# Identifying key *Phytophthora infestans* effectors as targets for more durable late blight resistance

I. Hein, S.C. Whisson, M. Armstrong, E.M. Gilroy, A.O. Avrova, Z. Tian, S. Chapman, P.R.J. Birch and G. Bryan. SCRI, Invergowrie, Dundee DD2 5DA.

*Phytophthora infestans*, the causal agent of potato and tomato late blight disease, is a pathogen with high evolutionary potential. Rapid pathogen population shifts often undermine breeding efforts towards resistance and hinder effective control of this devastating disease. A new strategy in the fight against late blight is to identify universally expressed, essential pathogen effectors and tailor resistances towards recognition of these important pathogen molecules and all know alleles.

## Results:


**Figure 1:** Infectivity of effector silenced *P. infestans* lineages on susceptible potato cultivar Craigs Royal.
Infection symptoms and lesions were observed on plants infected with wild type *P. infestans* strain 88069 [left] but not on plants infected with *P. infestans* strain silenced for *Avr2* or *Avr3a* [right].

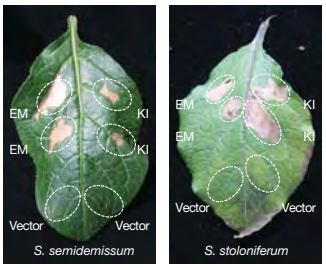


**Figure 2:** Recognition of *Avr3* alleles in wild potato accessions.
Recognition of *Agrobacterium tumefaciens* based expression of $Avr3a^{E80M103}$ [left] and $Avr3a^{K80I103}$ [right] in CPC accessions from *S. semidemissum* and *S. stoloniferum*. An empty vector control [bottom] was used to discriminate between true recognition and false-positive *Agrobacterium* responses.

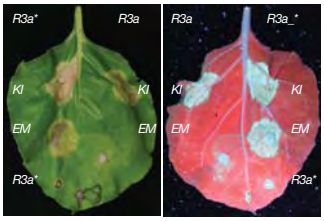


**Figure 3:** Recognition of *Avr3a* alleles by wild type R3a and shuffled version R3a* in the model plant *Nicotiana benthamiana*. R3a strongly recognises $AVR3a^{K80I103}$ but not $AVR3a^{E80M103}$. Shuffled version R3a*, however, displays a hypersensitve reaction (HR) upon co-infiltration with $AVR3a^{K80I103}$ and $AVR3a^{E80M103}$. The picture on the left is taken from the top of the leaf surface and the picture of the right from underneath while using UV-light to highlight the extend of autofluorescence assocatied with the HR.

The *P. infestans* genome contains more than 500 predicted RxLR containing effector candidates. Transient and stable silencing of 30 highly expressed effectors in *P. infestans* identified 20 that are functionally essential and include *Avr3a* and *Avr2* [**Figure 1**]. A diversity study of *Avr3a* in 82 Mexican *P. infestans* isolates revealed that every isolate contains either the avirulent allele $Avr3a^{K80I103}$, recognised by the cognate resistance protein R3a, and/or the virulent allele $Avr3a^{E80M103}$ that evades recognition by R3a.

We are using a two-pronged approach to tailor more durable resistance based on recognition of both $AVR3a^{K80I103}$ and $AVR3a^{E80M103}$. Screening the Commonwealth Potato Collection (CPC) for very resistant potato accessions has yielded accessions such as *S. semidemissum* and *S. stoloniferum* that recognise both *Avr3a* alleles [**Figure 2**].

Furthermore, shuffling the leucine rich repeat (LRR)-encoding region of functional R3a has yielded multiple R3a variants with enhanced recognition specificity towards $AVR3a^{K80I103}$ that have 'gained' recognition of $AVR3a^{E80M103}$ [**Figure 3**]. A second round of shuffling will recombine advantageous mutations whilst discriminating against deleterious changes.

## Conclusions:

Unlike some pathogenic bacteria, *P. infestans* appears to contain functionally non-redundant and thus essential effectors. Wild potato accessions contain a mechanism to identify such effectors and known alleles and display, as predicted, good resistance towards the late blight pathogen. Furthermore, the recognition specificity of cloned *R* genes can be extended *in vitro* to yield variances that recognise previously avirulent and virulent effector alleles. Our effector recognition-driven approaches provide new and exciting opportunities to obtain more durable *R* genes that target indispensable pathogen molecules.