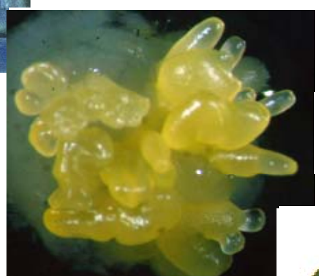
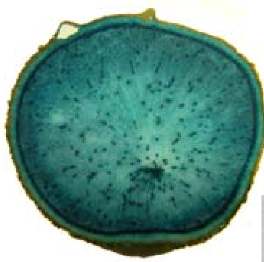
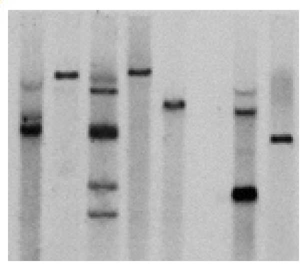
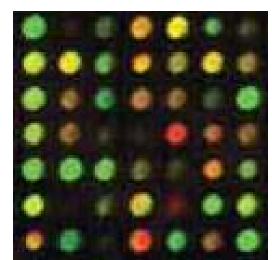
How to Add Value to Cassava?

- Products and Markets
- Biodiversity
- Transgenics

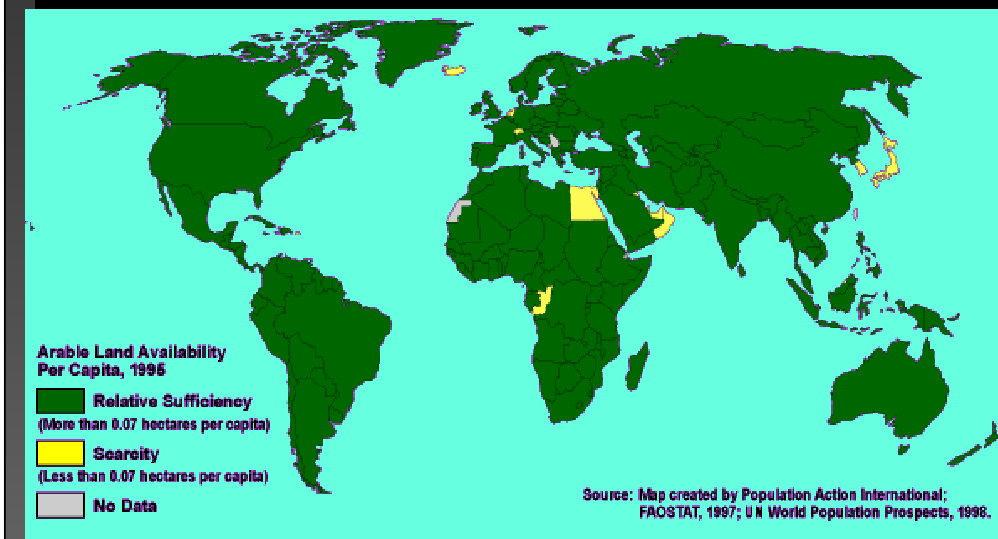







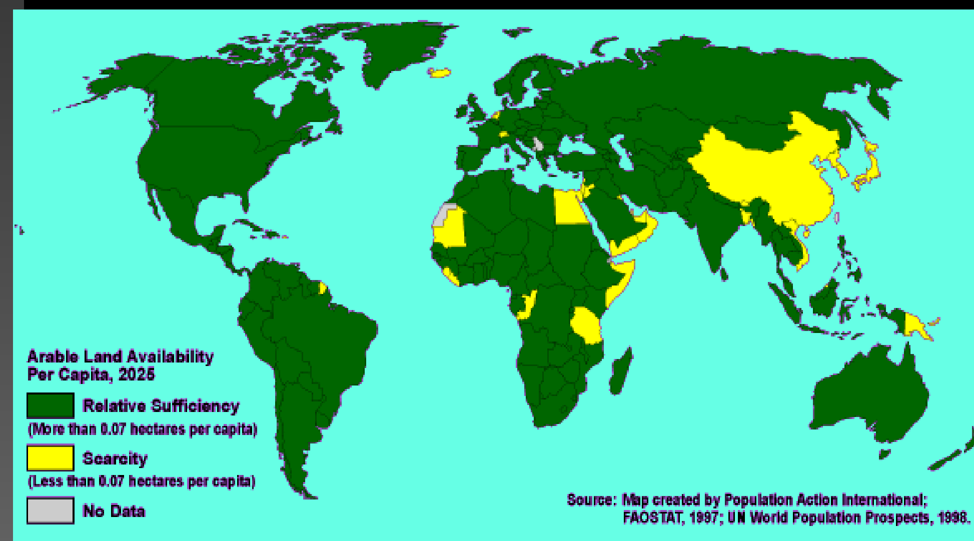

Why do we have to improve agricultural production and focus on crops such as cassava?

Land usable for agriculture becomes scarce

Survey 1995



Expectation for 2025

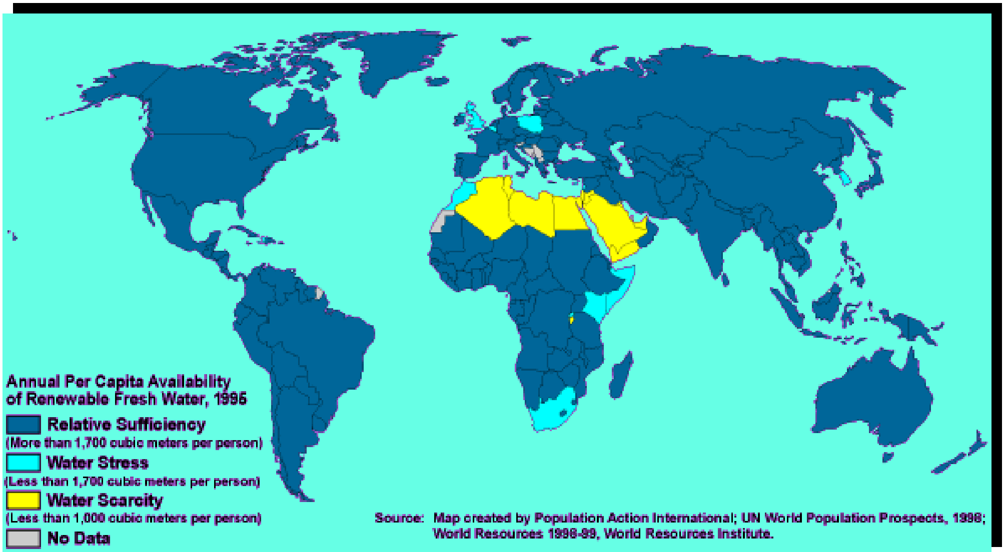


http://www.dayof6billion.org

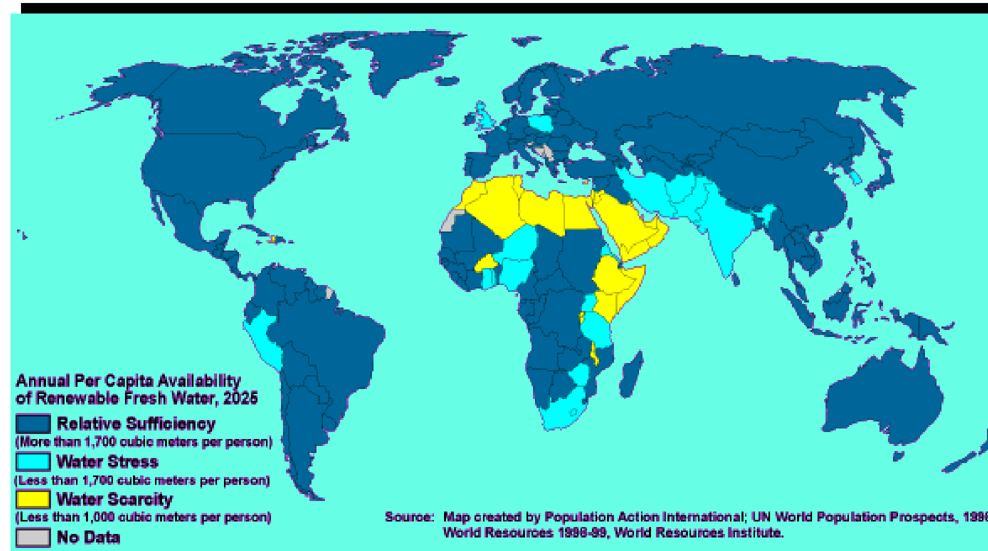
Why do we have to improve agricultural production and focus on crops such as cassava?

