The effect of expressing an anti-HIV lectin, Griffithsin, in different plant cellular compartments

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our future through science

HIV in Africa

- Sub-Saharan Africa remains the region most heavily affected by HIV
- Majority of new infections from heterosexual contact in women
- The cervical/vaginal mucosa is the main entry point of HIV in women (WHO/UNAIDS: Report on Global AIDS Epidemic.May 2006b and December 2009)

Microbicides

- A ***microbicide*** is any compound or substance whose purpose is to reduce the infectivity of microbes, such as viruses or bacteria

- Mode of action
  - Destabilizing virus structures
  - Preventing docking/entry
  - Preventing HIV replication
  - Combination drugs

  (Stone and Jiang, ww.thelancet.com Vol 368 August 5, 2006)

- Lectins have a unique interaction with the virus and presents a novel approach to target the virus

- Need for cost effective production for distribution to poorer populations

Griffithsin

- Griffithsin (GRFT) was isolated from the red algae *Griffithsia* sp.(Mori *et al*, 2004)
- GRFT is effective in low concentrations against both primary as well as laboratory-adapted HIV isolates of different clades
- GRFT is very stable and potent in cervical/vaginal lavage fluid
- GRFT did not show toxicity to the mucosal epithelial cells
- The molecular target is the gp120 envelope glycoprotein
- GRFT has multiple mannose binding sites

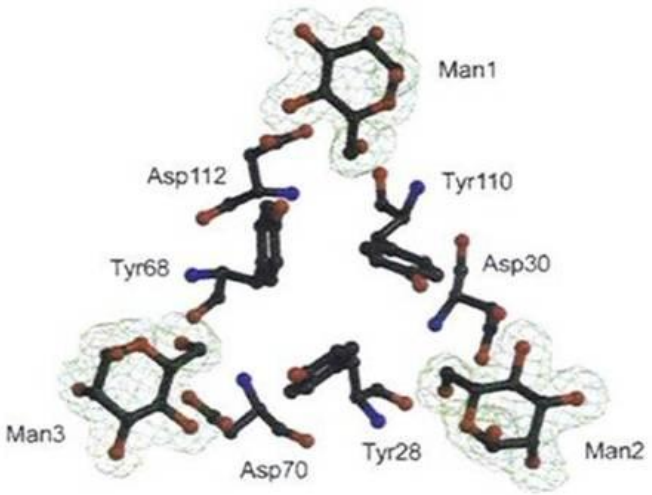


Figure 4. Mannose Binding Sites 1–3, Created Principally by Molecule A of Griffithsin

Ziolkowska *et al*., 2006

Advantages of plant production systems

- Protein processing tools
- Versatile
- Short turnover period
  - Stable transformation
  - Transient expression
- More cost effective
- Ease of scale up
- Commercially competent

