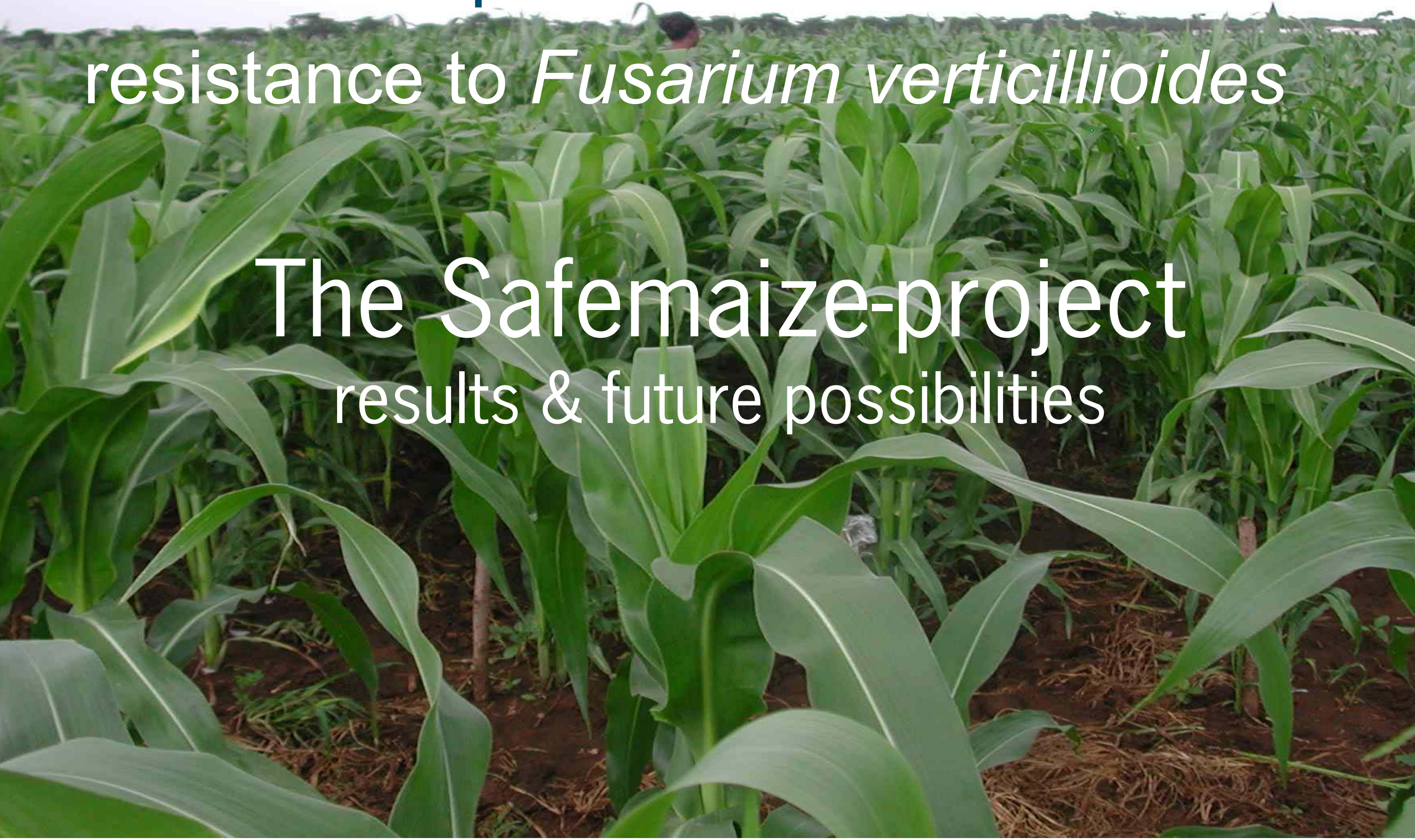
Towards improved maize with resistance to *Fusarium verticillioides*



The Safemaize-project

results & future possibilities

PLANT RESEARCH INTERNATIONAL

The Safemaize-project

Genetic improvement of maize to enhance food safety by introducing resistance to *Fusarium verticillioides*

EU-INCO Project January 2001 - June 2004

PLANT RESEARCH INTERNATIONAL

Why Safemaize-project?

