New plant models for the production of recombinant proteins: why not the carnivorous plants?

Estelle Nisse[1,&], Sissi Miguel[1,&], Benoît Mignard[1], Alain Hehn[2,3], Frédéric Bourgaud[1,2,3]

1- Plant Advanced Technologies SA, 13, Rue du bois de la Champelle, 54500 Vandœuvre-lès-Nancy, France.
2- INRA UMR 1121, Laboratoire Agronomie et Environnement, 2 avenue de la forêt de Haye TSA 40602 54518, Vandœuvre-lès-Nancy, France.
3- Université de Lorraine UMR 1121, Laboratoire Agronomie et Environnement, 2 avenue de la forêt de Haye TSA 40602 54518, Vandœuvre-lès-Nancy, France.
&: Equal contributions to this work

Introduction

- Carnivorous plants are able to attract, trap, retain, kill, and digest prey thanks to traps differentiated from leaves (Juniper *et al*., 1989).
- *Drosera* and *Nepenthes* are two carnivorous plant genera able to produce and excrete out of their tissues a digestive fluid containing proteins which are mostly hydrolytic enzymes (Juniper *et al*., 1989). Natural protein concentration can reach up to 200 $mg.L^{-1}$ in the digestive fluid.
- This natural ability of carnivorous plants to secrete proteins has been exploited to develop a *Drosera* and *Nepenthes* based plant platform to produce recombinant proteins. The method has been patented as the Friday® technology (Biteau *et al*., 2008).
- Producing recombinant proteins *via* carnivorous plants makes extraction easier since the digestive fluid is readily accessible and facilitates purification because the digestive fluid contains only a dozen of native proteins (Hatano and Hamada, 2008).

Fig. 2: Bending of the tentacles following the catching of a prey by Drosera.

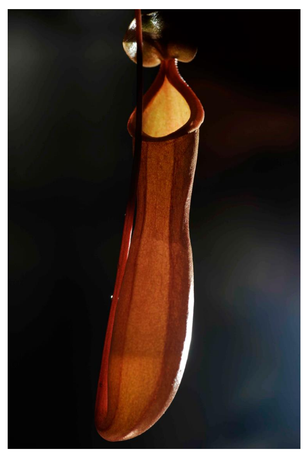

Fig. 1: Nepenthes pitcher.

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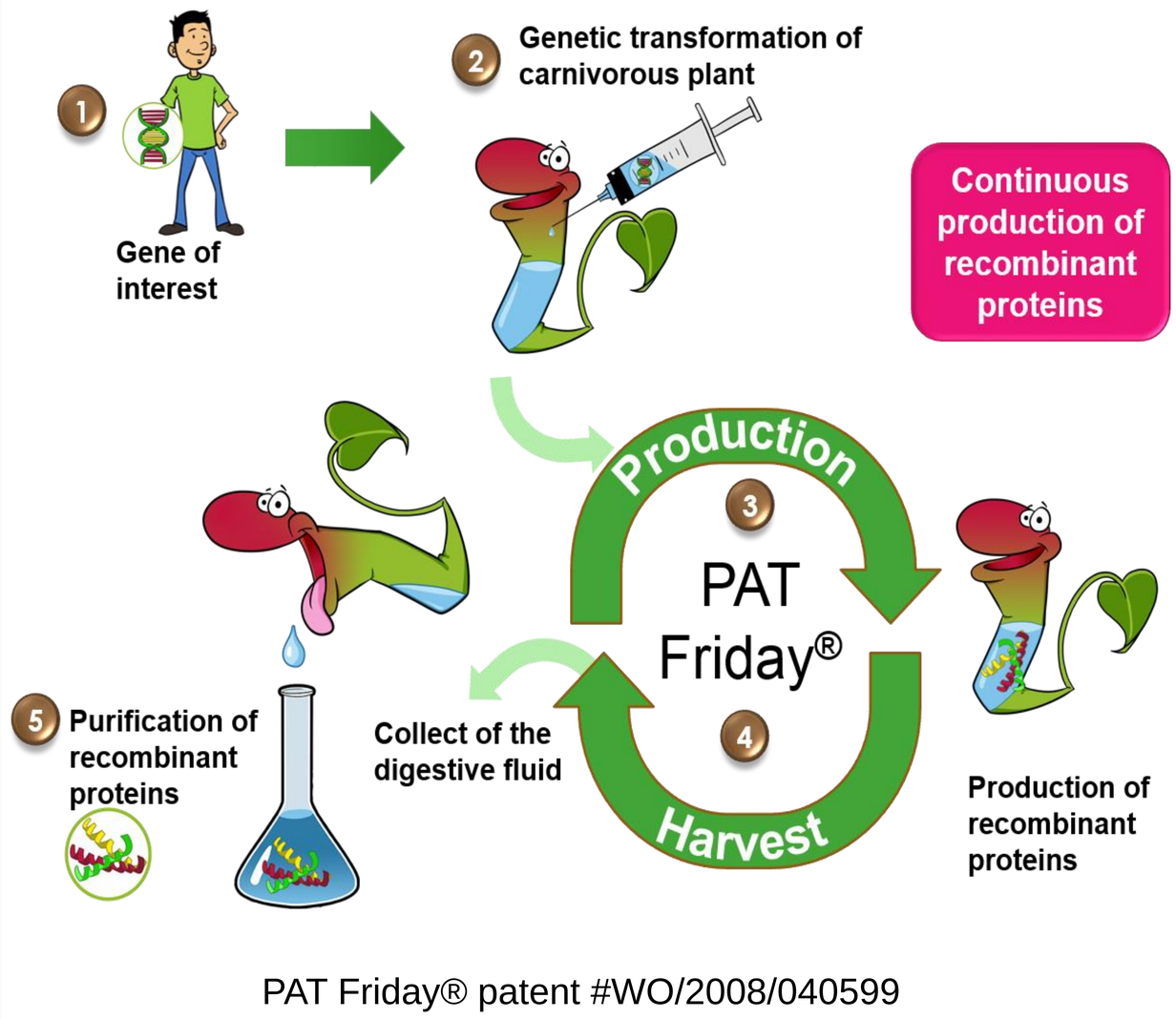

