Increased African cassava mosaic virus resistance in transgenic cassava plants expressing different viral antisense RNAs

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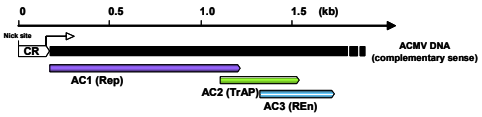
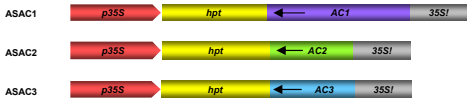
Introduction and Strategies

African cassava mosaic virus (ACMV) is a vital component leading to the epidemics of cassava mosaic disease, the most important and devastating disease of cassava in Africa[1]. We developed cassava plants with increased ACMV resistance using improved antisense-RNA technology by targeting the viral mRNAs of Rep (AC1), TrAP (AC2) and REn (AC3), three nonstructural proteins encoded on the complementary-sense strand of ACMV DNA-A with key roles in viral replication and transcriptional regulation in the life cycle of the virus[2]. Viral antisense-RNAs was expressed as 3'-untranslated regions of a selectable hygromycin phosphotransferase gene, regulated by the CaMV 35S promoter. Expression of these viral antisense-RNAs could interfere with ACMV DNA replication and accumulation to achieve resistance in ACMV inoculated transgenic cassava plants. The transcriptional viral gene silencing via siRNA were likely involved in the mechanism of ACMV resistance in these transgenic cassava plants.

Three overlapping genes on ACMV DNA-A



Constructs:

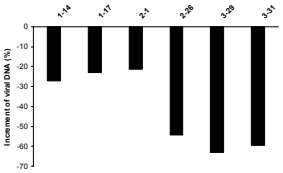


Challenge of transgenic cassava plant lines with ACMV

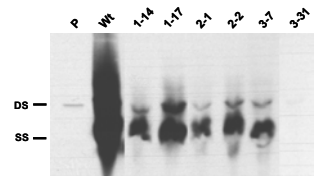
1. Reduced viral DNA accumulation in leaf disks of transgenic lines using two ACMV isolates from Kenya and Cameroon

Viral DNA replication in ACMV-inoculated leaf disks was analyzed as described by Zhang and Gruissem (2003)[3]. Plant lines transformed with the antisense orientation of ACMV AC1, AC2 and AC3 are identified by 1-, 2- and 3-, respectively.

Decreased viral DNA in cassava leaf disks of transgenic lines in comparison with wildtype after biolistic inoculation with ACMV Kenya isolate



Reduced viral DNA accumulation in cassava leaf disks of transgenic lines in comparison with wildtype (Wt) after biolistic inoculation with ACMV Cameroon isolate



2. Pressure-dependent resistance in transgenic plants by inoculation with ACMV Nigeria isolate

Four-week-old plants were inoculated with ACMV Nigeria isolates (100 or 200 ng viral DNA per plant) to allow systemic infection. The symptom severity on fully expanded leaves was recorded by a scale of 0-4 as indicated right:

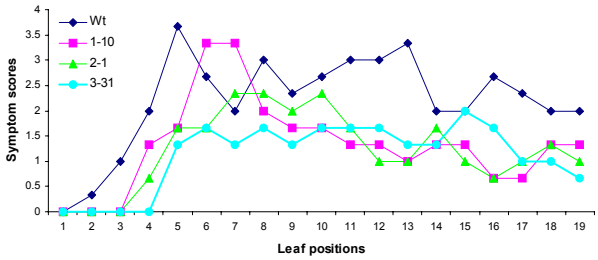
A. Inoculation with 100 ng viral DNA of ACMV

Systemic infection occurred in wildtype plants. Several transgenic lines showed resistance (symptomless) at this pressure level. We found that all these resistant lines have a strong expression of corresponding transgenes. The lines are: 1-10, 2-1, 2-26, 3-8 and 3-31.

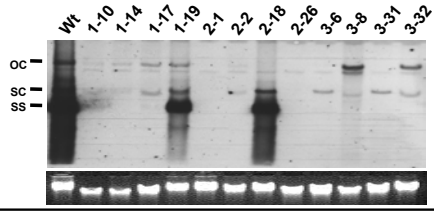
ACMV resistant transgenic cassava

B. Inoculation with 200 ng viral DNA of ACMV

Systemic infection occurred in wildtype plants. Some of transgenic plants showed delayed and attenuated symptoms of ACMV disease as shown in the figure.

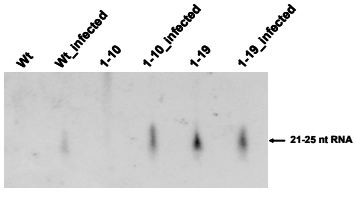


3. Reduced ACMV DNAs in infected transgenic plant lines



Five μg of undigested cellular DNA isolated from three young leaves on the top of the infected plants was used for detection of the viral DNA accumulation with a AV1 probe in Southern analysis.

4. Detection of siRNA in viral antisense-expressing plants



RNA gel blot analysis of siRNAs in ASAC1 transgenic plants.

Note: Low molecular weight RNA isolated from wildtype plant (Wt), ACMV infected wildtype plant (Wt_infected), transgenic lines 1-10 and 1-19 (1-10, 1-19), and ACMV infected transgenic lines 1-10 and 1-19 (1-10_infected, 1-19_infected). Approximately 30 μg of low molecular weight RNA per sample was loaded. SiRNAs were detected by hybridization with fragmented ^{32}P-labeled riboprobe corresponding to the sense strand of the AC1 gene.

Conclusions

- ACMV resistant cassava plants were produced using improved antisense RNA technology expressing non-structural ACMV antisense genes.
- Resistance is correlated with transgene expression levels and infection pressure.
- Transgenic plants conferring ACMV resistance to different ACMV isolates.
- SiRNA may play a role in the ACMV resistance in these transgenic lines.
- Field test needed to confirm the resistance of transgenic plants in Africa.

References:

1. Tresh, J.M., Otim-Nape, G.W., Legg, J.P. & Fargette D. African cassava mosaic virus disease: the magnitude of the problem. *African J Root Tuber Crops* **2**, 13-19 (1997).
2. Hanley-Bowdoin, L., Settlage, S.B., Orozco, B.M., Nagar, S. & Robertson, D. Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. *Crit. Rev. Plant Sci.* **18**, 71-106 (1999).
3. Zhang, P. & Gruissem, W. Efficient replication of cloned African cassava mosaic virus in cassava leaf disks. *Virus Res.* **92**, 47-54 (2003).