Fresh water for agriculture becomes a scarce commodity

Survey 1995



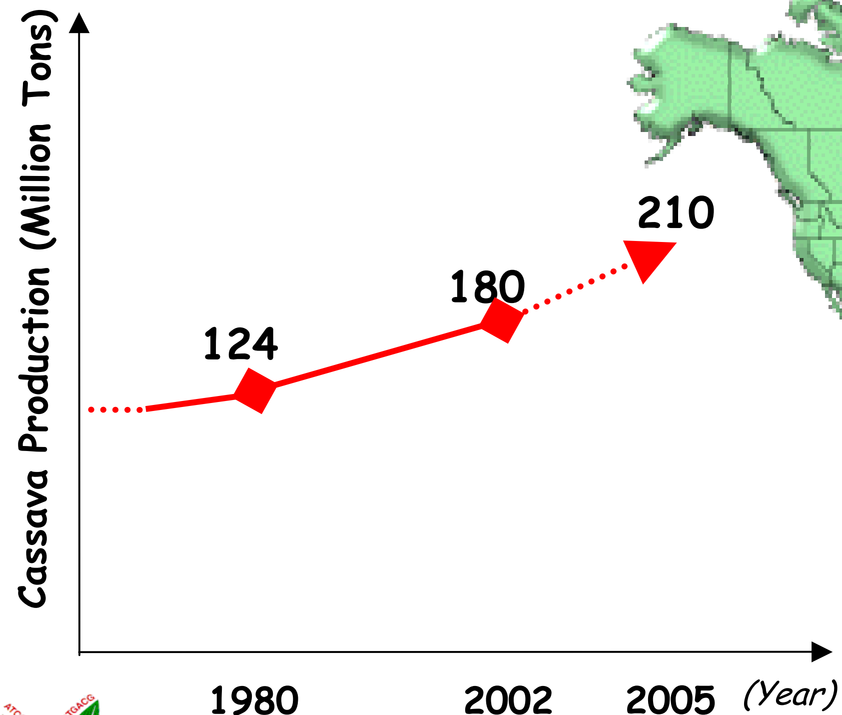
Expectation for 2025

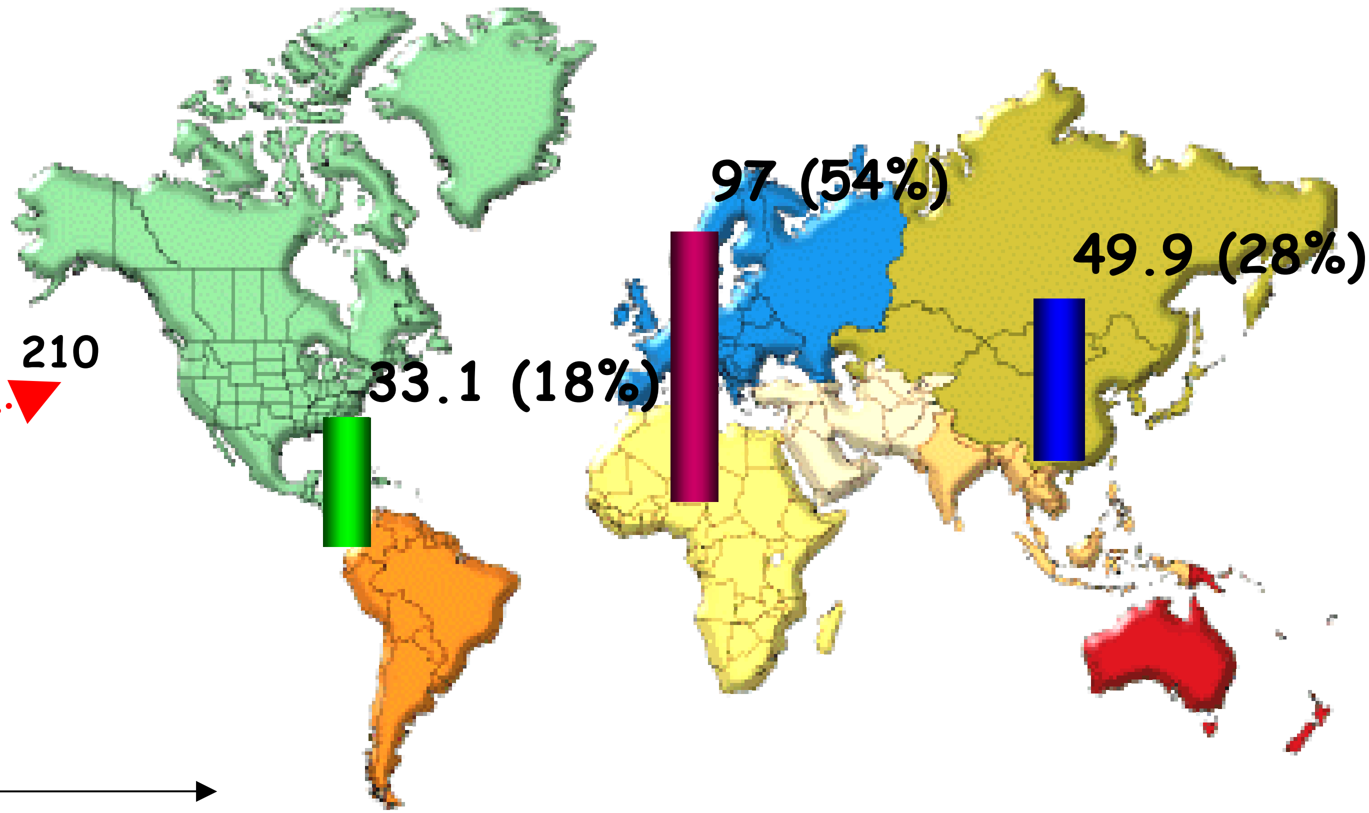


http://www.dayof6billion.org

Cassava
(*Manihot esculenta* Crantz)

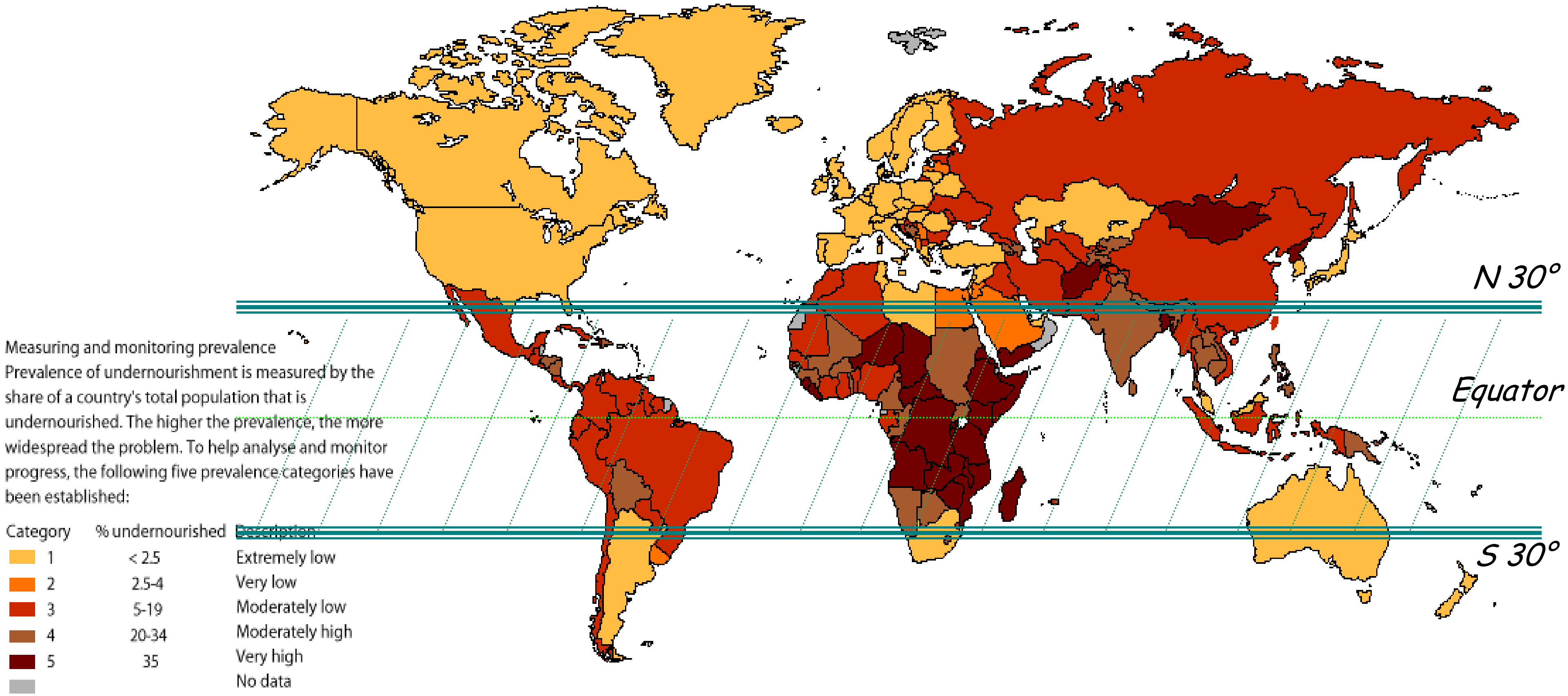
- Third most important calorie source in the tropics
- Food for >600 million people worldwide





(Source: FAO, 2002)

Cassava Distribution and Global Prevalence of Undernourishment



- ***A key role in food security of developing countries***

(Source: FAO)

Cassava
(*Manihot esculenta* Crantz)

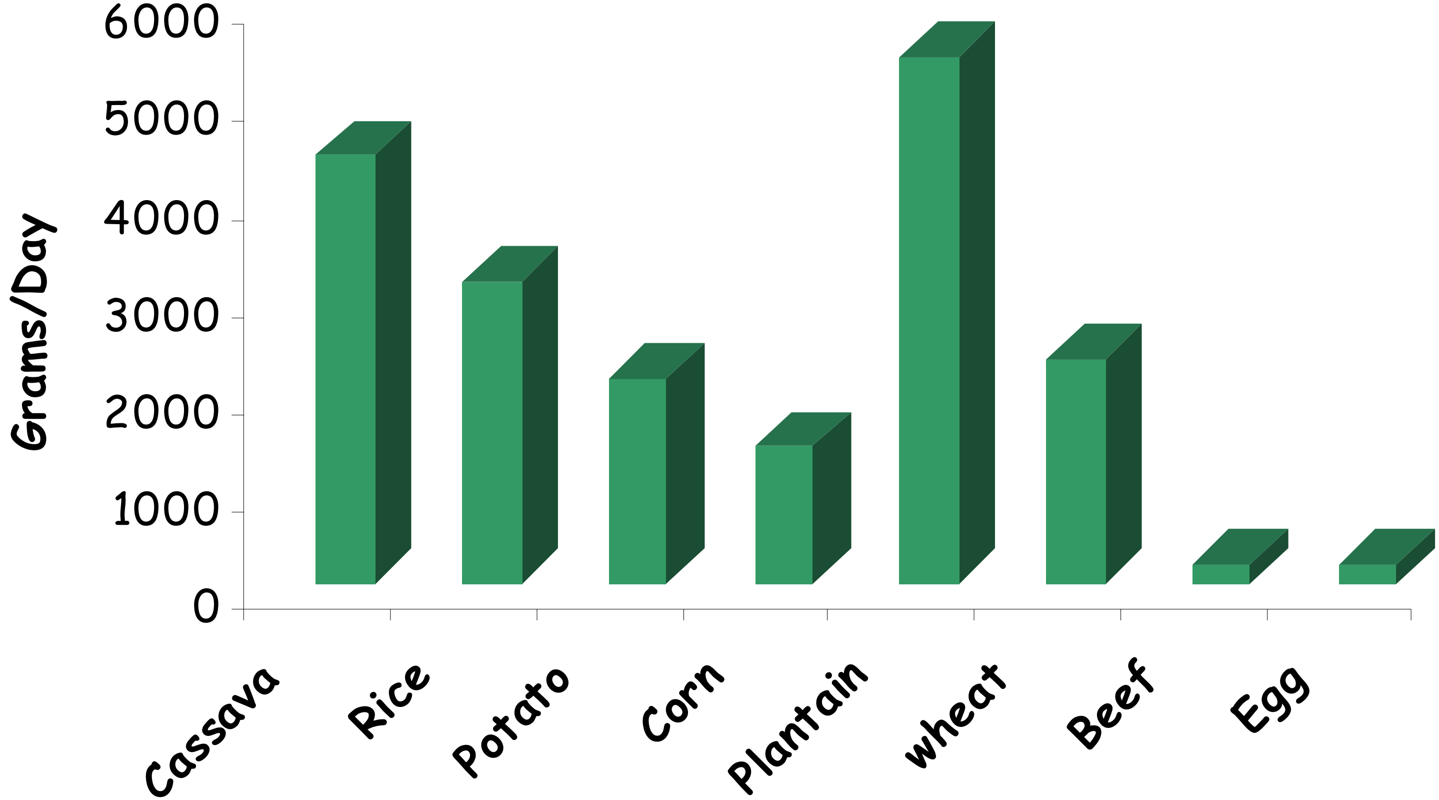
Advantages:

- Cheapest dietary carbohydrate source
- Tolerant of unfavourable ecological conditions
- Reliable yield and flexible harvesting time
- Vegetative propagation mode

Bottleneck problems:

- Plants can be attacked by various insect pests and virus diseases
- Storage roots are rich in starch but low in protein
- Mature leaves contain appreciable quantities of protein and vitamins but have a short life
- Storage roots suffer a rapid post-harvest deterioration
- Traditional breeding is difficult and time consuming

The amount of food products which must be consumed (g/day) in order to meet the minimum daily requirement of all essential amino acids



Strategies for Value Addition to Cassava-a Graded Biotechnology Approach

- Tissue culture-based breeding
  germplasm collection, virus-free plant material, in vitro mutation, doubled haploid etc.

