RNA-mediated broad-spectrum resistance to cassava geminiviruses in transgenic cassava

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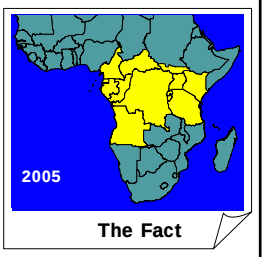
The African Cassava Mosaic Disease Pandemic

- First reported in 1894
- Most recent outbreak in Uganda 1988
- Spreading at 15-20Km/year
- Loss of 36% of total production
- Many abandoned fields
- No complete resistant variety

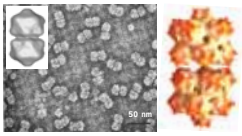


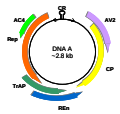
The Fact

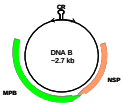
Symptoms:
- Mosaic bleaching of leaves
- Leaf deformation
- Reduction of leaf size
- Stunted growth
- Loss of storage root formation

African Cassava Mosaic Geminiviruses

African cassava mosaic virus (ACMV)
East African cassava mosaic virus (EACMV)
East African cassava mosaic virus-Ug (EACMV-Ug)
South African cassava mosaic virus (SACMV)





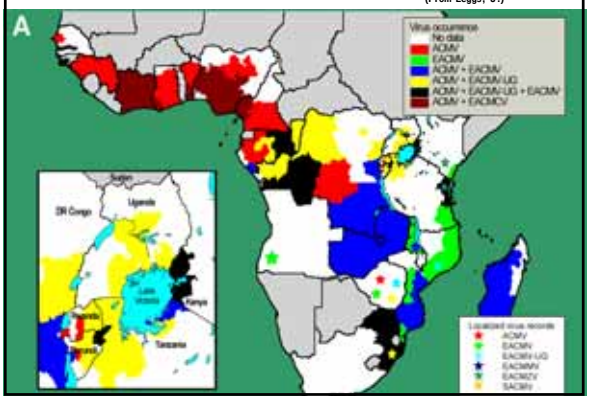


Begomovirus of the family *Geminiviridae*
Geminate particles
Bipartite circular ssDNA genomes: DNA A and DNA B
Common Region (CR) conserved between DNA A and DNA B

Transmitted by whitefly (*Bemisia tabaci*)

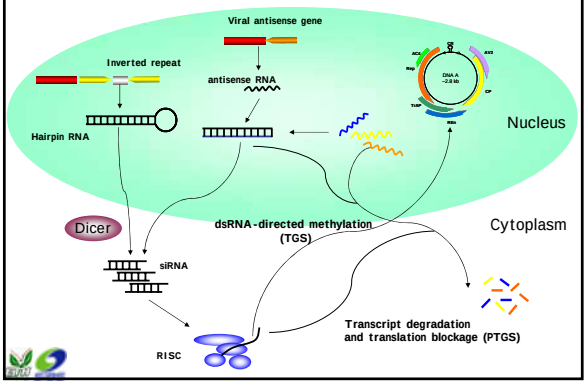
CMG Distribution in sub-Sahara Africa

(From Leggs, J.)



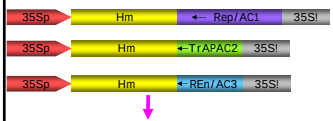
RNA-mediated Virus Resistance

Antisense and RNA-interference (RNAi) Strategy



Interference with ACMV Replication by Expression of Viral Antisense RNAs

Antisense RNA Constructs



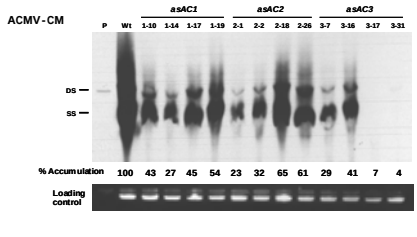
Transgenic cassava

Advantages:

- Stable expression of antisense RNAs
- Only one promoter used
- Reduction the workload, e.g. tissue culture and analysis of putative transgenic plants
- Silencing viral genes only upon infection