- Maize is the single most important staple food in countries of the Southern African Development Community
- Often, maize produced in SADC countries such as Zambia, Zimbabwe and South Africa, contains too high toxin levels due to infection with *F. verticillioides*

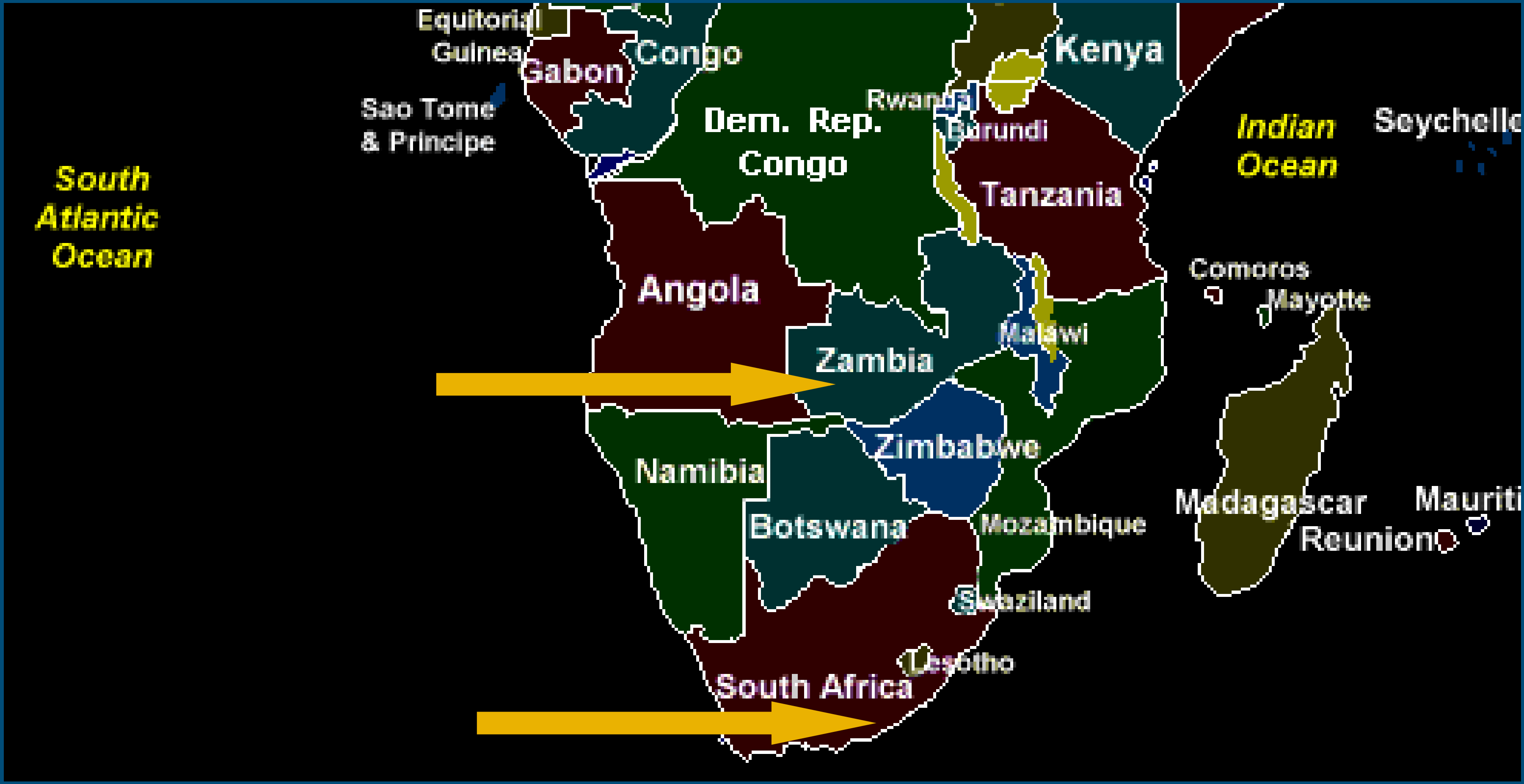
The Safemaize Project Partners

- University of Pretoria (co-ordinator)
- CSIR, Pretoria
- ARC-Roodeplaat, Pretoria
- University of Zambia
- University of Rome
  - Maize breeding, Bergamo
- PRI

General aims of the Safemaize project

- To characterize *Fusarium verticillioides* isolates from Africa.
- To develop screening methods to evaluate maize genotypes for resistance to the fungus.
- To produce transgenic maize with stable inheritance of selected plant anti-fungal genes, and evaluate resistance in the field.

