

Novel Platforms for Essential Biomolecules: Expression of Antibodies in Plants.

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Challenges of conventional protein expression platforms

Protein based drugs fastest growing class of new drugs for treatment and prevention of human disease.

But we face these barriers:

- Capacity:
 - *Insufficient capacity for drugs in the pipeline*
- Cost
 - *Cost of goods*
 - *Capital for manufacturing facilities*
- Safety
 - *Risk of contamination with mammalian pathogens*
 - *Many require needles, which are risky, costly*
- Efficacy

Our Goal

To develop a transgenic plant platform for the production of molecules to prevent and treat infectious disease, at levels that are relevant for commercial development



Advantages of the plant expression platform

Plants are the most efficient producers of proteins on earth

- **Plants are scalable bioreactors**
- **Plants provide cost advantages**

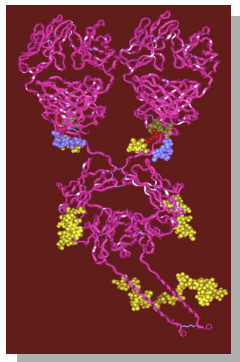


Plants cells are similar to human cells in many ways

- **Similar protein synthesis machinery**
- **Read the same genetic code**
- **Assemble, fold and secrete complex proteins**



A Novel Approach to Pharmaceutical Production



Identify gene for virus neutralizing monoclonal antibody gene

Transform plant cell with antigen gene



Product Formulation



Picture courtesy Charles Arntzen



Picture courtesy Charles Arntzen

our future through science

Biosafety

- **Cognisant of the need to protect the environment and preserve the sanctity of the food supply, our group will exercise the highest level of stewardship,**
- **Where maize is used as a host plant, and in light of its role as a staple crop in Southern Africa, the work with maize will be implemented under strict genetic containment. Biosafety measures will be built into every aspect of the project, including bulking up of select events under strict containment and breeding of select events with elite male sterile lines.**
- **In the expectation that the results from this study could go forward eventually to cGMP manufacturing and Phase I and II clinical trials, all the relevant work will be carried out under Good Laboratory Practice (GLP).**

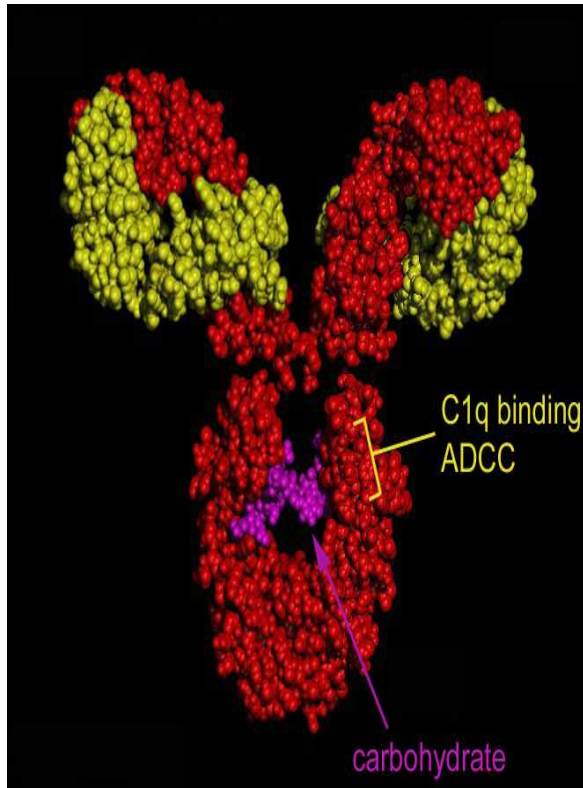
Target molecules for the CSIR Transgenic plant platform

**Microbicidal
peptides**

Antibodies

**Subunit
vaccines**

Focus on Recombinant Monoclonal Antibodies (MAbs)



- Inherently stable human mucosal surface defense proteins
- High specificity; low toxicity
- Injectable, topical and oral applications
- Appropriate for chronic conditions
- Potential long-lasting benefits