Viral replication assay
Virus infection test

Transient ACMV Replication of Antisense Plants



Homology between KE/CM: *AC1*, 96.7%; *AC2*, 94.9%; *AC3* 92.8%

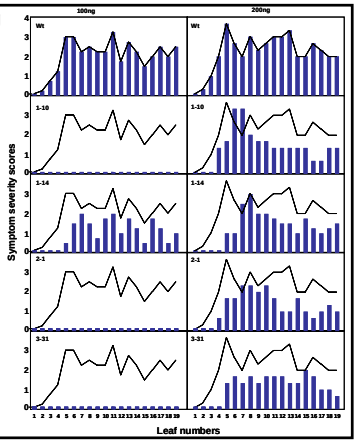
Infection of Transgenic Cassava with ACMV-NOg

Homology between KE/NOg: *AC1*, 97.3%; *AC2, 97.8%; AC3*, 97.3%

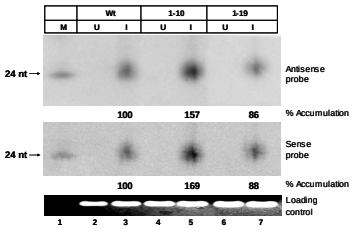
Analysis of ACMV-NOg Inoculated Plants

Severity Scores

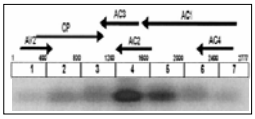
ACMV-NOg Inoculated Plants



Detection of siRNA in Viral Antisense-expressing Plants

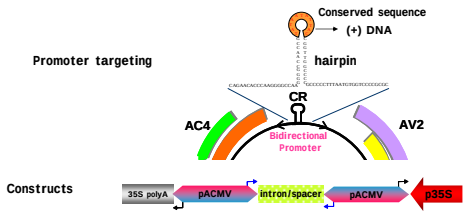


ACMV-CM-induced PTGS

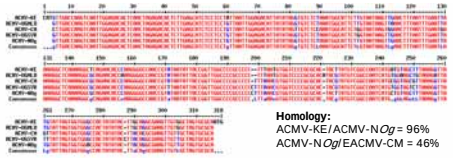


(Chellappan et al. 2004)

Transcriptional Gene Silence of ACMV Expression



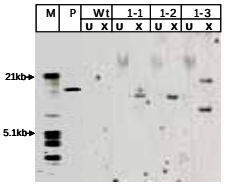
ACMVs



Homology:
ACMV-KE/ACMV-N*Og* = 96%
ACMV-N*Og*/EACMV-CM = 46%

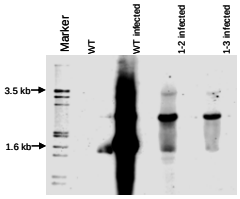
TC expressing hairpin RNA homologous to ACMV promoter sequence shows reduced viral DNA accumulation

Southern blot



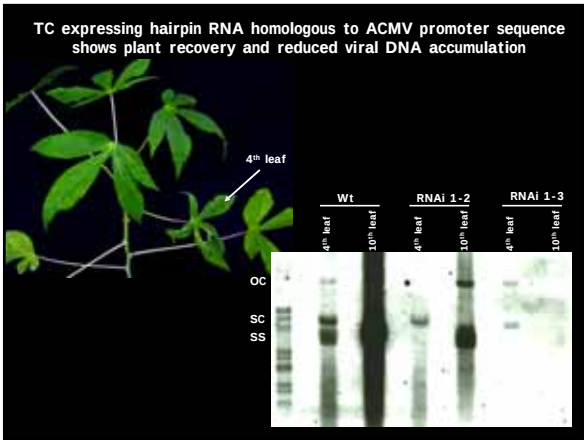
M, molecular marker; P, plasmid; Wt, wildtype; 1-1, 1-2 and 1-3, different transgenic lines; U, undigested DNA; X, *Xba*I

