Interactions between *Phytophthora infestans* RxLR effectors and potato host proteins

Yordan MUHOVSKI & Jean-Louis ROLOT

Breeding and Biodiversity Unit, Life Sciences Department, Walloon Agricultural Research Centre, 5030 Gembloux, Belgium /e-mail: y.muhovski@cra.wallonie.be/

Introduction: Potato (*S. tuberosum* L.) is one of the world's most important food crops for humans and has a high value-added component. However, as each crop, potato suffers from various diseases like late blight caused by the oomycete *Phytophthora infestans* (*Pi*). Like all successful pathogens, oomycetes are able to evade the defense reactions of their hosts. The *Pi* genome encodes large number of host-translocated effectors (more than 500), among them RxLR effector family being the best-studied group . To date the main virulence target of any eukaryotic effector protein is still not very well depicted [1,2].
The goal of the present study is to gain a better insight into the function of RxLR-effectors using the interaction of potato with *Phytophthora infestans* and to identify their molecular targets in the host plant.

Results: Yeast-2-Hybrid (Y2H) system offers an experimental approach to investigate protein-protein interactions *in vivo*. For the beginning, we conducted a Y2H screen against a "pray" cDNA library prepared from *P. infestans*-infected *Solanum pinnatisectum* CGN17743 accession (a wild diploid potato relative species, known as source for resistance to various *Pi* isolates) with *AVR3a-KI*, the avirulent form of *AVR3a* effector, as "bait" in order to identify potential host targets (Figure 1) [3]. After screening of $1x10^7$ yeasts diploid transformants with *AVR3a-KI*, 17 clones expressing interacting proteins and growing on high-stringency medium were identified. The determined interactants belonged to different gene families implicated in photosynthesis, carotenoid biosynthesis, transcription and protein folding (Table 1).

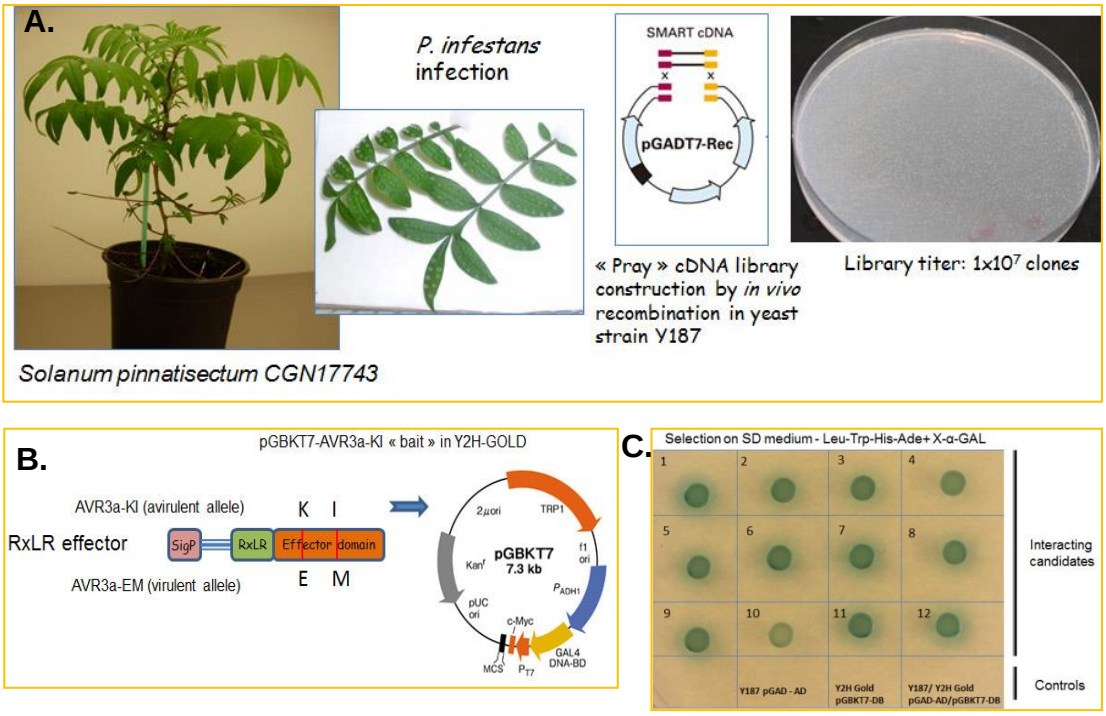

Figure 1. Yeast-2-Hybrid screening.
(A) « Pray » cDNA construction
(B) « Bait » preparation
(C) A positive interaction of two proteins reconstitutes GAL4-transcriptional factor and this in turn leads to the transcriptional activation of reporter genes, from which two of them (ADE and HIS) enable transformed cells to grow on particular drop-out medium. The third activated reporter, confirming protein-protein interaction, is MEL1, giving a blue colour after utilizing X-α-GAL as substrat.

Table 1. *Solanum pinnatisectum* proteins interacting with AVR3a-KI effector.

Clone ID	GenBank accession number	Annotation for plant proteins interacting with AVR3a-KI
Spinna_1	XM_006338195	Chloroplast manganese stabilizing protein-II (*S. tuberosum*)
Spinna_2	XM_006351733	Peroxidase N1-like (*S. tuberosum*)
Spinna_3	XM_004233367	33 kDa precursorprotein of oxygen evolving complex (*S. lycopersicum*)
Spinna_4	NM_001246896	CONSTANS interacting protein 3 (CIP3) (*S. lycopersicum*)
Spinna_5	XM_006364530	Geranylgeranyl diposphate reductase (*S. tuberosum*)
Spinna_6	KF573426	Elongation factor 1A protein (*S. chacoense*)
Spinna_7	XM_006351679	Peptidyl-prolylcis-transisomerase (*S. tuberosum*)
Spinna_8	XM_006361425	Uncharacterized protein (*S. tuberosum*)

Future work:

- ✓ Characterization of the identified putative interactants by over-expression or gene-silencing in appropriate model plant.
- ✓ Uning the virulent allelic form of AVR3a, AVR3a-EM as "bait".
- ✓ Testing additional *P. infestans* effector molecules.

References:
[1] Vleeshouwers V *et al*., 2011. Annu Rev Phytopathol 49, 25.1-25.25
[2] Haas BJ *et al*., 2009. Nature 461, 393-398
[3] Bos J *et al*., 2010. PNAS 107, 9909-9914

Centre wallon de Recherches agronomiques

Wallonie