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The effect of subcellular targeting on the expression and accumulation of Griffithsin in *N. benthamiana*

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INTRODUCTION

Due to the increasing demand for pharmaceuticals and the high cost of production in traditional fermentor systems, plants are being considered as alternative production platforms for therapeutic molecules. One of the considerations for production of a recombinant protein in a plant is the choice of plant host, vector system and targeting of the protein to a specific cell compartment. All these can hugely influence the yield, functionality and downstream purification of the recombinant protein. Griffithsin (GRFT), a lectin isolated from the red algae *Griffithsia*, has shown extreme potency against the Human Immunodeficiency Virus (HIV) *in vitro* in very low quantities (Mori *et al*., 2005). The molecular target of GRFT is the high mannose residues on the viral gp120 coat protein. Low cost production of GRFT and other anti-HIV drugs at minimal costs is crucial seeing that the high demand for anti-HIV drugs reside in poor developing countries.

This study investigates the production of GRFT in *N. benthamiana* tobacco and evaluates the effect of subcellular targeting on yield, functionality and toxicity on the host system.

VECTOR SYSTEMS

Two vector systems were employed to drive transient expression in *N. benthamiana*.

The pTRA vector system (**Figure1**) governs the following:
- Expression of GRFT is under the control of the 35S CaMV promoter
- GRFT protein targeting to cytosol, apoplast, chloroplast and retention in the endoplasmic reticulum (ER).

The Icon deconstructed viral vector system (**Figure 2**) governs the following:
- GRFT expression is under the control of the ACT2 promoter
- Protein is targeted to cytosol or apoplast
- Combination of different modules that recombine to give gene expression.

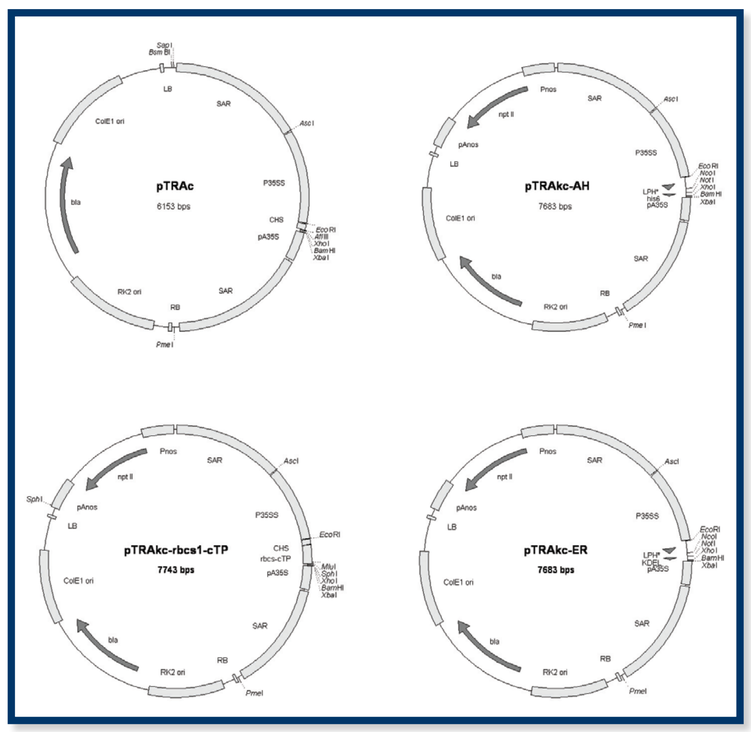

Figure 1: pTRA vectors used to express GRFT transiently in *N. benthamiana*. The pTRA vectors govern expression under the 35S CaMV promoter and directs expression to the cytosol (pTRAc), the apoplast (pTRAkc-AH), chloroplast (pTRAkc-rbcs1-cTP) or retention in the endoplasmic reticulum (pTRAkc-ERH)

