Identification of Key Genes Involved in Cassava Post-harvest Physiological Deterioration

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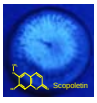
Abstract

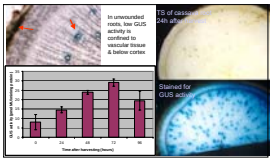
The rapid post-harvest physiological deterioration (PPD) of the starchy storage roots of cassava (*Manihot esculenta* Crantz) is a serious constraint to the development of this major crop. PPD is an active response involving changes in gene expression and the synthesis of novel proteins. Reactive oxygen species (ROS) and the enzymes and compounds that modulate them play central roles in PPD. Microarrays of ~ 11,000 cDNA clones to mRNA isolated from cassava roots over a deterioration time course were hybridised with cDNA populations in order to identify genes whose expression patterns were significantly altered during PPD. Analysis of these data has led to the identification and characterisation of key genes involved in a range of relevant functions, including: ROS modulation, programmed cell death, control of gene expression and signal transduction.

Post-harvest physiological deterioration

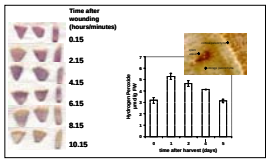


Transverse root section- vascular streaking and fluorescence

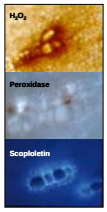
- PPD limits shelf life of cassava roots to 24 – 72h
- Staining of vascular tissue & fluorescence of the roots is due to the accumulation & oxidation of phenolic compounds. Scopoletin is particularly abundant [1]
- Reactive oxygen species (ROS) including H_2O_2 and O_2 are produced in response toPPD [2]
- Enzymes and compounds that modulate ROS play central role in PPD [2]
- Other changes include increases in respiration, ethylene biosynthesis, phenolic biosynthesis, enzyme activity (e.g. PAL, CAT, peroxidase, PPO, invertase), and changes in membrane lipids and sterols [3]
- Resembles wound responses in other plants, but lacks adequate wound repair.



Studies with PAL promoter constructs. PAL – a key entry point into phenylpropanoid metabolism is induced by wounding.



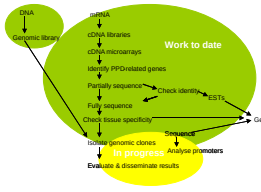
ROS accumulation in injured cassava roots. Superoxide anion (left) detected according to Vallelian-Bindschedler et al. [4] Hydrogen peroxide detected and quantified as in [2]



H_2O_2, peroxidase and scopoletin accumulate in the vascular tissue.

Experimental strategy

Flow chart of experimentation

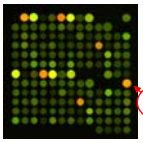


Hybridisation strategy for microarray experiments

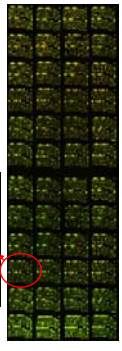
- "Early" and "late" cDNA libraries constructed from pooled mRNA 0, 6 and 12h or 24, 24 and 96h after harvest
- 11,136 cDNA clones (7680 from the early library and 3456 from the late library) hybridised for each time point
- 7 slides hybridised for each time point
- cDNA probes prepared over a PPD time-course

Probes	Slides							
0 vs 12h	Early Slide 1	Early Slide 2	Early Slide 3	Early Slide 4	Early Slide 5	Late Slide 1	Late Slide 2
0 vs 24h	Early Slide 1	Early Slide 2	Early Slide 3	Early Slide 4	Early Slide 5	Late Slide 1	Late Slide 2
0 vs 48h	Early Slide 1	Early Slide 2	Early Slide 3	Early Slide 4	Early Slide 5	Late Slide 1	Late Slide 2
0 vs 72h	Early Slide 1	Early Slide 2	Early Slide 3	Early Slide 4	Early Slide 5	Late Slide 1	Late Slide 2
0 vs 96h	Early Slide 1	Early Slide 2	Early Slide 3	Early Slide 4	Early Slide 5	Late Slide 1	Late Slide 2

Microarray hybridisations



Example array hybridisation – Early Slide 1 0 vs 12h



Aim: to identify, isolate & characterise genes, components of which could be used as tools to modulate PPD

- Identification of genes whose expression changes during PPD using cDNA microarrays
- PCR products of cDNA clones spotted by robot onto slide
- 4 technical & 3 biological replicates of target DNA + controls
- Early time point cDNA probe (e.g. time 0) labelled with Cy3
- Late time point cDNA probe (e.g. 24 hours) labelled with Cy5
- Selection based on normalised, background corrected ratios
- Clones selected on the basis of at least 2 fold up-regulation or 2.8 fold down-regulation for at least 2 time-point hybridisations (e.g. 0 vs 12h and 0 vs 24h)