- Genetic enhancement
  up- or down-regulation of cassava genes of interest, introduction of genes for new traits

- Genomics and proteomics
  molecular markers, development of physical map and ESTs, genome sequencing, gene regulation and discovery, protein structure and function

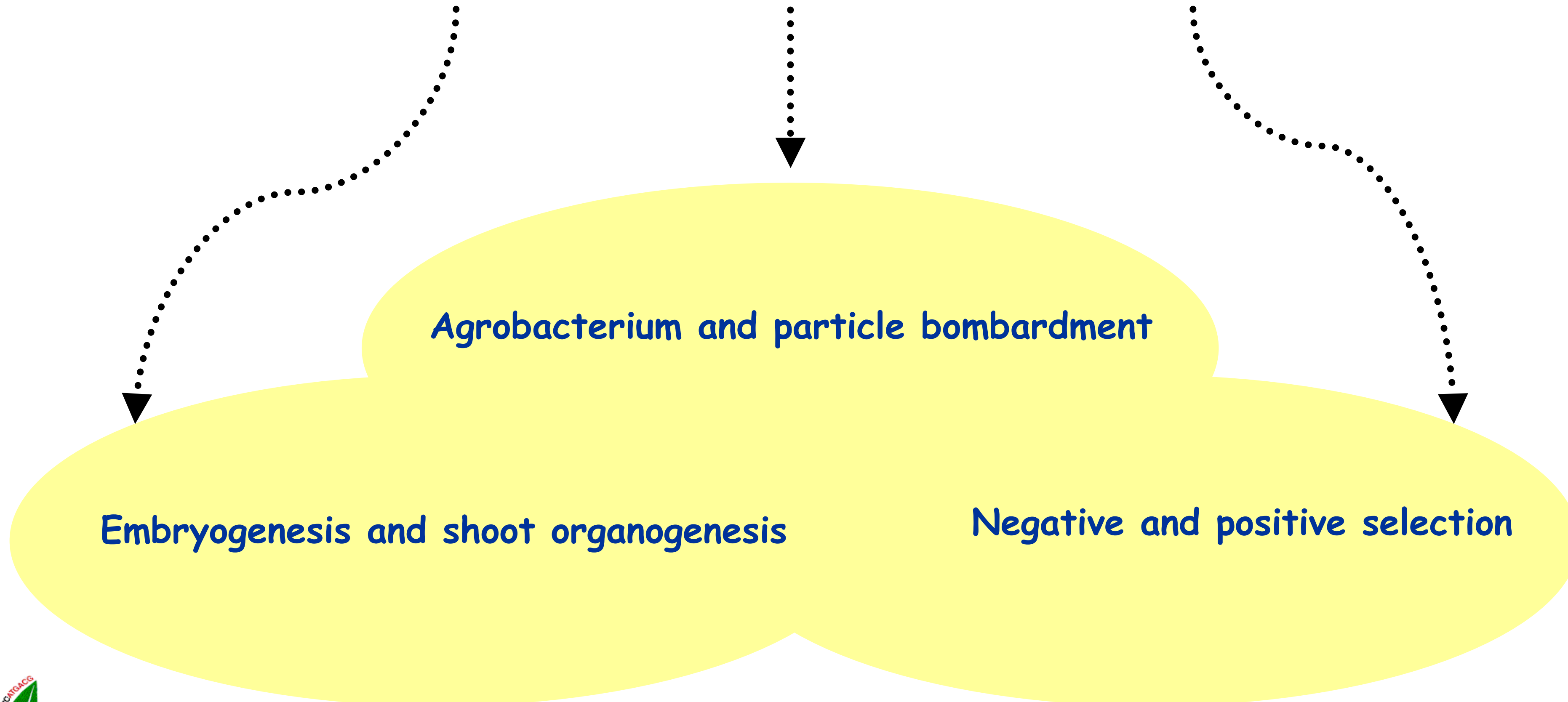
Peng Zhang and Wilhelm Gruissem
Plant Biotechnology
Swiss Federal Institute of Technology

Cassava biotechnology for developing countries– prospects and challenges

Biotechnology
a valuable tool for cassava genetic improvement

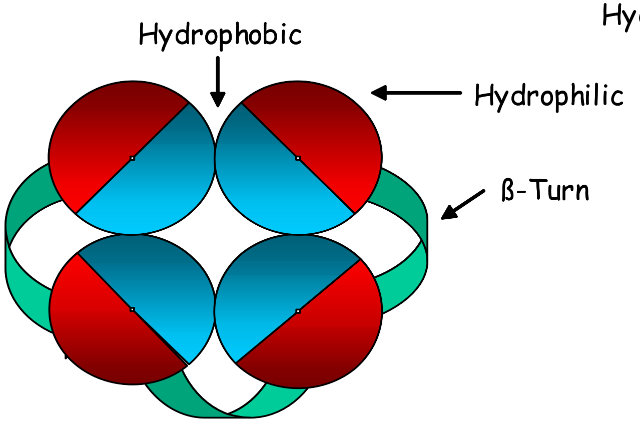
Efficient transformation system

compatible tissue culture, transformation and selection methods

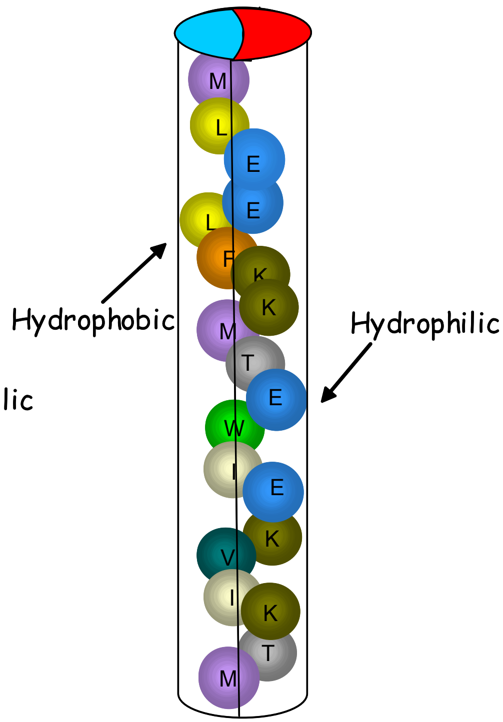


Artificial Storage Protein (ASP1)

Monomer
MLEELFKKMTEWIEKVIKTMG P
G
Dimer
P GMTKIVKEIWETMKKFLEELMR
ß-Turns
G
Trimer
RMLEELFKKMTEWIEKVIKTMG P
G
Tetramer
MTKIVKEIWETMKKFLEELMR

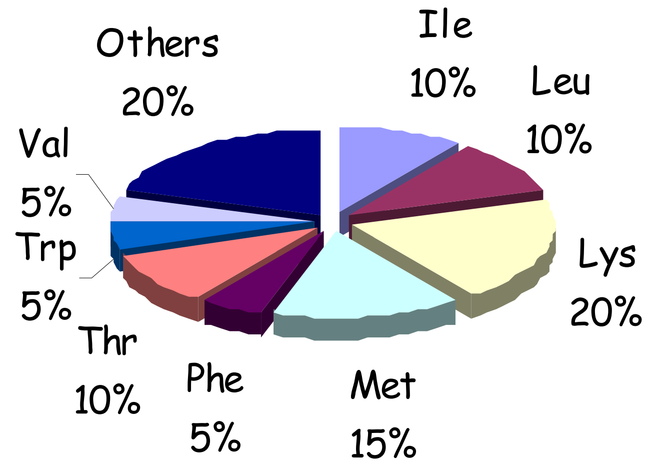


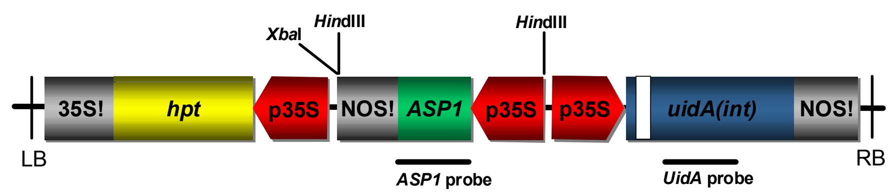
Tetramer (11.2KD)



