Post-harvest deterioration in cassava: from understanding towards control

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The starchy storage roots of cassava (*Manihot esculenta* Crantz) deteriorate within 24 – 72 hours of harvest rendering them unpalatable and unmarketable. With growing urbanization and the entry of cassava into the cash economy this post-harvest physiological deterioration (PPD) has become a major constraint to the development of this important crop, affecting farmers, processors and consumers alike. The physiological changes that occur during PPD are due to the oxidation of phenolic compounds and involve the formation of reactive oxygen species (ROS), alterations in gene expression, protein synthesis and the accumulation and oxidation of a range of secondary metabolites. Biochemical and molecular data confirm that the production and reactions of ROS are central to PPD, and a model suggesting that PPD is a ROS-mediated programmed cell death (PCD) response has been proposed. This model implies that enhancing the anti-oxidant or anti-PCD status of the cassava root at or shortly after harvest may limit the reactions, damage and changes induced by ROS. We are testing this model in transgenic cassava in which we have separately introduced five anti-oxidant genes and three anti-PCD gene driven by a root-specific promoter. The resultant data will be invaluable both for confirming and refining the model, and for moving towards the identification of means by which to usefully modulate the PPD response of cassava roots, as the ultimate goal of this research is to benefit the sustainable livelihoods of resource-poor farmers.

Keywords: Cassava, post-harvest physiological deterioration, reactive oxygen species, programmed cell death, transgenic.

Introduction

The Green Revolution of the 1960s and 1970s achieved spectacular results in increasing the production of the world's major cereal crops, thereby saving millions from famine and keeping pace with burgeoning population increases; however, the recent recognition of a global food crisis, in addition to the energy and climatic crises, suggest a pressing need to focus again on global crop production in addition to addressing other contributory factors (Schmitz & Kavallari, 2009). Moreover, there is increasing evidence that the gains achievable through classical breeding may have been reached for the major crops and that both new technologies and ignored crops have to become included in improvement programmes (Borlaug, 2000). In addition, Africa, a continent that was bypassed by the Green Revolution and much of whose population suffer from poverty and inadequate diet, desperately needs major research effort focused on improving its important crops, including cassava; crops which may prove more amenable to biotechnological solutions than conventional breeding (Thomson, 2008). It is particularly in Africa that cassava, both as a crop and a resource, has a major contribution to play.

Cassava (*Manihot esculenta* Crantz) is cultivated throughout the humid tropics for its starch-rich storage roots, which yield more energy per hectare than other major crops (Montagnac *et al.*, 2009). It is the world's sixth most important crop in terms of production (Mann, 1997), serves as the staple food for over 500 million, and is particularly important for the sustainable livelihoods of resource-poor farmers in sub-Saharan Africa (Cock, 1985). Increasingly, cassava is also becoming a cash-crop and is being used as input for higher value commodities such as starch or biofuels. Despite its many advantages as a crop, cassava suffers from several constraints that limit its production and its value as a food. In Africa cassava is predominantly cultivated by poor farmers, often on impoverished soils where other crops do not grow well; this severely limits productivity to well below the maximum recorded yield of 90 tonne/ha (El-Sharkawy, 2003). Average productivity for Africa was 8.8 tonne/ha for 2007 - as a point of reference, India's productivity was 32.9 tonne/ha for the same year (FAOSTAT, consulted on 15/07/2009). Therefore, while the distribution of improved germplasm could contribute to improving productivity, it is largely available soil quality and agronomic practices that severely limit cassava productivity in Africa. In addition, cassava suffers from biotic and abiotic constraints. These include: pathogens, particularly viral and bacterial; the possession of cyanogenic glycosides; a short shelf-life of the harvested roots of 24 – 72 hours; and poor nutritional content in terms of quantity and quality of protein, and low abundance of important micronutrients. Due to breeding difficulties with cassava, as a result of high heterozygosity, poor

flowering ability and clonal propagation via stem cuttings, many of these constraints may be more amenable to resolution via biotechnological approaches. Moreover, the production of some novel or high value products from cassava roots could be also facilitated through genetic manipulation.

A major constraint to the development of cassava as a crop is the short shelf-life of its roots, which deteriorate within 24 – 72 hours of harvest (Figure 1; Beeching *et al.*, 1998). This post-harvest physiological deterioration (PPD) is becoming increasingly important due to urbanisation in producing countries and the resultant increase in distances and time between farm and market or processor. A recent *ex ante* impact analysis of the economic benefits to African countries of developing cassava cultivars with delayed PPD indicates that this would have a major impact (Rudi, 2008). The physiological changes that make cassava storage roots unpalatable and unmarketable during PPD are due to the oxidation of phenolic compounds and involve the formation of reactive oxygen species (ROS), alterations in gene expression, protein synthesis and the accumulation and oxidation of a range of secondary metabolites (Beeching *et al.*, 1998; Buschmann *et al.*, 2000a; Buschmann *et al.*, 2000b; Han *et al.*, 2001; Reilly *et al.*, 2001). Biochemical and molecular data confirm that the production and reactions of ROS are central to PPD, and a model of PPD as a ROS-mediated programmed cell death (PCD) response has been proposed (Reilly *et al.*, 2003; Reilly *et al.*, 2007). This model predicts that enhancing the anti-oxidant or anti-PCD status of the cassava root at or shortly after harvest may limit the reactions, damage and changes induced by ROS. In this paper we describe experiments to test this hypothesis in transgenic cassava plants. The resultant data from these plants will be invaluable both for confirming and refining the model, and for moving towards the identification of means by which to usefully modulate the PPD response of cassava roots for the benefit of farmers and consumers.

