Expression of affordable microbicides to combat the spread of HIV pandemic

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INTRODUCTION

HIV prevalence is over 33 million worldwide with 68% of AIDS sufferers residing in sub-Saharan Africa (1). Currently the available HIV prevention tools are feasible but women cannot insist on these preventive measures due to social, cultural and economic issues. Therefore, there is a strong need to find appropriate HIV prevention measure that women can initiate, and microbicides are one such measure.

Microbicides are products that are applied topically inside the vagina or rectum to prevent the transmission of HIV and other sexually transmitted diseases (2). Previous microbicide candidates have failed due to lack of safety and efficacy, new candidates will need to be appropriate for use (3).

Some human chemokines, including RANTES (regulated upon activation, normal T expressed and secreted) show anti-HIV activity through their ability to block the HIV coreceptor CCR5 (**Figure 1**), and a number of N-terminally modified analogues of these proteins with much higher antiviral potency have been developed to generate potentially new low-cost preventatives or medicines (**Figures 2 and 3**) (3). These molecules have strong potential for use as microbicides, and it is imperative that they be produced in a cost effective manner. Plants offer an alternative method of cost effective production of protein therapeutics, and in this work, we test their effectiveness in expressing two RANTES analogues, 5P12 and 6P4 RANTES.

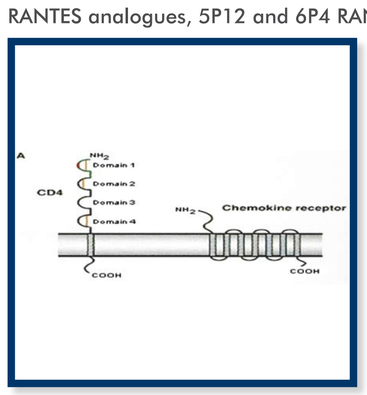


Figure 1. Schematic representation of the structure of CD4, showing four immunoglobulinlike domains and chemokine receptors (CXCR4 and CCR5) (4).

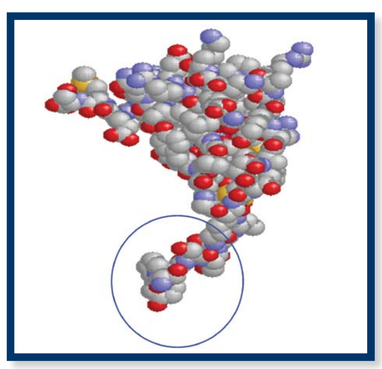


Figure 2. Structure of RANTES chemokine showing the N-terminal end with antiviral potency (5).

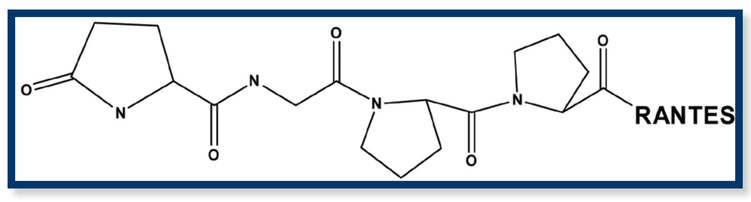


Figure 3. A structure of a fully recombinant RANTES analogue carrying N-terminal Gln^0-Gly^1-Pro^2 motif. Two examples of fully recombinant chemokines include 5P12-RANTES, Q^0-[G^1-P^2-P^3-L^4-M^5-A^6-T^7-Q^8-S^9] RANTES and 6P4-RANTES (Q^0-[G^1-P^2-P^3-G^4-D^5-I^6-V^7-L^8-A^9] RANTES)

MATERIALS AND METHODS

Expression of microbicides can be achieved via the stable transformation of tobacco with the genes encoding the relevant molecules. More commonly, transient expression is used. Transient expression of the microbicides was achieved in a tobacco plant distant relative *Nicotiana benthamiana* by introducing the microbicidal genes under the control of the CaMV35S promoter and the de-constructed TMV virus system from Icon Genetics (IconMagn™) (**Figure 4**). The expression of these molecules in plants was evaluated using western blot, dot blot and ELISA assays.