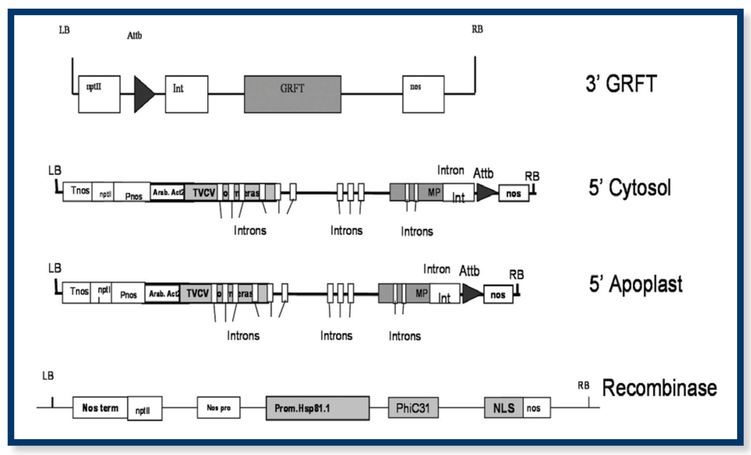

Figure 2: Icon deconstructed viral vectors used for transient expression of GRFT in *N. benthamiana*. GRFT was cloned into the Icon 3' provector. The GRFT module can recombine with a 5' cytosol or 5' apoplast targeting module.The recombinase facilitates combination of the different modules to affect the expression of any gene in the 3'module, in this case GRFT

GRFT EXPRESSION LEVELS AND THE TOXIC EFFECTS OF GRFT ACCUMULATION IN LEAF TISSUE

The levels of expression of GRFT in *N. benthamiana* as well as toxic side-effects of GRFT accumulation in leaves were evaluated for the different vector systems and subcellular locations of GRFT were evaluated using gp120 binding Enzyme-Linked Immunosorbent Assay (ELISA) and visual observations respectively (**Figures 3** and **4**).

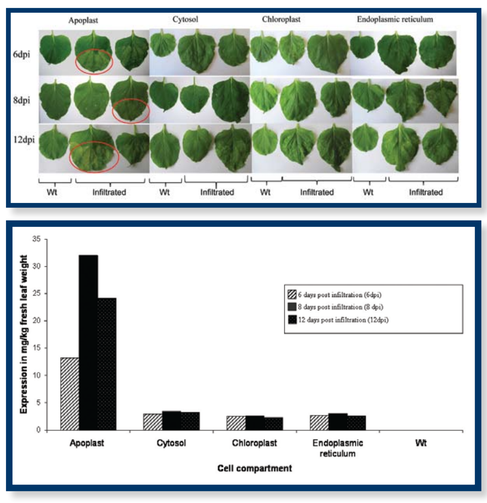

Figure 3: Toxicity effect (visual observation) and GRFT expression levels (ELISA graph) in mg/kg fresh leaf weight of *N. benthamiana* infiltrated with pTRA GRFT vectors. The highest expression was obtained when GRFT was targeted to the apoplastic space; targeting GRFT to this location also resulted in clear toxicity effects on the leaf.

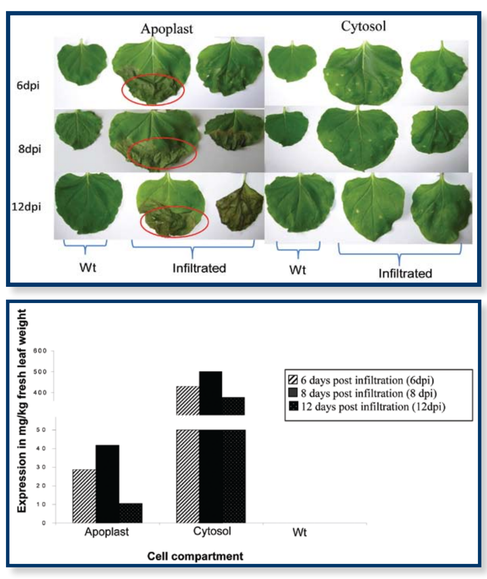

Figure 4: Toxicity effect (visual observation) and GRFT expression levels (ELISA graph) in mg/kg fresh leaf weight in *N. benthamiana* infiltrated with the GRFT module combined with the apoplast or cytosol module. Toxicity was evident when GRFT was targeted to the apoplastic space. No toxicity was observed when GRFT was expressed in the cytosol, here GRFT accumulated more than 15 fold higher than in the apoplast

COOMASSIE STAIN AND IMMUNOBLOT ANALYSIS OF *N. BENTHAMIANA* PRODUCED GRFT

Coomassie staining and immunoblot analysis (**Figures 5A** and **B** respectively) of tobacco produced GRFT shows the presence of both monomeric (15kDa) and dimeric (30kDa) forms of GRFT.