Southern Africa: isolates collection



Fusarium verticillioides isolates from Africa

- Collection of *Fusarium* isolates
  - 60 isolates from Zambia
  - 23 isolates from MRC (mainly from Transkei)
- Characterisation
  - species specific primers (Waalwijk)
  - AFLP

Species specific primers

Origin	n	Fvert	Fsub	Fprol
		Species specific primers		
UNZA	58	43	1	1
MRC	23	22		

Fvert=*F. verticillioides*, Fsub=*F. subglutinans*, Fprol=*F. proliferatum*

- *Acknowledgements: C. Waalwijk (PRI), A. Logrieco & G. Mulè (ITEM)*

Species specific primers

		Species specific primers			Fumonisin production					
Origin	**n**	**Fvert**	**Fsub**	**Fprol**	**+++**	**++-**	**-++**	**-+-**	**--+**	**---**
UNZA	43	43			32	1	4	5*		1
	13				1	1	1			10*
	2		1	1					2	
MRC	23	22			22					1

Fvert=*F. verticillioides*, Fsub=*F. subglutinans*, Fprol=*F. proliferatum*,

Fum prod=fumonisin producer (FUMF/R, VERTF-1/2, PKS-exon5F6R)

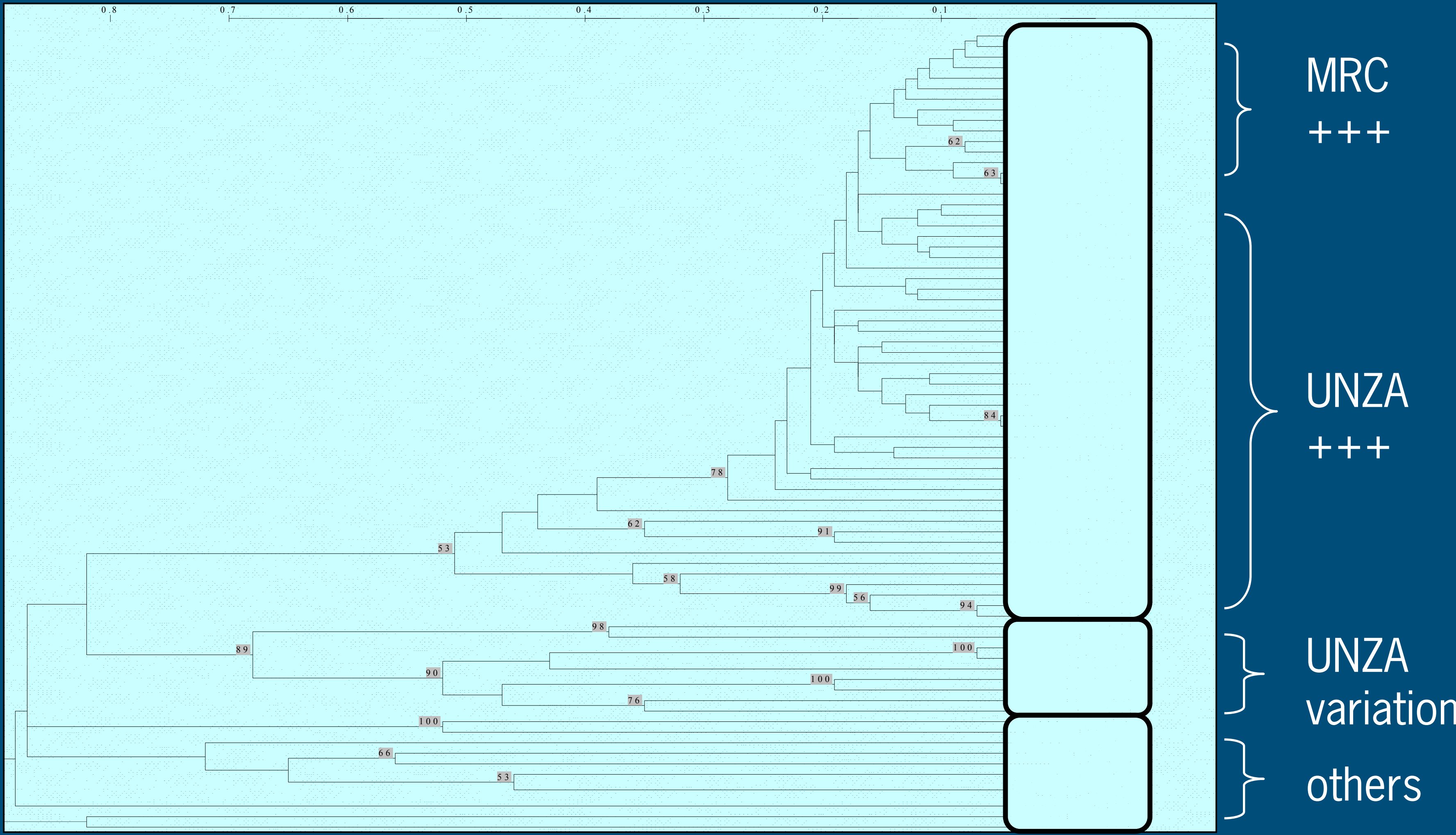
* *F. longipes*, *F. equiseti*, *F. graminearum*, unknown, ** *F. sambucinum*, *F. subglutinans*