Figure 1. PPD in cassava. A, section through cassava root at harvesting. B, cassava root at 48 hours after harvesting showing symptoms of PPD.

Experimental

Reactive oxygen species (ROS) play multiple roles in plants: as signalling molecules, in development, and defence, as well as being toxic and damaging. Their role depends largely upon species, concentration and localisation. To protect themselves against the damaging effects of ROS plants produce anti-oxidant enzymes and compounds (Figure 2).

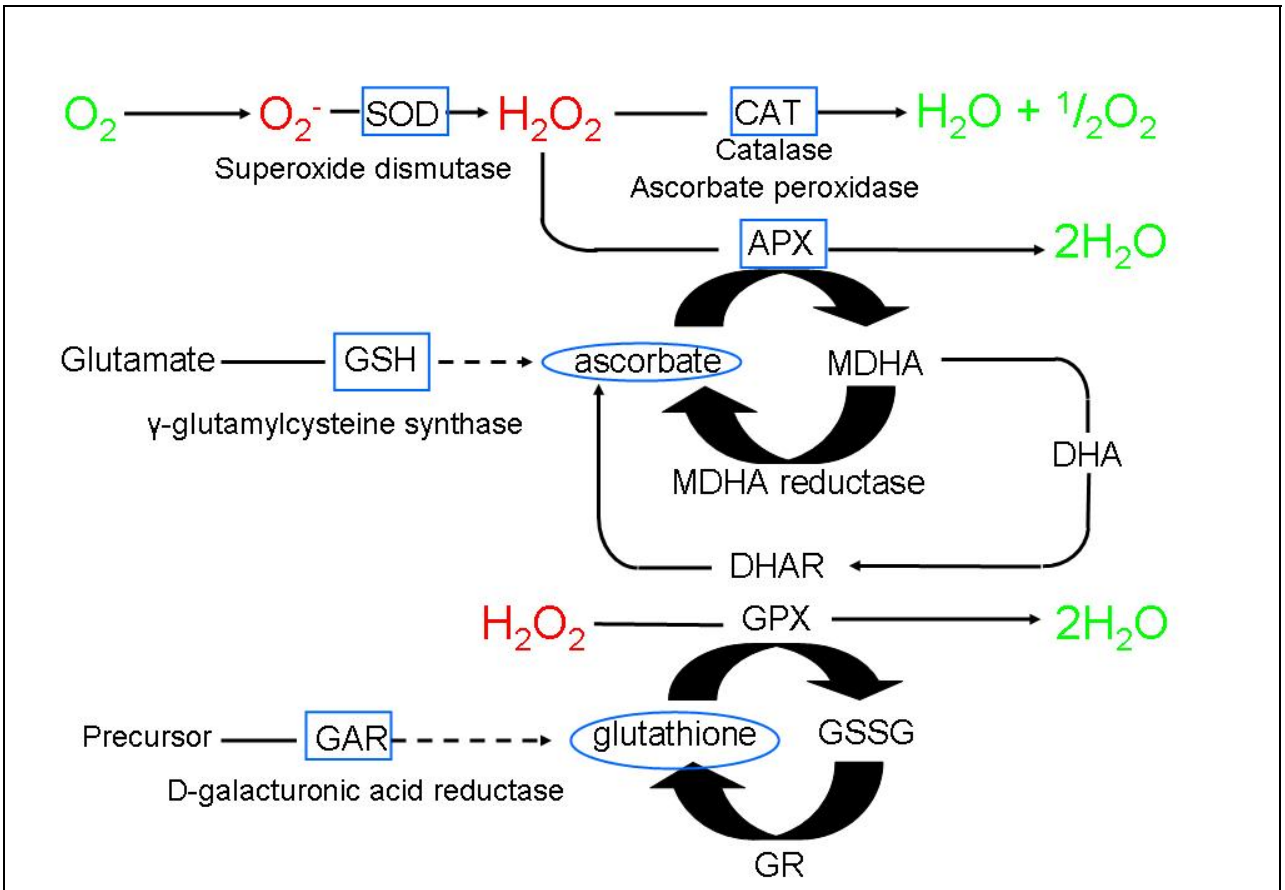

Figure 2. Major anti-oxidant defences in plants. Indicated in blue boxes are the enzymes used in this study

