enrichment of the set of the contigs upregulated in holm oak. The percentage of the most specific gene ontologies of biological processes, molecular functions and cell components are shown for the test set (holm oak upregulated contigs) and the reference set (all contigs).

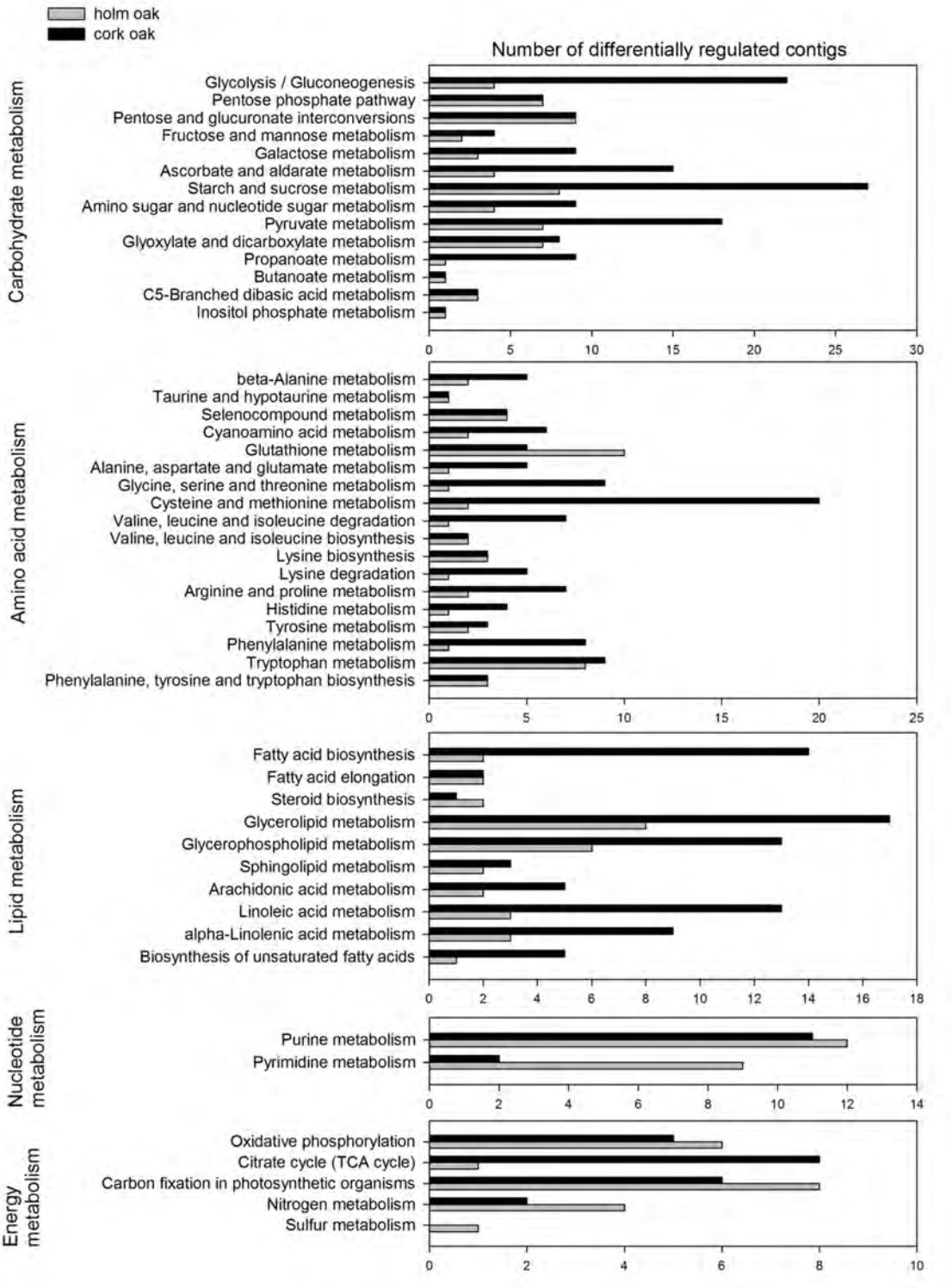

Fig. S4. Distribution of the contigs differentially regulated into KEGG pathways of the primary metabolism.

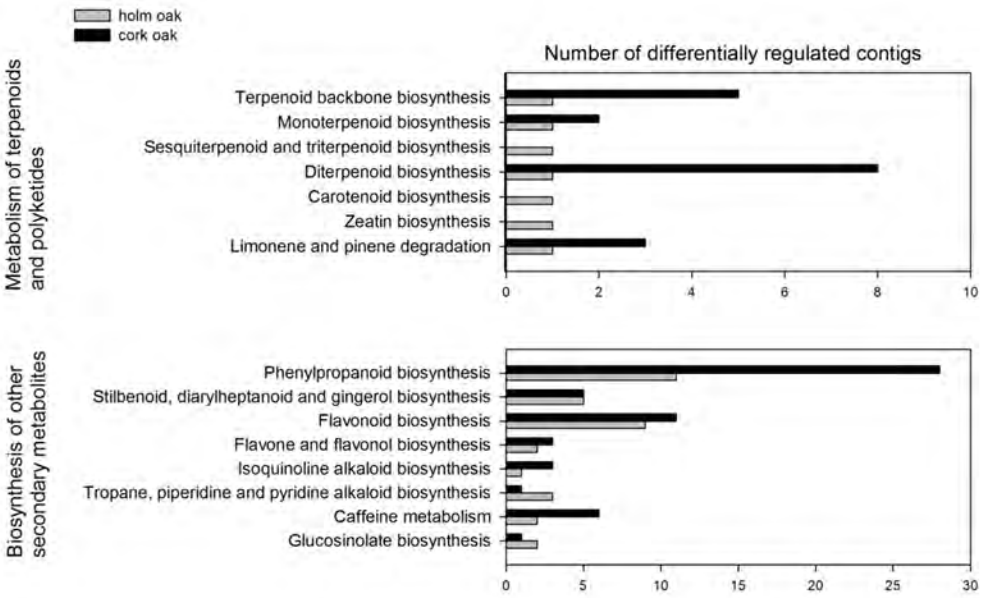

Fig. S5. Distribution of the contigs differentially regulated into KEGG pathways of the secondary metabolism.

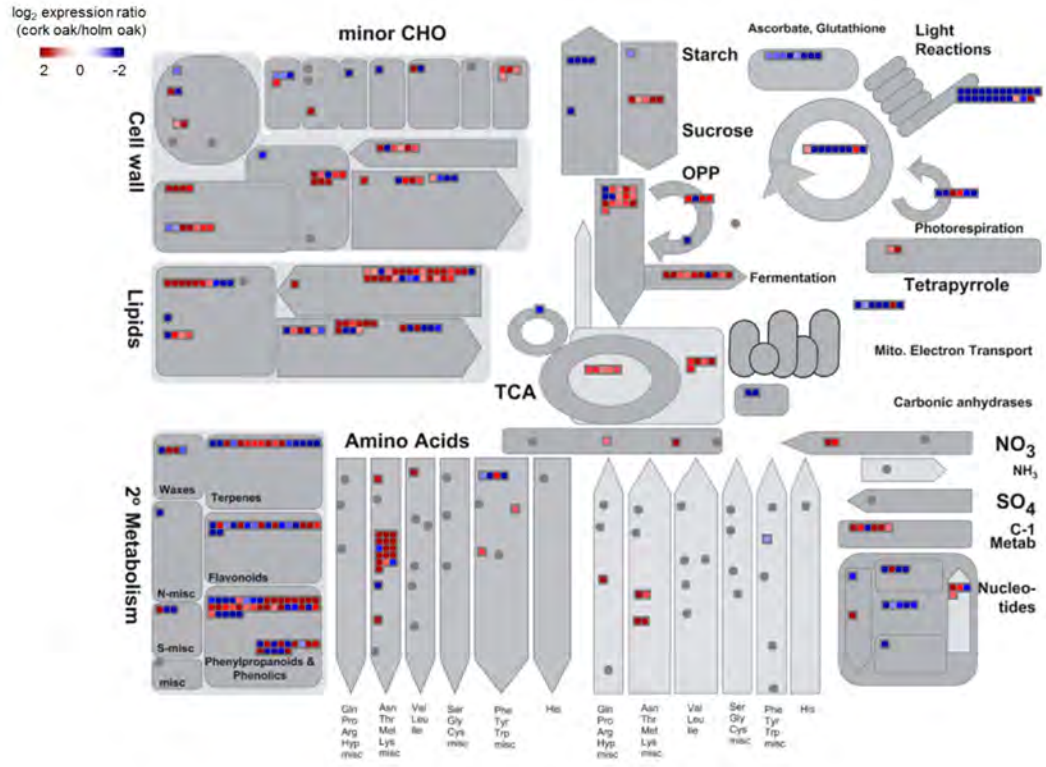

Fig. S6. MapMan metabolism overview of the contigs differentially regulated. Squares represent contigs. In red, the contigs more induced in holm oak. In blue, the contigs more induced in holm oak. The darker the colors, the higher the fold changes (log2 expression ratio (cork oak/holm oak).

