RNAi-mediated resistance to cassava mosaic virus in cassava

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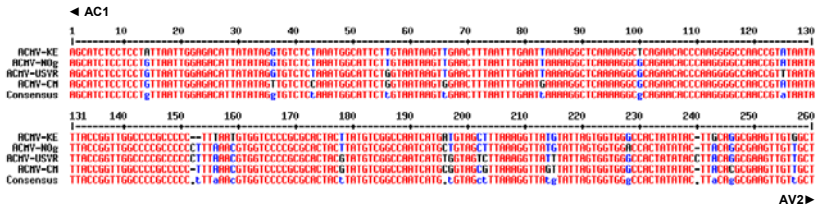
Introduction

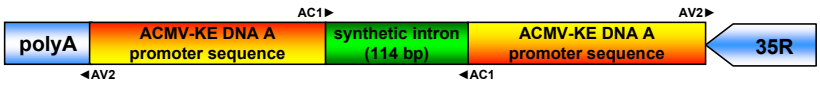
RNA interference is a promising tool to reduce virus replication and virus spreading in plants (1, 2). Small RNAs derived from the degradation of dsRNA are involved in transcriptional and post-transcriptional gene silencing (TGS and PTGS). In TGS, short RNAs trigger methylation of almost all cytosine residues within a region of RNA-DNA sequence identity (3). In PTGS, small interfering RNAs are integrated into a RNA-induced silencing complex (RISC) and the antisense siRNAs in the complex pair with cognate mRNAs causing their degradation. Our project aims at using both mechanisms to reduce viral protein expression of African cassava mosaic virus and hence producing transgenic cassava plants with increased resistance to ACMV.

Targeting viral promoter region of ACMV

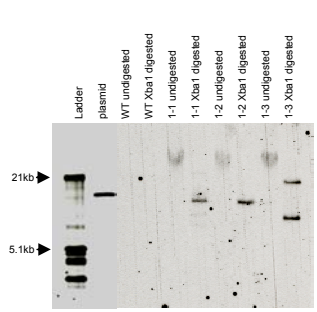
➢ **DNA A promoter region shows high homology among different ACMV strains**



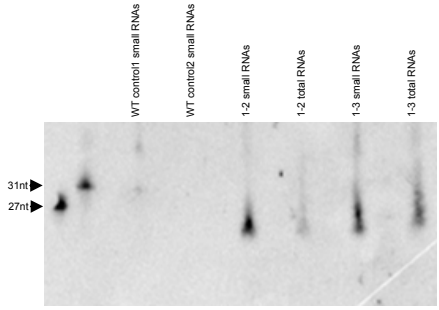
➢ **Expression of hairpin dsRNA complement to promoter region in cassava**



➢ **Insertion number of transgene and detection of siRNA in transgenic plants**

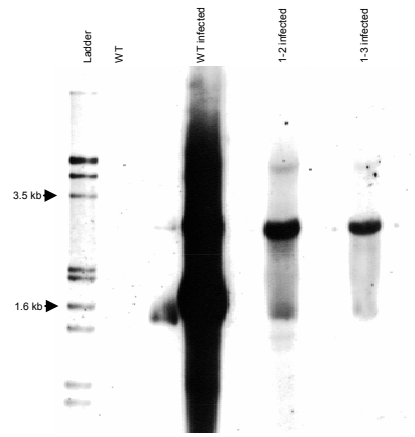


20 µg of DNA digested with 200U of Xba1 was loaded per line.
SB was hybridized with ACMV-KE promoter sequence probe.



40 µg of total RNA and 20 µg of small RNA were loaded on polyacrylamide gel. Transfer to HyBond membrane and hybridization with fragmented ACMV-KE promoter ^{32}P riboprobe.

➢ **Reduced replication of ACMV-NOg in transgenic cassava**



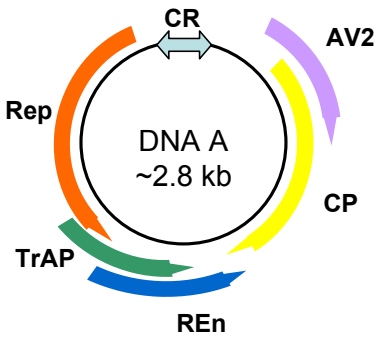
Transient viral replication assays were performed according to the leaf disks protocol (4).

Bombardment of 12 replicates per line (1 shot /replicate) with ACMV-NOg virus (200ng DNA A + 200ng DNA B).

5 µg of digested total DNA (50U Sph1 + 100U Dpn1) loaded per line. SB membrane was hybridized with ACMV-NOg AV1 probe.

Targeting viral coding sequences of ACMV

➢ **DNA A encoded proteins**



➢ **Targeting the partial and entire coding sequences of the viral proteins with hairpin dsRNA to inhibit viral protein expression by TGS and PTGS**

- Elaborating construct for the expression of hairpin dsRNA using pHellsgate plasmid (1)
- Two types of constructs will be made per targeted sequence
  - The first 125 bp in the 5' region of Rep, TrAP, REn and CP
  - The complete sequence of Rep (1076bp), TrAP(408bp), REn (405bp) and CP (777bp)

➢ **Producing transgenic cassava plants to determine the most effective RNAi constructs**

Perspectives

➢ **Ongoing experiments on transgenic plants using TGS to assess their resistance to different ACMV strains**

➢ **Assessment of the best strategy (TGS and PTGS) to reduce virus replication and systemic spreading**

➢ **Production of transgenic plants with a transgene under a tissue-specific cassava promoter (e.g. vascular-specific promoter)**

References

(1) Tenllado et *al*., BMC Biotechnol. 2003 Mar 20; 3(1):3.
(2) Pandolfini et *al*., BMC Biotechnol. 2003 Jun 25; 3(1):7.
(3) Aufsatz et *al*., Proc Natl Acad Sci U S A. 2002 Dec 10; 99 Suppl 4:16499-506.
(4) Zhang & Gruissem, Virus Res. 2003 Mar; 92(1):47-54.
(5) Helliwell & Waterhouse, Methods. 2003 Aug; 30(4):289-95.