High potential as microbicidal molecules

Antibodies are effective drugs for many indications

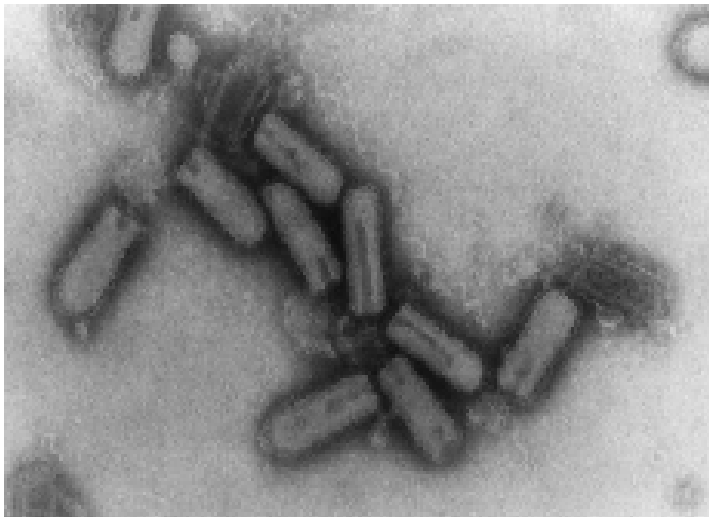
<u>Antibody</u>	<u>Indication</u>	<u>Developer</u>	<u>Launch</u>
<i>Reopro</i>	Coronary restenosis	Centocor	1995
<i>Rituxan</i>	NHL	IDEC	1997
<i>Remicade</i>	RA and Crohn's	Centocor	1998
<i>Synagis</i>	RSV	MedImmune	1998
<i>Herceptin</i>	Breast Cancer	Genentech	1998



Production of the rabies monoclonal antibody E559 as model antibody in transgenic plants

Rabies: The disease

- acute viral disease of the central nervous system
- affects humans and other mammals
- If post exposure treatment is not administered, disease is 100% fatal



Caused by the caused by a rod- or bullet-shaped virus in the family *Rhabdoviridae*

patient is vaccinated immediately administration of human rabies immune globulin (HRIG) or equine (ERIG) for passive immunization in wound and muscle, followed by active immunization.

Rabies antibodies: developing the plant platform

- Rabies is an important disease in the developing countries, particularly in Asia but also in Africa.
- 10 Million people receive ERIG or HRIG prophylaxis annually
- Key target disease for Pharmaplanta,

Rabies antibody production in plants to be used as model for other high value antibody targets