Differentially expressed genes

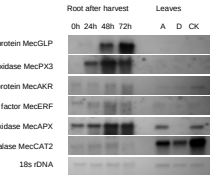
- Single pass sequencing to generate EST sequence data for selected clones
- To date 47 non redundant clones showing differential expression identified
- A subset of cDNA clones selected for full sequencing and Northern blotting

PPD up-regulated clones

PUTATIVE IDENTITY	BLAST HIT	E VALUE	POSSIBLE FUNCTION
Xiloxanine oxidase	NP_189953	2e-14	Cell wall remodelling
Ascorbate peroxidase	AAD14118	7e-80	ROS scavenging
M.esculenta allergenic related protein	AY150375	0.0	Unknown
PIP 1 type aquaporin	AAF60846.1	5e-85	Transport
Putative TIP type aquaporin	CAE53861	6e-26	Transport
PIP2 type aquaporin	AAD20607	e-106	Transport
PIP2 type aquaporin	AAC17529	1e-65	Transport
Elongation factor EF1a	O49169	1e-74	Protein translation machinery
Ethylene response factor	CAC54581	1e-069	Signalling
Auxin induced protein	P49691	e-115	Unknown
Oligosaccharide-binding protein LBP1	CAB75428	3e-61	Transcription
Vacuolar proton inorganic pyrophosphatase (H+ PPase)	AB097115	0.0	Transport
Cysteine protease	P22541	5e-86	Possible role in PCD
Papain	AAF04147	3e-37	Possible role in PCD
Class IV chitinase	NP_191019	5e-78	Possible role in PCD
Phospholipase	P80423	8e-25	Signalling
At2g23490	B84746	2e-22	Unknown
Arabidase	CAA09851	2e-94	Cell wall remodelling (also others)
ACC oxidase	AAP13098	1e-81	Signalling
Germin Like protein	AAM76228	6e-7	Cell wall remodelling
N-hydroxylating Cytochrome P450 CYP79D2	AAF27290	3e-59	Glucosinolate biosynthesis
Cytochrome P450 CYP79D1	AAF27289	e-121	Glucosinolate biosynthesis
Cytochrome P450 CYP71E	AAP57704.1	e-136	Unknown
Cytochrome P450	CAC24713.1	1e-60	Unknown
60S ribosomal protein S29	AAF68880	2e-28	Protein translation machinery
Rice ribosomal protein S3	AAR10054	1e-107	Protein translation machinery
UDP glucosyltransferase	NP_173553	2e-53	Cell wall remodelling (also others)
Catalase	AAD09274.1	0.0	ROS scavenging
Poly dRNA synthetase	Q81969	2e-77	Protein translation machinery
Immunoglobin	AAO49356.1	8e-55	Signalling
Gamma adaptin	NP_176215.1	2e-55	Transport
MN19 protein	BAB02146.1	2e-66	Unknown
Heat shock protein	AAR12194	6e-39	Signalling

PPD down-regulated clones

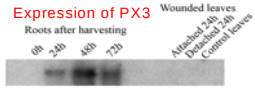
PUTATIVE IDENTITY	BLAST HIT	E VALUE	POSSIBLE FUNCTION
Putative senescence associated protein	BAB13410	4e-25	PCD
Translationally controlled tumour protein	Q05091	3e-77	PCD
PWWP domain protein	NP_191865	3e-09	Transcription factor
Auxin repressed protein	AAK25768	7e-33	Unknown
Auxin repressed protein	AAK25768	6e-20	Unknown
Unknown protein	AAG46152	6e-44	Unknown
RNA transglutaminase	AAL25174	3e-65	Protein translation machinery
ADP ribosylation factor	BAB85255	6e-96	GTP binding protein
60S ribosomal protein L7A	P35685	4e-91	Protein translation machinery
Expressed protein	NP_565499	2e-22	Unknown
Expressed protein	NP_564601	6e-39	Unknown
Hypothetical protein	NP_200518	3e-10	Unknown
Expressed protein	NP_568985	1e-8	Unknown



To validate and extend the microarray data, the expression of a subset of clones of interest was examined in roots and wounded leaves by Northern blotting. Root = Time after harvest (hours), Leaves = Wounded leaves 24h after injury, A = wounded attached, D = wounded detached, CK = control leaves.

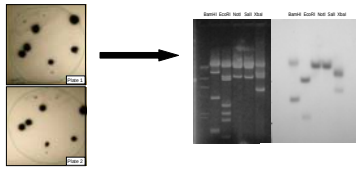
Tools – PX3 a candidate promoter

- Secretory peroxidase
- Expressed early during PPD
- Not expressed in:
  - Mature roots
  - Leaves
  - Wounded leaves
- Strong candidate to drive constructs in transgenic cassava



Isolation of selected genomic clones

- Genomic library constructed in λ DASH II
- Hybridisation screening with cognate cDNA clones
- λ DNA preparation, restriction digestion and Southern blotting
- Sub-cloning of selected restriction fragments



Subclone hybridising internal genomic DNA restriction fragments to plasmid vector and sequence

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