Cyanogenic Glucosides Participation in Nitrogen Transport in Cassava: Implication for the Generation of a Cyanogen-free Cassava Plant

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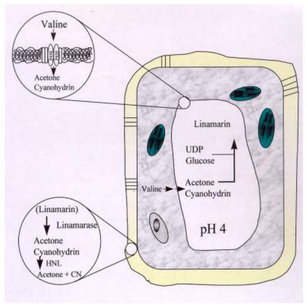
INTRODUCTION

Cyanogenic glucosides are present in many crop plants including cassava, sorghum, barley, cherry, apricot, plum, peach, mango and lima beans. These compounds yield cyanide following de-glycosylation and are thought to protect plants from herbivores. Among the cyanogenic crops, cassava is the most agronomically important. Cassava roots are consumed as a major source of carbohydrates by over 600 million people. The leaves, roots and stems of cassava, however, contain potentially toxic levels of cyanogenic glycosides (linamarin (95%) and lotaustralin (5%)).

Chronic, low-level cyanide exposure has been associated with the development of goiter and tropical ataxic neuropathy, whereas acute cyanogen poisoning, particularly during famines, has been associated with outbreaks of Konzo, a paralytic disorder, and in some cases death (Osuntokun, 1981; Bourdoux et al., 1978; Howlett et al., 1990).

While both leaves and roots have the ability to synthesize linamarin *in vitro*, it has been reported that linamarin can be transported from leaves to roots as well (Selmar, 1994; Du et al., 1995). The first dedicated step in linamarin synthesis is the conversion of valine to its oxime catalyzed by two highly similar cytochrome P450s, CYP79D1 and CYP79D2. (Andersson et al., 2000). **We present here the selective inhibition of linamarin synthesis in cassava by expressing the *CYP79D1* and *CYP79D2* genes in an antisense orientation in leaves or roots using either an *Arabidopsis thaliana* leaf-specific Cab1 promoter or the *Solanum tuberosum* tuber-specific class I patatin promoter, respectively.** Our objective in generating transgenic plants in which linamarin synthesis was selectively inhibited in roots or leaves was to determine the relative contribution of leaf- and root-synthesized linamarin to the total root linamarin content.

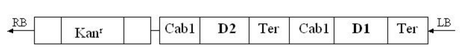
Figure 1: A model of linamarin synthesis and cyanide production following rupture of a cassava leaves



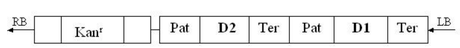
MATERIAL AND METHODS

❒ Binary vector pBI121 was modified to the following:

T-DNA region of the Cab1 construct having CYP79D1 and CYP79D2 in anti-sense orientation under the control of the leaf-specific Cab1 promoter:



T-DNA region of the Patatin construct having CYP79D1 and CYP79D2 in anti-sense orientation under the control of the tuber-specific Patatin promoter:



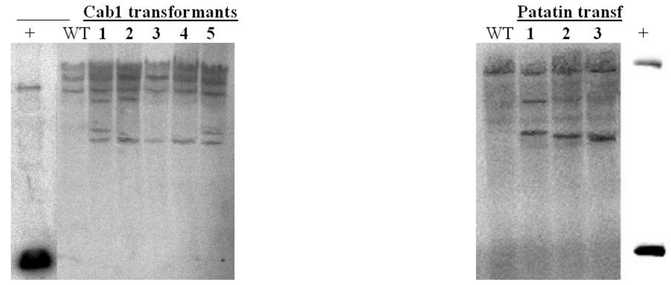
❒ Cassava transformation, tissue culture, DNA/RNA analysis was performed as described in Siritunga and Sayre (2003).

❒ Linamarin quantification was performed by a Thermo-Finnigan Trace 2000 instrument, on a 30 meter long, 0.25 micron film thickness Restek XTI-5 capillary gas chromatography column as described in Siritunga and Sayre (2003). Each of the runs was normalized for the internal standard (phenyl β-D-glucoside) and linamarin is expressed as a % of the quantity present in wild-type untransformed plants.

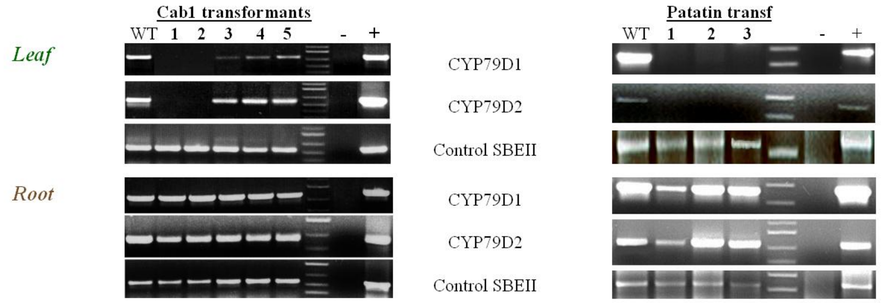
❒ Growth analysis on ammonium-free media was conducted by growing nodal cuttings of wild-type and transformants in normal Murashige Skoog media (Murashige and Skoog, 1962) in the presence or absence of ammonia. MS media without ammonium nitrate (Caisson Laboratories, Rexburg, ID) was supplemented with 38 mM potassium nitrate.

