Contribution of Cassava Biotechnology on Sustainable Development in Tropical Agriculture

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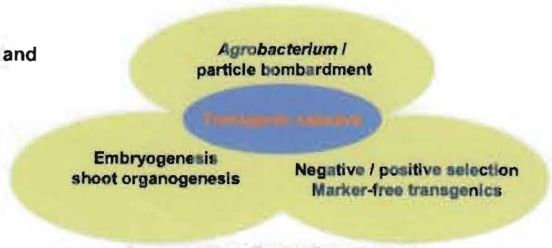
Cassava (*Manihot esculenta* Crantz) is an important root crop in the tropics. Traditional cassava breeding is extremely difficult and time consuming. Biotechnology provides a valuable tool for genetic improvement of the crop to complement its traditional breeding, such as increased disease resistance, improved root quality, etc. Over the last decade, Cassava Biotechnology Group in ETH-Zürich has been committed to develop improved cassava cultivars based on state-of-the-art strategies and transgenic technologies. Here we present several contributions on cassava genetic improvement.

Development of cultivar-independent and marker-free gene transfer

Bottlenecks:

- Incompatible plant regeneration and genetic transformation
- Strong cultivar-dependent
- Low transformation frequencies
- Time-consuming



Cassava transformation system

Our objectives:

- Reliable cassava transformation protocols
- Adaptable to other elite varieties or farm-preferred cultivars
- Reduced plant transformation and regeneration time
- Transferable to other laboratories in Africa
- Generation of marker-free transgenic cassava

Quality and yield improvement

Problems: low protein content in cassava storage roots and short life of cassava leaves

1. Improvement of protein content in cassava storage roots

1) High expression of storage protein in cassava storage roots

Current problem: Low expression of a storage protein gene ASP1 has been observed in transgenic cassava plants using the constitutive promoter CaMV 35S.

a) Leaves b) Primary roots

New Strategy: Expressing the storage protein gene under the control of cassava root specific promoters

2) Accumulation of protein in storage organelles amyloplast and vacuole

Study of the different signal sequences for protein targeting in cassava protoplasts

CaMV 35S::EGFP *CaMV 35S::CPE-EGFP* *CaMV 35S::TP-EGFP*

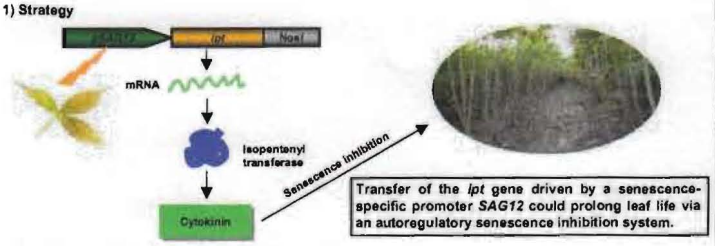
3) Enrichment of amino acids in cassava storage roots

Long tern strategy: Modification of amino acid metabolic pathways

2. Extension of cassava leaf life

Prolonging leaf life of cassava would allow higher root yields, improved root quality and more frequent leaf harvesting.

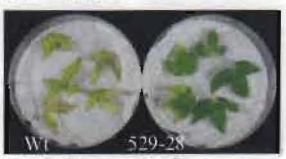
1) Strategy



Transfer of the *Ipt* gene driven by a senescence-specific promoter *SAG12* could prolong leaf life via an autoregulatory senescence inhibition system.

2) IPT expression in mature leaves from transgenic cassava lines

3) Prolonged leaf life in transgenic cassava line 529-28



Detached leaves on wet filter paper after 2 weeks in the dark.

Plants after one-month drought treatment

Cassava geminivirus disease resistance

African Cassava Mosaic Virus (ACMV)

- most important vector-borne disease in cassava
- up to 36% of cassava production losses
- severe epidemic currently in Africa

Strategy: Development of RNA-based ACMV resistance in transgenic cassava

- Expressing viral antisense RNAs of Rep, TrAP, and REn as 3' untranslated regions of a selectable marker gene.
- Expressing dsRNAs to target the promoter regions and coding sequences of ACMV proteins.

Reduced viral DNA replication in transgenic cassava expressing antisense genes

Resistance to ACMV-NOg infection

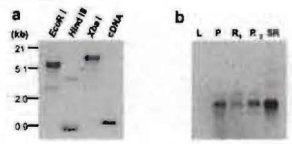
Root specific promoters of cassava

Cassava storage roots develop from primary roots by a peculiar secondary growth and accumulate starch up to 85% of their dry weight. To improve cassava root traits via genetic engineering, the availability of tissue specific promoters, especially storage root specific promoters, is a prerequisite.

Strategy: identifying tissue specific genes and promoters from a cassava storage root cDNA library by differential screening

Example: 1. Root specific cDNAs c54

cDNA c54:
Size, 902 bp;
Homology, Pt2L4;
Gene no., two or three



Southern analyses of genomic DNA (a) and expression patterns (b) in leaf (L), petiole (P), primary roots in diameter of 0-1 mm (R_1) and 1-2 mm (R_2), and storage root (SR) using c15 or c54 probe.

2. GUS expression driven by c54 promoter (p54/1.0)

Notes: a, leaf; b, petiole; c, stem; d, primary root; e, storage root.