Production of the rabies monoclonal antibody E559 in transgenic tobacco

Production of Pharmaceuticals in Tobacco

Advantages

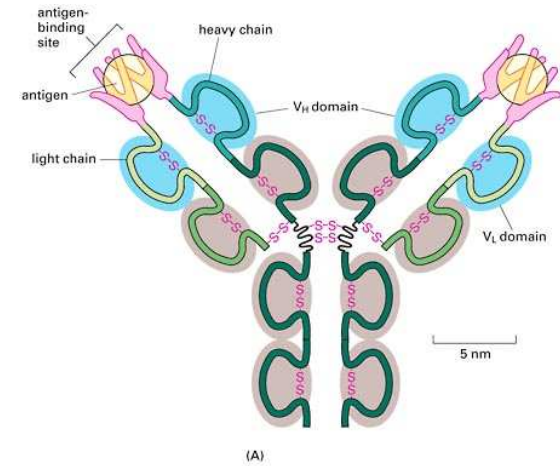
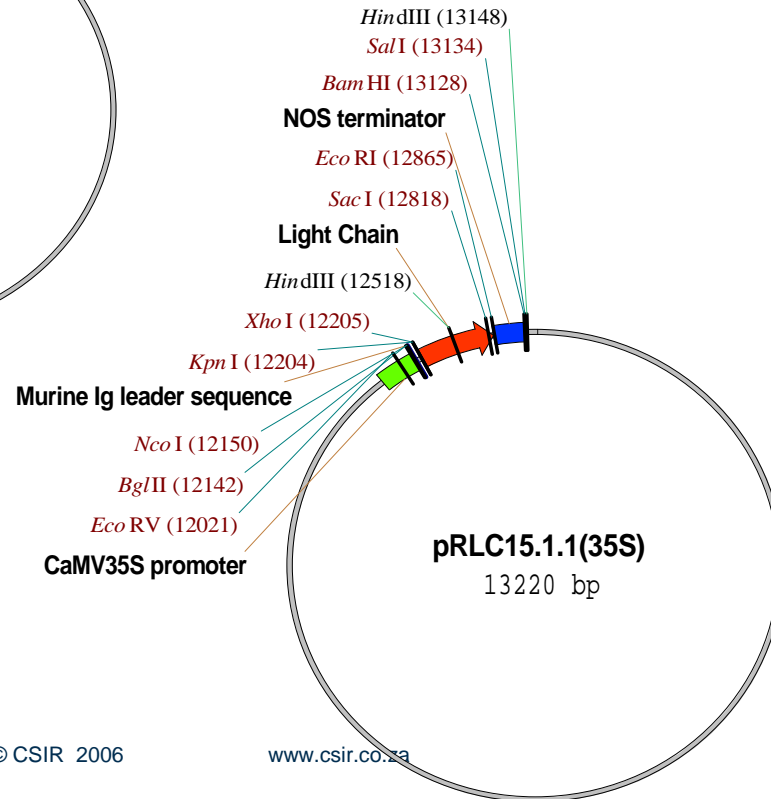
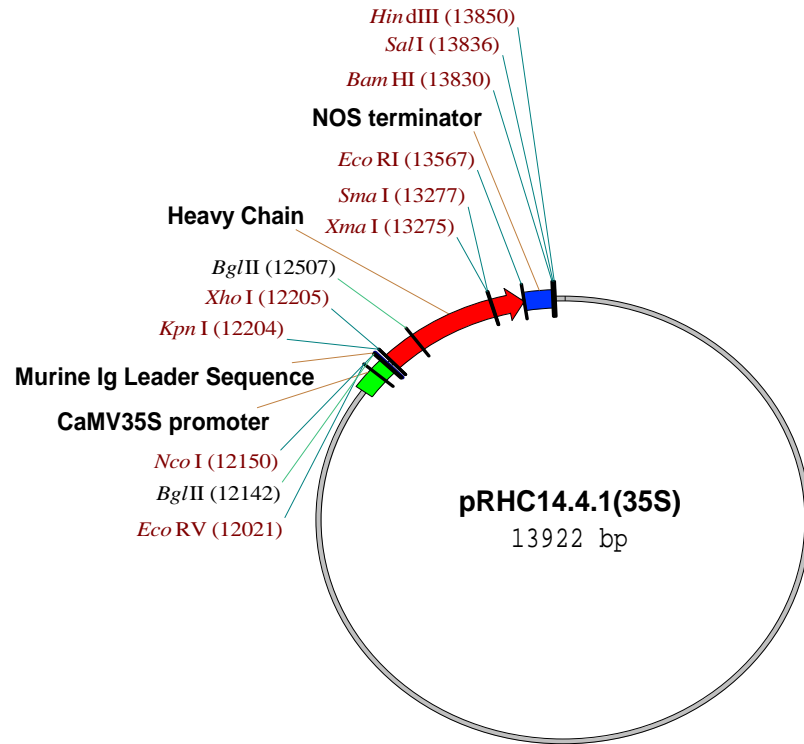
- Well established technology for gene transfer and expression
- High Biomass yield (100,000 kg/ha)
- Prolific seed production
- Good regulatory elements
- Large scale processing infrastructure
- Non food / feed crop



Key disadvantages:

- Requires immediate freezing, drying or other processing
- High Nicotine/alkaloid content making purification expensive