What to consider

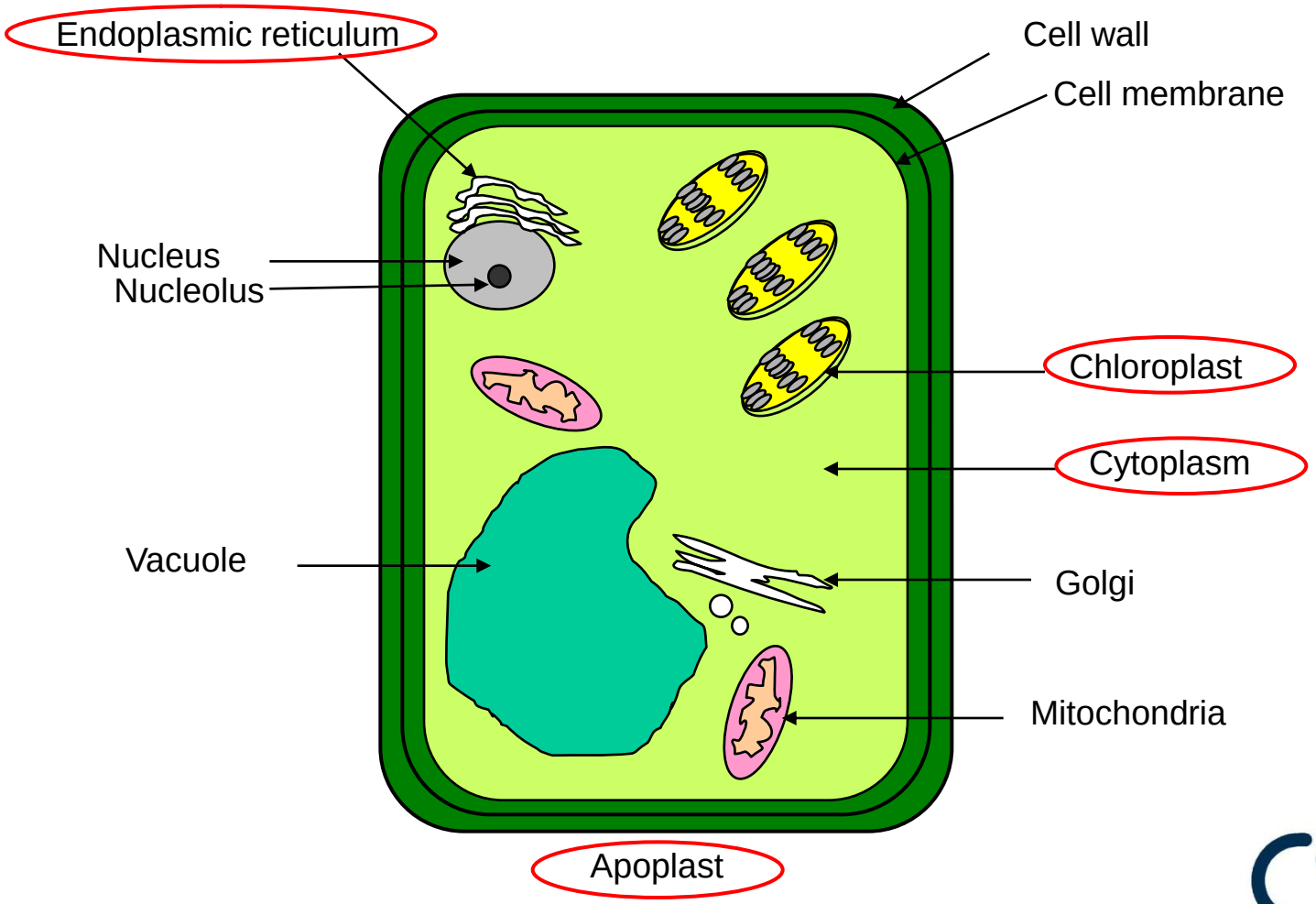
- Type of molecule
- Molecular tools
- Plant host
- Subcellular targeting
- Purification strategies

Aim of the study

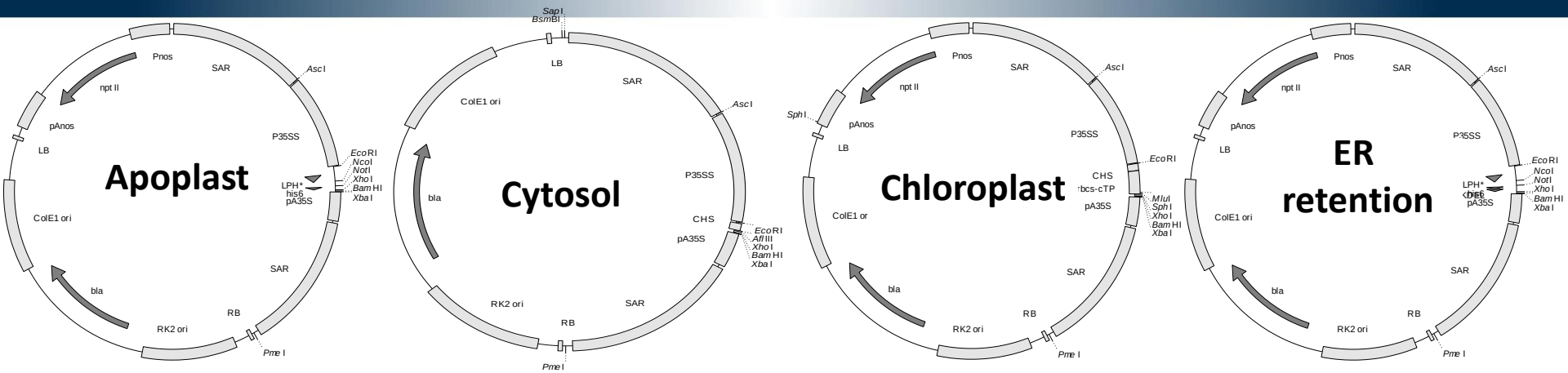
Investigate the effect of subcellular targeting of GRFT in tobacco on expression levels and plant cell viability

