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

Judith A. Owiti, Peng Zhang and Wilhelm Gruissem
(in collaboration with John Beeching)
Institute of Plant Sciences, ETH Zurich
Institute of Plant Physiology and Ecology, Chinese Academy of Sciences

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.

Post harvest physiological deterioration in cassava

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!

Control mechanisms

The process of PPD

Mechanical damage (wounding) to roots
Stress from wounding (wound response)
Signaling molecules
Production of defensive compounds
Wound repair
What happens during PPD? Two lines of thoughts already exist……

Biochemical level

Peroxidase + H₂O₂ + 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.

Molecular level

(Baichung et al., 2001)

Harvesting

Wounding

Pre-harvest pruning?

Oxidative burst

Other signals?

O₂ → H₂O₂ → OH⁻ formed via Haber-Weiss reaction

Membrane breakdown

Cell death

Lipid based signals e.g. JA?

Modulation of PPD related gene expression

PCD genes?

Antioxidant genes

Defence related gene

Modulation of H₂O₂ signalling?

Ethylene pathway

PAL, ACC oxidase

Catalase

Cysteine protease

Inhibitor (cystains)

Defence related genes

Antioxidant genes

Aspartic protease

Serine protease

Peroxidase

Phenylpropanoid metabolism

Pre-harvest pruning?

Exclusion of O₂

Cycloheximide

Wounding

Scopoletin

Wound healing?

Interaction with scopoletin?

H₂O₂ scavenging?

Modulation of PCD responses?

Role of ethylene or other signals in PPD development

- 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

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.
Protein profiling during PPD: SDS-PAGE

Proteins extracted from cassava roots over PPD time course

Protein profiling during PPD: 2D-PAGE

972 spots detected

Differentially expressed proteins

- Proteins unique to 0 hr PPD: 25 spots
- Down-regulated after 12 hr PPD: 49 spots
- Up-regulated after 12 hr PPD: 55 spots
- Proteins unique to 12 hr 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.
PPD associated proteins

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Name</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Allene oxide cyclase</td>
<td>gi5918749</td>
</tr>
<tr>
<td>8</td>
<td>Mannitol dehydrogenase</td>
<td>gi56925102</td>
</tr>
<tr>
<td>10</td>
<td>NADP-dependent malic enzyme</td>
<td>gi59927724</td>
</tr>
<tr>
<td>39</td>
<td>Ascorbate peroxidase</td>
<td>gi56920161</td>
</tr>
<tr>
<td>40</td>
<td>2-cys peroxiredoxine</td>
<td>gi75853361</td>
</tr>
<tr>
<td>53</td>
<td>Lactoyl glutathione</td>
<td>gi56919015</td>
</tr>
<tr>
<td>49</td>
<td>Podinase</td>
<td>gi56920912</td>
</tr>
<tr>
<td>52</td>
<td>Alpha 1,4-glucan phosphorylase</td>
<td>gi56923016</td>
</tr>
<tr>
<td>66</td>
<td>β- amylase</td>
<td>gi67214858</td>
</tr>
</tbody>
</table>

Next step: Integrating protein candidates to pathway
Cassava Plant Regeneration System

- Somatic Embryogenesis
- FEC & Suspension
- Shoot Organogenesis
- 24 cultivars
- 3 cultivars
- 14 cultivars

Cassava Transformation

(Zhang & Gruissem, 2004)

Main candidate ROS genes and their corresponding enzymatic activities

- Superoxide dismutase (SOD)
- Glutathione peroxidase (GPx)
- Ascorbate peroxidase (APX)
- Catalase (CAT)
- Glutathione reductase (GR)
- Dihydronicotinamide reductase (DHAR)
- Glucose-6-phosphate dehydrogenase (G6PDH)
- Glutathione S-transferase (GST)
- Peroxidase (POD)
- Thioredoxin (Trx)

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

Functional genes to modulate PPD process by transgenic approach

Our targets: GSH peroxidase and GSH reductase

ROS scavenging pathways in plant cells

Mittler et al. (2004) Trends Plant Science
Acknowledgements

In collaboration with:
BioCassava Plus Consortium

ETH Zurich:
Judith A. Owiti
Wilhelm Gruissem

University of Bath:
Simon Bull
John Beeching

Supported by:
Bill and Melinda Gates Foundation
Rockefeller Foundation
ETH Zurich
Chinese Academy of Sciences

Thank you!