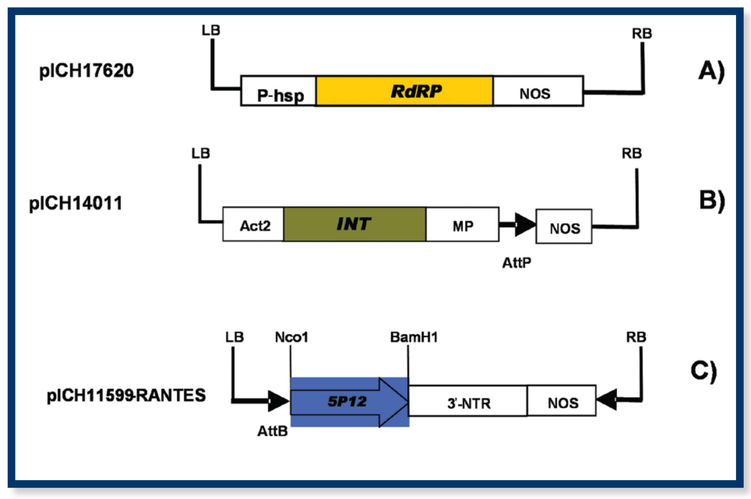


Figure 4. Schematic representation of the T-DNA regions of the ICON constructs used in this study. This is a modular vector system consisting of modules A-C. A) RNA-dependent RNA Polymerase (RdRP) catalyzes the replication of RNA from a RNA template. B) INT, Streptomyces phage PhiC31integrase mediates recombinational joining of the provectors. (A and C) 5′ and 3′ viral provectors; LB and RB, left and r ight borders of the T-DNA region; MP, movement protein; NOS, nopaline synthase terminator; Act2, *Arabidopsis* actin 2 promoter; AttP and AttB, PhiC31 integrase recombination sites; P-hsp, *Arabidopsis* heat shock protein 81.1 promoter; 3′-NTR, 3′ non-translated region.

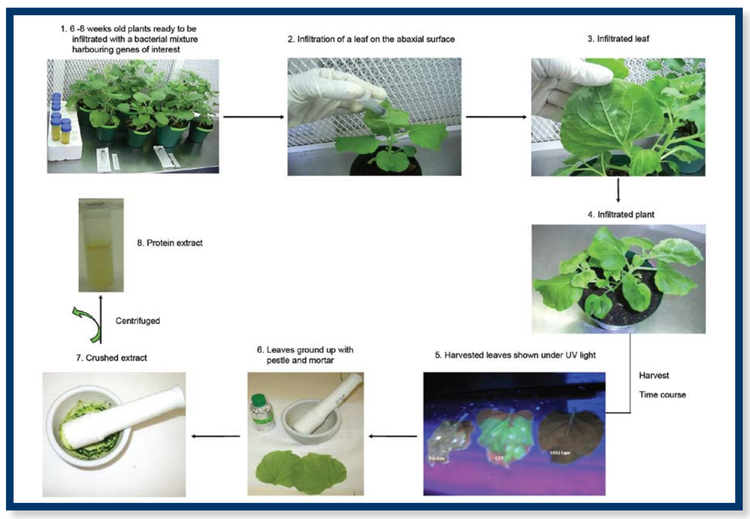
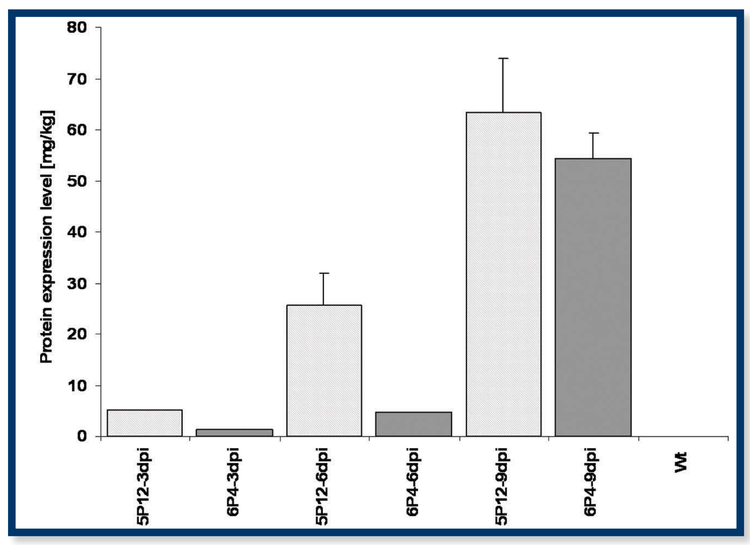


Figure 5. The flow diagram showing processes of transient Agro – mediated transformation of *N. benthamiana* with *A. tumefaciens* harbouring the microbicidal genes, harvesting and protein extraction.