■ *Ackn: C. Waalwijk (PRI), A. Logrieco & G. Mulè (ITEM) , J. Rheeder (MRC)*

UPGMA tree

Nei and Li distance

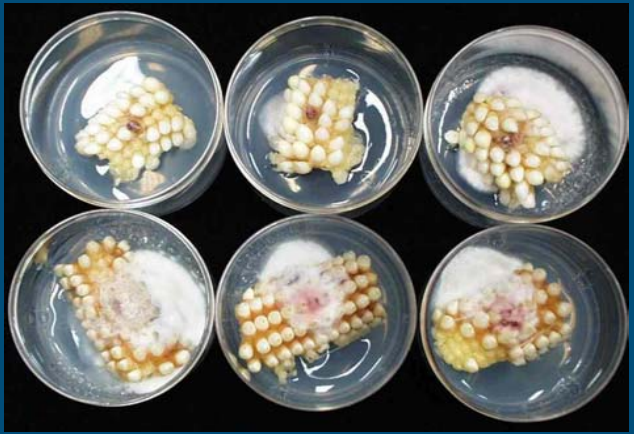
Bootstrap values above 50%



Screening methods

- Greenhouse/in vitro test in the Netherlands
- Field test in Zambia
- Aims:
  - to screen germplasm for resistance
  - to screen transgenic maize