Plasmids encoding antibody chains for tobacco transformation

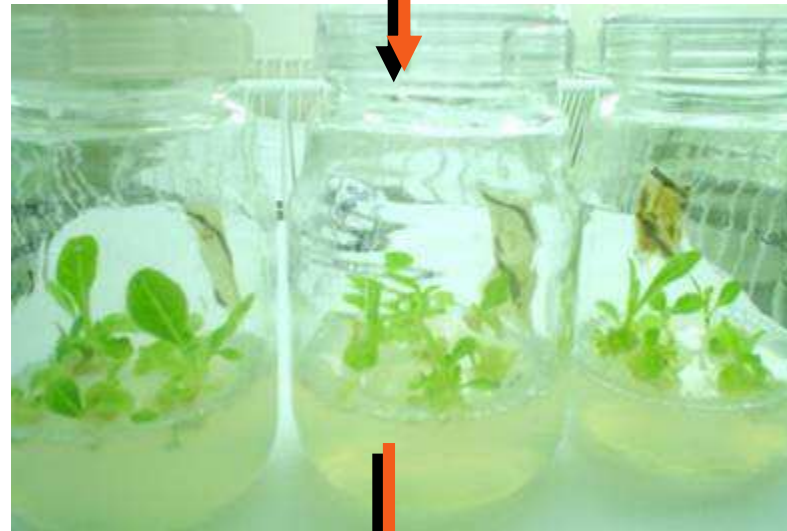


Tobacco Transformation

Agrobacterium transformed tobacco leaf discs



Transgenic plants expressing heavy and light antibody chains separately

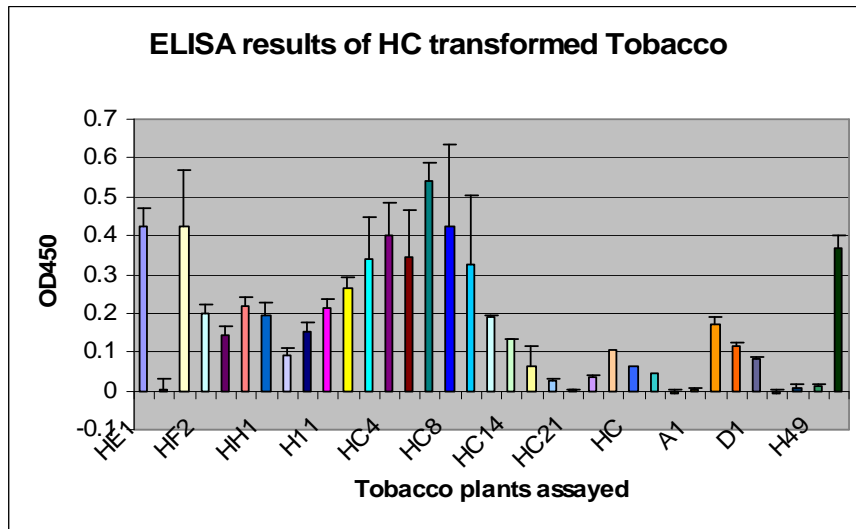


Lines expressing heavy and light chains transferred to the green house. Plants with HC and LC crossed, and seedlings growing *in vitro*.