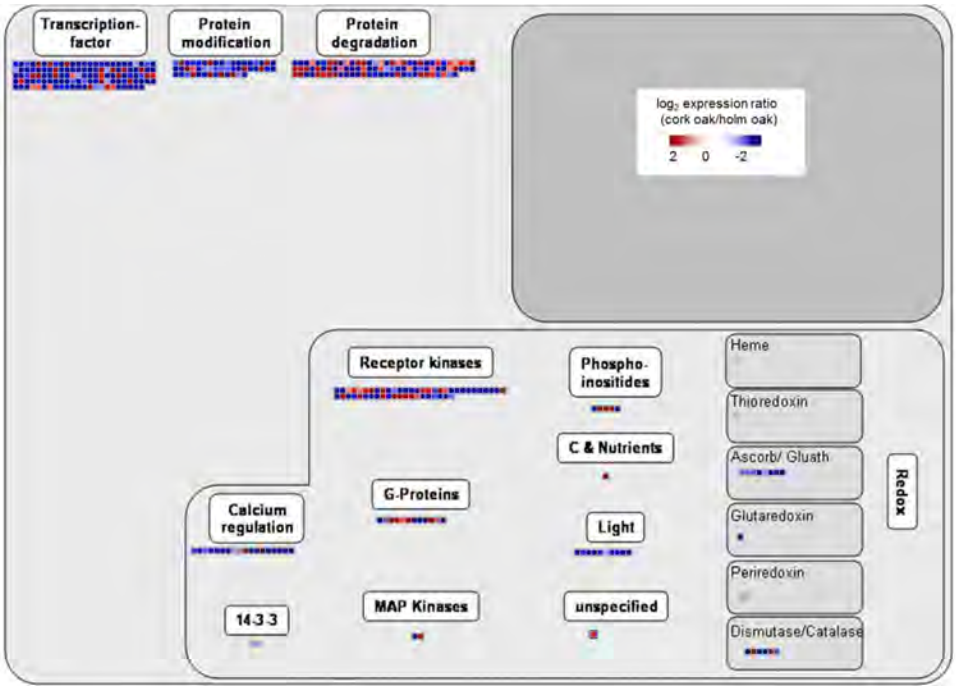

Fig. S7. MapMan regulation overview of the contigs differentially regulated. Squares represent contigs. In red, the contigs more induced in holm oak. In blue, the contigs more induced in holm oak. The darker the colors, the higher the fold changes (log2 expression ratio (cork oak/holm oak).

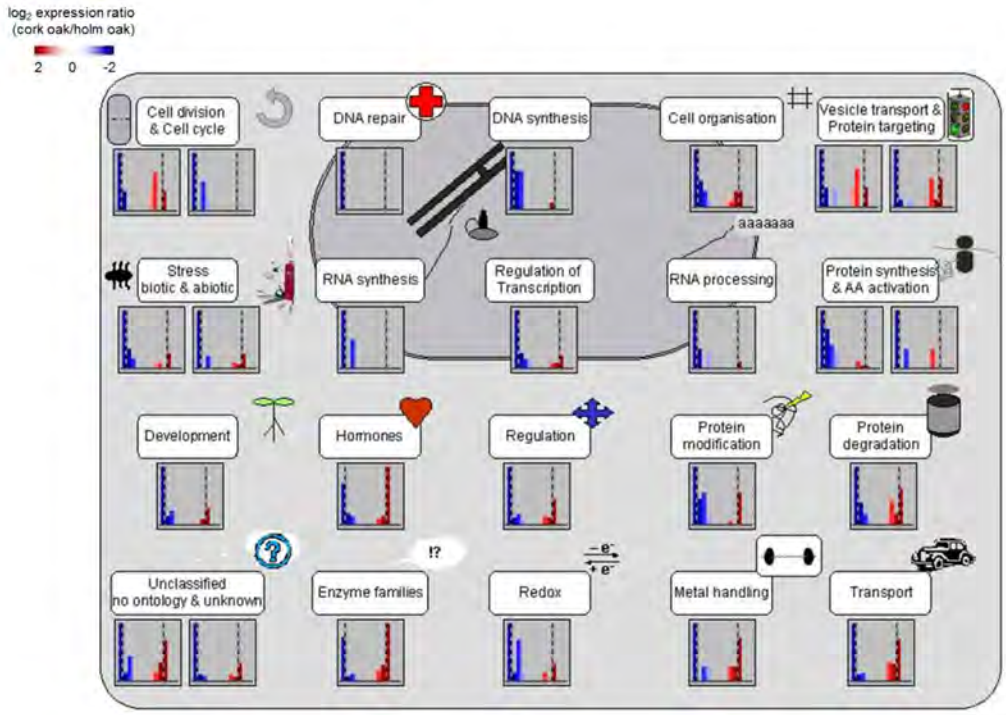

Fig. S8. MapMan cell functions of the contigs differentially regulated. Squares represent contigs. In red, the contigs more induced in holm oak. In blue, the contigs more induced in holm oak. The darker the colors, the higher the fold changes (log2 expression ratio (cork oak/holm oak).

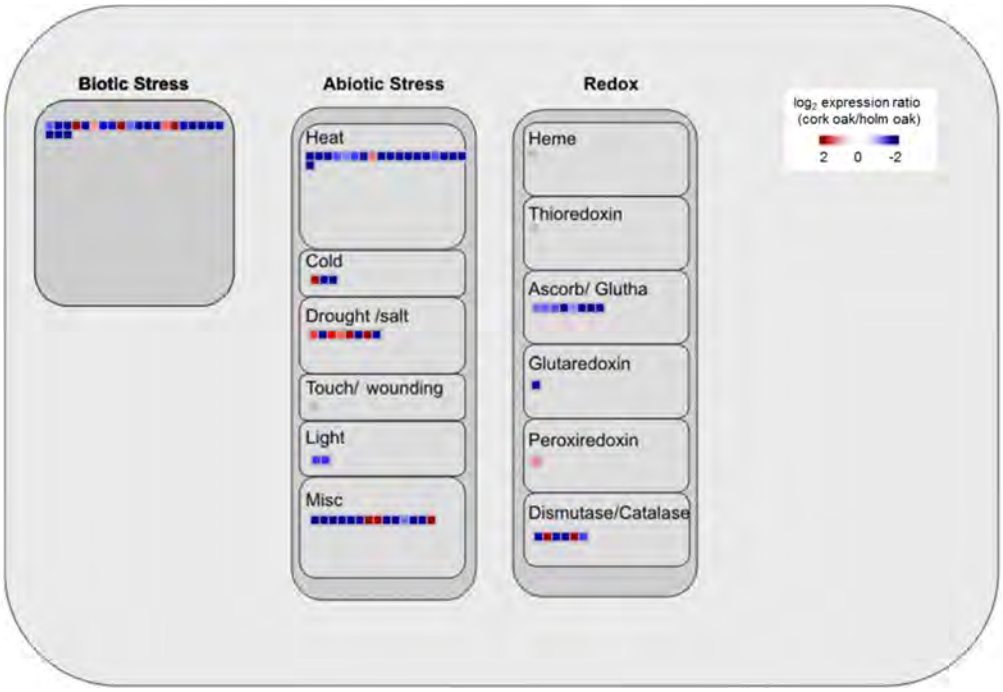

Fig. S9. MapMan cellular response of the contigs differentially regulated. Squares represent contigs. In red, the contigs more induced in holm oak. In blue, the contigs more induced in holm oak. The darker the colors, the higher the fold changes (log2 expression ratio (cork oak/holm oak).

Supplementary tables

Table S1. Summary of the bioinformatic analysis.

454 sequencing	**Total**	**Cork oak**	**Holm oak**
Raw nucleotides	476960933		
Raw reads	1151927	499569	584310
Cleaned and processed reads	482677	345190	137487
Transcriptome assembly			
contig number (n)	16865		
contig total size (nt)	13881688		
mean contig length (nt)	818.4		
contigs N50	1063		
contigs present in libraries (n)	-	14616	13911
Functional annotation	**no. contigs**	**%**	
BLASTX hit Swissprot, Refseq_prot and Refseq_rna	13429	79.6	
BLASTX hit nr (GenBank)	13653	80.9	
BLASTX hit TAIR 10 (Arabidopsis proteome)	12716	75.3	
BLASTX hit Ptrichocarpa 210 v3.0	12998	77.1	
BLASTN hit CorkOakDB	16040	95.1	
Gene ontology (GO)	10454	61.9	
EC number (KEGG pathways)	4006	23.8	
Mercator functional categories (bins)	9168	54.3	
Protein conserved domains (CDD)	7854	46.6	

Table S11A. Selection of the most co-expressed genes with suberin biosynthetic genes. Selection of the co-expressed Arabidopsis homologs with genes identified in the RNA-seq. The table contains the Pearson's correlation coefficient (*r*) between the query genes identified and the suberin candidate genes used as a bait genes. All genes shows co-expression values greater than 0.55 with at least two suberin bait genes.