- Integration vector
- Deconstructed viral vector

Subcellular location



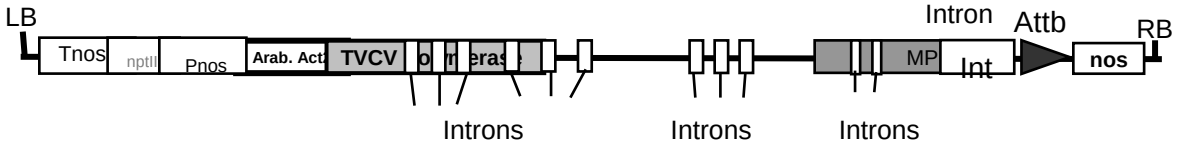
Tobacco integration vectors- pTRA



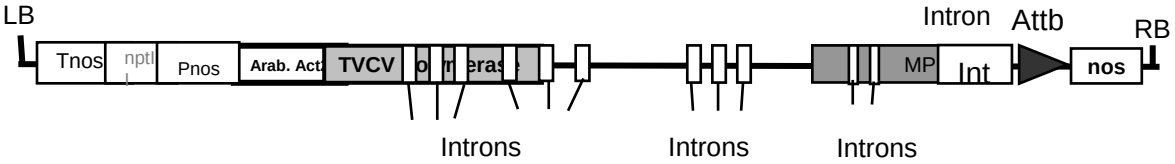
- Expression under the control of the 35S CaMV promoter.
- Targeting to Apoplast, Cytosol, Chloroplast and retention in the Endoplasmic Reticulum(ER).