RESULTS

Figure 6. Quantification of expression levels of 5P12-RANTES and 6P4-RANTES crude extracts using ELISA. The highest expression of about 63.4 mg/kg of 5P12-RANTES proteins was observed 9 days post infiltration (dpi)

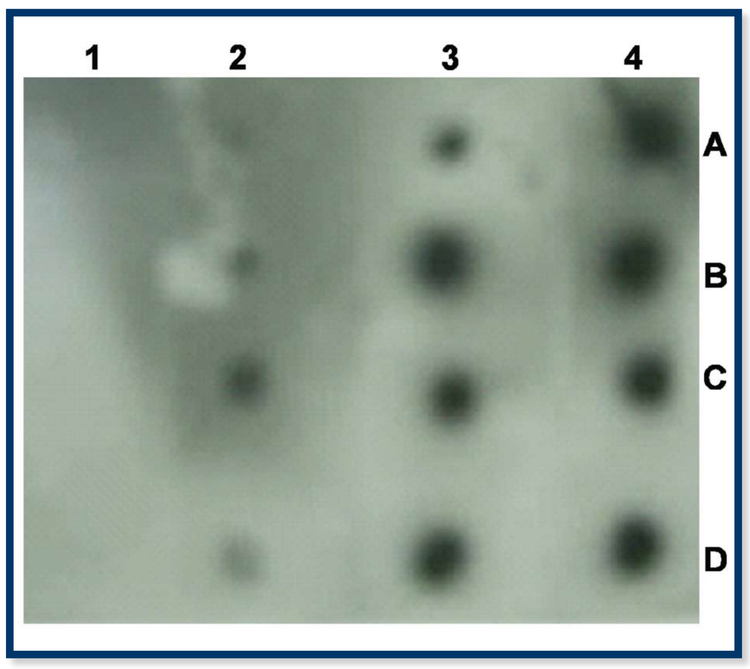


Figure 7. The dot blot ECL autoradiography film scan of the purified 5P12-RANTES and 6P4-RANTES proteins. Proteins were detected, analysed and identified with anti-RANTES antibodies by spotting through circular templates with varying concentrations directly onto the PVDF membrane. 1, wild type; 2, 278RN-rhCCL5/RANTES (positive control); 3, 5P12-RANTES; 4, 6P4-RANTES. A, 25 ng of sample; B, 50 ng of sample; C, 75 ng of sample; D, 100 ng of sample.

More effective delivery of affordable microbicides to control HIV transmission - tobacco plant as vehicle to express the anti-HIV proteins to be formulated as topical microbicides.

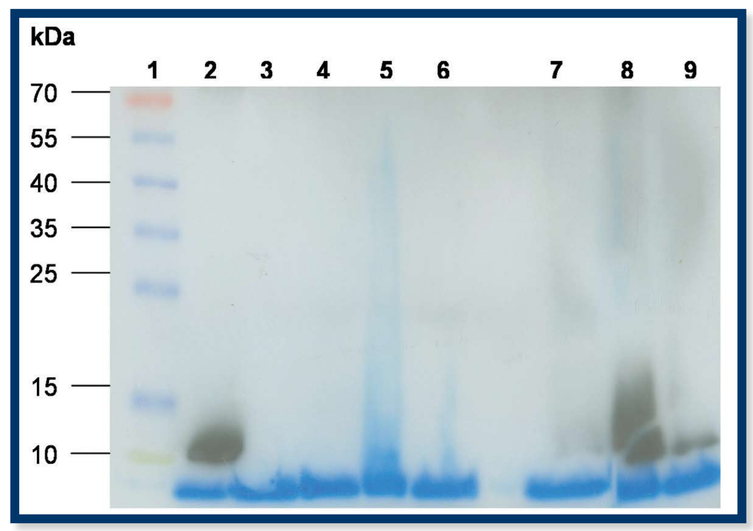


Figure 8. Western blot ECL autoradiography film scan of the transiently expressed RANTES proteins. 1, marker protein; 2, 278RN-rhCCL5/RANTES; 3, extraction buffer; 4, wild type (crude); 5, wild type (purified); 6, 5P12-RANTES (crude); 7, 5P12-RANTES (purified); 8, 6P4-RANTES (crude); 9, 6P4-RANTES (purified). Very little expression of 5P12-RANTES was observed on this run, but substantive expression and some purification recovery was observed for 6P4-RANTES.

DISCUSSION AND CONCLUSION

The objective of this work was top determine the feasibility of expressing RANTES analogues which are candidate microbicidal molecules in plant tissues (6).

5P12 and 6P4-RANTES proteins were successfully expressed in *N. benthamiana* as confirmed by ELISA, dot and western blot techniques. The expression levels of 5P12-RANTES appeared low as reflected by analyses of the crude samples by western blot analyses and as such could be barely detected.

Future work will include improved expression, extraction and purification of mg amount of RANTES analogues and then determining their efficacy *in vitro* and later in animal models.
Transgenic plants will also be generated via Agrobacterium-mediated transformation.

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