List of query genes			List of bait genes						
AGI	Symbol	Description	251944_at ABCG20	249881_at CYP86B1	259282_at GPAT5	250239_at ABCG6	247765_at CYP86A1	249289_at ASFT	**Mean *r*.**
AT1G24430	AT1G24430	HXXXD-type acyl-transferase family protein	0.703	0.582	0.632	0.726	0.713	0.642	**0.67**
AT1G78990	AT1G78990	HXXXD-type acyl-transferase family protein	0.696	0.705	0.681	0.735	0.778	0.608	**0.70**
AT1G74460	AT1G74460	GDSL-like Lipase/Acylhydrolase superfamily protein	0.764	0.804	0.767	0.862	0.953	0.871	**0.84**
AT2G23540	AT2G23540	GDSL-like Lipase/Acylhydrolase superfamily protein	0.744	0.797	0.741	0.824	0.951	0.847	**0.82**
AT4G36610	AT4G36610	alpha/beta-Hydrolases superfamily protein	0.812	0.816	0.798	0.873	0.823	0.866	**0.83**
AT5G37690	AT5G37690	SGNH hydrolase-type esterase superfamily protein	0.768	0.730	0.719	0.850	0.916	0.782	**0.79**
AT3G06390	CASPL1D2	CASPL1D2	0.734	0.674	0.707	0.839	0.840	0.761	**0.76**
AT4G20390	CASPL1B2	CASPL1B2	0.752	0.749	0.734	0.857	0.930	0.809	**0.81**
AT5G44550	CASPL1B1	CASPL1B1	0.737	0.734	0.715	0.814	0.935	0.795	**0.79**
AT1G68850	AtPrx11	Peroxidase superfamily protein	0.816	0.858	0.809	0.854	0.899	0.848	**0.85**
AT2G35380	AtPrx20	Peroxidase superfamily protein	0.718	0.780	0.750	0.816	0.918	0.774	**0.79**
AT5G14130	AtPrx55	Peroxidase superfamily protein	0.714	0.779	0.718	0.759	0.798	0.768	**0.76**
AT5G66390	AtPrx72	Peroxidase superfamily protein	0.516	0.585	0.573	0.667	0.740	0.810	**0.65**
AT1G05260	AtPrx03	Peroxidase superfamily protein	0.550	0.594	0.554	0.657	0.701	0.795	**0.64**
AT5G67400	AtPrx73	root hair specific 19	0.525	0.527	0.523	0.623	0.689	0.710	**0.60**
AT2G30210	LAC3	laccase 3	0.570	0.635	0.595	0.690	0.771	0.811	**0.68**
AT2G40370	LAC5	laccase 5	0.778	0.735	0.709	0.732	0.681	0.671	**0.72**
AT3G09220	LAC7	laccase 7	0.553	0.600	0.621	0.717	0.788	0.749	**0.67**

Table S11B. Selection of the most co-expressed genes with suberin biosynthetic genes. The expression values and the contigs with significant homology are shown. Contigs upregulated in cork oak (↑) and holm oak (↓) are indicated.

AGI	Symbol	ESTs/100,000 ESTS		Contigs with BLASTX hits (*E*-value < 10^{-10})
		Cork oak	Holm oak	to Arabidopsis proteins
AT1G24430	AT1G24430	1064.1	393.5	Q_05974↓,Q_15270↑,Q_15271↑,Q_15273↑,Q_15274↑,Q_15275↑
AT1G78990	AT1G78990	116.5	96.0	Q_07765,Q_10155↓,Q_12234
AT1G74460	AT1G74460	458.3	202.9	Q_09397↑
AT2G23540	AT2G23540	669.2	195.7	Q_10256↑,Q_11119↑,Q_11120↑,Q_12005↑,Q_14346
AT4G36610	AT4G36610	83.1	27.6	Q_06582↑
AT5G37690	AT5G37690	201.6	218.9	Q_10689↑,Q_13451,Q_13453
AT3G06390	CASPL1D2	167.7	38.5	Q_03294↑
AT4G20390	CASPL1B2	83.7	21.8	Q_10798↑
AT5G44550	CASPL1B1	162.2	61.1	Q_14678↑
AT1G68850	AtPrx11	186.6	64.7	Q_08706↑,Q_15849↑
AT2G35380	AtPrx20	5.2	16.7	Q_12041↓
AT5G14130	AtPrx55	9.8	11.6	Q_05213
AT5G66390	AtPrx72	44.3	12.4	Q_14245↑
AT1G05260	AtPrx03	7.2	8.7	Q_04165,Q_04820↑,Q_12454
AT5G67400	AtPrx73	21.1	0.0	Q_07983↑
AT2G30210	LAC3	2.9	10.2	Q_15678↓
AT2G40370	LAC5	97.9	19.6	Q_14541↑
AT3G09220	LAC7	27.2	0.7	Q_05454↑

Table S13. Selection of candidates genes representative of primary metabolism, secondary metabolism, cell wall, stress, cell growth, hormones signaling, meristem regulation, integrators of environmental cues and chromatin-modifying enzymes. Contigs are annotated with their best BLASTX hit against Arabidopsis proteins (TAIR10) (BLASTX with cutoff *E*-value < 10^{-10}). To enable comparison of cork oak EST levels to holm oak EST levels, EST counts are normalized by libraries size (ESTs/100,000 ESTs). There may be more than one contig/TAIR10 locus ID in which case EST numbers for multiple contigs that were assigned to the same protein are summed in this table. The corresponding contigs are shown and the contigs significantly upregulated in cork oak (↑) and holm oak (↓) are indicated.

Functional role	TAIR10 ID	aliases	Gene Description	ESTs/100,000 ESTs		Contigs homologs
				cork oak	holm oak	
Primary metabolism						
Plasma-membrane sugar transporters	AT2G02860	SUC3/SUT2	sucrose transporter 2 (SUT2)	4	1	Q_14558↑
	AT1G09960	SUC4/SUT4	sucrose transporter 4 (SUT4)	7	2	Q_16829↑
	AT3G18830	PMT5	polyol/monosaccharide transporter 5 (PMT5)	152	22	Q_15010↑
	AT1G77210	STP14	sugar transport protein 14 (STP14)	13	1	Q_14941↑
	AT1G54730	MFS	Major facilitator superfamily protein	4	38	Q_04477,Q_15717↓,Q_15718,Q_15719↓
Sucrose metabolism	AT3G43190	SUS4	sucrose synthase 4 (SUS4)	661	96	Q_16688↑,Q_16689,Q_16690↑
	AT4G02280	SUS3	sucrose synthase 3 (SUS3)	26	12	Q_16685,Q_16686↑,Q_16687
	AT4G09510	CINV2	cytosolic invertase 2 (CINV2)	37	19	Q_06441
	AT4G34860	A/N-INVB	Plant neutral invertase family protein	5	1	Q_12688↑
Glycolysis	AT2G36530	ENO2	ENOLASE 2	147	99	Q_03401,Q_03402↓,Q_03403↑,Q_03405↑,Q_03406↑,Q_15923
	AT4G26270	PFK3	phosphofructokinase 3 (PFK3)	27	15	Q_09934,Q_14725,Q_14726↑
	AT2G22480	PFK5	phosphofructokinase 5 (PFK5)	11	6	Q_03659↑,Q_10134
	AT5G56630	PFK7	phosphofructokinase 7 (PFK7)	44	16	Q_04252↑
	AT1G76550	PFP-α	Phosphofructokinase family protein	62	28	Q_10997↑
	AT5G56350	PK	Pyruvate kinase family protein	15	3	Q_14039↑
Plastid carbon metabolism & transporters	AT3G22960	PKp1	PKP-ALPHA	114	50	Q_03673↑,Q_03674,Q_04352↑
	AT1G79750	NADP-ME4	NADP-malic enzyme 4 (NADP-ME4)	180	72	Q_10321↑,Q_10323,Q_10325↑,Q_15891↑
	AT5G33320	PPT1	CAB UNDEREXPRESSED 1 (CUE1)	30	10	Q_11194↑
	AT5G64290	DIT2.1	dicarboxylate transport 2.1 (DIT2.1)	8	1	Q_11920↑
	AT5G16150	GLT1	plastidic GLC translocator (PGLCT)	18	38	Q_03713↓
	AT5G54800	GPT1	glucose 6-phosphate/phosphate translocator 1 (GPT1)	54	23	Q_14323↑
Fatty acid biosynthesis	AT1G30120	PDH (E1β)	pyruvate dehydrogenase E1 beta (PDH-E1 BETA)	23	15	Q_12340
	AT1G01090	PDH (E1α)	pyruvate dehydrogenase E1 alpha (PDH-E1 ALPHA)	144	51	Q_15592↑
	AT3G16950	LPD1 (E3)	lipoamide dehydrogenase 1 (LPD1)	44	20	Q_04661↑
	AT3G25860	LTA2 (E2)	LTA2	46	22	Q_07116↑,Q_07117,Q_14047↑
	AT2G30200	MCMT	catalytics	33	15	Q_00121↑
	AT1G62640	KASIII	3-ketoacyl-acyl carrier protein synthase III (KAS III)	31	7	Q_10195↑
	AT5G46290	KASI	3-ketoacyl-acyl carrier protein synthase I (KAS I)	117	30	Q_06706↑
	ATCG00500	accD	acetyl-CoA carboxylase carboxyl transferase subunit beta (ACCD)	4	0	Q_00611↑
	AT1G08510	FATB	fatty acyl-ACP thioesterases B (FATB)	185	61	Q_05932↑,Q_12953↑,Q_15949↑,Q_15950
	AT1G77590	LACS9	long chain acyl-CoA synthetase 9 (LACS9)	17	7	Q_07889↑,Q_07890,Q_07891
	AT1G43800	SAD6	Plant stearoyl-acyl-carrier-protein desaturase family protein	34	3	Q_13824↑
Amino acid metabolism	AT3G47340	ASN1	glutamine-dependent asparagine synthase 1 (ASN1)	30	10	Q_03864↑,Q_13282
	AT5G04140	GLS1	glutamate synthase 1 (GLU1)	18	2	Q_03728↑,Q_03729
	AT5G53460	GLT1	NADH-dependent glutamate synthase 1 (GLT1)	67	21	Q_13185,Q_16076↑,Q_16077
	AT2G27820	ADT3	prephenate dehydratase 1 (PD1)	45	17	Q_10989↑
	AT3G06350	MEE32	MATERNAL EFFECT EMBRYO ARREST 32 (MEE32)	33	69	Q_03356,Q_04180↓,Q_05833↑,Q_05834,Q_14283,Q_15711↓
	AT4G39980	DAHP	3-deoxy-D-arabino-heptulosonate 7-phosphate synthase 1 (DHS1)	30	63	Q_06641↓
	AT1G02500	SAM1	S-adenosylmethionine synthetase 1 (SAM1)	238	118	Q_10361↑,Q_10363
	AT4G01850	SAM2	S-adenosylmethionine synthetase 2 (SAM-2)	484	288	Q_10359↑,Q_10360,Q_15752↓
	AT5G17920	MS1	METHIONINE SYNTHESIS 1	430	124	Q_08497,Q_10349,Q_10352,Q_10353↑,Q_10354,Q_15560↑