Tobacco deconstructed viral vectors - Icon

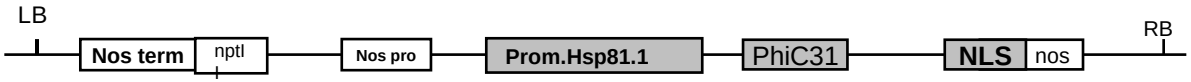
3’ GRFT



5’ Cytosol

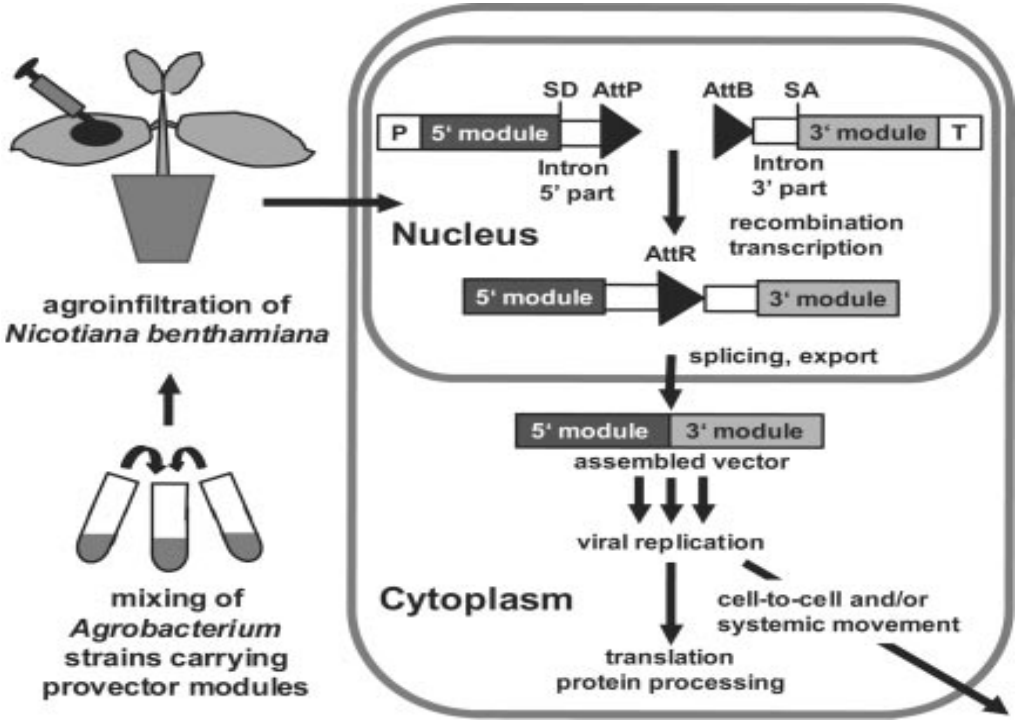


