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Biotechnological approaches to modulate post harvest physiological deterioration of cassava storage roots

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ETH

Post harvest physiological deterioration in cassava

- Cassava deteriorates rapidly after harvest;
- Physiological /biochemical changes in cassava root;
- Deterioration due to microorganisms;
- Occurs as a result of wounding and infection during harvesting;
- Observed as vascular streaking in the surface of cassava roots;
- Dependent on environmental conditions and genetics.


0 hr


After 72hrs

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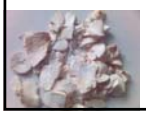
Control mechanisms

- Processing the roots immediately after harvest;
- Exclusion of oxygen e.g. coating with wax or storage in plastic bags etc.;
- Storage in low temperature, freezing of cut roots;

Laborious, costly, time-consuming and unsuitable for large amount!



ETH

The process of PPD

Mechanical damage (wounding) to roots

↓

Stress from wounding (wound response)

↓

Signaling molecules

↓

Production of defensive compounds

✕

Wound repair

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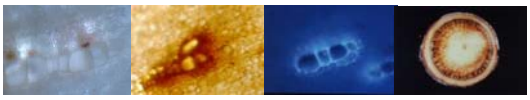
What happens during PPD? Two lines of thoughts already exist……

Biochemical level

Peroxidase + H_2O_2 + Scopoletin = " Vascular Streaking"



Localization data provide support for the hypothesis of Tanaka *et al*. and Wheatley *et al*. that PPD results from peroxidase-mediated oxidation of scopoletin.

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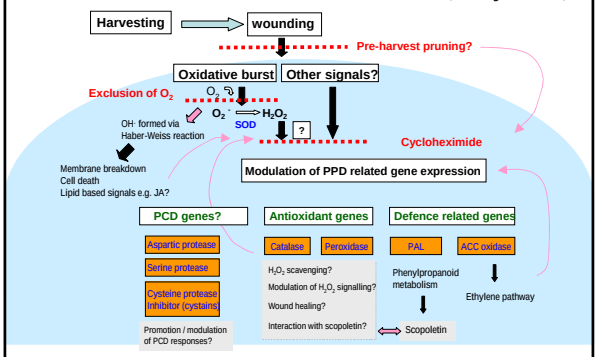
Molecular level

(Beeching et al., 2001)



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Research activities

- Metabolites/chemical signals produced during PPD, e.g. role of ethylene;
- Proteins differentially expressed during PPD course;
- Metabolism involved during PPD course;
- Transgenic approaches to modulate PPD via e.g. modification of ROS scavenging system;
- Cassava transformation and validation of transgene function in transgenic plants.

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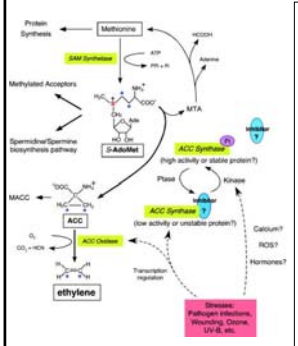
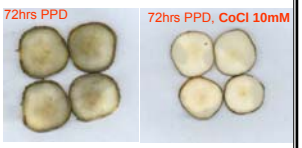
Role of ethylene or other signals in PPD development



Biosynthetic Pathway and Regulation of Ethylene

Wang, K. L.-C., et al. Plant Cell 2002;14:131-S151

- **Ethylene induction by signals such as wounding?**
- **Ethylene biosynthesis/function inhibitors?**
- **Ethylene production during PPD?**



Other inhibitors: Silver nitrate/Sodium Thiosulphate, Aminovinyl glycine HCl etc.

Large scale screening platform

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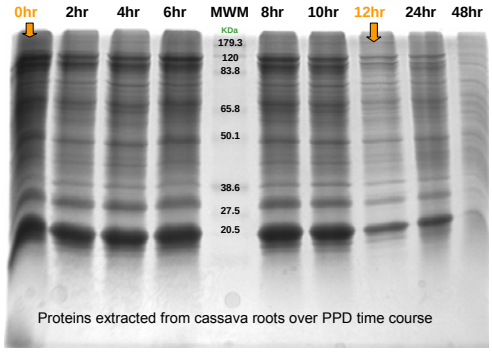
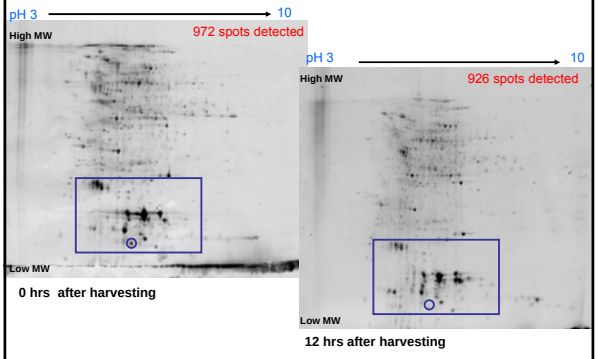
Protein profiling during PPD: SDS-PAGE