Regulation of carbon metabolism	AT3G03780	MS2	methionine synthase 2 (MS2)	98	29	Q_10348↑,Q_10350↑,Q_10376
	AT2G36880	M3	methionine adenosyltransferase 3 (MAT3)	131	20	Q_10364↑
	AT3G54320	WRI1	WRINKLED 1 (WRI1)	20	11	Q_15556
	AT4G34590	BZIP11	G-box binding factor 6 (GBF6)	7	1	Q_04754↑
	AT1G68020	TPS6	ATTPS6	35	9	Q_05675↑
	AT1G23870	TPS9	trehalose-phosphatase/synthase 9 (TPS9)	14	4	Q_12940,Q_15064↑,Q_15989
Stress						
Biotic and abiotic stress response	AT1G54100	ALDH7B4	aldehyde dehydrogenase 7B4 (ALDH7B4)	31	5	Q_00080↑,Q_05692↑,Q_16412↑
	AT3G24503	ALDH2C4	aldehyde dehydrogenase 2C4 (ALDH2C4)	17	6	Q_15203↑,Q_15204
	AT1G44170	ALDH3H1	aldehyde dehydrogenase 3H1 (ALDH3H1)	101	39	Q_13322↑
	AT5G47120	BI1	BAX inhibitor 1 (BI1)	50	68	Q_03859,Q_12414
	AT1G20630	CAT1	catalase 1 (CAT1)	0	32	Q_00388↓,Q_00389↓
	AT4G35090	CAT2	catalase 2 (CAT2)	65	107	Q_00390↑,Q_00392↓
	AT1G08830	CSD1	copper/zinc superoxide dismutase 1 (CSD1)	32	81	Q_16609↓
	AT5G18100	CSD3	copper/zinc superoxide dismutase 3 (CSD3)	9	1	Q_12531↑
	AT4G17260	LDH	Lactate/malate dehydrogenase family protein	40	7	Q_05363↑,Q_05364↑,Q_14262
	AT5G54960	PDC2	pyruvate decarboxylase-2 (PDC2)	21	8	Q_10982↑
	AT5G17380	PDC	Thiamine pyrophosphate dependent pyruvate decarboxylase family protein	18	7	Q_14147↑
	AT3G02550	LBD41	LOB domain-containing protein 41 (LBD41)	58	11	Q_09782↑,Q_13888↑
	AT1G02930	GSTF6	glutathione S-transferase 6 (GSTF6)	50	149	Q_04187↓
	AT1G17180	GSTU25	glutathione S-transferase TAU 25 (GSTU25)	24	52	Q_03533↓,Q_03534,Q_14161,Q_14162↓
	AT2G29420	GSTU7	glutathione S-transferase tau 7 (GSTU7)	10	28	Q_04106↓,Q_05880,Q_10270↓
	AT2G34930	AT2G34930	disease resistance family protein / LRR family protein	17	55	Q_04356,Q_04496,Q_06128↓,Q_09313,Q_09314,Q_10135↓,Q_10260↓,Q_10492,Q_11564,Q_15259,Q_15262,Q_15264
	AT5G17680	AT5G17680	disease resistance protein (TIR-NBS-LRR class), putative	11	27	Q_05409,Q_09210,Q_10726↓,Q_12812↓,Q_12841,Q_13956,Q_15238↑,Q_15240↓,Q_16756
	AT2G34930	AT2G34930	disease resistance family protein / LRR family protein	17	55	Q_04356,Q_04496,Q_06128↓,Q_09313,Q_09314,Q_10135↓,Q_10260↓,Q_10492,Q_11564,Q_15259,Q_15262,Q_15264
Secondary metabolism						
Waxes and aliphatic suberin synthesis and transport	AT1G01120	KCS1	3-ketoacyl-CoA synthase 1 (KCS1)	159	43	Q_12651↑,Q_13818↑
	AT1G04220	KCS2/DAISY	3-ketoacyl-CoA synthase 2 (KCS2)	99	22	Q_10410↑
	AT5G43760	KCS20	3-ketoacyl-CoA synthase 20 (KCS20)	95	23	Q_07428↑
	AT5G58860	CYP86A1	cytochrome P450, family 86, subfamily A, polypeptide 1 (CYP86A1)	1056	196	Q_00360↑,Q_00361↑,Q_00362,Q_04141↑
	AT5G23190	CYP86B1	cytochrome P450, family 86, subfamily B, polypeptide 1 (CYP86B1)	423	106	Q_03337↑,Q_08965↑,Q_09620↑
	AT5G41040	ASFT/FHT	HXXXD-type acyl-transferase family protein	750	241	Q_03564↑,Q_03565↑,Q_03571,Q_10185↑,Q_10186↑,Q_10187,Q_15945↑
	AT3G11430	GPTA5	glycerol-3-phosphate acyltransferase 5 (GPAT5)	300	53	Q_03513↑,Q_12912↑,Q_13743↑
	AT2G39350	ABCG1	ABC-2 type transporter family protein	71	11	Q_14646↑
	AT3G53510	ABCG20	ABC-2 type transporter family protein	343	90	Q_00027↑,Q_14780↑
Acyl-lipid miscellanous	AT2G38110	GPAT6	glycerol-3-phosphate acyltransferase 6 (GPAT6)	25	6	Q_11648↑
	AT4G00400	GPAT8	glycerol-3-phosphate acyltransferase 8 (GPAT8)	126	38	Q_09055↑
	AT1G55020	LOX1	lipoxygenase 1 (LOX1)	454	12	Q_05712↑,Q_09356↑,Q_09826↑,Q_10286↑,Q_10287↑,Q_10288↑,Q_10289↑
	AT3G22400	LOX5	LOX5	361	12	Q_09827↑,Q_10291↑,Q_10292,Q_14498
	AT2G23540	AT2G23540	GDSL-like Lipase/Acylhydrolase superfamily protein	669	196	Q_10256↑,Q_11119↑,Q_11120↑,Q_12005↑,Q_14346
	AT1G24430	AT1G24430	HXXXD-type acyl-transferase family protein	1064	393	Q_05974↓,Q_15270↑,Q_15271↑,Q_15273↑,Q_15274↑,Q_15275↑
Aromatic suberin synthesis and cross-linking	AT2G37040	PAL1	PHE ammonia lyase 1 (PAL1)	126	15	Q_10961↑,Q_10962↑,Q_10967↑
	AT3G53260	PAL2	phenylalanine ammonia-lyase 2 (PAL2)	390	89	Q_03830↑,Q_10960↑,Q_10965↑,Q_10970,Q_13950,Q_15639↑
	AT5G04230	PAL3	phenyl alanine ammonia-lyase 3 (PAL3)	157	41	Q_13949↑
	AT3G10340	PAL4	phenylalanine ammonia-lyase 4 (PAL4)	27	4	Q_10963↑
	AT2G30490	C4H	cinnamate-4-hydroxylase (C4H)	505	98	Q_08088↑,Q_16723↑
	AT1G51680	4CL1	4-coumarate:CoA ligase 1 (4CL1)	187	76	Q_00019↑,Q_00020
	AT4G38620	MYB4	myb domain protein 4 (MYB4)	7	41	Q_09542↓,Q_10829↓,Q_10832
	AT5G48930	HCT	hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (HCT)	144	62	Q_03620,Q_03621,Q_03623↑,Q_15778↑
	AT4G34050	CCoAOMT1	caffeoyl coenzyme A O-methyltransferase 1 (CCoAOMT1)	243	89	Q_00378,Q_00379↑,Q_00380↑
	AT5G54160	COMT/OMT1	O-methyltransferase 1 (OMT1)	191	79	Q_00492↑,Q_00493,Q_05475↓,Q_10900↑
	AT4G37980	CAD4	elicitor-activated gene 3-1 (ELI3-1)	0	10	Q_10382↓
	AT4G37990	CAD5	elicitor-activated gene 3-2 (ELI3-2)	0	9	Q_14059↓
	AT1G72680	CAD9	cinnamyl-alcohol dehydrogenase (CAD1)	4	17	Q_04339↓,Q_04340,Q_09521↓,Q_09522,Q_15546
	AT2G33590	CCR-like3	NAD(P)-binding Rossmann-fold superfamily protein	12	25	Q_04069,Q_05737↓,Q_12401
	AT5G58490	CCR-like5	NAD(P)-binding Rossmann-fold superfamily protein	3	9	Q_05785↓

