RNA-mediated Cassava Geminivirus Resistance in Transgenic Cassava

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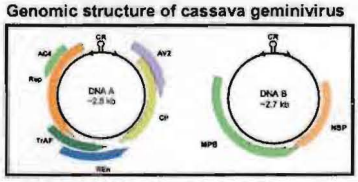
Introduction and Strategies

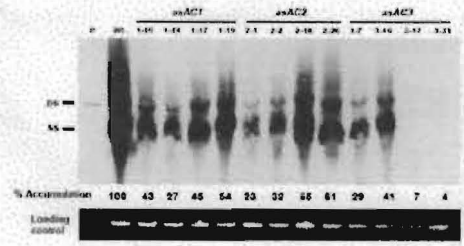
Genomic structure of cassava geminivirus



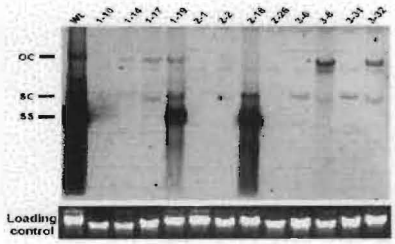
Cassava geminiviruses are a vital component leading to the epidemics of cassava mosaic disease (CMD), the most important and devastating disease of cassava in Africa. Cassava-based food security has been unstablized by the detrimental situation of CMD[1]. We have developed RNA-mediated resistance strategies in transgenic cassava to combat CMD in Africa. The first strategy is to express each full length antisense sequence of *AC1(Rep), AC2 (TrAP)* and *AC3 (REn)* from African cassava mosaic virus (ACMV) as 3'-untranslated region of a selectable hygromycin phosphotransferase gene in transgenic cassava, regulated by CaMV 35S promoter[2]. Another is to develop RNA interference (RNAi)-mediated cassava geminivirus resistance. The hairpin dsRNAs homologous to either the bidirectional promoter of ACMV or protein-coding sequences are being introduced into cassava. Different hairpin dsRNA expression cassettes have been constructed to target one or more viral sequences. Besides, AC2-inducible and vascular specific promoters are also used to minimize the potential negative effect on cassava development from expressed siRNAs.

ACMV resistance in antisense-transgenic cassava plants

1. Reduced viral DNA replication and accumulation in leaf disks and plants after ACMV inoculation



Reduced viral DNA accumulation in cassava leaf disks of transgenic lines in comparison with wildtype (Wt) after biolistic inoculation with ACMV-CM, using the method 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. ss, single-stranded DNA; ds, double-stranded DNA.



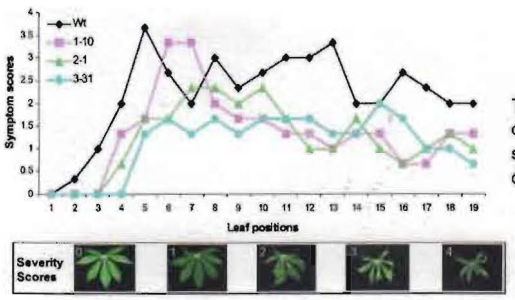
Reduced viral DNA accumulation in leaves of resistant transgenic lines from ACMV-N*Og* systemic infected plants. Viral single-stranded (ss), supercoiled (sc) and open-circled (oc) DNA forms are indicated.

2. Pressure-dependent resistance in transgenic plants

A. Inoculation with 100 ng viral DNA of ACMV-NOg

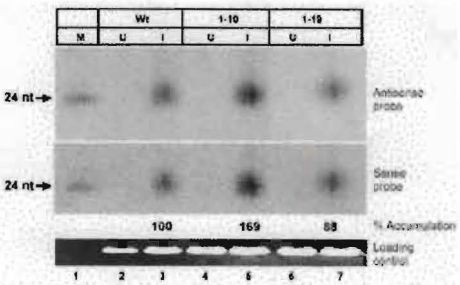
Systemic infection occurred in wild-type plants. Several transgenic lines showed resistance (symptomless) at this pressure level, e.g. 3-31. All these resistant lines have a strong expression of corresponding transgenes. The lines are: 1-10, 2-1, 2-2, 3-8 and 3-31.

B. Inoculation with 200 ng viral DNA of ACMV-N*Og*



Transgenic plants showed delayed and attenuated symptoms of ACMV disease.

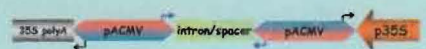
3. siRNA may have a role in ACMV resistance lines



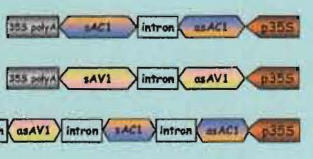
SiRNAs were detected in all infected plants (I, lane 3, 5, 7) but not uninfected plants (U, lane 2, 4, 6) using both antisense and sense probes. The resistant *AC1* line 1-10 produced more siRNAs than susceptible *AC1* line 1-19 and wild-type plants (Wt).

Box: RNAi constructs and their efficacies against cassava geminivirus replication and systemic infection in host plants

Promoter targeting:



Protein-coding sequences:



Other promoters:

AC2 inducible promoter: At1g13610, hypothetical protein of *Arabidopsis*

Vascular specific promoter: p54/1.0, glutamic acid-rich protein of cassava

Testing systems: Cassava cell cultures, cassava and *N. benthamiana* plants

Cassava geminiviruses: ACMV-N*Og*, ACMV-CM and EACMV-CM

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 pressures.
- Transgenic plants confer ACMV resistance to different ACMV isolates.
- Small molecular RNAs may play a role in ACMV resistance in these antisense lines.
- Field test is needed to confirm the resistance of antisense transgenic plants in Africa.
- New approaches using RNAi-mediated resistance to cassava geminivirus are in progress.

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. Zhang, P., Vanderschuren, H., Fütterer, J. and Gruissem, W. (2005) Resistance to cassava mosaic disease in transgenic cassava expressing antisense RNAs targeting virus replication genes. *Plant Biotechnol. J.* 3 (in press)
3. Zhang, P. & Gruissem, W. Efficient replication of cloned African cassava mosaic virus in cassava leaf disks. *Virus Res.* **92**, 47-54 (2003).

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