Proteins extracted from cassava roots over PPD time course

Protein profiling during PPD: 2D-PAGE

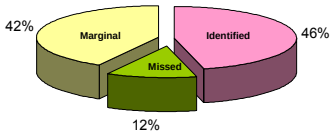


Differentially expressed proteins

- Proteins unique to 0hr PPD…… 25 spots
- Down-regulated after 12hr PPD…….. 49 spots
- Up-regulated after 12hr PPD…….. 55 spots
- Proteins unique to 12hr PPD…….. 60 spots

Protein identification

- Spots unique to 0 hr time point and those down regulated after 12 hrs (74 spots).



Parameters: Identified-score >60; Marginal-score 60-50; Missed-score <50.

Database used: Cassava dbESTs and Arabidopsis sequences from NCBI and Swissprot.

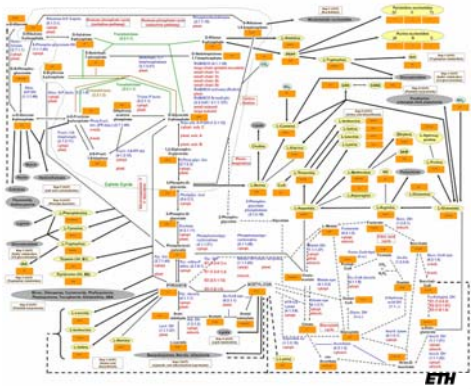
Spot ID/No	Protein Name/class	Accesssion No.	EST score	MW kDa	pI	Unique to 0 hr	Downreg. in 12hrs
4	Heat shock protein	gi56921781	135	17.7	8.18	#	
5	Actin 3	gi56924258	136	10.7	8.89	#	
8	Mannitol dehydrogenase	gi56925102	252	16.2	7.22	#	
9	heat shock protein	gi56921781	78	17.7	8.16	#	
10	Malic enzyme	gi56927724	69	14.8	8.07	#	
13	Allene oxide cyclase	gi56918749	143	23.6	9.44	#	
14	2-cys peroxiredoxin	gi56917946	145	13.7	5.34	#	
16	Heat shock protein	gi56927357	162	20.1	8.79	#	
20	Heat shock protein	gi67208020	125	27	9.21	#	
23	Heat shock protein	gi67215572	300	15.8	9.46	#	
24	Dehydrin ERD 14	gi75853367	167	30.6	5.71	#	
25	Heat shock protein	gi67215572	208	15.8	9.46	#	
36	Elongation factor 1-β	gi56922467	118	19.2	4.74		+
38	Elongation factor 1-β	gi56926827	141	23.3	4.81		+
39	L-ascorbate peroxidase	gi56920161	174	19.5	9.16		+
40	2-cys peroxiredoxin	gi75853361	283	30.4	5.65		+