5’ Apoplast



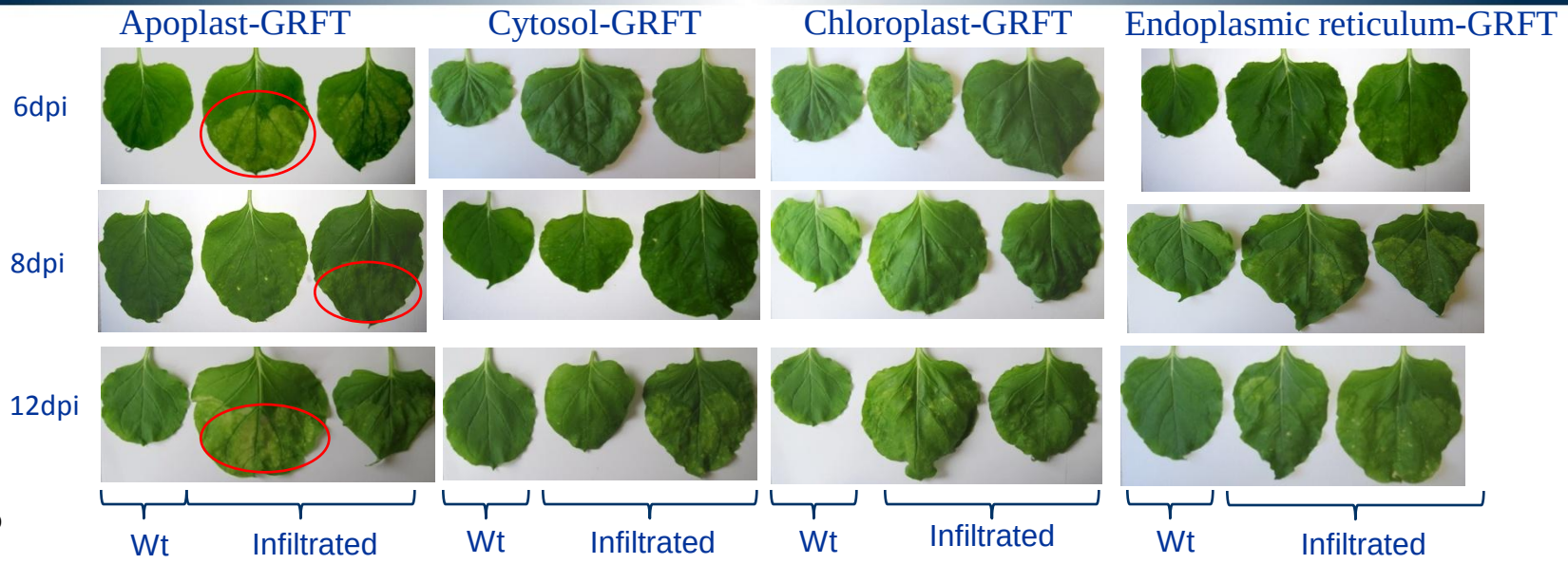
Recombinase

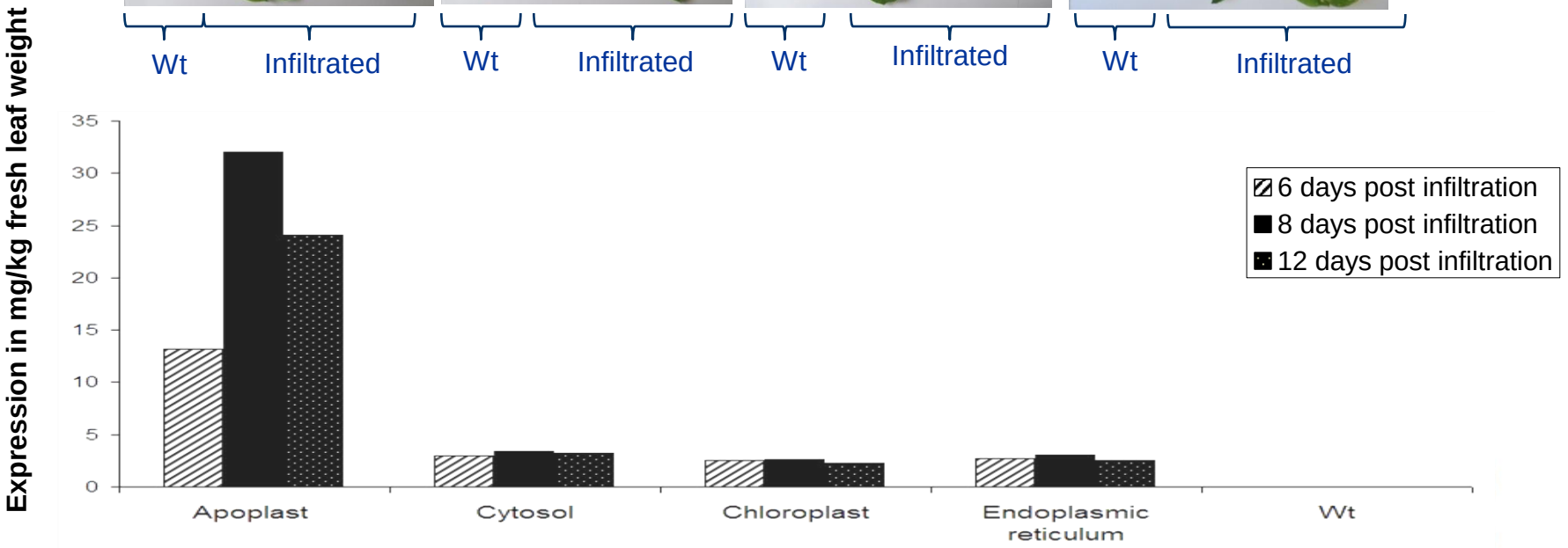
Assembly of viral pro-vector modules in plants



