

Molecular Approaches to Elucidate the Importance of Cyanogenic Glucosides in Cassava

Dimuth Siritunga, PhD
University of Puerto Rico Mayaguez
Mayaguez, PR 00681-9012
siritunga@uprm.edu

Cassava (*Manihot esculenta*) is a staple food for 500 million people world-wide. It is the most agronomically important of the cyanogenic food crops. Cyanogenic compounds function in plants as protective agents against herbivores as well as the translocable form of reduced nitrogen. Cyanogenic glycosides can release cyanide after enzymatic breakdown. Certain health disorders such as tropical ataxic neuropathy and konzo, a paralytic disorder, have been associated to poorly processed cassava diets. Thus, cassava foods must be processed extensively to remove cyanogens prior to consumption. Transgenic strategies utilized to silence CYP79D1 and CYP79D2, key enzymes in the cyanogen synthesis pathway, demonstrated the importance of cyanogens as a mobile source of reduced nitrogen to the cassava plants. These mobile cyanogens are converted to invaluable amino acids through a pathway whose primary enzyme is β -cyanoalanine synthase (β -CAS). This enzyme belongs to the β -substituted alanine synthases (Bsas) family, and catalyzes the incorporation of cyanide to the asparagine and aspartate biosynthesis pathway. β -CAS is located in the mitochondria, where it prevents the lethal action of cyanide on the electron transport chain. Successful isolation of β -CAS gene showed that the cassava β -CAS has high homology to β -CAS from rubber tree (93%), Arabidopsis (79%) and potato (79%). Expression analysis of β -CAS using real-time PCR has revealed equal levels of expression in the root and leaves of *in vitro* grown 3 month old cassava. This is contrary to the expression pattern of other genes in the cyanogenic glucoside pathway where the leaves have a much higher expression pattern in comparison to roots. The β -CAS pathway provides an ideal way to develop a protein-rich transgenic cassava plant that has low cyanide content. In a separate project, transgenic strategies have been used to over-express hydroxynitrile lyase gene, which volatilizes cyanogens faster upon disruption of the cells resulting in a cassava food product with drastically reduced toxicity. These plants are currently in confined field trials in Puerto Rico.