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**“Metabolic Engineering of Cyanogenesis in the Tropical Root
Crop, Cassava”**

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ABSTRACT

Cassava is the major food-crop for subsistence farmers in many regions of the tropics. Cassava, however, contains potentially toxic levels of the cyanogenic glucoside, linamarin. The cyanogen content of cassava foods can be reduced to safe levels by processing, however, short-cut processing techniques can yield toxic food products. Our objectives have been to eliminate cyanogens from cassava so as to eliminate the need for food processing. To achieve this goal two independent strategies were assessed; 1) inhibition of linamarin synthesis, and 2) acceleration of cyanide volatilization during processing. We will describe the phenotypes of transgenic acyanogenic cassava plants in which the expression of the cytochrome P450 genes (CYP79D1 and CYP79D2), that catalyze the first-dedicated step in linamarin synthesis, has been inhibited in leaves and roots. Significantly, when linamarin steady-state levels in leaves are reduced by 60-100% the linamarin levels in roots were reduced by more than 95% relative to wild-type levels. In turn, inhibition of CYP79D1 and CYP79D2 expression in roots had no effect on root linamarin levels. These results suggest that linamarin is transported from leaves to roots and that a threshold level of leaf linamarin production is required for linamarin accumulation in roots. Evidence will be presented that linamarin functions in nitrogen transport to roots and plays an important role in post-harvest physiological deterioration. A second strategy to detoxify cassava foods is to accelerate cyanogenesis and cyanide volatilization. To accelerate cyanide volatilization during root processing we have over-

expressed the gene encoding hydroxynitrile lyase (HNL) in roots. HNL catalyzes the conversion of acetone cyanohydrin (de-glycosylated linamarin) to cyanide. Significantly, wild type plants do not express HNL in roots. We observed a direct correspondence between the level of HNL expression in roots and the reduction in acetone cyanohydrin levels in processed roots. We propose that cassava plants expressing HNL in roots will provide a safer food product for consumers.