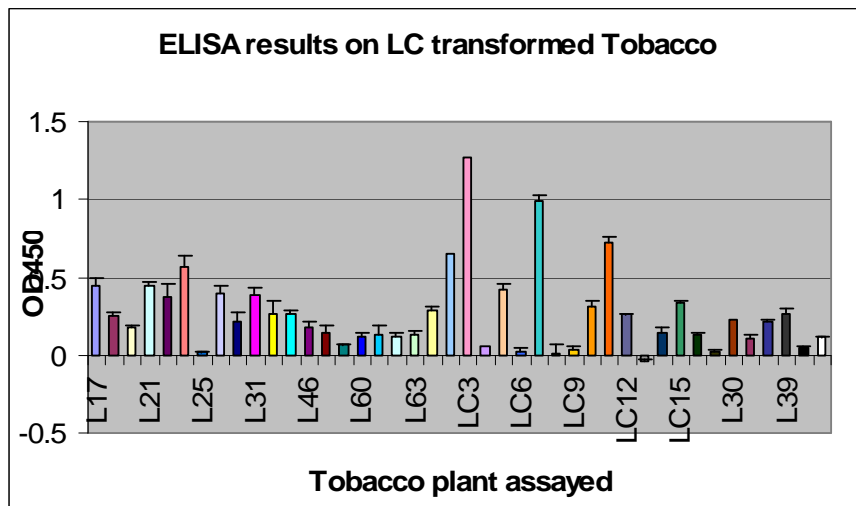
Non-transformed tobacco leaf discs

Screening of MAb E559 Expression in Tissue Culture Plants



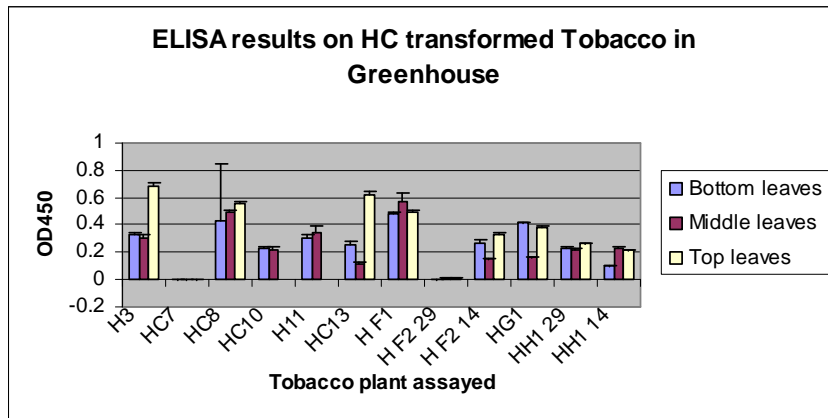
A total of 35 plants assayed for heavy chain expression 23 showed expression

All data corrected for background absorbance. New standard acquired for quantification of expression levels by ELISA



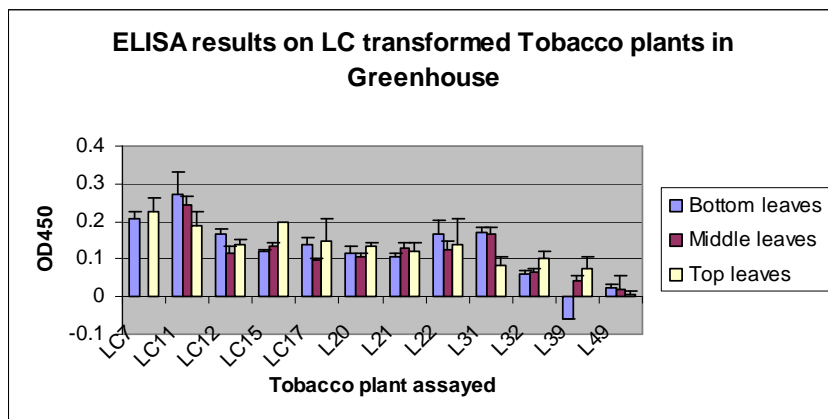
A total of 42 plants assayed for heavy chain expression 33 showed expression

Screening of MAb E559 Expression in Greenhouse Plants



Only some of the plants survived transplantation into greenhouse. Not all plants showed expression once moved to the greenhouse.

All data corrected for background absorbance, three leaves at different stages assayed per plant. New standard acquired for quantification of expression levels by ELISA



For the LC, plants show consistent expression between all three leaves

Future perspectives

1. Evaluation of hybrids expressing heavy and light chains
2. Breeding programme to develop lines expressing high levels of functional antibody (target of 0.1% of total soluble protein)
3. Extraction of functional antibody from crude extracts
4. Evaluation of efficacy of the plant made antibody against infective rabies virus in collaboration with other partners

Progress on the Expression of the Rabies MAb E559 in Maize

Production of Pharmaceuticals in maize

Advantages:

- Seed allow for long term storage
- Seed are specialized are protein accumulating organs
- Dry grain concentrates product
- Simplified processing and purification



