Tropentag, September 14-16, 2010, Zurich

"World Food System —
A Contribution from Europe"

Analysis of Cassava (*Manihot esculenta* Crantz) Stress-inducible Promoters and Modulation of Antioxidant Expression in Cassava Storage Roots

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Abstract

Cassava (*Manihot esculenta* Crantz) is the 5th staple crop for over 800 million people in tropic and subtropics. The rising economic importance of cassava has led to increased efforts to stabilise yields by developing varieties with enhanced resistance to biotic and abiotic constraints. An important constraint that limits cassava's full potential is the short shelf life of harvested roots. The roots undergo rapid deterioration 24–48 hours after harvest, the so-called post harvest physiological deterioration (PPD) which renders the roots unpalatable and unmarketable. Use of genetic transformation, circumvents the limitations of traditional breeding for vegetatively propagated crops with high degree of heterozygosity such as cassava. Engineering cassava genotypes for reduced PPD, requires a reliable transgenic expression system in desired tissues at the desired level. Seven proteomic based PPD-inducible promoters were selected, isolated and fused to the uidA reporter gene in binary vectors for the production of transgenic cassava and Arabidopsis. Functional activities of each promoter segment by GUS staining and quantification of uidA gene proteins in various plant organs are being emphasised on a set of transgenic lines.

Additionally, strategies to reduce PPD in cassava roots have been tested using constitutive root-specific expression of candidate genes. PPD is caused when wounds are created as a result of harvesting cassava storage roots. The wounds initiate an oxidative "burst" with subsequent over-accumulation of reactive oxygen species (ROS). Inadequate wound healing and the "rare return to homeostasis from stress" cause prolonged and oxidative damage spreading from the site of injury. Up-regulation of defense-related genes occurs in cassava storage roots post-harvest; but this is insufficient in magnitude and timing to prevent deterioration. To examine the potential of ROS scavenging enzymes (*i.e.* glutathione peroxidase (GPX) and dehydroascorbate reductase (DHAR)) to reduce the oxidative stress, we have developed transgenic cassava plants constitutively over-expressing either GPX or DHAR under the control of the patatin promoter. Integration of the GPX and DHAR expression cassettes in the cassava genome has been confirmed by PCR and southern blots. The transgenic lines are currently being evaluated for their level of tolerance to PPD, salt and drought.

Keywords: Oxidative stress, post harvest physiological deterioration, promoters, tropical crop

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