

Towards the bioengineering of *Nepenthes* pitchers for pharming

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Synopsis:

It has become possible that any protein of critical function, either natural or bioengineered, can be produced through plants provided with only simple nutrients, water and sunlight. The first plant-derived recombinant pharmaceutical protein (PMP) has received commercial approval, and many more are expected to come. Imagine the future of protein farming (pharming) whereby this can be achieved in a more sustainable, convenient and cheaper way through the development of a new expression system based on our local plants. *Nepenthes* pitcher plants belong to the species-rich family of *Nepenthaceae*, which is found to be endemic in Southeast Asia, and are especially diverse in Borneo. The unique pitcher glands, which secrete proteins into pitcher fluid, could potentially be exploited for the production of pharmaceutically important recombinant proteins. We have previously established a successful tissue culture system for *Nepenthes* conservation. Transcriptomic and proteomic studies to profile the proteins present in the pitcher fluids at different physiological stages are currently on-going. The next step will be attempt to engineer *Nepenthes* plant as a protein producer through transgenic approach. This involves the identification of secretory proteins and characterisation of their signal peptide (SP) sequences for protein secretion. Next is to combine these different SP sequences with reporter protein (GUS-GFP) to be expressed in a *Nepenthes* species most amenable to genetic transformation. The comparative characterisation of GUS activities in these transgenic pitcher plants will determine which signal peptide is the most suitable for future engineering of secretory recombinant protein using pitcher plant system.

Objectives:

1. To optimise tissue culture for *Nepenthes* spp.
2. To identify signal peptide sequences from proteins secreted into pitcher fluid
3. To establish a gene transformation system for a *Nepenthes* sp.

Research Activities:

Collection, cultivation, propagation and tissue culture selection of target *Nepenthes* spp.