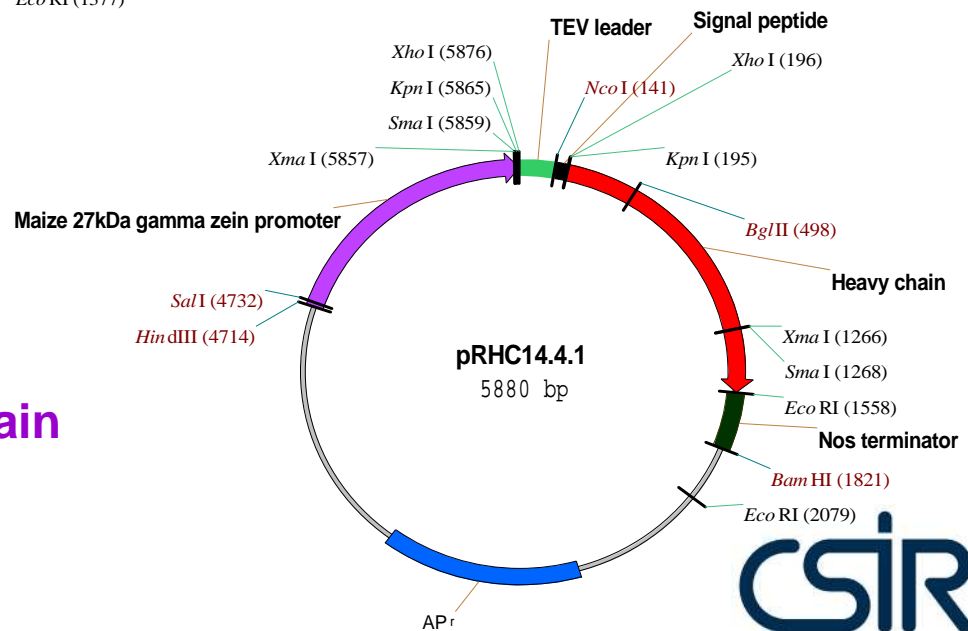
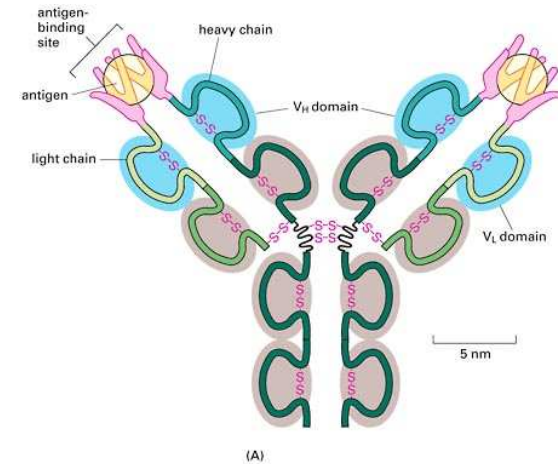
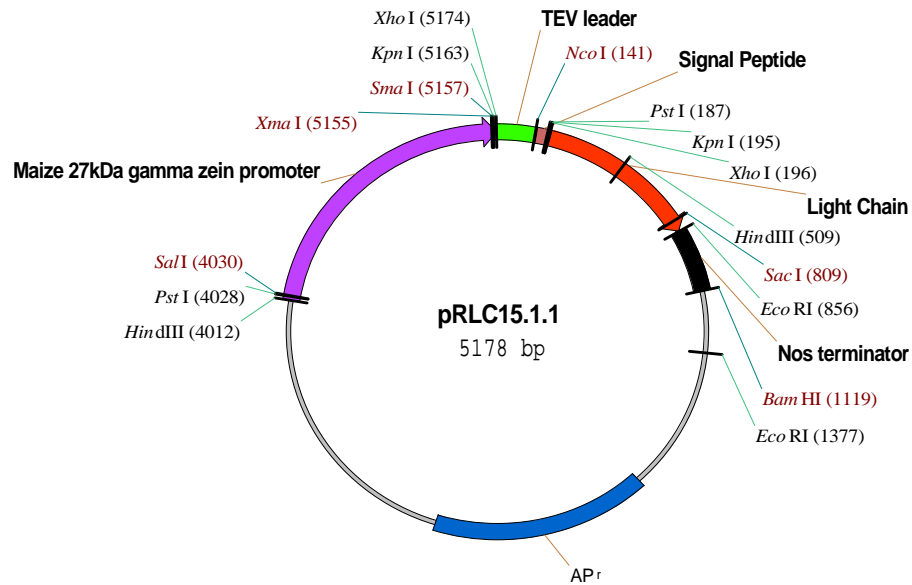
Key disadvantages:

- Biosafety issues with pollen dispersal and potential for contamination of food/feed crops
- Food crop/public perception issues

Proactive biosafety measures:

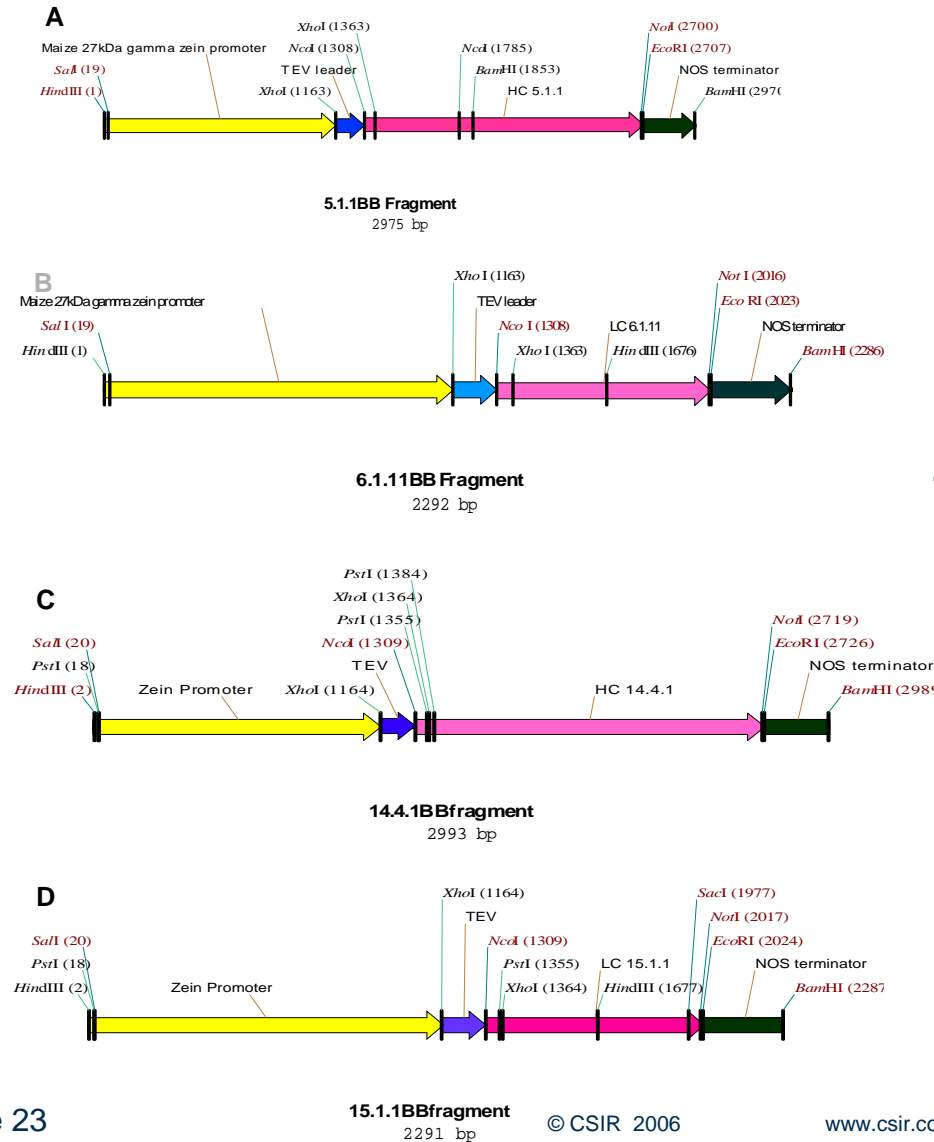
- All crossing to be done under containment in green houses
- Field bulking facilities also under containment
- In future, color markers, male sterile lines will be employed

Cloning vectors for maize transformation



Designed both heavy and light chain constructs to be transformed into maize

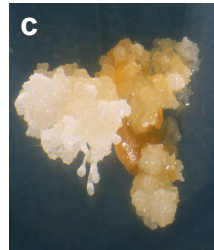
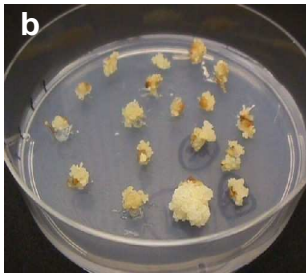
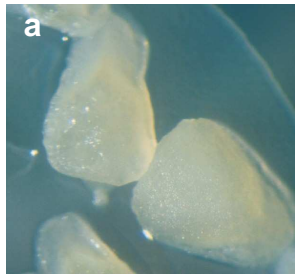
Minimal cassettes used for transformation