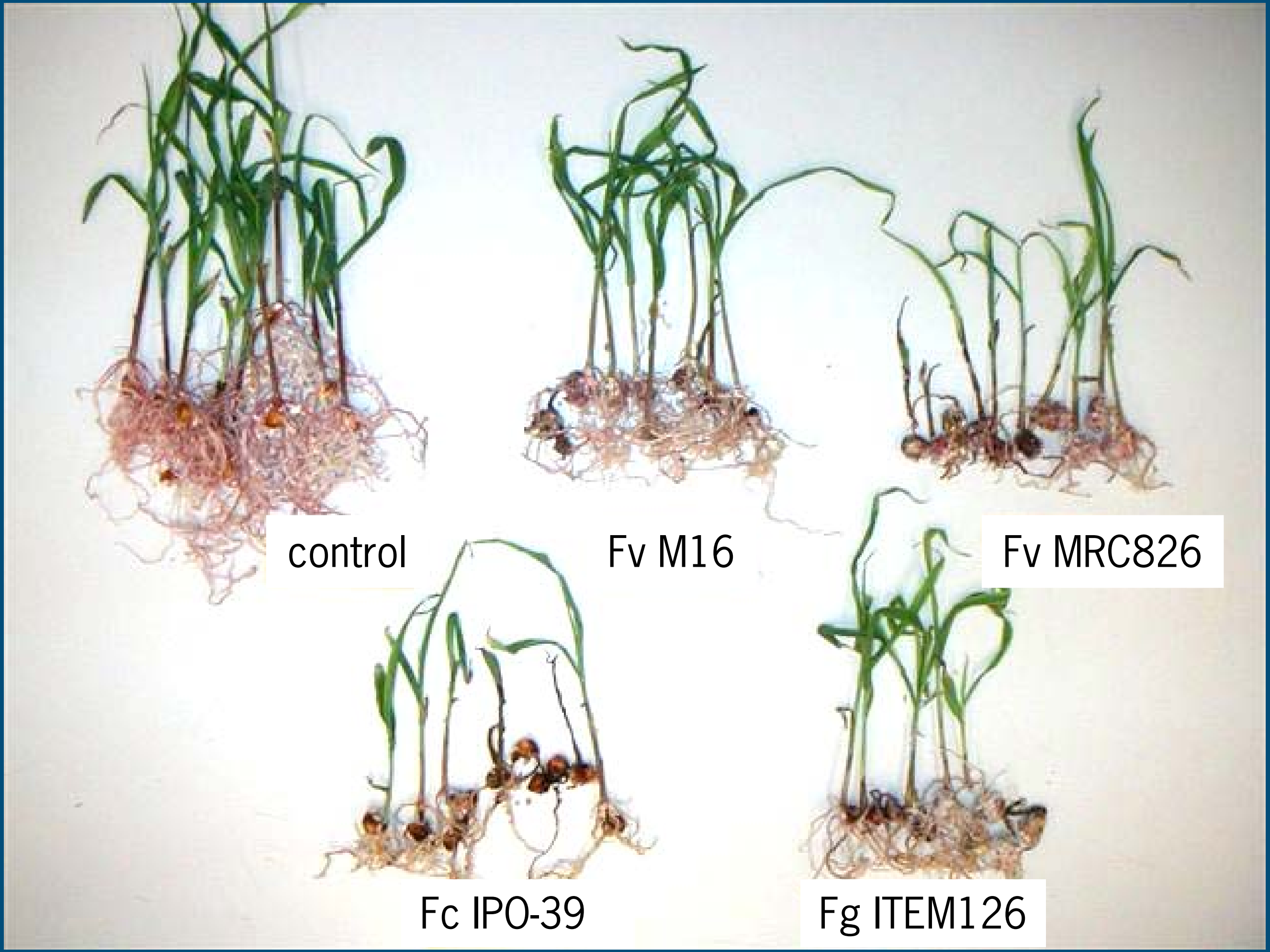
Greenhouse test in the Netherlands



Good correlation

between years (r^2=0.77)

Seedling assay: aggressiveness



Field experiment in Zambia

- Carried out at the Golden Valley Research Trust
- Maize growing season Nov- April
  - 2001/2002 very dry
  - 2002/2003 good
  - 2003/2004 good