monomer

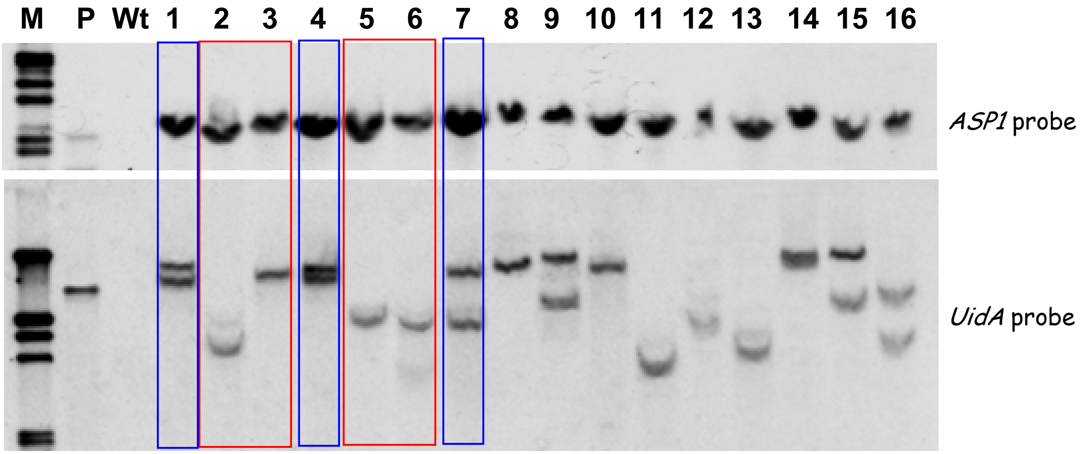
EAA composition of ASP1



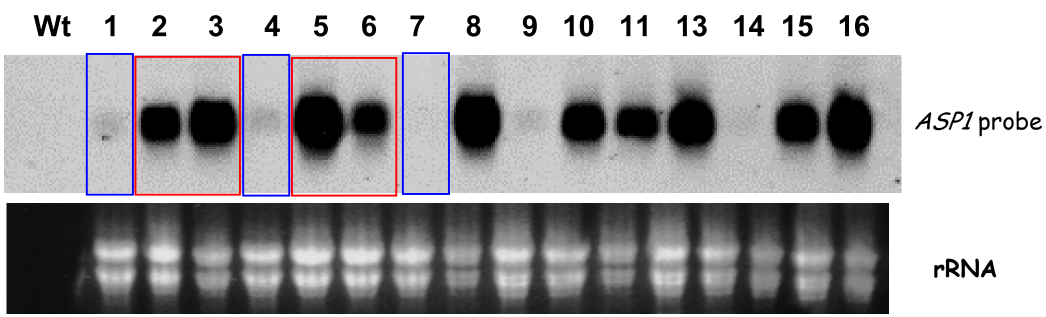


Integration and expression of *ASP1* (Poster #61)

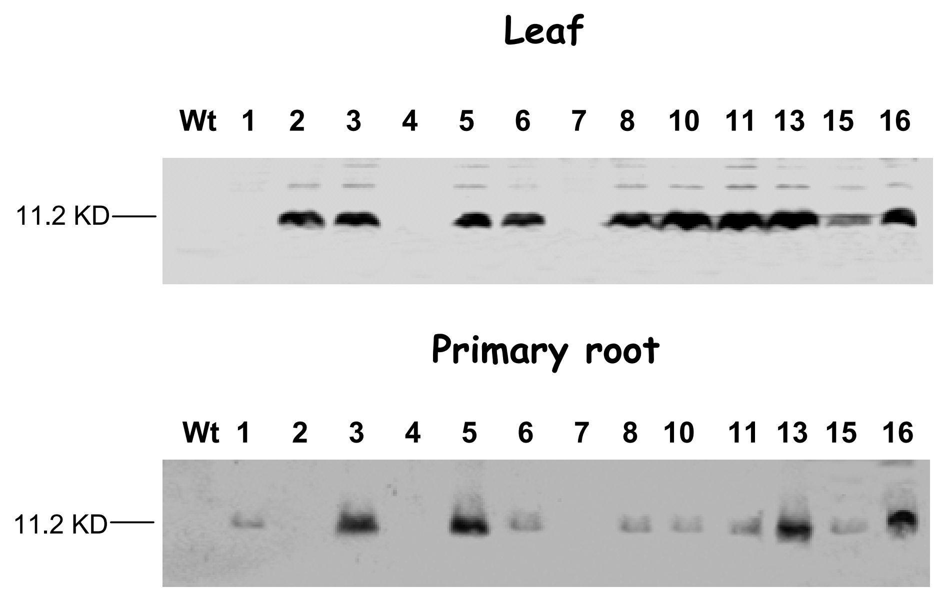
Southern



Northern



Western



Zhang et al. (2003) Transgenic Research, 12: 243-250

Gruissem/CBN VI

Perspectives:

1. Amino acid composition of storage roots and leaves
2. Protein content of storage roots and leaves
3. Field trials (IITA, Nigeria)

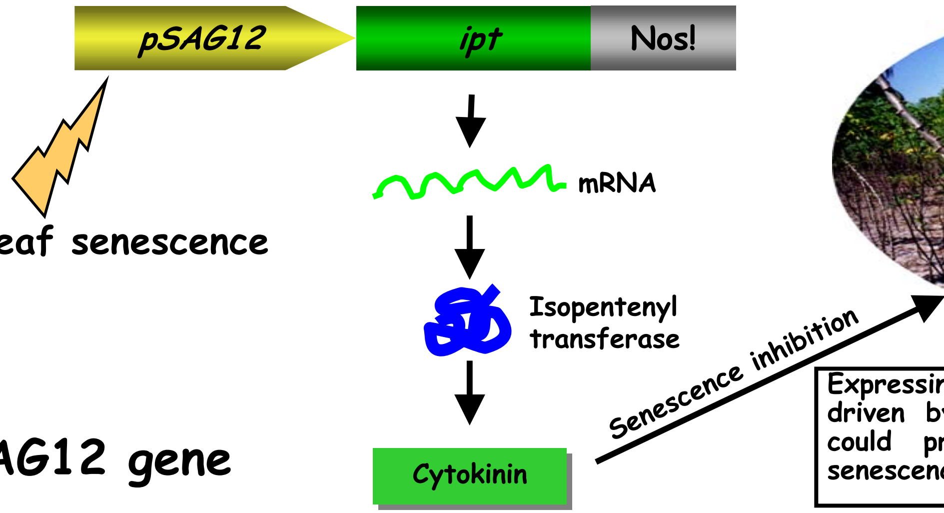
New Approach:

Targeting ASP1 to cell organelles in cassava storage roots for nutritional improvement

Plastids e.g. amyloplast
Vacuole

Leaf retention (Poster #14)

Strategy



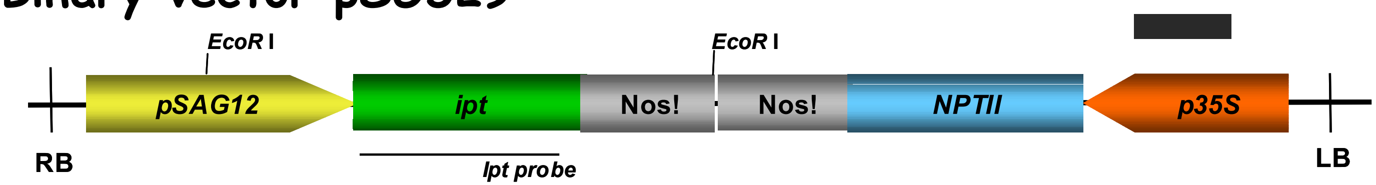
Expressing the isopentenyl transferase (ipt) gene driven by a senescence-specific promoter *SAG12* could prolong leaf life via an autoregulatory senescence inhibition system.

Expression of SAG12 gene

Developmental stage

SAG12

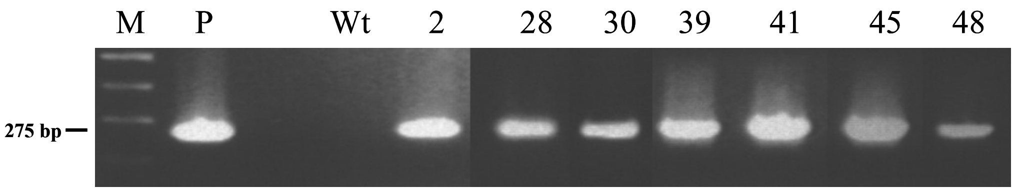
Binary vector pSG529



Wt Transgenic

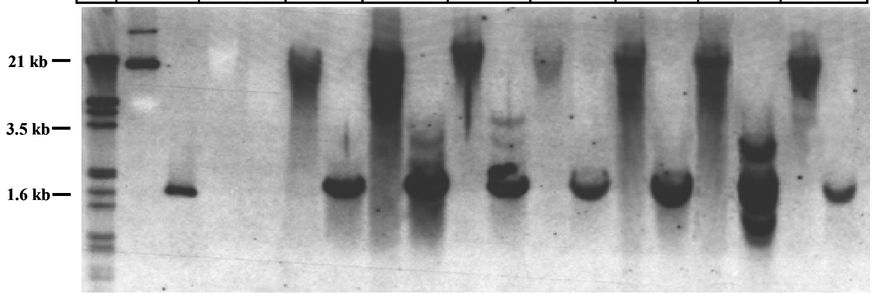
Molecular Analysis of P_{SAG12}-*IPT* Transgenic Plants

PCR using *ipt* primers



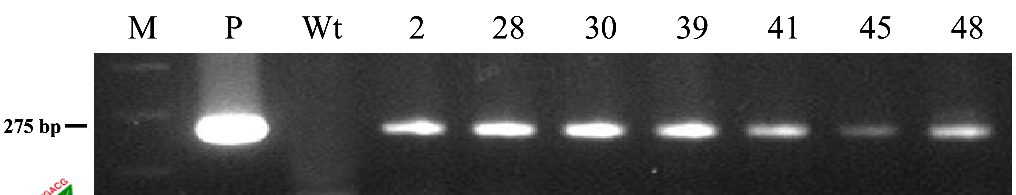
Southern analysis with *ipt* probe

M	P		Wt		2		28		30		39		41		45		48	
	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E	U	E



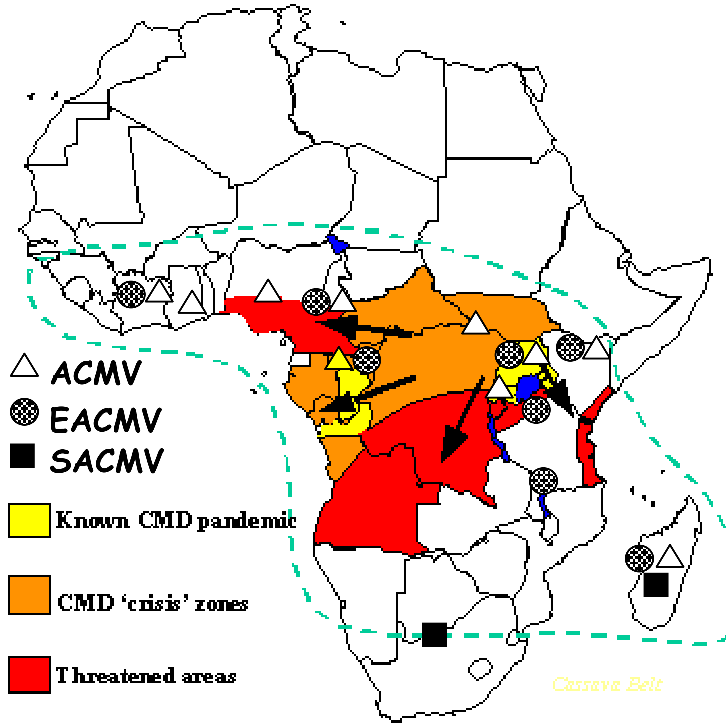
U: undigested; E: *Eco*RI

RT-PCR using *ipt* primers



Gruissem/CBN VI


Wildtype
529-28
erspectives:



Disease Resistance
(African Cassava Mosaic Disease)

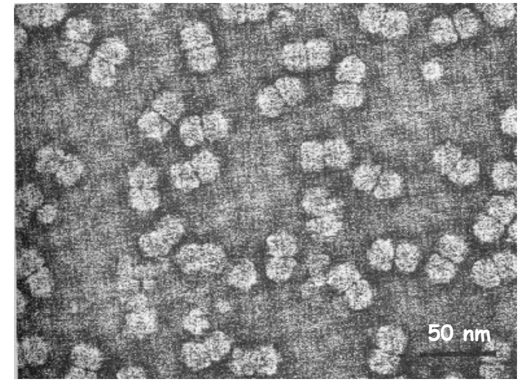
(Source: IITA)

- First reported in 1884
- Most recent outbreak in Uganda 1988
- Spreading N-S at 15-20 Km/year
- Loss of 36% of total production
- Many abandoned fields
- No fully resistant variety

Symptoms:

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

African Cassava 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)

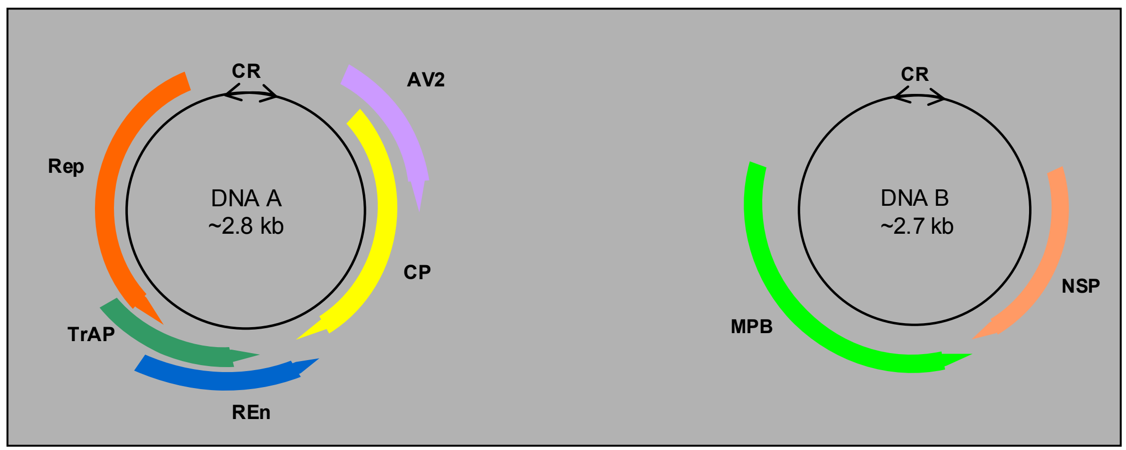
Begomovirus of the family *Geminiviridae*

Geminate particles

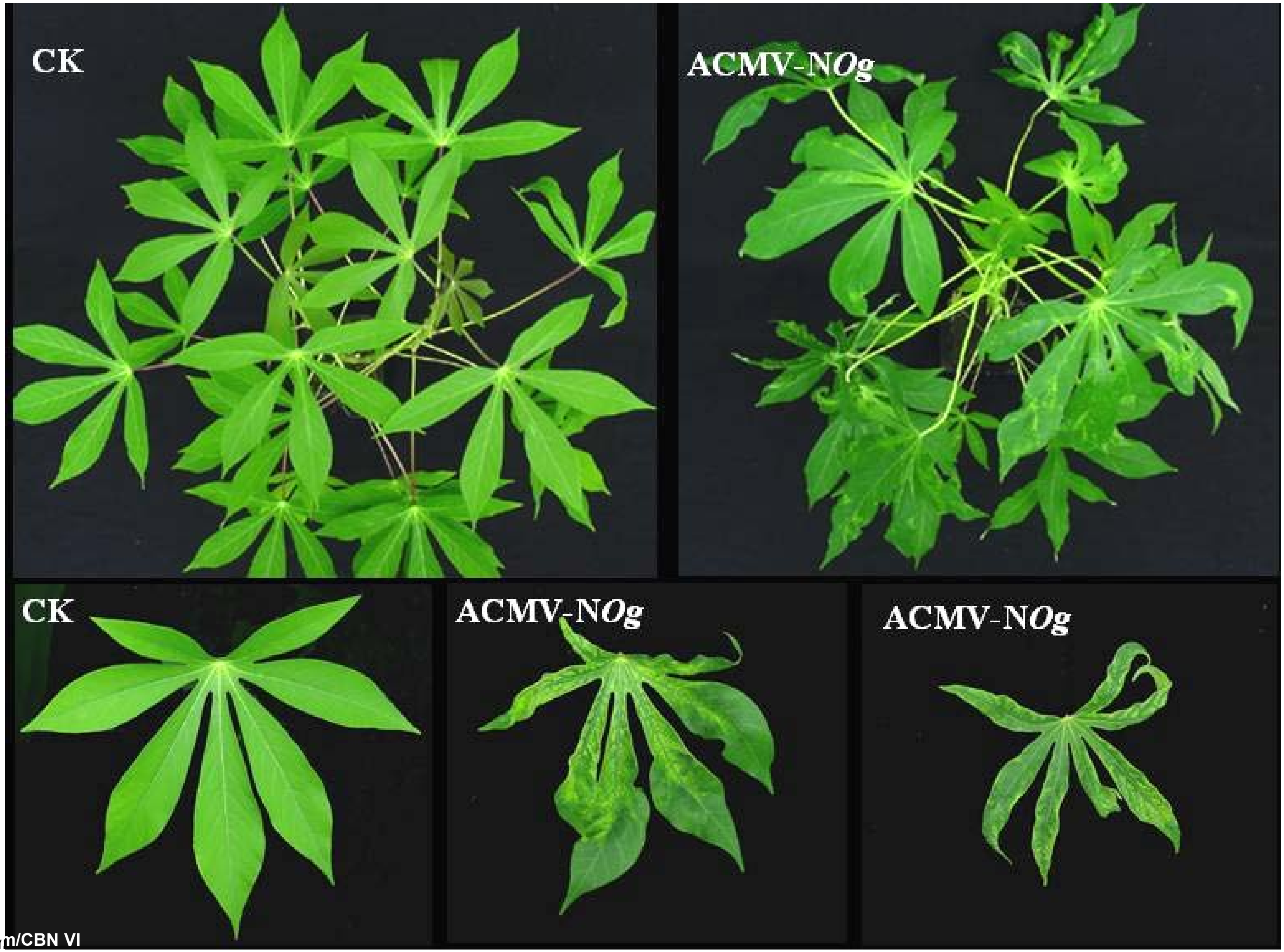
Bipartite circular ssDNA genomes: DNA A and DNA B

Common Region (CR) conserved between DNA A and DNA B

Transmitted by whitefly (*Bemisia tabaci*)

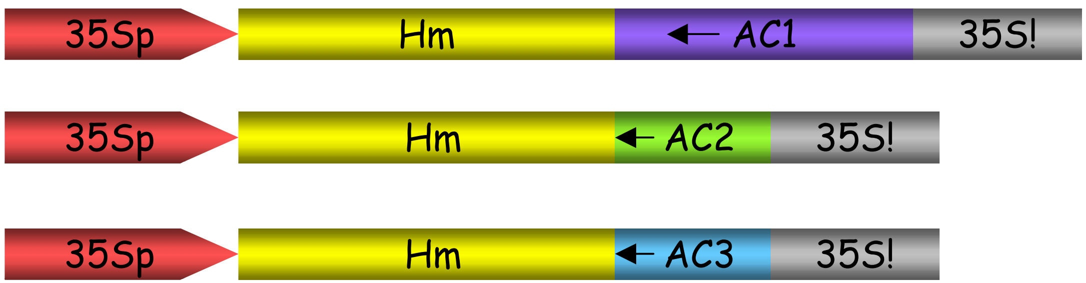


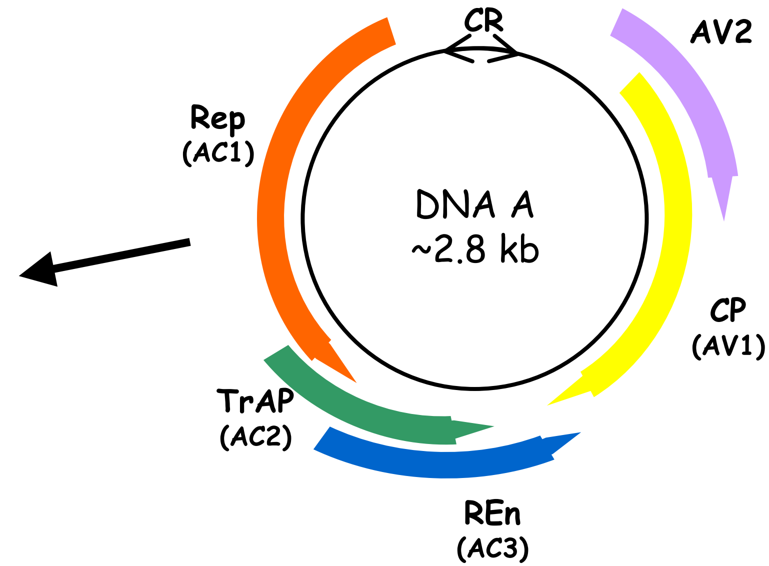
ACMV Infection of Cassava Plants



Interference with ACMV Replication by Expression of Viral Antisense RNAs (Poster #15)