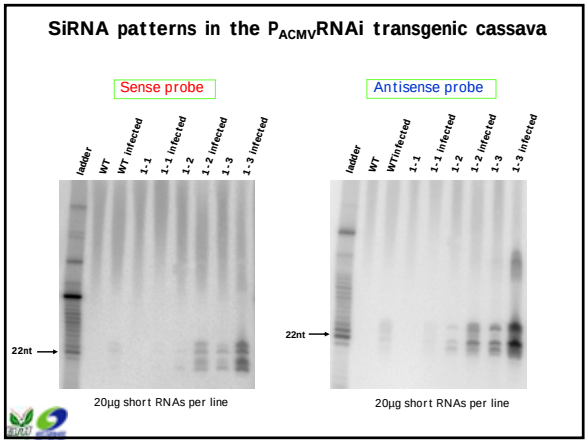
Transient virus replication assay

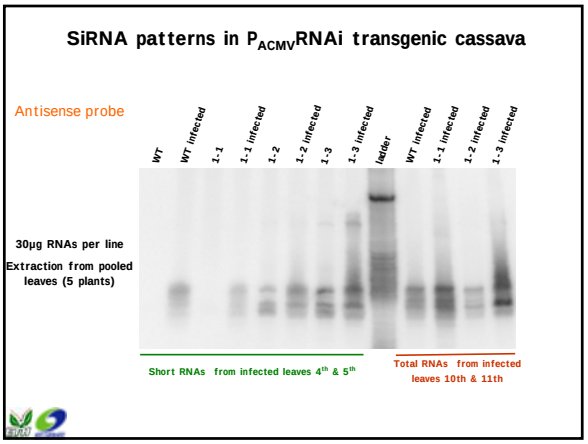


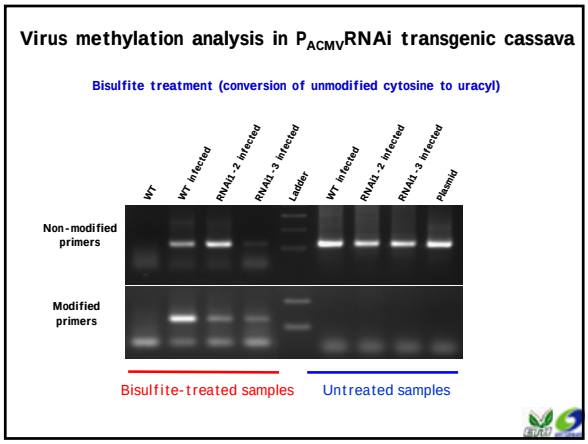
Bombardment of 12 replicates per line (1 shot /replicate) with ACMV-NOg virus (200ng DNA A + 200ng DNA B).

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

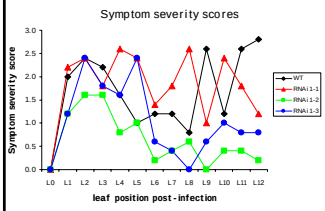

TC expressing hairpin RNA homologous to ACMV promoter sequence shows plant recovery and reduced viral DNA accumulation
4th leaf
Wt
RNAi 1-2
RNAi 1-3
4th leaf
10th leaf
4th leaf
10th leaf
4th leaf
10th leaf
OC
SC
SS

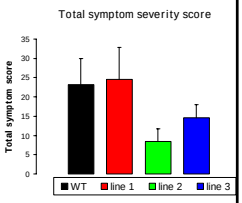

SiRNA patterns in the P ACMV RNAi transgenic cassava
Sense probe
Antisense probe
ladder
WT
WT infected
1-1
1-1 infected
1-2
1-2 infected
1-3
1-3 infected
22nt
20µg short RNAs per line
20µg short RNAs per line


SiRNA patterns in P ACMV RNAi transgenic cassava
Antisense probe
WT
WT infected
1-1
1-1 infected
1-2
1-2 infected
1-3
1-3 infected
ladder
WT infected
1-1 infected
1-2 infected
1-3 infected
30µg RNAs per line
Extraction from pooled leaves (5 plants)
Short RNAs from infected leaves 4th & 5th
Total RNAs from infected leaves 10th & 11th


Virus methylation analysis in P ACMV RNAi transgenic cassava
Bisulfite treatment (conversion of unmodified cytosine to uracyl)
WT
WT infected
RNAit-2 infected
RNAit-3 infected
Ladder
WT infected
RNAit-2 infected
RNAit-3 infected
Plasmid
Non-modified primers
Modified primers
Bisulfite-treated samples
Untreated samples

ACMV-N*Og* Challenge of P_{ACMV}RNAi transgenic cassava





5 cassava plants per line were biolistically infected with ACMV-NOg DNA A and DNA B (100ng each)

Total severity symptom score is obtained by summing up the symptom severity score of the emerging leaves post biolistic delivery of the virus

