

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

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*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 Craig's 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].

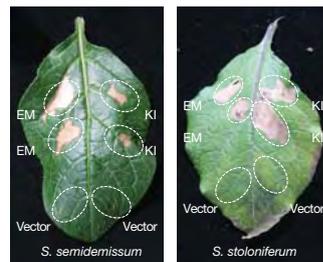


Figure 2: Recognition of Avr3 alleles in wild potato accessions. Recognition of *Agrobacterium tumefaciens* based expression of Avr3a<sup>E80M103</sup> [left] and Avr3a<sup>K80I103</sup> [right] in CPC accessions from *S. semidivissum* and *S. stoloniferum*. An empty vector control [bottom] was used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

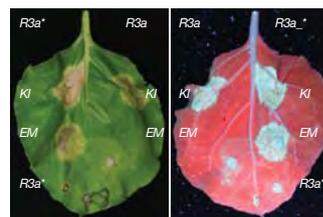


Figure 3: Recognition of Avr3a alleles by wild type R3a and shuffled version R3a\* in the model plant *Nicotiana benthamiana*. R3a strongly recognises AVR3a<sup>K80I103</sup> but not AVR3a<sup>E80M103</sup>. Shuffled version R3a\*, however, displays a hypersensitive reaction (HR) upon co-infiltration with AVR3a<sup>K80I103</sup> and AVR3a<sup>E80M103</sup>. 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 associated 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<sup>K80I103</sup>, recognised by the cognate resistance protein R3a, and/or the virulent allele Avr3a<sup>E80M103</sup> that evades recognition by R3a.

We are using a two-pronged approach to tailor more durable resistance based on recognition of both AVR3a<sup>K80I103</sup> and AVR3a<sup>E80M103</sup>. Screening the Commonwealth Potato Collection (CPC) for very resistant potato accessions has yielded accessions such as *S. semidivissum* 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<sup>K80I103</sup> that have 'gained' recognition of AVR3a<sup>E80M103</sup> [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.