Antisense RNA Constructs





Transgenic cassava

Viral replication assay
Virus infection test

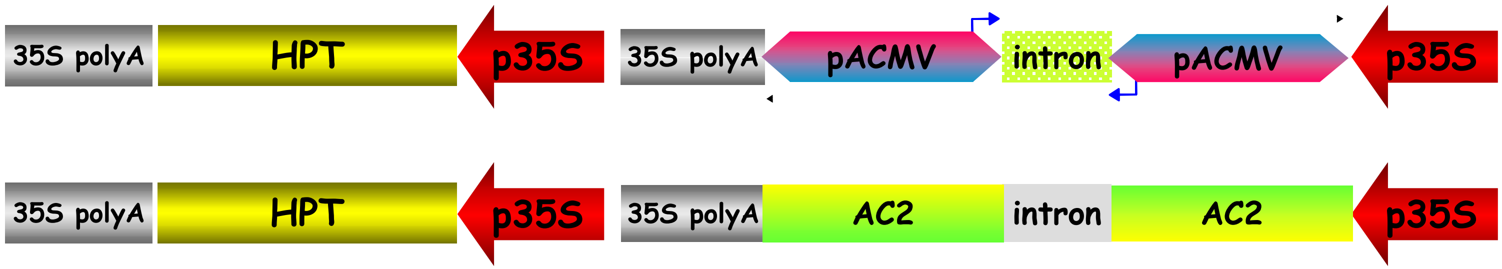

ASAC3-16


ASAC3-8


ASAC3-31

Gene Silencing-based Virus Resistance (Poster #15)

RNA interference (RNAi)



Perspectives

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

- Virus infection of cassava plants
- Mechanism of resistance (e.g. PTGS)
- Field trial of virus resistance of the transgenic plants (IITA, Nigeria)

Genetic Resources: Gene Isolation via Differential Screening of A Tuber cDNA Library

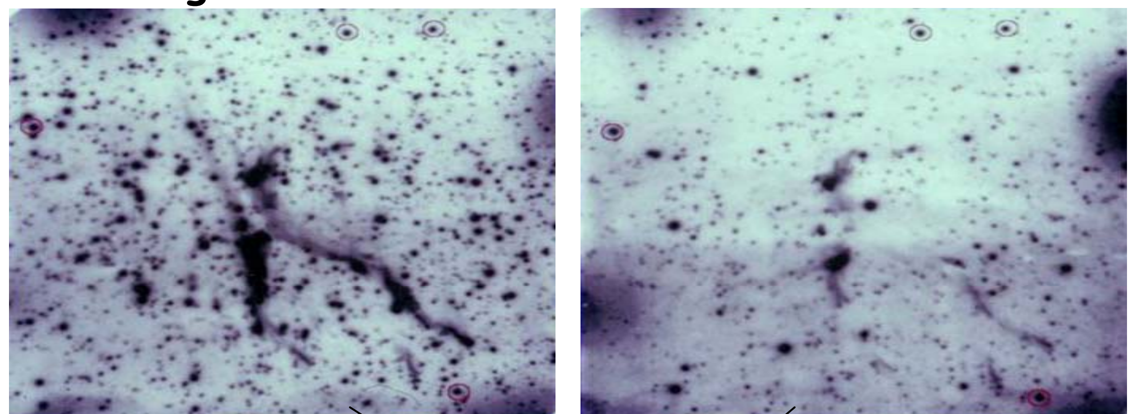
Potential root specific cDNAs

cDNA	#	homology
csp 1	11	Glycin/Prolin rich protein
csp 2	43	no homology in database
csp 3	14	no homology in database
csp 4	4	Cytochrome P_{450}
csp 5	1	Sucrose Synthase
csp 6	1	Arabidopsis mRNA
csp 7	1	rice mRNA
csp 8	1	Glutathione S-transferase
csp 9	1	Pyrophosphatase

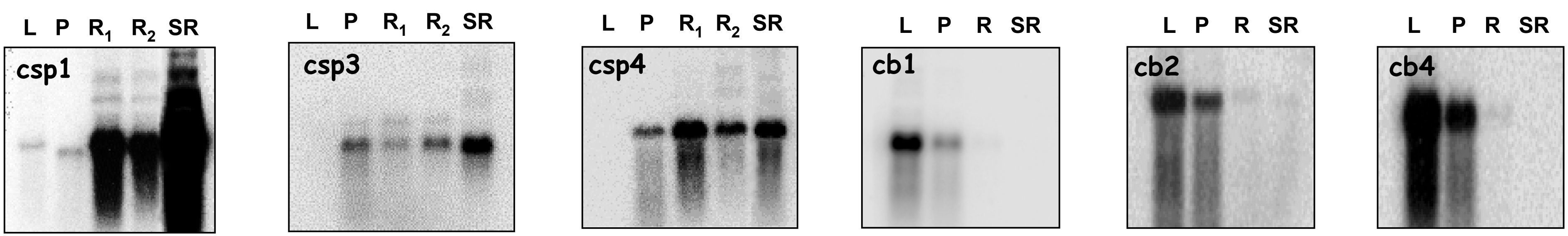
Strongly leaf induced cDNAs

cDNA	#	homology
Cb1	1	fructose 1,6-bisP aldolase
Cb2	1	subunit, oxygen-evolving complex
Cb3	1	cholophyll a/b binding protein
Cb4	1	photosystem I subunit
Cb5	2	ribulose biphospte carboxylase

*storage-root mRNA *leaf mRNA



Identical cDNA replica filters

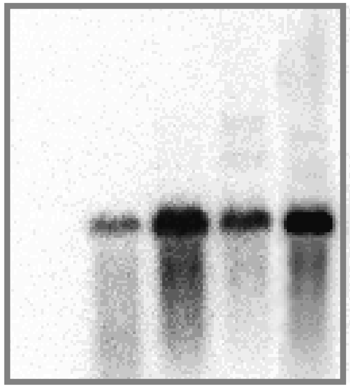


Northern blots showing the expression patterns of some isolated putative tissue-specific cDNAs. (L) leaf; (P) petiole; R) primary root; (R_1) primary root 0-1 mm ∅; (R_2) primary root 1-2 mm ∅; (SR) storage root

cDNA c15 (Poster #13):

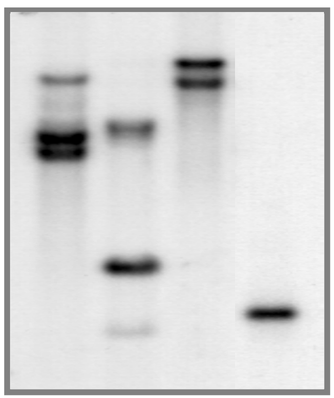
Size: ~1.55 kb (511 aa)
Homology: Cytochrome P_{450}
Two or three genes
Genbank access. no.AY217351

Northern



L: leaf
P: petiole
R_1: primary root 0-1 mm ∅
R_2: primary root 1-2 mm ∅
SR: storage root

Southern



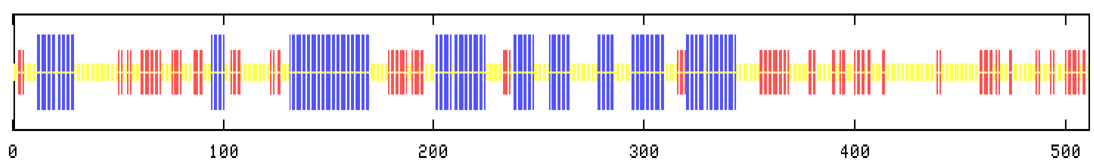
E: *Eco*RI
H: *Hin*dIII
X: *Xba*I
C: control cDNA

C15 protein showed highest similarity to cytochrome P450 CYP71E1 of *Sorghum bicolor*

CYP71E1 is involved in the biosynthesis of L-tyronsine-derived cyanogenic glucoside dhurrin

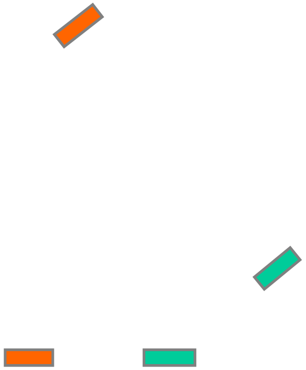
NCBI Conserved Domain Search

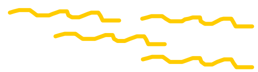
```
C15 :   1    MSVAILTSLPPQWLSILAVFLLPILTLLLFRGKDDNQKKGLKIPPGPRQLPLIGNLHQLG
P450:   1    -----------------------------------------------------------
                                                     Cluster of prolines

C15 :   60   GGQXYVDFWKMAKKYGPVMYLQLGRCPTVVLSSTETSKELMKDRDVECCSRPLSVGPGQL
P450:   1    KGILHSVLTKLRKKYGPIFRLYLGSRPVVVLSGPELVKEVLIKRGEDFSGRPDRAWFATG

C15 :   120  SYNFL--DVAFSPYSDYWREMRKLFIFELLSMRRVQTFWYAREEQMDKMIEILDG--AYP
P450:   61   HGPFRGKGILFSDGPR-WRALRRLLTPTFTS-FGKLSLEPLVQEEADDLVERLRKLAGEP

C15 :   176  NPVNLTEKVFNMMDGIIGTIAFGRTTYAQQEFRDGFVKVLAATMDMLDNFHAENFFPVVG
P450:   119  FSIDITPLLQRAALNVICSILFGVRFDLEDEEALPLVKLVQELLLLVSSPDHQLLDLFPI

C15 :   236  RFIDSLTGALAKRQRTFTDVDRYFEKVIEQHLDPNRPKPETEDIVDVLIGLM-KDESTSF
P450:   179  LLYL-PGPHLRKFKEARKELKDYLDKLIEER----RETLDDDSPRDFLDALLLAKEKDGS

C15 :   295  KITKDHVKAILMNVFVGGIDTSAVTITWAFSELLKNPKLMKKAQEEVRRAVGPNKRRVEG
P450:   234  ELTDEELAATVLDLLFAGHDTTSSTLSWALYLLAKHPEVQAKLREEIDEVLGRG-RSPTY
                    Oxygen binding and activation

C15 :   355  KEVEKIKYIDCIVKETFRKHPPVPLLVPHFSMKHCKIGGYDILPGTTIYVNAWAMGKDPT
P450:   293  DDLQNMPYLDAVIKETLRLHPPVPLLLPRVATKDTEIGGYLIPKGTLVIVNLYSLHRDPE
                                                         ERR triad

C15 :   415  IWENPEEYNPDRFMNSEVDFRGSDFELVPFGAGRRICPGLAMGTTAVKYILSNLLYGWDY
P450:   353  VFPNPEEFDPERFLDENGKFKKS-FAFLPFGAGPRNCLGERLARMELFLFLATLL-----
                                            Heme binding

C15 :   475  EMPRGKKFEDFPLIEEGGLTVHNKQDIMVIPKKHKWD
P450:   407  -----QRFELLPLPGVDPPPILETPGLVLPPKPYKLK
```