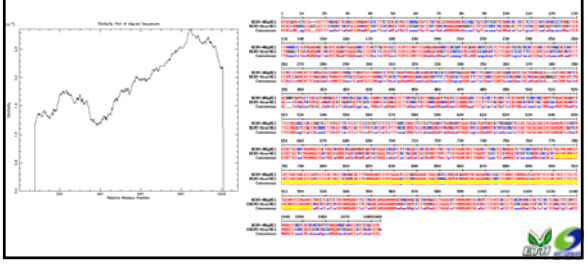
PTGS of Cassava Geminivirus Genes

Targeting genes: AC1, AC2 (overlap with AC1), and AV1

⇒ Designing RNAi constructs for a better homology between ACMV and EACMV isolates

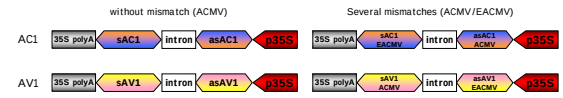
⇒Designing double constructs targeting both AC1 & AV1

⇒ Introducing mismatches in the RNAi constructs targeting AC1 and AV1

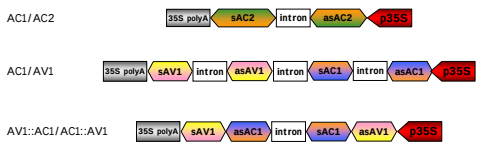


Constructs for RNAi-mediated viral gene silencing

Single target



Double targets



Inducible or local expression siRNA

⇒Use of *At1g13610 hypothetical protein* promoter to replace the CaMV 35S promoter in the single-target constructs

VIGS suppressor AC2-inducible promoters :

Gene/Promoter construct	Transactivator construct, AC2 from:	Basal transcript level on control chip(s)	Fold activation on AC2 chip	Relative CAT expression in *N.plumbaginifolia*	Relative CAT expression in *A. thaliana*
CaMV 35S promoter				100	100
	MYMV			76	99
	ACMV			70	95
At3g12460, Hypothetical 3'-5' exonuclease		44 (17 to 76)		0.9	0.6
	MYMV		43x	47	127
	ACMV		20x	4.3	7.1
At1g13610, Hypothetical protein		6 (3 to 9)		0.2	<0.01
	MYMV		56x	65	173
	ACMV		27x	25	36
At4g12140, Putative RING finger		43 (9 to 63)			5
	MYMV		9x		480
	ACMV		18x		410

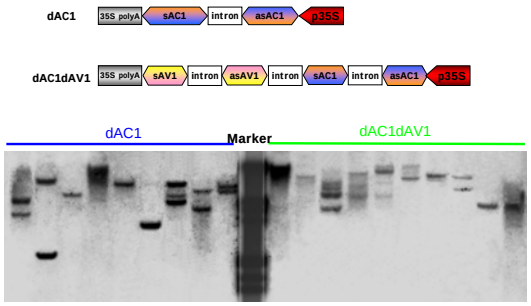
(T.Hohn's Lab, University of Basel)

⇒Using vascular specific promoters to express the dsRNA in vascular system of cassava

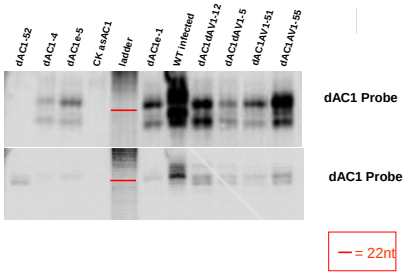
promoters p15/1.5 and p54/1.0

(Zhang et al., 2004, Planta 218, 192-203)

Production of transgenic cassava expressing dAC1 and dAV1dAC1



SiRNA processing in Nicotiana benthamiana



Conclusions and perspectives

- ACMV resistant cassava plants were produced using improved antisense RNA technology expressing non-structural ACMV antisense genes and using gene silencing by expressing double small RNAs cognate to viral non-coding sequence.
- Resistance is correlated with transgene expression levels and infection pressure;
- Transgenic plants showed ACMV resistance to different ACMV isolates;
- Short RNAs have been characterized in some ACMV infected transgenic cassava lines;
- Analysis of cassava transformed with dsRNA constructs are ongoing;
- Field tests are needed to confirm the resistance of transgenic plants in Africa;

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