We have isolated full length cDNA clones for Cu/Zn superoxide dismutase, catalase and ascorbate peroxidise from cassava (Gómez-Vásquez *et al.*, 2004; Reilly *et al.*, 2003; Reilly *et al.*, 2007) and cDNAs for γ-glutamylcysteine synthase and D-galacturonic acid reductase from *Arabidopis* and strawberry, respectively, were obtained (http://www.brc.riken.go.jp; Agius *et al.*, 2003). These cDNAs were cloned using Gateway® cloning (http://www.invitrogen.com) into pCambia1305.1 (www.cambia.org) that we had modified as follows: the CaMV35S promoter and the GUSPlus reporter gene had been removed and replaced by the *StPAT* patatin promoter, which has been shown to be root-specific in cassava (Ihemere *et al.*, 2006); and the vector had been modified to permit Gateway cloning after the *StPAT* promoter (Figure 3).

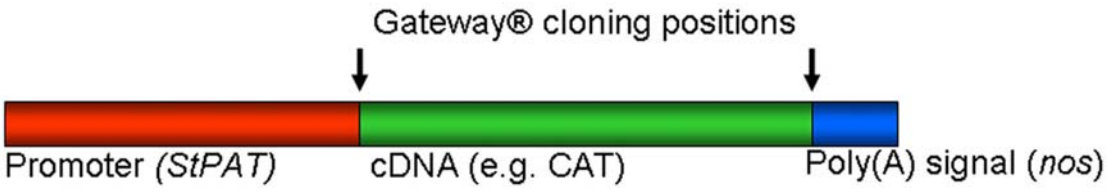

Figure 3. Typical gene construct inserted into pCambia1305.1

A cDNA microarray analysis of PPD had, in addition to ROS modulation, highlighted the expression of pro- and anti-programmed cell death (PCD) genes during the PPD response (Reilly *et al.*, 2007). PCD shares many features with apoptosis, which is much better characterised in animal systems (Delgado *et al.*, 2007). The expression of anti-apoptosis genes in plants has been shown to shown to inhibit oxidative stress induced PCD through

promoting the scavenging of free radicals (Chen *et al.*, 2003; Li & Dickman, 2004). Therefore, we have made gene constructs similar to the anti-ROS constructs above, in which three different anti-apoptotic genes were driven by the StPAT promoter and then transformed them into cassava. These PCD experiments are solely to test the effect of these genes on the plant and root in the laboratory, if the results suggest that this approach might offer a means to control PPD, we would employ plant functional homologues for further work.

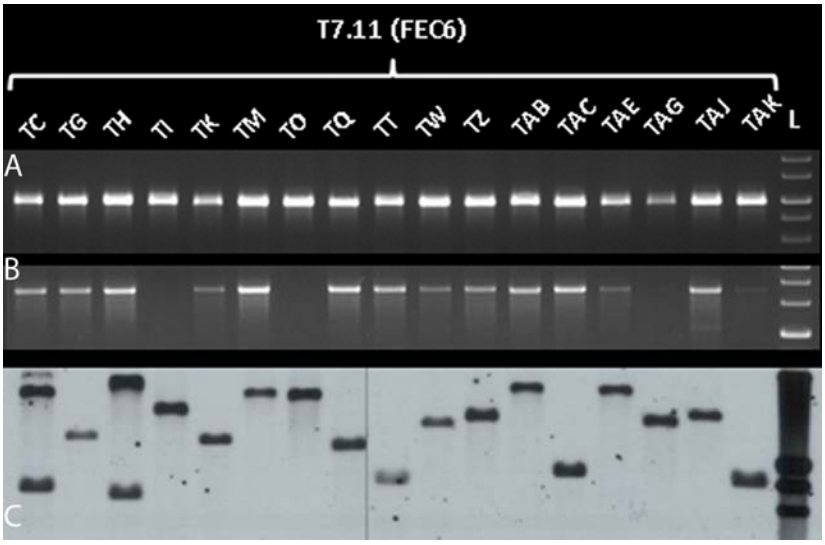

Figure 4. Example of confirmation of transgenesis. A, PCR with hygromycin primers; B, PCR with -glutamylcysteine synthase primers; Southern blot probed with hygromycin gene.

At least 30 transgenic cassava lines for each construct, five anti-ROS, three anti-PCD, plus the necessary controls, have been produced. These have been tested by PCR and Southern blotting to confirm the presence and copy-number of the transgenes (Figure 4). The transgenic plants are currently being grown to maturity so as to enable, phenotypic biochemical and molecular analyses of the developing plants and their storage roots.

Discussion

Cassava PPD is a signification limitation to the achievement of the full potential of this important crop. While it is a complex response the current understanding of the problem suggests experiments in which the expression of likely candidate genes involve in both anti-oxidant defence and the regulation of PCD pathways can be modulated using an organ-specific promoter. Sufficient lines of cassava plants containing eight candidate genes have been produced and their transgenic nature confirmed. The full analysis of these plants will not only increase our understanding of PPD by should also point to specific genes with the potential to control the deterioration response.

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