	AT1G68850	Prx11	Peroxidase superfamily protein	187	65	Q_08706↑,Q_15849↑
	AT2G35380	Prx20	Peroxidase superfamily protein	5	17	Q_12041↓
	AT5G05340	Prx52	Peroxidase superfamily protein	62	6	Q_06399↑,Q_09813↑
	AT5G67400	Prx73	root hair specific 19 (RHS19)	21	0	Q_07983↑
	AT5G05390	LAC12	laccase 12 (LAC12)	246	39	Q_03682↑,Q_07246↑
	AT5G09360	LAC14	laccase 14 (LAC14)	149	139	Q_03741,Q_05162↓,Q_05561,Q_05961↓,Q_07261↓,Q_07550,Q_13977,Q_13978↑,Q_13979↑,Q_13980,Q_14453↓
	AT2G30210	LAC3	laccase 3 (LAC3)	3	10	Q_15678↓
	AT2G40370	LAC5	laccase 5 (LAC5)	98	20	Q_14541↑
Isoprenoids (MVA pathway)	AT1G76490	HMGR1	hydroxy methylglutaryl CoA reductase 1 (HMG1)	450	125	Q_03577↑,Q_03578↑,Q_03580↑,Q_03581↑
	AT5G47720	AACT1	Thiolase family protein	7	1	Q_12205↑
	AT3G54250	MPDC2	GHMP kinase family protein	61	22	Q_14886↑
Transcription factors (TFs) candidates for suberin regulation						
NAC TFs	AT2G24430	NAC038	NAC domain containing protein 38 (NAC038)	35	49	Q_13893
	AT3G18400	NAC058	NAC domain containing protein 58 (NAC058)	22	14	Q_10840
	AT5G18270	ANAC087	Arabidopsis NAC domain containing protein 87 (ANAC087)	28	4	Q_07599↑
MYB TFs	AT1G17950	MYB52	myb domain protein 52 (MYB52)	13	8	Q_10822,Q_10825,Q_15425
	AT5G65790	MYB68	myb domain protein 68 (MYB68)	15	28	Q_10119
	AT1G34670	MYB93	myb domain protein 93 (MYB93)	52	41	Q_10814,Q_10815
	AT4G21440	MYB102	MYB-like 102 (MYB102)	13	2	Q_04178↑,Q_10812↑
	AT2G46130	WRKY43	WRKY DNA-binding protein 43 (WRKY43)	74	23	Q_12647↑
WRKY TFs	AT1G64000	WRKY56	WRKY DNA-binding protein 56 (WRKY56)	26	17	Q_16355
	AT1G29280	WRKY65	WRKY DNA-binding protein 65 (WRKY65)	6	6	Q_05353,Q_13163
Cell wall						
nucleotide-sugar interconversions	AT3G62830	AUD	AUD1	109	47	Q_08718↑,Q_08719
	AT1G08200	AXS2	UDP-D-apiose/UDP-D-xylose synthase 2 (AXS2)	27	10	Q_04907↑
	AT1G73250	GER1	GDP-4-keto-6-deoxymannose-3,5-epimerase-4-reductase 1 (GER1)	9	3	Q_15502↑
	AT5G28840	GME1	GDP-D-mannose 3',5'-epimerase (GME)	15	21	Q_03506,Q_03507↓
	AT2G28760	SUD3	UDP-XYL synthase 6 (UXS6)	49	24	Q_14144↑,Q_15046
	AT5G15490	UGD3	UDP-glucose 6-dehydrogenase family protein	31	15	Q_16814↑
	AT4G10960	UGE5	UDP-D-glucose/UDP-D-galactose 4-epimerase 5 (UGE5)	0	8	Q_03479↓
cellulose synthases	AT4G39350	CesA2	cellulose synthase A2 (CESA2)	117	49	Q_04056↑,Q_13093
	AT5G05170	CesA3	CONSTITUTIVE EXPRESSION OF VSP 1 (CEV1)	132	47	Q_06357↑,Q_11905↑
	AT5G03760	CslA9	ATCSLA09	15	1	Q_06869↑
	AT3G03050	CslD3	cellulose synthase-like D3 (CSLD3)	39	11	Q_13239↑,Q_15575↑
callose synthases	AT3G07160	GSL10	glucan synthase-like 10 (GSL10)	18	8	Q_03284↑,Q_09697,Q_10484
	AT2G31960	GSL3	glucan synthase-like 3 (GSL03)	36	14	Q_00373↑,Q_06279↑
	AT4G03550	GSL5	glucan synthase-like 5 (GSL05)	25	11	Q_15367↑
	AT2G01850	XTH27	endoxyloglucan transferase A3 (EXGT-A3)	51	17	Q_09900,Q_11521,Q_12469↑
xyloglucan endotransglucosylase-hydrolase	AT1G14720	XTH28	xyloglucan endotransglucosylase/hydrolase 28 (XTH28)	37	14	Q_12253↑,Q_12254↑
	AT1G32170	XTH30	xyloglucan endotransglucosylase/hydrolase 30 (XTH30)	77	35	Q_10643↑,Q_15551↑
	AT5G13870	XTH5	xyloglucan endotransglucosylase/hydrolase 5 (XTH5)	53	12	Q_15765↑
	AT4G03210	XTH9	xyloglucan endotransglucosylase/hydrolase 9 (XTH9)	12	23	Q_11842↓
	AT5G57560	XTH22	Touch 4 (TCH4)	47	66	Q_03787,Q_05725,Q_06312↓
	AT4G25810	XTH23	xyloglucan endotransglycosylase 6 (XTR6)	21	25	Q_09505,Q_11813↓
glycoside hydrolase family 17	AT5G55180	AT5G55180	O-Glycosyl hydrolases family 17 protein	76	19	Q_09379↑,Q_11333↑
Expansins	AT2G40610	EXPA8	expansin A8 (EXPA8)	6	1	Q_14549↑
arabinogalactan-proteins	AT5G56540	AGP14	arabinogalactan protein 14 (AGP14)	18	7	Q_11457↑
	AT5G11740	AGP15	arabinogalactan protein 15 (AGP15)	13	5	Q_09587↑
	AT4G37450	AGP18	arabinogalactan protein 18 (AGP18)	23	10	Q_01757↑
CASP-like proteins	AT5G44550	CASPL1B1	CASP-LIKE PROTEIN 1B1	162	61	Q_14678↑
	AT4G20390	CASPL1B2	CASP-LIKE PROTEIN 1B2	84	22	Q_10798↑
	AT3G06390	CASPL1D2	CASP-LIKE PROTEIN 1D1	168	39	Q_03294↑
	AT4G25040	CASPL1F1	CASP-LIKE PROTEIN 1F1	17	39	Q_03289↓

Cell growth						
Cell growth regulation	AT5G60920	COB	COBRA (COB)	32	22	Q_07498↑,Q_07593,Q_13061↑
	AT3G44200	NEK6	NIMA (never in mitosis, gene A)-related 6 (NEK6)	60	16	Q_06584↑,Q_06585↑
	AT4G23496	SP1L5	SPIRAL1-like5 (SP1L5)	8	0	Q_12994↑
	AT1G70140	FH8	formin 8 (FH8)	52	14	Q_06072↑
Hormone-related genes						
auxin	AT1G02520	ABCB11	P-glycoprotein 11 (PGP11)	137	40	Q_08124↑,Q_15603↑
	AT3G62150	ABCB21	P-glycoprotein 21 (PGP21)	144	58	Q_15602↑
	AT2G47000	ABCB4	ATP binding cassette subfamily B4 (ABCB4)	17	7	Q_08125,Q_11319,Q_11858,Q_12405↑
	AT3G53480	ABCG37	pleiotropic drug resistance 9 (PDR9)	34	7	Q_06275↑
	AT1G70940	PIN3	PIN-FORMED 3 (PIN3)	6	1	Q_08270↑,Q_11014
	AT1G76520	PIN-LIKES 3	Auxin efflux carrier family protein	32	9	Q_14386↑
	AT3G62980	TIR1	TRANSPORT INHIBITOR RESPONSE 1 (TIR1)	8	3	Q_14281,Q_14282↑
	AT3G26810	AFB2	auxin signaling F-box 2 (AFB2)	22	68	Q_07511↓
	AT5G62000	ARF2	auxin response factor 2 (ARF2)	26	36	Q_00251,Q_05355↑,Q_08254
	AT5G20730	ARF7	NON-PHOTOTROPHIC HYPOCOTYL (NPH4)	11	7	Q_06502↑,Q_07554,Q_07555
	AT1G19220	ARF19	auxin response factor 19 (ARF19)	11	4	Q_14598↑,Q_14599
	AT2G33860	ARF3	ETTIN (ETT)	8	22	Q_04466↓,Q_08907↓
	AT5G60450	ARF4	auxin response factor 4 (ARF4)	0	9	Q_04151↓
	AT1G30330	ARF6	auxin response factor 6 (ARF6)	11	20	Q_04087↓,Q_04088,Q_14170
	AT4G23980	ARF9	auxin response factor 9 (ARF9)	1	15	Q_07829↓,Q_15529
	AT3G04730	IAA16	indoleacetic acid-induced protein 16 (IAA16)	1	12	Q_03630↓,Q_12984
	AT5G65670	IAA9	indole-3-acetic acid inducible 9 (IAA9)	9	49	Q_13299↓,Q_13876↓
	AT4G29080	IAA27/PAP2	phytochrome-associated protein 2 (PAP2)	1	12	Q_05901↓
	AT3G16500	IAA26/PAP1	phytochrome-associated protein 1 (PAP1)	12	4	Q_08049↑
ethylene	AT2G19590	ACO1	ACC oxidase 1 (ACO1)	29	4	Q_06509↑,Q_06510↑
	AT1G05010	ACO4/EFE	ethylene-forming enzyme (EFE)	13	1	Q_00035↑
	AT2G40940	ERS1	ethylene response sensor 1 (ERS1)	5	1	Q_03419,Q_09346↑
	AT3G23150	ETR2	ethylene response 2 (ETR2)	35	3	Q_03421↑,Q_14181↑
	AT3G14230	RAP2.2	related to AP2 2 (RAP2.2)	79	60	Q_04879↑,Q_04881
	AT3G16770	RAP2.3/EBP	ethylene-responsive element binding protein (EBP)	27	13	Q_05248,Q_15396↑
	AT1G78080	RAP2.4	related to AP2 4 (RAP2.4)	24	10	Q_07158↑,Q_15152
	AT1G53910	RAP2.12	related to AP2 12 (RAP2.12)	200	128	Q_13746,Q_13747
	AT4G25480	DREB1A	dehydration response element B1A (DREB1A)	0	8	Q_15916↓
	AT3G15210	ERF4	ethylene responsive element binding factor 4 (ERF4)	3	13	Q_03413↓,Q_05020↓
	AT5G47230	ERF5	ethylene responsive element binding factor 5 (ERF5)	6	15	Q_03411↓
	AT5G44210	ERF9	erf domain protein 9 (ERF9)	3	28	Q_10599↓
ABA	AT4G08920	CRY1	cryptochrome 1 (CRY1)	7	19	Q_13298↓
	AT1G20630	CAT1	catalase 1 (CAT1)	0	32	Q_00388↓,Q_00389↓
	AT1G35670	CPK11	calcium-dependent protein kinase 2 (CDPK2)	2	15	Q_14534↓
	AT4G33950	OST1	OPEN STOMATA 1 (OST1)	5	7	Q_09423,Q_14851↓,Q_16671
	AT5G19330	ARIA	ARM repeat protein interacting with ABF2 (ARIA)	7	20	Q_05141↓,Q_05142↓
	AT5G47100	CBL9	calcineurin B-like protein 9 (CBL9)	2	9	Q_00415↓
	AT3G11410	PP2CA	protein phosphatase 2CA (PP2CA)	17	33	Q_10692↓,Q_12271
	AT1G32640	MYC2	MYC2	17	9	Q_05358↑,Q_05359
	AT3G63520	NCED1	carotenoid cleavage dioxygenase 1 (CCD1)	5	14	Q_00421↓
gibberellin	AT3G63010	GID1B	GA INSENSITIVE DWARF1B (GID1B)	9	3	Q_11531↑
	AT5G27320	GID1C	GA INSENSITIVE DWARF1C (GID1C)	5	4	Q_15715
	AT1G02400	GA2OX6	gibberellin 2-oxidase 6 (GA2OX6)	1	1	Q_03473
	AT5G24910	CYP714A	cytochrome P450, family 714, subfamily A, polypeptide 1 (CYP714A1)	45	9	Q_03311,Q_03312↑,Q_03313↑,Q_05187,Q_08623,Q_15128↑
brassinosteroids	AT3G19820	DWF1	DWARF 1 (DWF1)	24	7	Q_03361↑
	AT5G13710	SMT1	sterol methyltransferase 1 (SMT1)	4	12	Q_07524↓
	AT3G13380	BRL3	BRI1-like 3 (BRL3)	16	19	Q_07849,Q_12352,Q_13446
	AT4G39400	BRI1	BRASSINOSTEROID INSENSITIVE 1 (BRI1)	4	2	Q_00350,Q_04498
	AT3G51550	FER	FERONIA (FER)	68	41	Q_00094,Q_03447↑,Q_03803,Q_03805,Q_03806↓,Q_03807,Q_03808,Q_08355,Q_08421,Q_10611↑,Q_11652,Q_14168,Q_15211↑
	AT5G54380	THE1	THESEUS1 (THE1)	11	8	Q_11767,Q_11768,Q_13936↑
cytokinin	AT5G56970	CKX3	cytokinin oxidase 3 (CKX3)	7	2	Q_15180↑
	AT5G21482	CKX7	cytokinin oxidase 7 (CKX7)	1	8	Q_04550↓
	AT1G27320	AHK3	histidine kinase 3 (HK3)	14	15	Q_04742
	AT2G01830	CRE1	WOODEN LEG (WOL)	4	13	Q_00072,Q_05923,Q_05924,Q_15338
	AT3G16857	ARR1(B)	response regulator 1 (RR1)	3	4	Q_13761