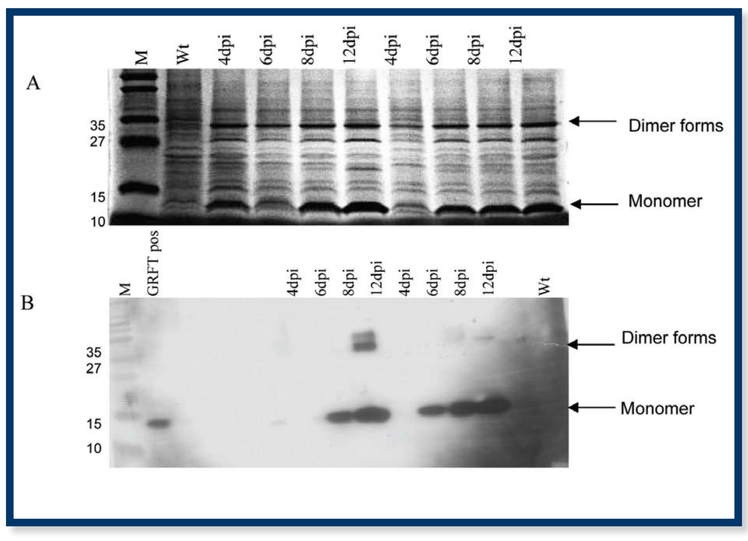

Figure 5: Coomassie stain (A) and immunoblot (B) of tobacco produced GRFT after 4, 6, 8 and 12 days post infiltration showing monomeric and dimeric forms

EFFICACY TEST

Tobacco produced GRFT was evaluated for anti-HIV activity in HIV-1 neutralisation assay in TZMbl (**Table 1**).

Table 1: Efficacy evaluation of *N. benthamiana* produced GRFT. The ID50 for plant GRFT indicates (0.1) equivalent potency to bacterial *E.coli* produced GRFT (0.2)

	N.benthamiana produced GRFT $_{ID50}$	Untransformed Tobacco $_{IC50}$	E. coli GRFT $_{IC50}$	PBS
QH0692.42	0.1	<20	0.2	<20
VSV-G	<20	<20	<20	<20

Plants have the potential to produce more cost effective anti-HIV therapeutics.

CONCLUSIONS

Vector systems used

Different expression levels were obtained for the different vector systems. The Icon vector system exhibited expression levels more than 15 fold higher than the pTRA vector system. The pTRA vectors are standard integration vectors, while the Icon system consists of deconstructed viral modules and results in greater accumulation of the target protein.

Subcellular targeting

Targeting to different cell compartments affected yield and protein accumulation related toxicity effect on plants. Both vector systems indicated that targeting GRFT to the apoplastic space was toxic to the plant. However, whereas the toxicity and highest expression were associated with the apoplastic space for pTRA, the highest expression in Icon was obtained in the cytosol which showed not toxicity. The apoplast targeting with the Icon system resulted in severe leaf toxicity which translated to very poor expression as a result of cell death.

Combined effect

There is an indication that a combination of both load on cellular machinery and destined cell compartment influence the yield of the final product as well as cell physiological integrity.

Biochemical characterisation

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.

Functionality

GRFT from *N. benthamiana* recognised gp120 in ELISA analysis and was able to neutralise HIV sub-type C *in vitro*.

Levels of expression

Commercially viable levels of expression obtained with both systems 30 mg/kg fresh weight and 500 mg/kg for pTRA and Icon respectively, but the Icon vector system is likely to be more cost effective. The plant host expression system is thus a viable platform for production of functional GRFT.

ACKNOWLEDGEMENTS

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