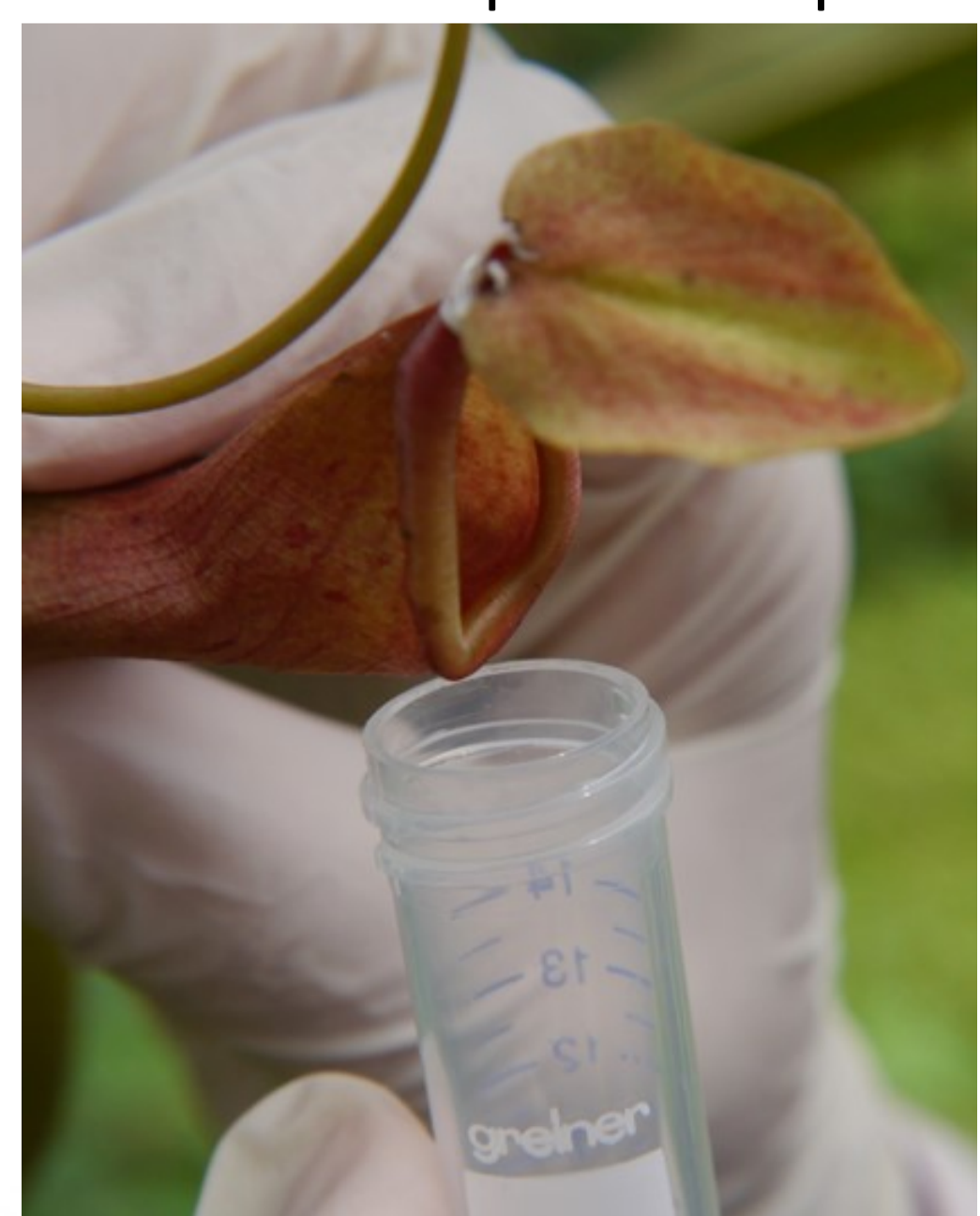
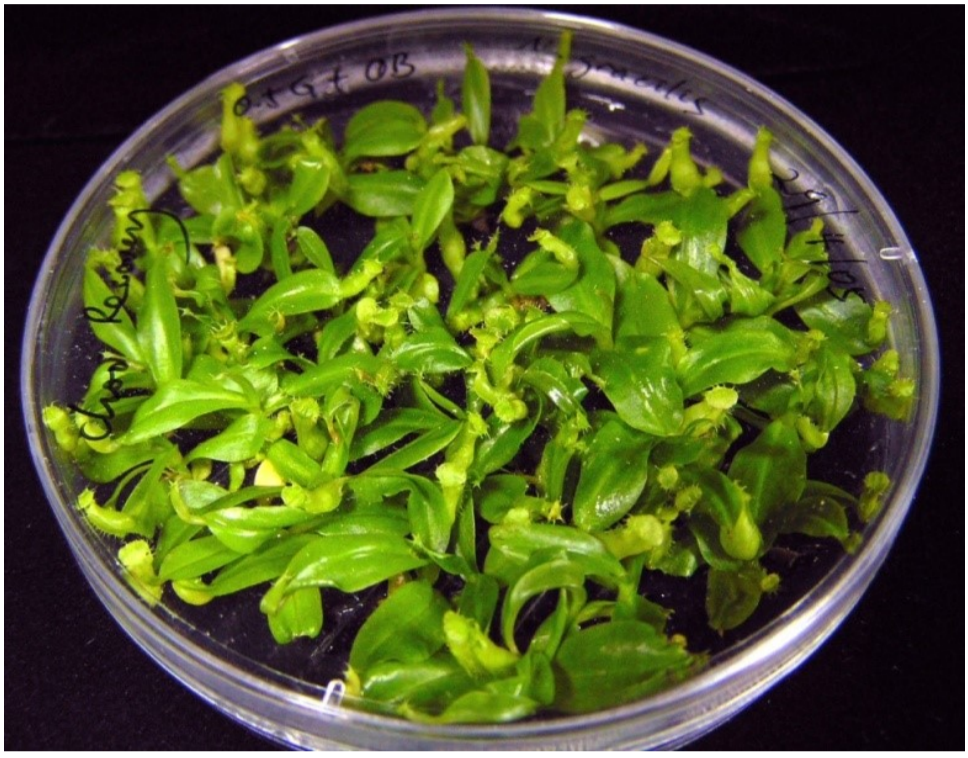
Identification of secretory proteins in pitcher fluid and consensus sequences of signal peptide (SP)

Design and molecular cloning of different SP-GUS-GFP fusion reporter protein constructs

Transformation, tissue culture, selection and regeneration of transgenic *Nepenthes* spp.

Characterisation of transgenic *Nepenthes* adult plants and pitcher fluids

Established proof-of-concept experiment based on reporter protein on the use of local *Nepenthes* plants as a novel bioreactor for recombinant protein expression



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