Field test in Zambia

- Silk channel inoculation method

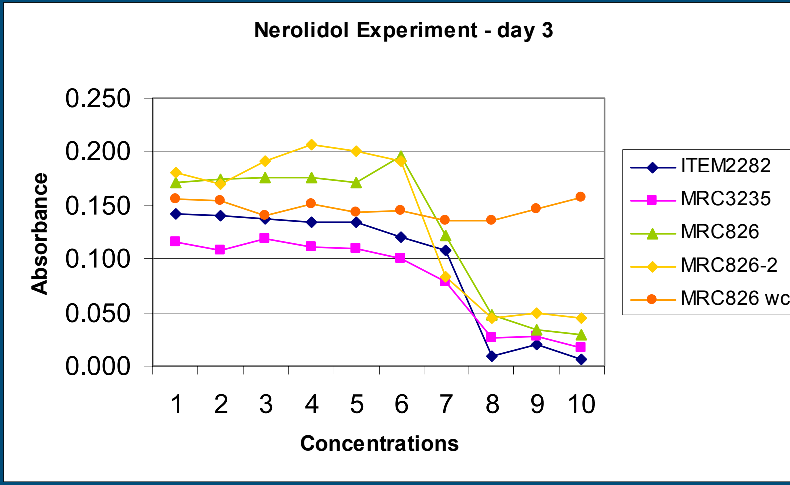
Field test in Zambia 203/2004

		Blotter	PDA
CO255	Netherlands	7.5	10.0
LO 1096	Italy	15.0	15.0
6705-60	Mexico	10.0	15.0
A722-2	Zimbabwe	12.5	17.5
1725-29	Mexico	15.0	25.0
LO1010	Italy	11.7	20.0
A722-4	Zimbabwe	20.0	23.3
6705-56	Mexico	16.7	23.3
PAN 6363	Zambia	13.3	26.7
MRI 455	Zambia	20.0	30.0
MRI 624	Zambia	16.7	33.3
LO1124	Italy	30.0	33.3
F113	Netherlands	21.7	35.0
GV 412	Zambia	20.0	36.7
MRI 514	Zambia	20.0	36.7
MM 502	Zambia	16.7	40.0
MRI 734	Zambia	20.0	43.3

Genes available for transformation

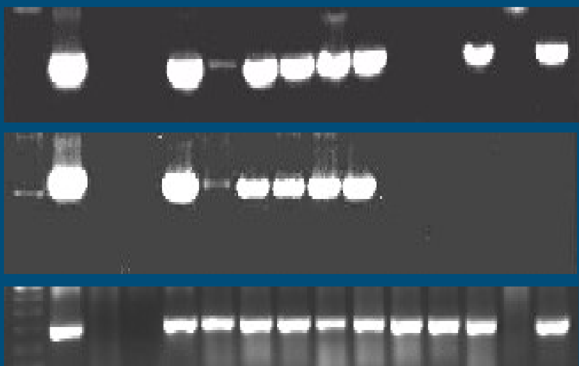
- *Pvpgip2* gene (polygalacturonase inhibitor gene)
- maize *b-32* gene (ribosome inhibiting protein)
- chitinase gene
- terpene synthase genes

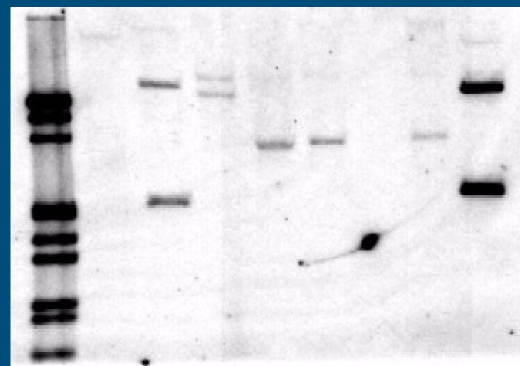
M. Jongsma PRI



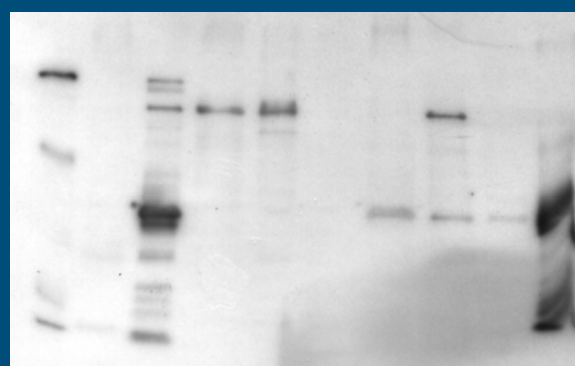
Plant transformation: B32 and bean *Pgip2*

