Screening wild potato accessions for resistance to the virulent allele of the *Phytophthora infestans* effector *avr3a* EM

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The perception of the *Phytophthora infestans* avirulence gene product *Avr3a* by the cognate potato resistance gene *R3a* results in a localised form of programmed cell death termed the hypersensitive response (HR), which inhibits pathogen development and mediates resistance [1]. Pathogen effectors such as *Avr3a*, if not recognized by the plant, are thought to play a positive role during infection and in mediating susceptibility, and are therefore maintained by the pathogen. To prevent recognition, the pathogen is under constant selective pressure to alter the form of these effectors. Screening of *P. infestans* isolates has identified two alleles from *Avr3a* that display 100% correlation with either avirulence (*Avr3a* with the amino acids C^{19} K^{80} and I^{103}) or virulence (*avr3a* with the amino acids S^{19} E^{80} and M^{103}) in potato plants harbouring *R3a*. Our aim was to screen wild potato accessions, resistant to *P. infestans*, that recognise the mature virulent allele from *Avr3a* (*avr3a* EM) and/or the mature avirulent allele (*Avr3a* KI).

Screening the Commonwealth Potato Collection to identify potato accessions that recognise *avr3a* (EM)

We have screened 52 potato accessions from the Commonwealth Potato Collection (CPC), which have been identified as resistant or very resistant to *P. infestans*, by overexpressing *avr3a* EM and *Avr3a* KI utilising *Agrobacterium tumefaciens-Potato Virus X* (PVX) or *A. tumefaciens* only. In total, 22 accessions were responsive, of which 14 recognised both the virulent and avirulent alleles, 7 the virulent allele only and one the avirulent form only. The most consistent results were obtained with *Solanum verrucosum, S. stoloniferum* and *S. demissum* (Table 1). Co-bombardment of the *Avr3a* alleles with GFP has been used to confirm the recognition of *avr3a* EM and *Avr3a* KI in *S. stoloniferum* and *S. microdontum* (Figure 2). *R3a*-like sequences have been PCR amplified from the diploid *Solanum* species, *S. verrucosum* and are currently being analysed (Figure 3).

	Species	Country	Series	EM	Accessions
	S.commersonii	Arg/Bra/Uru	Commersoniana	1:1	5858 (1:3)
5x	*S.semidemissum*	Mex	Demissa	1:1	7103 (3:5)
6x	*S.brachycarpum*	Mex	Demissa	0:1	
6x	*S.demissum*	Mex	Demissa	1:11	2098 (4:9)
6x	*S.iopetalum*	Mex	Demissa	1:1	7055 (2:5)
	S.brevidens	Arg	Etuberosa	1:1	2451 (3:9)
4x	*S.fendleri*	Mex/USA	Longipedicellata	1:2	4020 (3:7)
4x	*S.hjertingii*	Mex	Longipedicellata	1:1	3029 (2:2)
4x	*S.papita*	Mex	Longipedicellata	1:2	7085 (2:6)
4x	*S.polytrichon*	Mex	Longipedicellata	1:2	3984 (2:4)
4x	*S.stoloniferum*	Mex	Longipedicellata	4:10	2619 (4:9), 4013 (2:6), 2711 (3:4), 2220 (5:5), 1331 (2:2)
	S.cardiophyllum	Mex	Pinnatisecta	0:1	
	S.polyadenium	Mex	Polyadenia	0:1	
	S.alandiae	Arg/Bol/Chi	Tuberosa	0:1	
	S.berthaultii	Bol	Tuberosa	1:1	5701(1:4)
	S.microdontum	Arg	Tuberosa	3:7	4048 (1:3) , 7175 (3:6), 7176 (2:3)
	S.okadae	Arg	Tuberosa	0:1	
	S.venturii	Arg	Tuberosa	0:1	7327 (1:4)
2x	*S.vernei*	Arg	Tuberosa	0:1	
	S.verrucosum	Mex	Tuberosa	2:2	7091(1:2), 7213 (3:5)
	S.chacoense	Chi/Arg/Par	Yungasensia	1:4	7211(2:5)
	Σ 21	8	8	21:52	14x EM&KI, 7x EM, 1x KI

Table 1. A selection of 52 potato accessions comprising 21 potato species from 8 countries and 8 series, all resistant to *P. infestans* race 1,2,3,4,6,7 were screened and analysed for their response to overexpression of *Avr3a* KI and *avr3a* EM. The number of accessions responding to one or both alleles is shown in column 4. The CPC accession identifier and the number of independent, positive responses are shown in column 5. Accessions responding to *avr3a* EM are shown in BLUE, those responding to Avr3a KI shown in RED and those responding to both alleles in GREEN.