PAT Friday® patent #WO/2008/040599

Materials and Methods

- The protocol for genetic transformation of *Drosera rotundifolia* (Hirsikorpi *et al*., 2002) was adapted to *Drosera capensis*. A new protocol was set up for *Nepenthes mirabilis*.
- Transgenic plants overexpressing the Green Fluorescent Protein (GFP), were grown in a confined greenhouse, without insects or other prey, and the digestive secretions were collected.
- The presence of GFP was highlighted in the digestive fluids either by immunodetection (for *Nepenthes mirabilis*), or by direct observation of the fluorescence (for *Drosera rotundifolia* and *Drosera capensis*).

Results

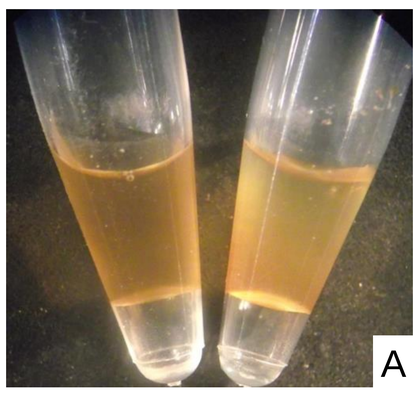

Fig. 3: Drosera capensis secretions. A: Normal light, B: UV light. From Nisse 2014.

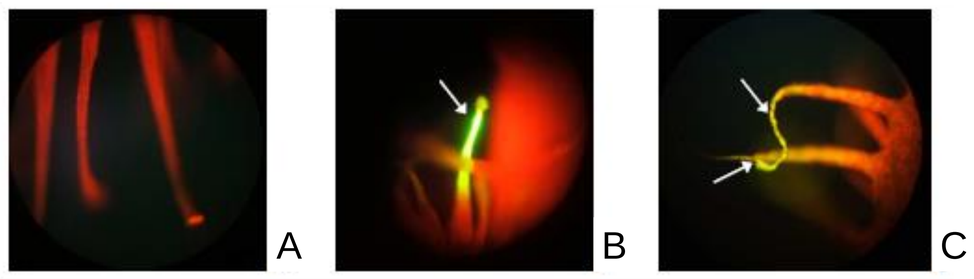

Fig. 4: Leaf tentacles of Drosera rotundifolia producing GFP. The leaves were observed with a fluorescence microscope (x400). A: Tentacles from Wild Type Drosera. B and C: Tentacles from transgenic plants. From Biteau 2009.

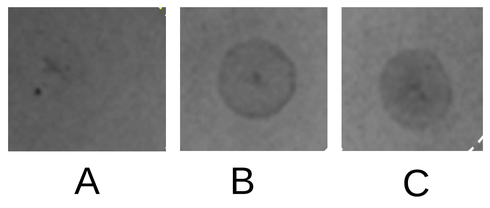

Fig. 5: Dot Blot immunodetection of the GFP produced in the digestive secretions of transgenic Nepenthes mirabilis. A: Wild Type plant. B and C: Transgenic plants. From Miguel 2013.

Conclusion

- The system we developed allows the production and the secretion of functionnal GFP in transgenic *Drosera* and *Nepenthes* digestive fluids.
- PAT Friday ® will now be extended to the expression of relevant recombinant therapeutic proteins such as monoclonal antibodies, cytokines, and gastric proteins.

About PAT

Plant Advanced Technologies is a French plant biotech company which develops innovative Technologies to produce natural active compounds & recombinant proteins for cosmetic & pharmaceutical industries.

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