RESULTS

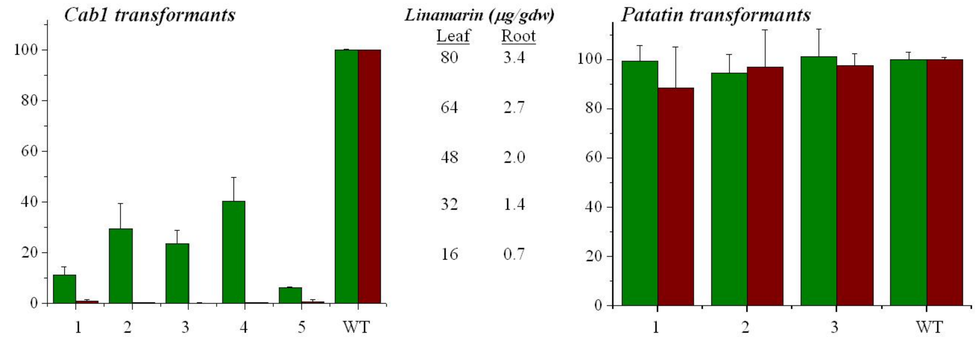
Southern blot analysis shows the integration of the T-DNA (CYP79 genes) into the genome of each transformant.



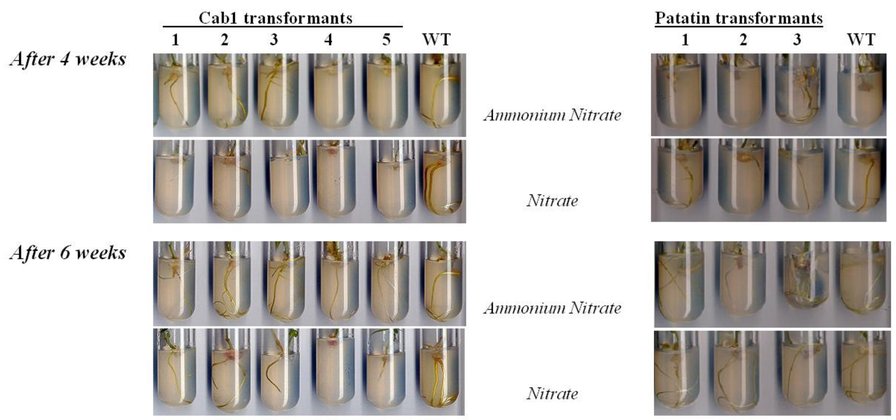
RT-PCR demonstrates the complete loss or reduction of CYP79D1 and CYP79D2 transcripts



Average leaf (green) and root (brown) linamarin content of wild-type and Cab1 *CYP79D1/CYP79D2* antisense transformed plants or patatin *CYP79D1/CYP79D2* antisense transformed plants



In vitro root growth in the presence of ammonium plus nitrate or nitrate alone of Cab1and Patatin transformants



CONCLUSSIONS

❒ **Transgenic cassava plants were generated with organ-specific inhibition of CYP79D1 and CYP79D2 by utilizing either an *Arabidopsis thaliana* leaf-specific Cab1 promoter or the *Solanum tuberosum* tuber-specific class I patatin promoter.**

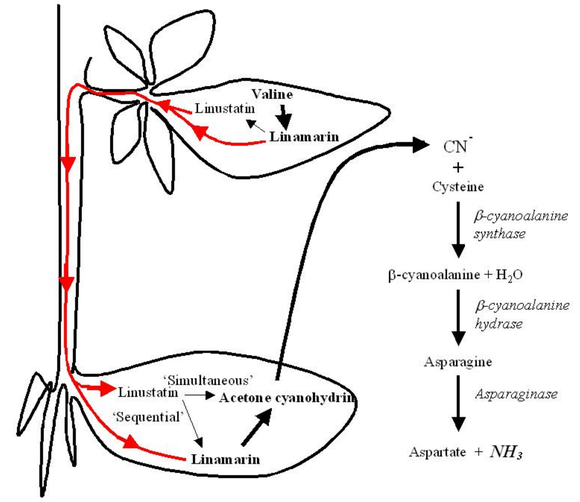
❒ **Root linamarin content was unaltered in transformants in which CYP79D1/D2 transcripts were reduced to non-detectable levels in roots.**

❒ **In contrast, root linamarin content of transformants having substantially reduced CYP79D1/D2 transcripts in leaves was less than 1% of wild-type levels.**

❒ **These results suggest that linamarin made in the leaves is transported to the roots (Figure 2).**

❒ **Analysis on the growth of transgenic cassava in media lacking ammonia suggests that cyanogenic glucosides may function as an important mobile nitrogen source in young plants, in addition to their proven ability to deter herbivory (Figure 2).**

Figure 2. Model for the movement of cyanogenic glucoside as a source of reduced nitrogen in cassava.



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