Transgenes are inserted into maize genome: PCR, Southern blot



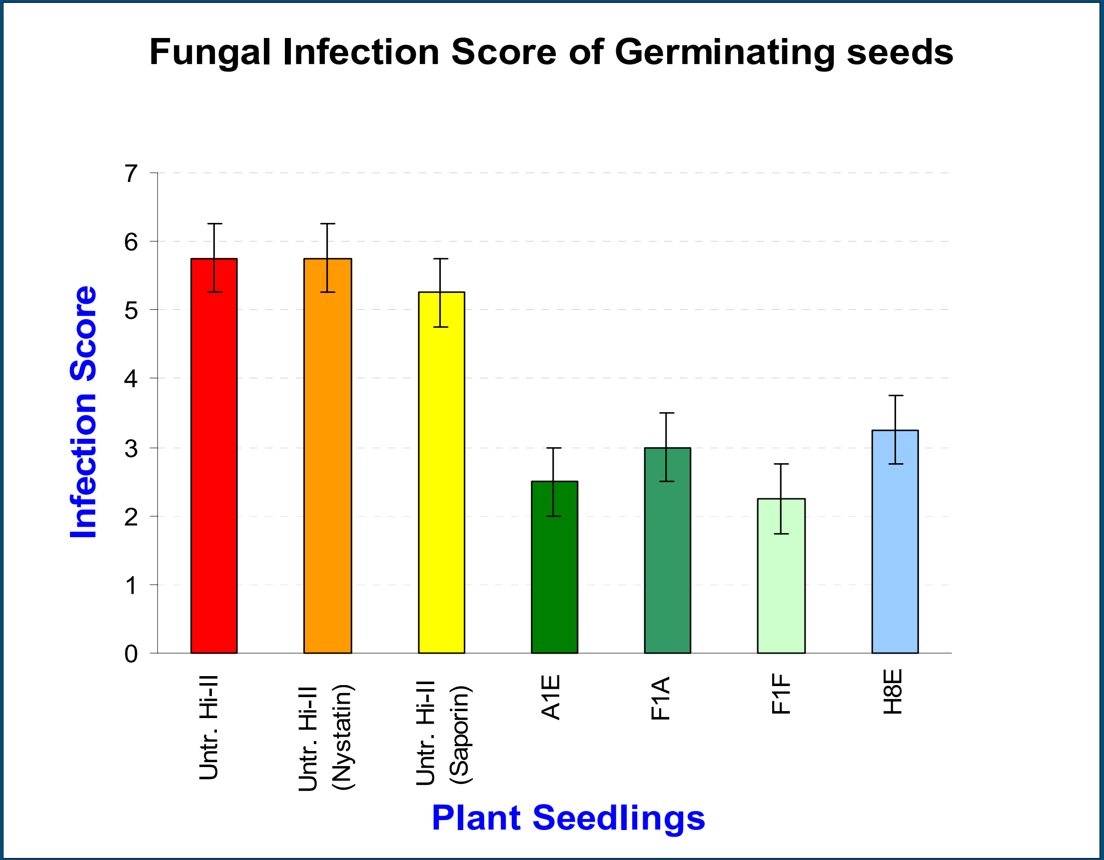


Transgenes are expressed: Northern blot, Western blot



Plant transformation: achieved

Transgenic maize shows fungal resistance: laboratory test



Plant transformation: achieved

- Putative T0 transgenics in pots under greenhouse conditions [plants are dwarfed due to tissue culture stress]

Plant transformation: achieved

T1 plants in greenhouse

T3 plants in larger greenhouse, 3 m old

Field Experiment 2003/2004

- GMO Field experiment has been carried out in South Africa instead of Zambia, because it is not allowed to grow GMO maize in Zambia
- Results: look promissing, wait for plating results

Safemaize project training

- Guest workers
  - from UNZA to PRI
  - from University of Pretoria & ARC to University of Rome
- Training included
  - involvement in plant pathological aspects
  - molecular biology

Safemaize conclusions

- Collection of Fusarium isolates
- Screening assays have been developed
- Variation in resistance levels is present > breeding!
- GMO-plants with additional plant defense genes have been obtained
- Lab-assays show improved levels of resistance
- Training

How to improve the situation?

Further research

Plant
GMO/non GMO
resistance genes
selectable markers
cultural measures

Fungus and toxin
infection process
isolates/species
aggressiveness
mixtures of toxins

Medical research
influence of other food types
relationship between diet and health
toxin mixtures

Social impact
awareness of toxins
acceptance new varieties and other food