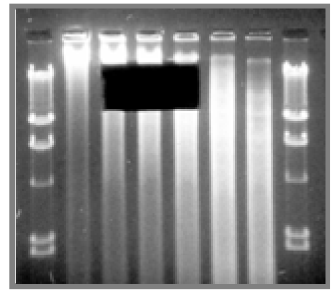


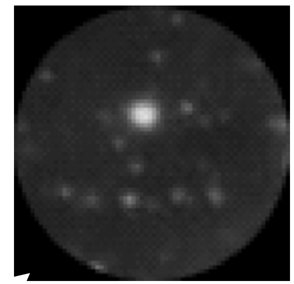
Gruissem/CBN VI

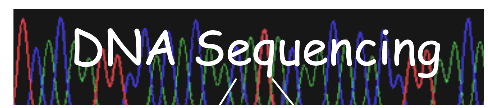
Zhang et al. (2003) Planta. DOI 10.1007/s00425-003-1098-0



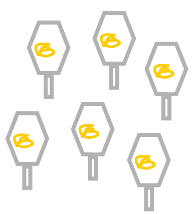








genomic library:



Promoter p15/1.5

Genbank access. no.AY217351

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1     AAATTATATT AAATTTATTT ATAATATAAT AAGTTTAAAT TCGAGTTTAA ATAAATTTAA
61    ATTTAAATTT TTTAAATAGA ATTTAACTCG TTAATATAAT AAATGAATTT ATTACGAATA
121   GAGTTGGAAT CAAATTCAAA TTTAATATTT ATTCTATGTT TATAAATGAA ATTTAAATTT
181   TTAAATTTAT TTTTTAATAA ATTTATTTTA TCAAGATTTA GTTTAGTTGG ATTTGATTAG
241   AGGTGAATAT AAATTGTTTT TAAATTTTAT GTATTTAAAA TAAAGAAATA AATGATTAAT
301   AAGCACAGAA GTGAAATTTT GTAAAAAAAT TAATGAAATT TAAGTCCCCA TGCGTTGGTG
361   AAGGCAGGTG CAATAATTCA AACGTGCGTG CGGATATATG ATAAAACAGT TAGCAAATTA
       Athb-1 <---------------                          -------------> MYB.Ph
421   TAACACGTGG CGCTAGAGAA TCCCAACTAC CTTCCGTTTT CTCTATTGTC ACGAGTTACA
481   AATGAAATCA CCTACCAAAT CTGTAACTAT TAATTCTTTA TTTTATTTAC TTTTATCGTT
                ---------> P
541   TATTATTTTA CAGACGTGGT AGCTGGTGCT GTACCACCAC GTGCCAATAA TTTTAATGTT
                     P <---------               Athb-1  <--------------
601   TAACGGAGTA TTCAATATTA CGTATAAAAT AAATATTTAT TAAAAATATT TTATTCAATT
661   TATAATATAT AAATTTATCT CAATATTTAC TTTATTTATA AAAATCAAAC TTAAACAAAT
721   TCGTCAAAAG TACAGAATTT TAATTTGTTC TCATATGCAA GAAGTCTATT CTCGTAGGAT
781   GAGTTTAGTG CACGAACTTC AATGACATAG GTGTTGAGCT CCTTGTCATC GATTTGATAT
841   TACAAATTTT ATTTTTTTAA AAATAAAAAA TATAAAAATT ATTATATAAT TTTATTAAAT
901   TTTTAATTTA AAATTTATAA TTTAATATAA AACATTTAAT TTAATTTTAT ATTTAAATAA
961   TATCTCATAA CATCATATCA TTTATTAATA AATTTTAATA GGCAAATTGA GTAAATTTGA
1021  AATTTATAGT GGGAGTAAAT GAAAGCTTTG GCAACTTGCC TCGATGATTG AAGTTCAATA
1081  ATCAGAAAAG TACGAAAAAG CCAGAAAGTC ATAAGGGAAA CTAGAGGAAA AATTAAATAG
1141  AATTGGCGTA TACATCATTA AATTTCCACC AAACAAATTG CACAATCTTC ATTTGGAATC
1201  TTATTTCTTG GCTTTAATTA TATCAGCATC TTTCCTTCCT TTCTTTTAAA TAAAATAAAA
1261  TACATACACT CCTGCCTTTA TTAGCCGAAG GTTTTGGGCT TTTCCTTTTT TTATTTTGGT
1321  ATTTAGTCTG TATATAAAAA TGGTATTAAA ATAAAAACGT GCTCACCCAA CTCTTGATAT
1381  AAGTAAATTT TTAGAGTCGA TTATATAAAG GGCGTGGGGG GTGCTTGCAA CTCCNCAAGG
1441  GCAGGAAACC AAAACAAAAT TAAAGATG
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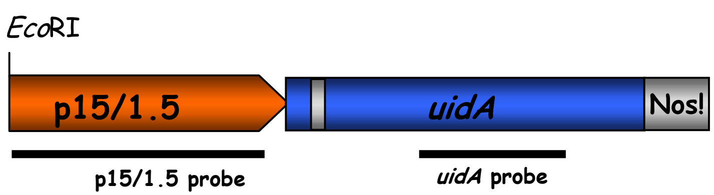
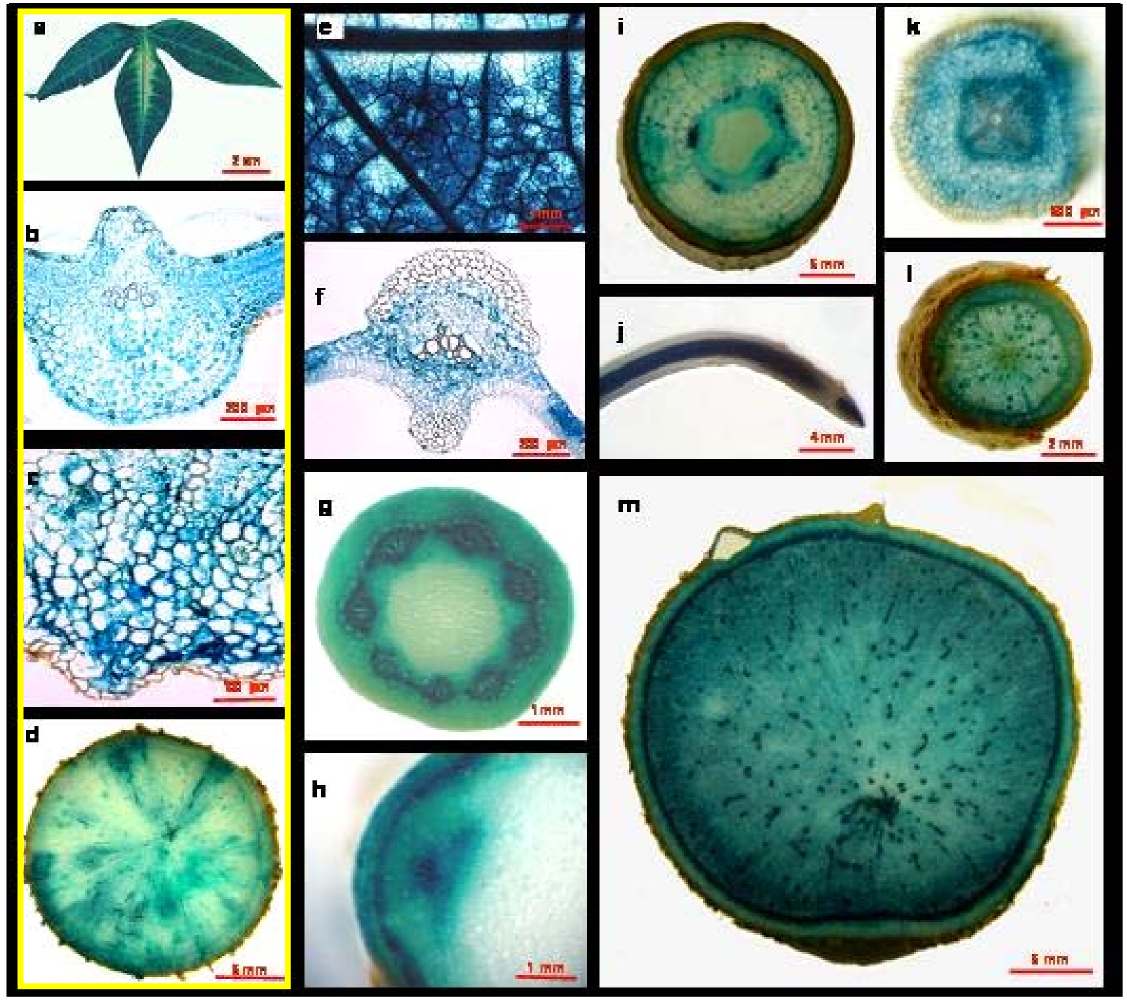
TFSEARCH: Searching Transcription Factor Binding Sites (ver 1.3)

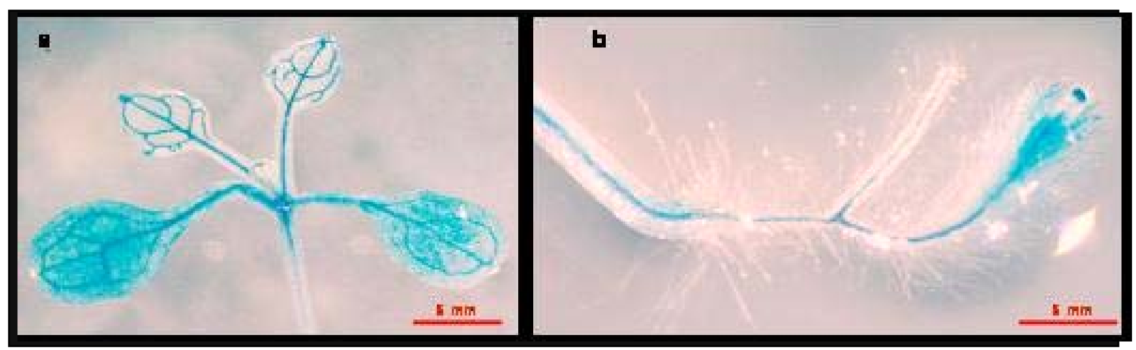
Athb-1: *Arabidopsis thaliana* homeo box protein 1.
MYB.Ph: Myb-like protein of *Petunia hybrida*.
P: maize activator P of flavonoid biosynthetic genes.