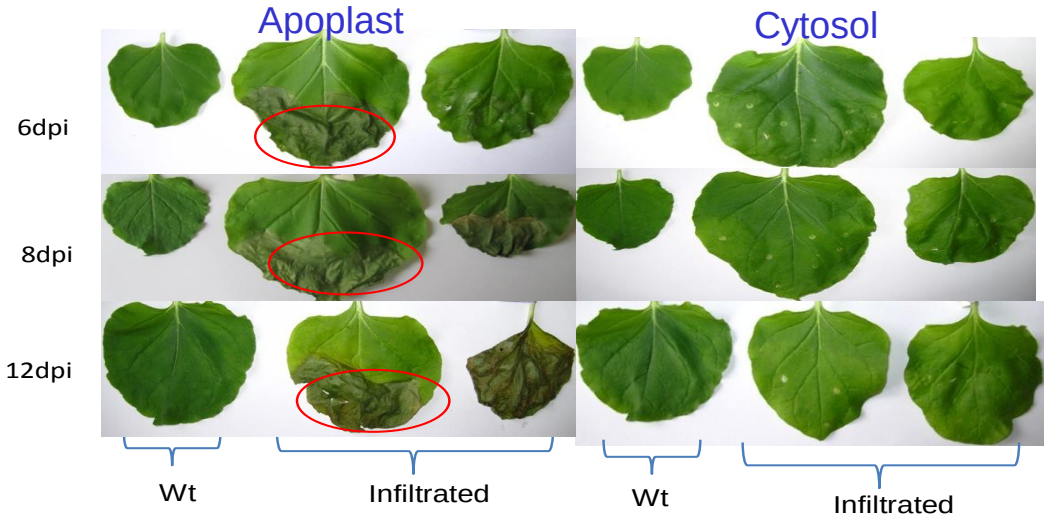
Marillonnet *et al*., 2003

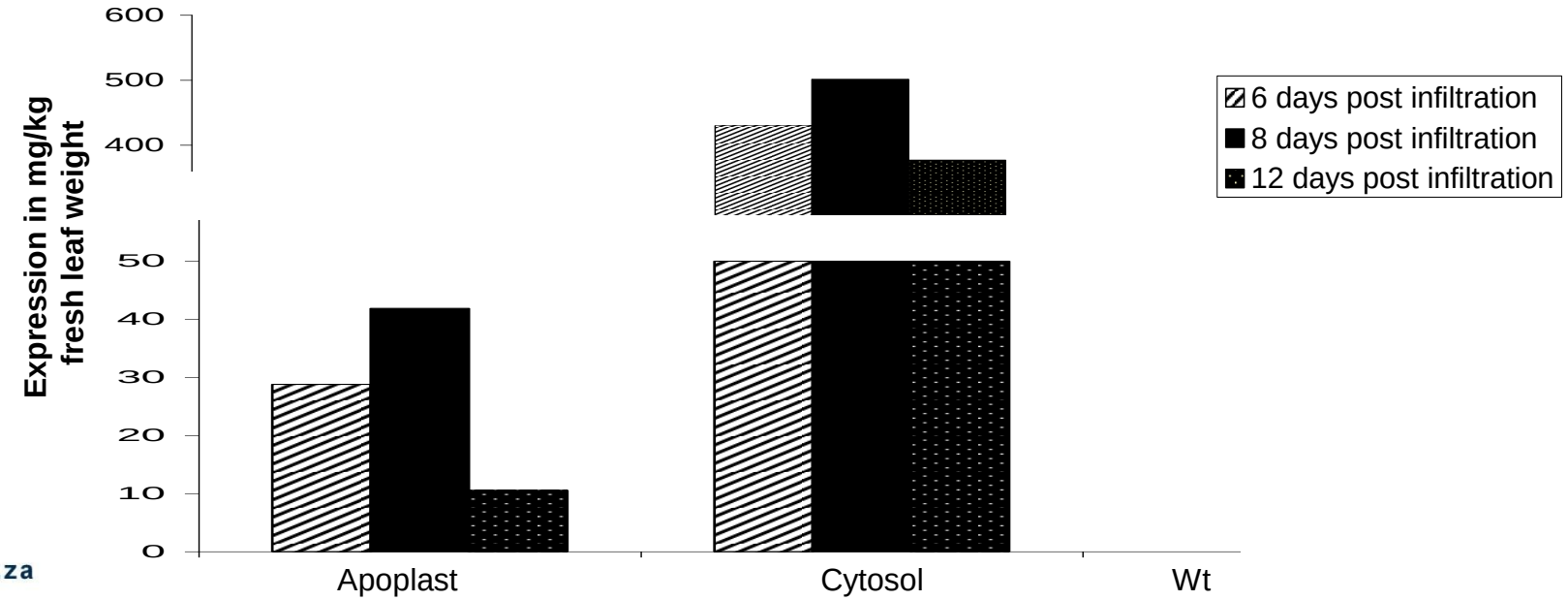
GRFT integration vector infiltrated



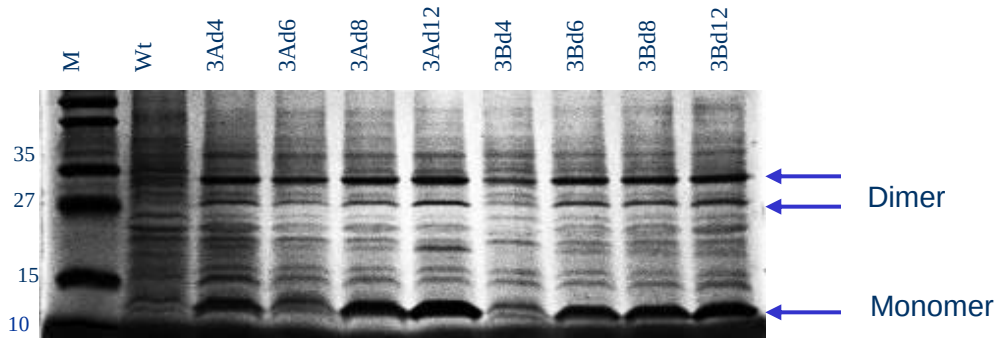


GRFT deconstructed viral vector infiltrated

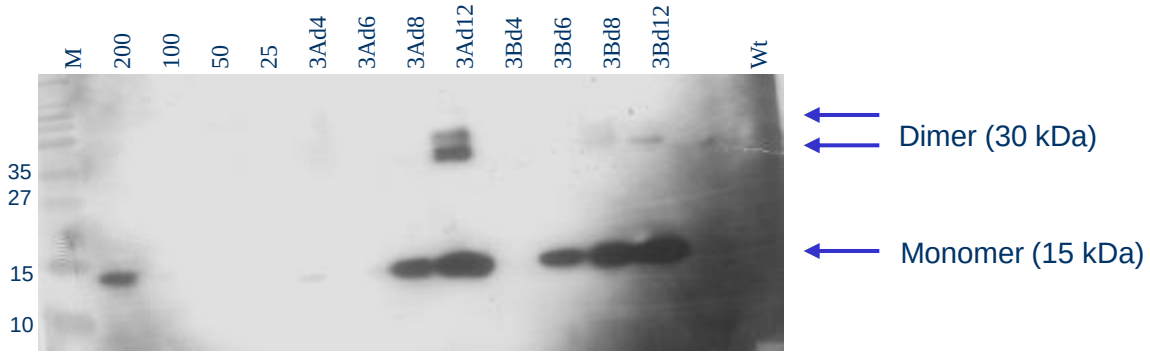




Coomassie and Immunoblot of GRFT expressed in the cytosol



Coomassie stain



Western blot

- Coommassie indicates expression of GRFT and MW
- Western blot analyses confirm exclusive recognition by antibody and MW

Efficacy data

	N.benthamiana produced GRFT ID_{50}	Untransformed Tobacco IC_{50}	*E. coli* GRFT IC_{50}	PBS
QH0692.42	0.10	<20	0.2	*<20*
VSV-G	*<20*	*<20*	*<20*	*<20*

- IC_{50} (ng/mL) and ID50 (given as a ratio) needed to neutralize virus
- IC_{50} is the concentration of substance that provides 50% inhibition
- ID_{50} the dose that will infect 50% of the experimental group
- IC_{50} of Nevirapine = 10 ng/ml

Conclusions

- Different expression levels were observed for the different vector systems. The Icon vector system exhibited expression levels more than 15 fold higher than the pTRA vector system.
- Targeting to different cell compartments affected yield and protein accumulation; in both vector systems
  - Apoplast associated with toxicity as reflected by leaf tissue death in both vector systems
  - Some accumulation observed (40mg/kg) prior to cell death
- *N. benthamiana* produced GRFT was detected by the polyclonal antibody and had the same molecular weight size as *E.coli* produced GRFT. Both monomeric and dimeric forms of GRFT were detected.
- GRFT from *N. benthamiana* recognized viral coat protein gp120 in ELISA analysis and was able to neutralize HIV sub-type C *in vitro.*
- Commercially viable levels of expression obtained with both systems 30 mg/kg fresh weight and 500 mg/kg for pTRA and Icon respectively (in plant systems levels of <100mg/kg considered potentially commercially viable, Evangelista *et al.,* 1998).

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Thank You

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