Abstract

Storage roots of cassava (*Manihot esculenta* CRANTZ) undergo a rapid post-harvest physiological deterioration (PPD). The process is an endogenous root disorder which can occur within 24-48 hours after harvest. It is initially observed as blue/black vascular streaking that develops from wound sites and along xylem strands, followed by browning of the storage parenchyma. Several lines of evidence suggest that PPD is an enzymatically mediated oxidative process. Therefore, molecular and biochemical approaches were used to study the generation of reactive oxygen species (ROS) in the cassava storage root after harvest, and to examine the expression of the primary ROS-scavenging enzymes--catalase, peroxidase and superoxide dismutase.

A cassava catalase, MecCAT1, was isolated from a root PPD-related cDNA library constructed 48 hours after harvest. The clone represents a full-length transcript of 1792 bp. It encodes a predicted protein of 492 amino acids with highest similarity to *Ricinus communis* CAT2 protein (91% pairwise amino acid identity). Southern hybridisation indicated the presence of at least two, probably three catalase genes in the cassava genome. The MecCAT1 transcript is expressed predominantly in roots, with low-level expression in leaves, and contains a conserved carboxy-terminal peroxisomal targeting signal, suggesting the protein may be targeted to glyoxysomes within the root. The transcript was up-regulated in response to pre-harvest pruning and ethylene treatment. Catalase protein activity and MecCAT1 transcript expression during the post-harvest period were compared in a range of cultivars showing differing susceptibility to PPD. These data suggest that high levels of catalase activity may play a role in delaying the deterioration process.

An oligo-nucleotide primer based on the conserved active site of plant peroxidases was used to allow screening of the cDNA library by a PCR-based approach. Five positive clones were obtained and two, designated MecPX1 and MecPX2, were further characterized. Both clones were truncated at the 5' and 3' ends and were identical except that MecPX2 was the larger transcript, having additional sequence at both the 3' and 5' ends. The MecPX2 transcript was 726 bp in size, and encoded a predicted protein of 241 amino acids with greatest similarity to a cationic wound and ethylene induced peroxidase, VIRPRX, of *Vincula angularis* (61% pairwise amino acid identity). (**Abstract** shortened by UMI.)