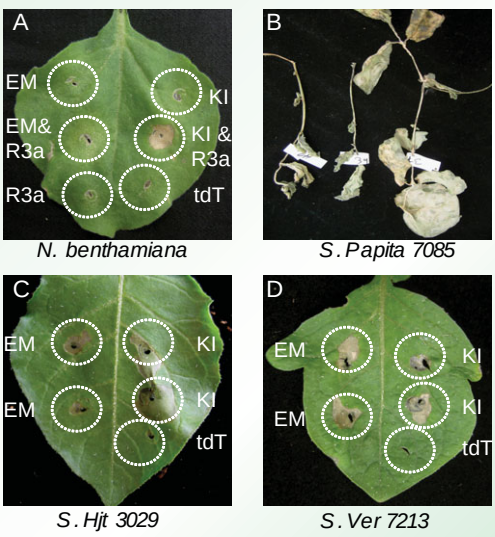

Figure 1. A) Transient expression and coexpression of *Avr3a* and *R3a* in *N. benthamiana*. Leaves were infiltrated with *A. tumefaciens* carrying pGR106::Avr3a KI (or EM) alone, or mixed with a *A. tumefaciens* strain carrying pBIN plus::R3a. Similar results were obtained with a PVX free overexpression system utilising a pGRAB::Avr3a KI (or EM) based delivery (results not shown). B: PVX resistance response in *Solanum papita*. Recognition of *avr3a* Em and *Avr3a* KI in *S. hjertingii* (C) and *S. verrucosum* (D). Expression of the red fluorescent protein tdTomato (tdT) was used as a control.

Independent confirmation of *Avr3a* (EM/KI) recognition and *R* gene cloning progress

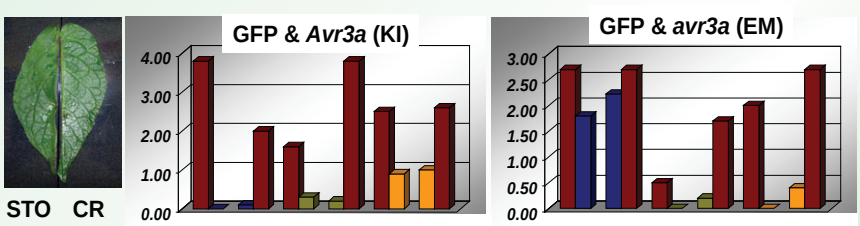

Figure 2. Preliminary co-bombardment results confirm recognition of *avr3a* EM and *Avr3a* KI in *S. stoloniferum* and *S. microdontum* but only recognition of *Avr3a* KI in *S. tuberosum* cv. Pentland Ace (*R3a*). *Avr3a* alleles were transiently coexpressed with GFP in different *Solanum* accessions, each compared with cv. Craigs Royal. In each histogram, the average number of GFP fluorescence cells is shown. This number was obtained by analysing 10 individual observation points (approximately 25 epidermal cells per point) for each bombarded leaf half. Co-bombardment with GFP and GUS resulted in comparable levels of GFP expressing cells in all potato accessions (results not shown).

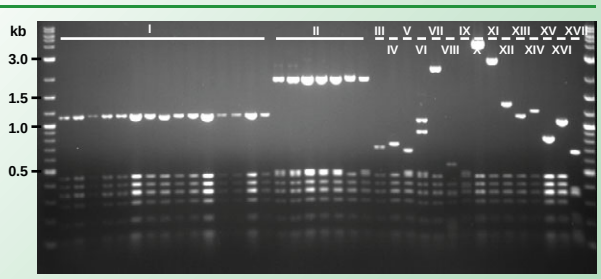

Figure 3. Restriction enzyme digest (*Hha*I) of *R3a*-like gene fragments from *S. verrucosum*. As the mature alleles of *Avr3a* differ only in two amino acids and *R3a*, which recognises *Avr3a* KI, has also been shown to weakly recognise *avr3a* EM [2] we used a PCR approach to amplify *R3a*-like sequences from *S. verrucosum*, which recognises both *Avr3a* alleles. Conserved primers, derived from the four known *R3a* paralogues [3], amplified a fragment of the expected size (3 kb) from genomic DNA comprising the ATG start codon at the 5'end but not the entire 3'UTR. A set of 77 cloned fragments were successfully digested and clustered into 17 groups according to their restriction enzyme pattern. Two main groups were identified (I) and (II) comprising 37 and 14 clones respectively. Group III comprised 5 clones. The remaining groups comprised between 1 and 3 members only. Sequencing of members from the different groups is currently in progress.

Literature cited:

[1] Armstrong,M.R. et al (2005) An ancestral oomycete locus contains late blight avirulence gene *Avr3a*, encoding a protein that is recognized in the host cytoplasm. *Proc.Natl.Acad.Sci.U.S.A*, 102:7766-7771.

[2] Bos,J.I.et al. (2006) The C-terminal half of *Phytophthora infestans* RXLR effector *AVR3a* is sufficient to trigger *R3a*-mediated hypersensitivity and suppress INF1-induced cell death in *Nicotiana benthamiana*. *Plant J*, 48:165-176.

[3] Huang,S. et al (2005) Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *Plant J*, 42:251-261.