	AT4G16110	ARR2(B)	response regulator 2 (RR2)	12	7	Q_15071
	AT1G67710	ARR11(B)	response regulator 11 (ARR11)	8	4	Q_04579
	AT2G25180	ARR12(B)	response regulator 12 (RR12)	1	4	Q_06802
Meristem regulation						
	AT1G62360	STM	SHOOT MERISTEMLESS (STM)	7	1	Q_11329↑
	AT1G46480	WOX4	WUSCHEL related homeobox 4 (WOX4)	9	1	Q_05377↑
	AT4G32880	HB-8	homeobox gene 8 (HB-8)	3	1	Q_13333
	AT1G52150	HB-15	ATHB-15	3	6	Q_06912,Q_15894
	AT5G60690	REV	REVOLUTA (REV)	7	6	Q_04132
	AT5G16560	KAN1	KANADI (KAN)	1	4	Q_04400
	AT1G65380	CLV2	clavata 2 (CLV2)	1	1	Q_09804
	AT5G65700	BAM1	BARELY ANY MERISTEM 1 (BAM1)	6	6	Q_00326,Q_10263,Q_12451
	AT4G37750	ANT	AINTEGUMENTA	14	24	Q_05644
	AT4G00150	HAM3	HAIRY MERISTEM 3 (HAM3)	1	4	Q_03519
	AT3G54220	SCR	SCARECROW (SCR)	1	5	Q_16053
	AT1G56010	NAC1	NAC domain containing protein 1 (NAC1)	5	0	Q_10856↑
	AT5G25220	KNAT3	KNOTTED1-like homeobox gene 3 (KNAT3)	10	30	Q_03668,Q_03669,Q_05938,Q_09364↓
	AT5G66870	ASL1	ASYMMETRIC LEAVES 2-like 1 (ASL1)	1	0	Q_10153
	AT2G41370	BOP2	BLADE ON PETIOLE2 (BOP2)	1	0	Q_03905
	AT4G28190	ULT1	ULTRAPETALA1 (ULT1)	9	5	Q_12825
	AT4G32551	LUG	LEUNIG (LUG)	17	36	Q_03697,Q_06787,Q_06788,Q_07929↓,Q_07930↓,Q_13089
	AT1G69120	AP1	APETALA1 (AP1)	3	1	Q_15855↑
Integrators of environmental cues						
	AT5G10140	FLC	FLOWERING LOCUS C (FLC)	1	17	Q_03453↓
	AT2G22540	SVP	SHORT VEGETATIVE PHASE (SVP)	6	18	Q_10319↓,Q_12462,Q_12463
	AT2G45660	SOC1	AGAMOUS-like 20 (AGL20)	26	27	Q_03704
Chromatin-modifying enzymes						
	AT4G16845	VRN2	REDUCED VERNALIZATION RESPONSE 2 (VRN2)	3	5	Q_13879
	AT4G02020	SWN1/EZA	SWINGER (SWN)	1	3	Q_06116
	AT2G23380	CLF	CURLY LEAF (CLF)	2	1	Q_09637
	AT3G20740	FIE	FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)	0	4	Q_04714
	AT3G12810	PIE	PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1 (PIE1)	3	6	Q_09077,Q_14040,Q_14041,Q_14042
	AT5G37055	SEF1	SERRATED LEAVES AND EARLY FLOWERING (SEF)	2	4	Q_15676
	AT2G25170	PICKLE	PICKLE (PKL)	19	20	Q_00455↓,Q_07104,Q_07105↑,Q_14981
	AT1G79730	ELF7	EARLY FLOWERING 7 (ELF7)	2	8	Q_06017↓
	AT2G06210	ELF8	EARLY FLOWERING 8 (ELF8)	7	16	Q_05663↓,Q_05664,Q_05665
	AT4G29830	VIP3	vernalization independence 3 (VIP3)	4	5	Q_06520
	AT5G61150	VIP4	VERNALIZATION INDEPENDENCE 4 (VIP4)	6	9	Q_04957,Q_15865
	AT1G62830	LDL1/SWP1	LSD1-like 1 (LDL1)	5	4	Q_09672,Q_11493,Q_15649
	AT2G45640	SAP18	SIN3 associated polypeptide P18 (SAP18)	5	9	Q_05343↓,Q_05344
	AT1G77300	EFS/SDG8	EARLY FLOWERING IN SHORT DAYS (EFS)	5	5	Q_04486,Q_04488,Q_09093
	AT1G79000	HAC1	histone acetyltransferase of the CBP family 1 (HAC1)	12	25	Q_02554,Q_03560,Q_05465,Q_05466,Q_08827↓
	AT5G03740	H2DC	histone deacetylase 2C (HD2C)	6	20	Q_05711↓,Q_06359
	AT5G08450	H2DC1	Histone deacetylation protein Rxt3-like	7	16	Q_08471,Q_09097↓
	AT3G44530	HIRA	homolog of histone chaperone HIRA (HIRA)	1	5	Q_07735
	AT1G74560	NAP1	NAP1-related protein 1 (NRP1)	4	15	Q_04409↓
	AT2G27040	(AGO4)	ARGONAUTE 4 (AGO4)	21	55	Q_03785↓
	AT2G16390	DMS1	DEFECTIVE IN RNA-DIRECTED DNA METHYLATION 1 (DRD1)	4	9	Q_06951,Q_08628↓
	AT3G23780	DMS2	nuclear RNA polymerase D2A (NRPD2A)	6	8	Q_12560,Q_14976

Table S15. List of target genes for the RT-qPCR analyses and the primers used.

