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

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)

CMG Distribution in sub-Sahara Africa

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

Transmitted by whitefly (Bemisia tabaci)
RNA-mediated Virus Resistance
Antisense and RNA-interference (RNAi) Strategy

Dicer
siRNA
RISC
Transcript degradation and translation blockage (PTGS)
Inverted repeat
dsRNA-directed methylation (TGS)

Nucleus
Cytoplasm

Hairpin RNA
DNA A
~2.8 kb
CR
Rep
TrAP
REn
CP
AV2

Viral antisense gene
antisense RNA

Antisense RNA Constructs
Transgenic cassava
Viral replication assay
Virus infection test

Interference with ACMV Replication by Expression of Viral Antisense RNAs

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
Transgenic cassava

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

Homology between KE/NOg: AC1, 97.3%; AC2, 97.8%; AC3, 97.3%
Analysis of ACMV-NOg Inoculated Plants

Severity Scores

Detection of siRNA in Viral Antisense-expressing Plants

Transcriptional Gene Silence of ACMV Expression

TC expressing hairpin RNA homologous to ACMV promoter sequence shows reduced viral DNA accumulation
TC expressing hairpin RNA homologous to ACMV promoter sequence shows plant recovery and reduced viral DNA accumulation.

SiRNA patterns in the PACMVRNAi transgenic cassava

Virus methylation analysis in PACMVRNAi transgenic cassava

Bisulfite treatment (conversion of unmodified cytosine to uracyl)
ACMV–Nog Challenge of P_{ACMV}RNAi transgenic cassava

Symptom severity scores

Total symptom severity score

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

Symptom severity scores

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

Constructs for RNAi-mediated viral gene silencing

Single target

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<th>asAC1</th>
<th>p35S</th>
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Double targets

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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:

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

promoters p15/1.5 and p54/1.0

(T. Hohn’s Lab, University of Basel)
Production of transgenic cassava expressing dAC1 and dAV1dAC1

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SiRNA processing in Nicotiana benthamiana

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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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