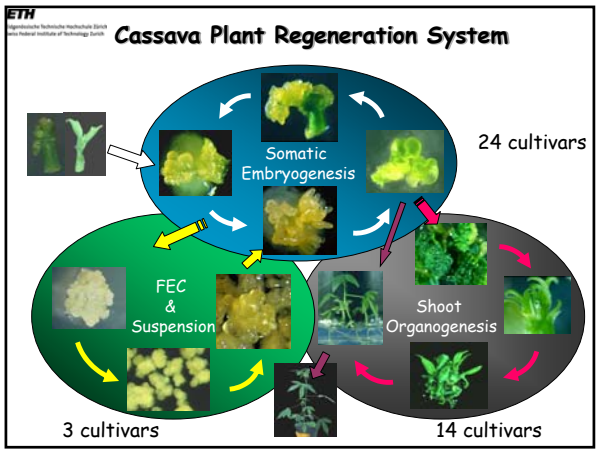
Spot No	Protein Name	Accesssion No.	EST score	MW kDa	pI	Unique to 0 hr	Downreg. in 12
47	Heat shock protein	gi56928361	88	20.2	9.79		+
48	Glyceradehyde 3-phosphate dehydrogenase	gi67212205	160	8.7	9.82		+
49	Pectinase	gi56920912	204	19.9	8.85		+
51	L-ascorbate peroxidase	gi56918960	204	17.3	5.76		+
52	α-1,4glucan phosphorylase	gi56923016	86	18.8	4.82		+
53	Lactoylglutathione	gi56919015	114	23.4	5.2		+
57	Actin isoform	gi56924258	146	10.7	8.89		+
58	fructose-bisphosphate aldolase	gi56919325	184	20	8.39		+
59	Tubulin α-chain	gi56921351	84	21.3	8.77		+
62	Heat shock protein	gi67215408	253	15.7	8.97		+
64	Phosphoprotein ECPP44	gi56925804	126	20.8	5.58		+
65	L-scorbate peoxidase	gi56918814	346	22.4	9.04		+
68	Actin	gi56924258	142	10.7	8.89		+
70	Glyceraldehyde 3-phosphate dehydrogenase	gi67216388	130	24.7	9.04		+
71	Dehydrin	gi56925373	139	15.5	5.92		+
72	Heat shock protein	gi67208738	87	13.3	9.85		+
74	Heat shock protein	gi67215572	201	15.8	9.46		+

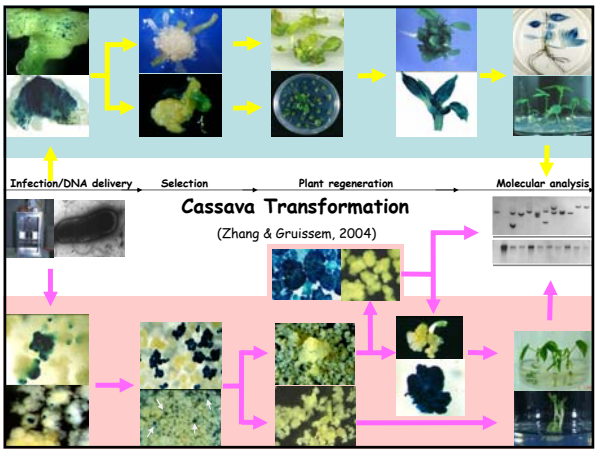
PPD associated proteins

Spot No.	Protein Name	Accession No.
13	Allene oxide cyclase	gi56918749
8	Mannitol dehydrogenase	gi56925102
10	NADP dependent malic enzyme	gi56927724
39	Ascorbate peroxidase	gi56920161
40	2-cys peroxiredoxine	gi75853361
53	Lactoyl glutathione	gi56919015
49	Pectinase	gi56920912
52	Alpha 1,4-glucan phosphorylase	gi56923016
66	β- amylase	gi67214858

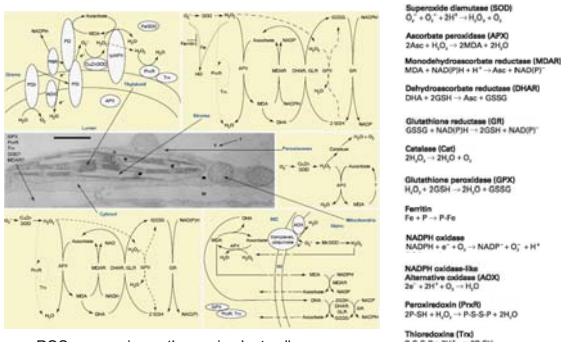
Next step: Integrating protein candidates to pathway







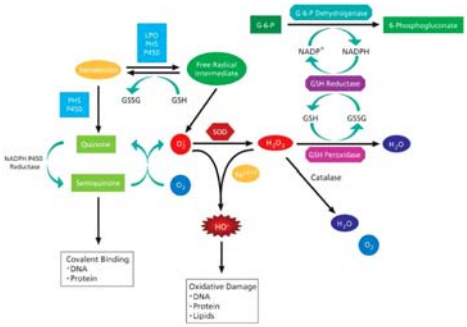
Main candidate ROS genes and their corresponding enzymatic activities



ROS scavenging pathways in plant cells

Mittler et al. (2004) Trends Plant Science

Functional genes to modulate PPD process by transgenic approach



Our targets: GSH peroxidase and GSH reductase

Expression: Tissue-specific (Patatin) or PPD-inducible (PX3)

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