Functional category	Contig ID	Gene Name	Best TAIR10 BLASTX	Primer sequence (5'-3')
Carbohydrate metabolism	Q_16690	SUS4	AT3G43190	fwd: TCTCAAGATAACGACGGCTCT rev: TACTGGGGTTTATGGCTTTTG
	Q_03673	PKP1	AT3G22960	fwd: ACTCTCCCAGAACGCACCAT rev: CGAACCTCACCATACCACCA
Secondary metabolism	Q_03337	CYP86B1	AT5G23190	fwd: CAATTTCCCTAAAGGACCATTC rev: TAGCTGAGTGGAATTCAATGCT
	Q_03578	HMGR1	AT1G76490	fwd: ATTGGTGAGGTAAAGTGGGAGT rev: TGAAAATGGGAGTGGGTCTACA
Cell wall biogenesis	Q_15575	CSLD3	AT3G03050	fwd: GTCCAGACCCTCAATGTCACT rev: CTAGTCCCTCCAATCAACCAA
	Q_14549	EXPA8	AT2G40610	fwd: GGGAGACACCATCTAGGATCA rev: GCCAAGGATATGGGACTAACA
Cell death	Q_16826	γ-VPE	AT4G32940	fwd: CCACTTTTGTGGGTGAAAACT rev: TGCTTCAACAAACTGCTTCTG
	Q_12414	BI1	AT5G47120	fwd: CAGAAGATAGCAAGCCACCA rev: GCGGTTGACCCAAGTATTTT
Hormone metabolism	Q_08270	PIN3	AT1G70940	fwd: TGAGGAGAAAGAGTAGGAGGGA rev: ACGGACAATCAAAACCAACACT
	Q_06510	ACO1	AT2G19590	fwd: GCATTCTCAGGGAGCAAAGG rev: GGGACTTGGTCATCTTGGAGT
	Q_00035	EFE/ACO4	AT1G05010	fwd: ACCAGTGTTGGTGCTGGATA rev: CCCAAACAGATGGAAACAGA
	Q_03361	DWF1	AT3G19820	fwd: ACCATGCTTTACAATCCAACTG rev: TGCTGTCTGTGCATACTGGTAG
Meristem regulation	Q_11329	STM	AT1G62360	fwd: GGTGGCAAGACTAGAACAAGC rev: TGGTCAGCATCTCACAGTAGG
	Q_05377	WOX4	AT1G46480	fwd: GCAATGGTGGTAATGGCAGTC rev: GCGAGAGGCAGAAGCAGAAG
Transcription Factors	Q_10829	MYB4	AT4G38620	fwd: CTCATGGTGAAGGGTGTTGG rev: CAGCAGCAGCCTCATTCATT
	Q_03453	FLC/AGL25	AT5G10140	fwd: AGTGCAGCATTGAATTGTCTCT rev: TGCGAGTCTAGAGGATGTGAGT
	Q_03704	SOC1/AGL20	AT2G45660	fwd: TATCAGTTCTTTGCGATGCAG rev: CGGTAGCGTTCTATTGTCTCC
	Q_15855	AP1/AGL7	AT1G69120	fwd: TTGTTGCTGAGGTTGGTTGC rev: TTATTGGGTTGTTGCCGATG

The following additional supplemental data of Chapter III can be found in electronic format (CD-ROM) attached to the end of this thesis:

Table S2. Functional annotation of contigs against several sequences databases. BLAST analyses were performed against generic non-redundant databases (Swissprot, Refseq and nr databases) and organism-specific databases: Arabidopsis (TAIR10) and poplar (P. trichocarpa v3.0) proteins and the cork oak transcriptome (CorkOakDB).

Tables S3A-D. Functional annotation of contigs with (A) Gene Ontologies, (B) KEGG pathways, (C) MapMan 'bins' and (D) Protein conserved domains (CDD).

Table S4. Assessment of the 454 contig sequences homology with the cork oak Sanger-sequenced sequences from Soler *et al.* (2007) analysed by BLASTN.

Table S5A-B. (A) Gene Ontology enrichment of the most abundant contigs in cork oak and (B) holm oak transcriptomes accumulating the 30% of the total ESTs mapped

Table S6. List of the contigs differentially expressed determined by the DEGseq statistical test (**q-value < 0.01, *q-value < 0.05).

Table S7. Functional analysis of the genes differentially regulated by (A) Gene Ontology enrichments, (B) PlantGSEA tool enrichment, (C) KEGG pathways, (D) MapMan 'bins', (E) Protein conserved domains (CDD).

Table S8. Annotation and expressed sequence tag (ESTs) levels for contigs annotated to Arabidopsis proteins. Contigs were assigned to Arabidopsis TAIR 10 proteins by BLASTX with cutoff *E*-value < E^{-10}. To enable comparison of cork oak EST levels to holm oak EST levels, EST counts are normalized by libraries size (ESTs/100,000 ESTs). There may be more than one contig/TAIR10 locus ID in which case EST numbers for multiple contigs that were assigned to the same protein are summed in this table. The corresponding contigs are shown and the contigs significantly upregulated in cork oak (↑) and holm oak (↓) are indicated.

Table S9. Classification of contigs into functional groups: (A) carbohydrate metabolism, (B) amino acid metabolism, (C) acyl-lipid metabolism, (D) isoprenoids metabolism, (E) CAZy enzymes, (F) cell wall proteins, (G) transcription factors families, (H) phytohormone-related genes, (I) meristem regulatory genes and (J) flowering time genes.

Table S10. List of genes showing *in silico* co-expression patterns with suberin biosynthetic genes based on Arabidopsis microarray data. In yellow are marked the suberin genes used as bait genes.

Table S12. *In silico* detection of sequences variants (SNPs and INDELs). The variants with coverage equal or greater than six sequences are shown.

Table S14. Contig ESTs levels normalized by library size (ESTs/100,000 ESTs).

Supplemental data for Chapter IV

Transcriptional characterization of a selected set of new cork candidate genes: mRNA abundance in phellem versus xylem tissue and effect of cork seasonal growth in their expression

This supplemental data include the list of the genes analysed and the primers used for the RT-qPCR analyses, the list of the genes from Soler *et al*. (2008) which were used for the principal component analysis and the hierarchical clustering analysis, and the correlation matrix of the expression profiles along the growing season of all the genes analysed in Chapter IV.

Supplementary Tables

Table S1. List of the primers used for RT-qPCR analyses performed for each gene

Functional category	Contig ID	Gene Name	Best TAIR10 BLASTX	Primer sequence (5'-3')
Carbohydrate metabolism	Q_16690	SUS4	AT3G43190	fwd: TCTCAAGATAACGACGGCTCT
				rev: TACTGGGGTTTATGGCTTTTG
	Q_03673	PKP1	AT3G22960	fwd: ACTCTCCCAGAACGCACCAT
				rev: CGAACCTCACCATACCACCA
De novo Fatty acid synthesis	Q_15592	PDH-E1α	AT1G01090	fwd: GGTTAGGGAGGTGGCAAAGG
				rev: TTCTCAGCAGGGTCACGAAG
	Q_15556	WRI1	AT3G54320	fwd: GGCATTTCTCTGGGTTGTGA
				rev: CCTGTGTTGGTTCTGTCATTCC
Suberin synthesis	Q_03337	CYP86B1	AT5G23190	fwd: CAATTTCCCTAAAGGACCATTC
				rev: TAGCTGAGTGGAATTCAATGCT
Isoprenoids metabolism	Q_03578	HMGR1	AT1G76490	fwd: ATTGGTGAGGTAAAGTGGGAGT
				rev: TGAAAATGGGAGTGGGTCTACA
	Q_15575	CSLD3	AT3G03050	fwd: GTCCAGACCCTCAATGTCACT
				rev: CTAGTCCCTCCAATCAACCAA
Cell wall	Q_14549	EXPA8	AT2G40610	fwd: GGGAGACACCATCTAGGATCA
				rev: GCCAAGGATATGGGACTAACA
	Q_10798	CASPL1B2	AT4G20390	fwd: GGGAAGGACGGAGGATGATT
				rev: AGGAGCAAAACAACTCGCTTG
Cell death	Q_12414	BI1	AT5G47120	fwd: CAGAAGATAGCAAGCCACCA
				rev: GCGGTTGACCCAAGTATTTT
Plant hormone metabolism	Q_08270	PIN3	AT1G70940	fwd: TGAGGAGAAAGAGTAGGAGGGA
				rev: ACGGACAATCAAAACCAACACT
	Q_11531	GID1B	AT3G63010	fwd: AAGCCCTCCAATACCAATCG
				rev: TCCTTCTTCATCCAATGTTTGC
	Q_00035	EFE/ACO4	AT1G05010	fwd: ACCAGTGTTGGTGCTGGATA
				rev: CCCAAACAGATGGAAACAGA
	Q_14181	ETR2	AT3G23150	fwd: AAGGGGAGCGATGAAGTGAA
				rev: ATTGGAAACCCGAAGCATTG
	Q_03361	DWF1	AT3G19820	fwd: ACCATGCTTTACAATCCAACTG
				rev: TGCTGTCTGTGCATACTGGTAG
	Q_12352	BRL3	AT3G13380	fwd: AGAAAGCAAAGGCGAAGGAA