For biosafety reasons, only the gene expression cassettes were used. No antibiotic markers introgressed into the transgenic plants. Mannose (sugar) selectable marker system used

Maize transformation

Blast genes with
particle gun into
embryos



Genes = Heavy Chain

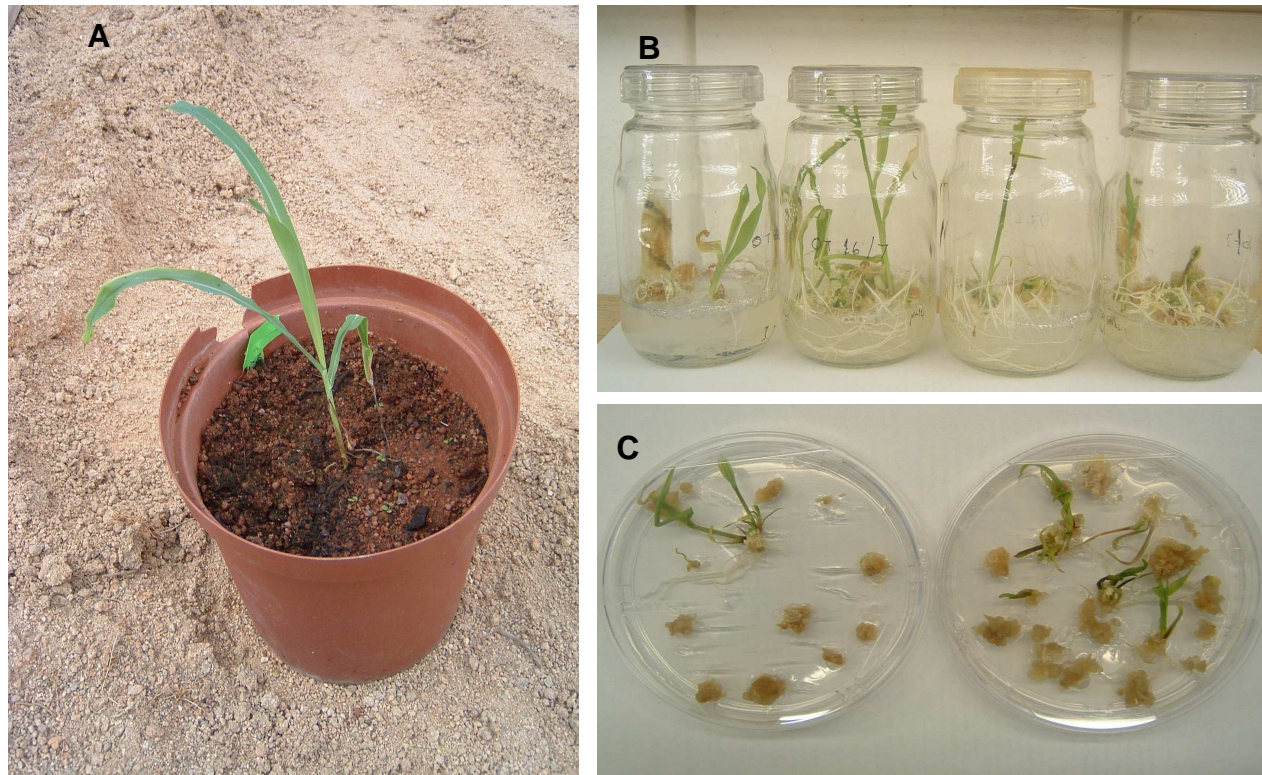
Light Chain

Mannose (marker)



Current Status:

Transgenic maize plants just recovered



- Transgenic plants co-bombarded with both HC and LC undergoing molecular analysis to confirm transgene integration.
- Evaluation of gene expression to be undertaken in maize seed

Future perspectives for the platform

- **New, relevant and high social impact targets**
- **New vectors for faster transient expression**
- **Custom made plant hosts**

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