PlantCARE: Determining plant cis-acting regulatory elements



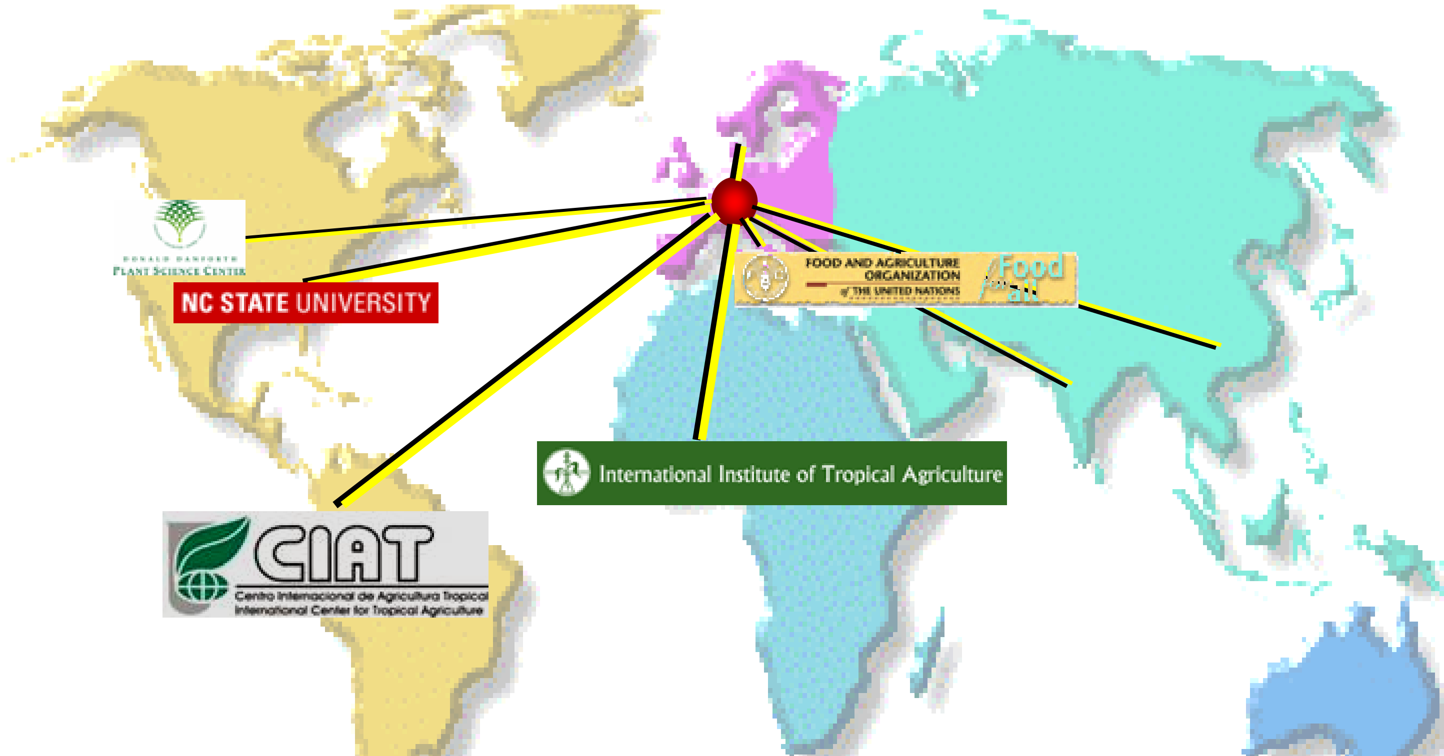
Gruissem/CBN VI





Zhang et al. (2003) Planta. DOI 10.1007/s00425-003-1098-0

The ETH Cassava Group is a member of the Cassava Biotechnology Network and the Global Partnership for Cassava Genetic Improvement



- Development of improved genotypes
- International scientist training
- Share of scientific information
- Biosafety and GMO acceptance

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

The ETH Cassava Team

Peng Zhang
Johannes Fütterer
Hervé Vanderschuren
Wilhelm Gruissem
Ingo Potrykus (Emeritus Professor)
Susanne Bohl-Zenger
Johanna Puonti-Kaerlas

http://www.pb.ethz.ch

In collaboration with:

CIAT (Colombia)
IITA (Nigeria)
Linda Hanley-Bowdoin (NCSU, Raleigh)
Jesse M Jaynes (NovaTero Foundation)
Thomas Hohn (Friedrich Miescher Institute)
Richard Amasino (University of Wisconsin-Masison)

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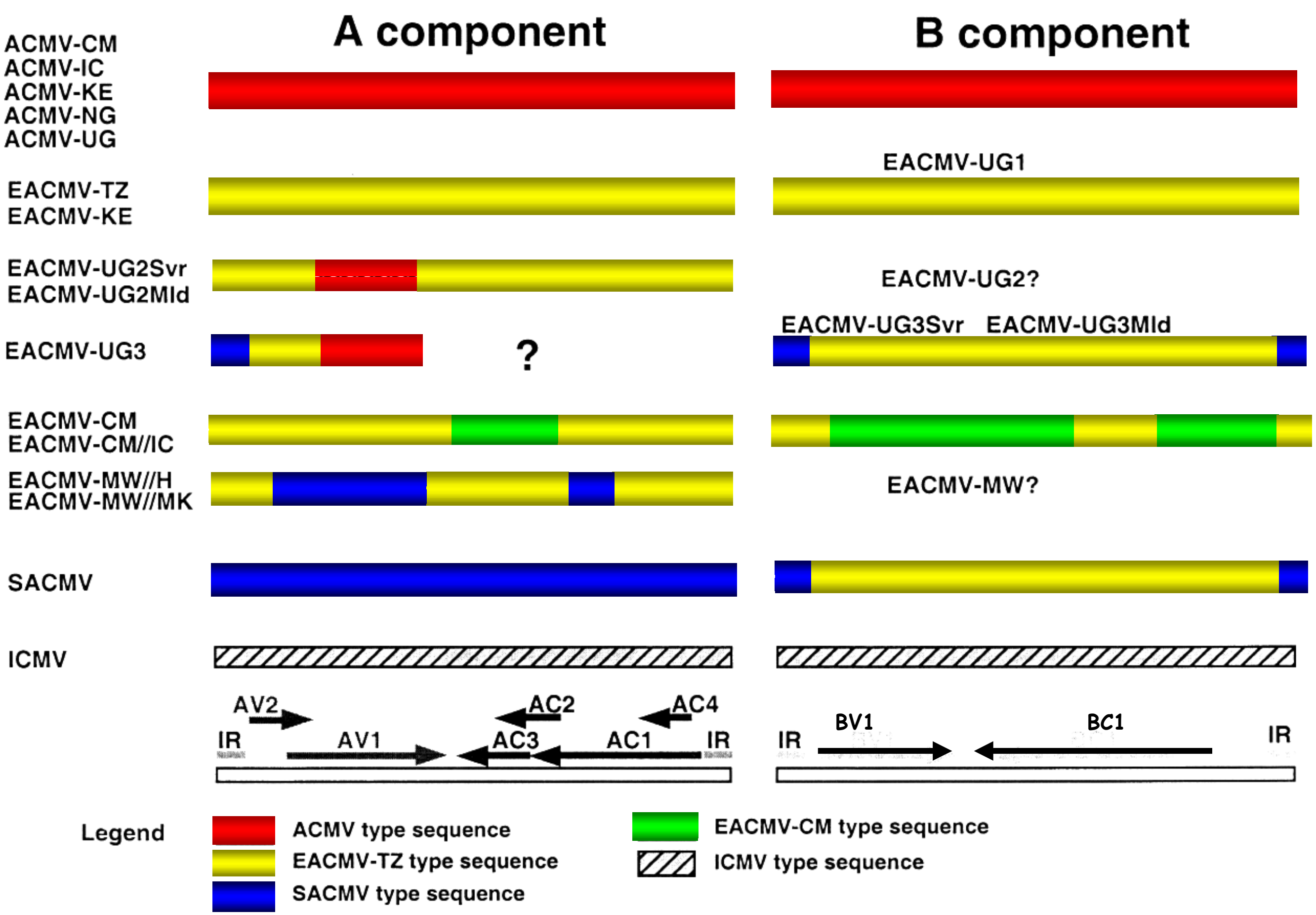
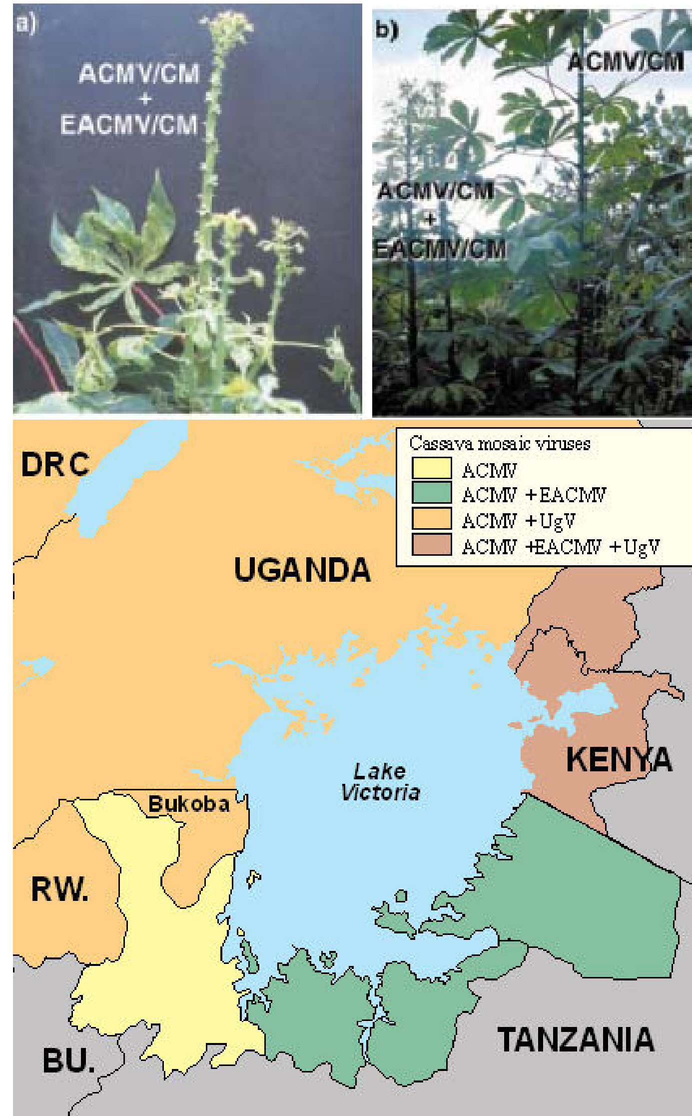
Natural Pseudorecombination and Synergistic Interaction between Cassava Geminiviruses





From *Legg & Thresh, 2000*