				rev: TGGGGAGAACAAAGTAATGGAG
	Q_04742	HK3	AT1G27320	fwd: CGACATCAAAGACACCACCA rev: TCTCCCCTCAAATGCTACCC
Meristem regulation	Q_11329	STM	AT1G62360	fwd: GGTGGCAAGACTAGAACAAGC rev: TGGTCAGCATCTCACAGTAGG
	Q_05377	WOX4	AT1G46480	fwd: GCAATGGTGGTAATGGCAGTC rev: GCGAGAGGCAGAAGCAGAAG
	Q_13333	HB8	AT4G32880	fwd: CAGCAGTTCCAGTAGCCTTCG rev: TCAACACCATCTGACACCTCA
NAC transcription factors	Q_07599	NAC087	AT5G18270	fwd: CTGGGGTTGAGGACTTTTTGA rev: AAGGATGAATGGGTTGTCTGC
	Q_13893	NAC038	AT2G24430	fwd: TGCTCTTGATTGGGTTGAGG rev: GCAGGATGAATGGGTGGTTT
	Q_15872	NAC100	AT5G61430	fwd: TGCGATACGAGGAAACTCCA rev: GCCCTGCTTCTCCAACTTCA
	Q_10854	NAC075	AT4G29230	fwd: GCCACCTTGTTTCACCACCT rev: TTGAGCAGGCATTTCTTCCA
	Q_10840/ EE743827*	NAC058	At3g18400	fwd: AACGCTGCCATCTCTTGAGTCT rev: CCCACTTGATGAATTTGCAATG
MYB transcription factors	Q_10814	MYB93	AT1G34670	fwd: TGAATAGTGAGATGGGTCAAGGTT rev: CAGGAGTGGGAAATGGGAAA
	Q_10822	MYB52	AT1G17950	fwd: GCAACGAAGCAAAGTTCCAAG rev: CGAAACCCCACTAAAGAAAGGA
	Q_04943	MYB67	AT3G12720	fwd: CCTCATCATCCTCTTCCGTGA rev: CTTCCAATCCTGGGTGCTTC
	Q_10119	MYB68	AT5G65790	fwd: TGAAGAGAGGGGTTGAGATGGT rev: TGGTTTGAGAATGGTATGTGTGC
MADS-box transcription factors	Q_03453	FLC/AGL25	AT5G10140	fwd: AGTGCAGCATTGAATTGTCTCT rev: TGCGAGTCTAGAGGATGTGAGT
	Q_03704	SOC1/AGL20	AT2G45660	fwd: TATCAGTTCTTTGCGATGCAG rev: CGGTAGCGTTCTATTGTCTCC
	Q_15855	AP1/AGL7	AT1G69120	fwd: TTGTTGCTGAGGTTGGTTGC rev: TTATTGGGTTGTTGCCGATG

*Sequence obtained from Soler *et al*. (2007)

Table S2. List of genes from Soler *et al*. 2008 and their annotation based on their best TBLASTX against Arabidopsis sequences.

Functional group	Gene name and gene description from (Soler *et al*. 2008)	EST GenBank Accession no.	Best TAIR TBLASTX	Arabidopsis annotation	Arabidopsis gene description
Acyl-lipids					
	FAT; Palmitoyl-acyl carrier protein thioesterase	EE743843	At1g08510	FATB	fatty acyl-ACP thioesterases B
	CYP86A1; Fatty acid omega-hydroxylase	EE743846	At5g58860	CYP86A1	cytochrome P450, family 86, subfamily A, polypeptide 1
	GPAT; Glycerol-3-phosphate acyltransferase	EE743865	At3g11430	GPAT5	glycerol-3-phosphate acyltransferase 5
Isoprenoids					
	bAS; Beta-amyrin synthase	EE743683	At1g78955	CAMS1	camelliol C synthase 1
Phenylpropanoids					
	F5H; Ferulate-5-hydroxylase	EE743847	At4g36220	F5H	ferulic acid 5-hydroxylase 1
	HCBT; N-Hydroxycinnamoyl/benzoyltransferase	EE743861	At5g41040	ASFT/RWP1	HXXXD-type acyl-transferase family protein
Flavonoids					
	ANR; Anthocyanidin reductase	EE743804	At1g61720	BAN	BANYULS
Regulatory					
	WRKY; WRKY transcription factor	EE743809	At2g46130	WRKY43	WRKY DNA-binding protein 43
	NAM; NAM transcription factor	EE743827	At3g18400	NAC058	NAC domain containing protein 58
Stress-related					
	HSP17.4; Heat shock protein 17.4	AJ000691	At5g59720	HSP18.2	heat shock protein 18.2
	ANN; Annexin	EE743878	At1g35720	ANN1	annexin 1
	APX; Acorbate peroxidase	EE743659	At3g09640	APX2	ascorbate peroxidase 2

Table S3. Correlation matrix among the relative transcript abundance values of the genes analysed (n = 24. *, P < 0.05; and **, P < 0.01)

	ANN1	BAN	APX2	CAMS1	F5H	NAc058	FATB	ASFT	CYP86A1	GPAT5	HSP18.2	WRKY43	BRL3	GID1B	HB-8	HK3	WRI1	MYB93	ETR2	PDH-E1α	WOX4	CASPL1B2	AP1	BI1	CSLD3	CYP86B1	DWF1	EXPA8	FLC	HMGR1	PIN3	SOC1	STM	SUS4
ANN1																																		
BAN	-.005																																	
APX2	.455*	.387																																
CAMS1	.437*	-.310	-.046																															
F5H	-.574**	-.044	-.037	-.265																														
NAC058	.066	.246	.345	.494*	.256																													
FATB	-.391	.386	.165	-.083	.619**	.492*																												
ASFT	.332	.023	.406*	.456*	.042	.612**	.397																											
CYP86A1	.473*	-.293	.244	.629**	-.046	.519**	.023	.808**																										
GPAT5	.053	-.140	-.017	.494*	.183	.486*	.320	.690**	.706**																									
HSP18.2	.843**	.023	.354	.325	-.736**	-.021	-.515*	.264	.382	-.100																								
WRKY43	-.129	.333	.341	.177	.374	.681**	.789**	.702**	.331	.420*	-.209																							
BRL3	.592**	-.018	.275	.078	-.270	-.133	-.453*	-.206	.011	-.192	.361	-.528**																						
GID1B	.405*	-.277	-.158	.402	-.457*	.053	-.500*	.071	.324	.136	.510*	-.243	.211																					
HB-8	.574**	-.297	.260	.639**	-.441*	.167	-.392	.285	.522**	.225	.576**	-.113	.204	.574**																				
HK3	.252	.003	.396	.174	.136	.148	.289	.285	.196	.093	.149	.210	.110	-.210	.190																			
WRI1	.227	.264	.498*	.158	.179	.557**	.471*	.563**	.390	.190	.219	.514*	.106	-.092	.076	.486*																		
MYB93	.342	-.100	.195	.378	-.084	.391	.109	.366	.449*	.353	.268	.132	.256	.424*	.285	.242	.552**																	
ETR2	.095	-.382	-.027	.481*	.016	.285	-.136	.130	.389	.358	-.048	.080	.122	.335	.400	.166	.147	.340																
PDH-E1α	.400	.252	.475*	.220	-.124	.430*	.129	.540**	.572**	.263	.382	.298	.238	.282	.387	.199	.617**	.418*	.098															
WOX4	-.211	.284	.248	-.615**	.412*	-.261	.207	-.263	-.352	-.274	-.348	-.025	.231	-.362	-.319	.273	.121	-.073	-.091	.140														
CASPL1B2	.481*	-.041	.333	.494*	-.141	.482*	.254	.695**	.572**	.376	.508*	.470*	-.156	.259	.368	.501*	.513*	.469*	-.005	.334	-.441*													
AP1	.029	.332	.411*	.171	.289	.686**	.727**	.499*	.194	.191	-.073	.807**	-.251	-.131	-.088	.284	.666**	.388	.071	.277	-.051	.575**												
BI1	.372	-.061	.574**	.239	.279	.423*	.267	.698**	.643**	.409*	.208	.416*	.142	-.050	.313	.369	.559**	.167	.081	.490*	.080	.444*	.356											
CSLD3	.729**	-.280	.357	.402	-.246	.157	-.333	.438*	.670**	.233	.609**	-.121	.546**	.478*	.618**	.308	.388	.363	.297	.594**	-.075	.398	-.071	.655**									
CYP86B1	.801**	-.180	.337	.558**	-.495*	.284	-.382	.534**	.712**	.332	.805**	-.048	.424*	.643**	.661**	.177	.388	.519**	.305	.522**	-.405*	.605**	.072	.466*	.840**									
DWF1	-.243	.354	-.027	-.302	.250	.073	.456*	-.002	-.306	.097	-.214	.254	-.243	-.310	-.481*	.171	.268	.039	-.146	-.306	.161	.120	.355	.009	-.307	-.213							
EXPA8	.523**	.106	.703**	.181	-.306	.305	-.131	.523**	.366	.001	.649**	.247	.067	.107	.388	.313	.429*	.202	.001	.258	-.279	.582**	.282	.456*	.399	.584**	-.058							
FLC	-.127	.297	.410*	-.111	.403	.225	.550**	.172	-.052	.009	-.228	.396	-.057	-.393	.075	.609**	.469*	.078	-.046	.339	.596**	.095	.379	.404	.009	-.253	.155	.054					
HMGR1	.516**	-.441*	-.042	.819**	-.362	.307	-.419*	.303	.643**	.371	.479*	-.084	.248	.604**	.712**	.078	.038	.214	.569**	.249	-.531**	.370	-.100	.286	.650**	.723**	-.381	.196	-.299				
PIN3	.128	.513*	.517**	-.172	.085	.231	.514*	.193	-.222	-.080	.058	.404	.022	-.468*	-.245	.541**	.488*	.069	-.297	-.001	.219	.401	.603**	.246	-.134	-.063	.655**	.357	.499*	-.363			
SOC1	-.040	.452*	.474*	.155	.346	.668**	.685**	.353	.031	.218	-.109	.658**	-.191	-.159	.032	.446*	.581**	.331	.111	.169	.090	.453*	.781**	.275	-.123	.002	.502*	.291	.528**	-.124	.675**		
STM	-.387	.355	.193	-.222	.591**	.373	.832**	.163	-.218	.150	-.532**	.647**	-.379	-.614**	-.478*	.327	.333	.053	-.090	-.212	.289	.170	.684**	.105	-.499*	-.483*	.651**	-.047	.521**	-.514*	.709**	.716**	
SUS4	.522**	-.147	.368	.246	.073	.252	.084	.386	.471*	.217	.244	.109	.404*	.225	.229	.343	.499*	.691**	.355	.338	.116	.330	.360	.494*	.556**	.497*	.043	.268	.140	.149	.139	.243	.047	