Understanding and Exploiting Late Blight Resistance in the Age of Effectors

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Abstract

Potato (*Solanum tuberosum*) is the world's third-largest food crop. It severely suffers from late blight, a devastating disease caused by *Phytophthora infestans*. This oomycete pathogen secretes host-translocated RXLR effectors that include avirulence (AVR) proteins, which are targeted by resistance (R) proteins from wild *Solanum* species. Most *Solanum R* genes appear to have coevolved with *P. infestans* at its center of origin in central Mexico. Various *R* and *Avr* genes were recently cloned, and here we catalog characterized R-AVR pairs. We describe the mechanisms that *P. infestans* employs for evading R protein recognition and discuss partial resistance and partial virulence phenotypes in the context of our knowledge of effector diversity and activity. Genome-wide catalogs of *P. infestans* effectors are available, enabling effectoromics approaches that accelerate *R* gene cloning and specificity profiling. Engineering *R* genes with expanded pathogen recognition has also become possible. Importantly, monitoring effector allelic diversity in pathogen populations can assist in *R* gene deployment in agriculture.

INTRODUCTION

Effectors: pathogen molecules that alter host cell structure and function thereby facilitating infection and/or triggering defense responses

The potato (*Solanum tuberosum* L.) is one of the three most consumed crops, along with wheat and rice (44). The regions of the globe with the largest areas of potato cultivation are in Asia and Europe, and production of potatoes approached 330 Megatons in 2009 (27). However, potato plants are susceptible to many pests and pathogens that seriously hamper crop production, and extensive use of chemicals is required to reach sufficient yield. The most devastating disease of potato is late blight, which results in global yield losses of 16% (44). Late blight is caused by the notorious oomycete pathogen *Phytophthora infestans* Mont. de Bary, which can infect the entire plant, including stems, leaves, and tubers (32).

P. infestans, renowned for triggering the Irish potato famine in the 1840s, is a destructive pathogen that when left unchecked can destroy a potato crop within a few days. This pathogen success is not only due to its elevated virulence but also to its remarkable capacity to rapidly adapt to resistant plants (32, 41, 84). Indeed, this feature has led authors to describe *P. infestans* as a pathogen with a "high evolutionary potential" (84) and an "*R* gene destroyer" (41). Remarkably, evolutionary and comparative analyses of the *P. infestans* genome revealed a peculiar architecture that underpins accelerated adaptation to host plants (41, 108). The *P. infestans* genome shows an unusual discontinuous distribution of gene density in which disease effector genes and other virulence factors are localized to repeat-rich and gene-sparse regions of the genome. In contrast, housekeeping genes occupy repeat-poor and gene-dense regions (41, 108). The repeat-rich, gene-sparse regions appear to promote evolutionary plasticity and enhance genetic variation of the subset of genes that determine pathogenicity and host-specificity (108).

P. infestans has the capacity to reproduce sexually, a feature that is associated with increased genetic diversity and survival in many parts of the world (32). However, late blight epidemics are often caused by asexual clones that rapidly spread and amplify through aerial dispersion of sporangia, the asexual spores that are abundantly produced on infected plants (33, 63). In agricultural settings, pathogen migration appears to define population dynamics of *P. infestans*. Population displacement by genotypes with increased fitness is a recurrent event. For instance, in the summer of 2007, the late blight season in Great Britain was the worst in 50 years due mainly to the emergence and rapid spread of an aggressive clone termed genotype_13 (Blue13) (33). In the United States and Canada, another *P. infestans* clone that is particularly aggressive on tomato first appeared in the summer of 2009 and spread further in 2010 (118). The impact of these epidemics is illustrated by the significant media and internet coverage. We used Google Trends to track the usage of the word "blight" as an internet search word. Web searches in the United Kingdom and the United States peaked in 2007 and 2009, respectively, during the late blight epidemics (**Figure 1**).

Resistance to *P. infestans* occurs in many tuber-bearing wild *Solanum* species that belong to the highly diverse section *Petota* Dumort. The center of origin of *Solanum* section *Petota*

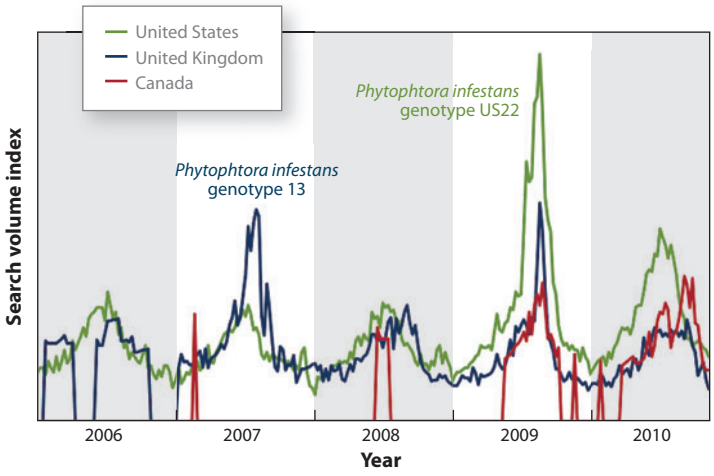

Figure 1

Public concern about late blight reflected in internet searches and news. The graphic displays the search volume index for the term "blight" by region (United Kingdom, United States, and Canada) from January 2006 to the end of 2010. Adapted from Google Trends. **http://www.google.co.uk/trends**.

is thought to be in central Mexico, from which species migrated southward and evolved in a separate gene pool in South America. A return migration to Mexico was followed by hybridizations and allopolyploidizations within Central American taxa (45). Currently, a large number of diploid and polyploid *Solanum* species occur from the most southern parts of Argentina and Chile to the southern United States (57) (**Figure 2*a***). *Solanum demissum*, a common species in central Mexico, has been used in the earliest potato breeding efforts since the first half of the twentieth century. Eleven *S. demissum* resistance (*R*) genes designated *R1-R11* are distinguished in a potato differential set by Black and Mastenbroek (8, 80). *R1*, *R3*, and *R10*, and to a lesser extent *R2* and *R4*, have widely been used for introgression in European breeding programs (62). The exploitation of new cultivars containing these *R* genes was initially successful, but rapidly changing populations of *P. infestans* overcame them (32, 84, 105, 148). Durability in the field of a particular *R* gene is, however, variable (74), and a wealth of novel *R* genes are being discovered from other wild *Solanum* species (23, 51, 113, 115, 122, 124, 140). To date, 21 *R* genes that confer differential resistance specificities to *P. infestans* isolates have been cloned from various *Solanum* species (**Table 1**, **Figure 2**). In relatively short time frames, it should be possible to deploy these *R* genes using genetic engineering approaches that circumvent the problem of linkage drag, which can be a serious problem during classical introgression breeding (46, 59, 98). Because the cloned *R* genes originate in wild species related

R: resistance

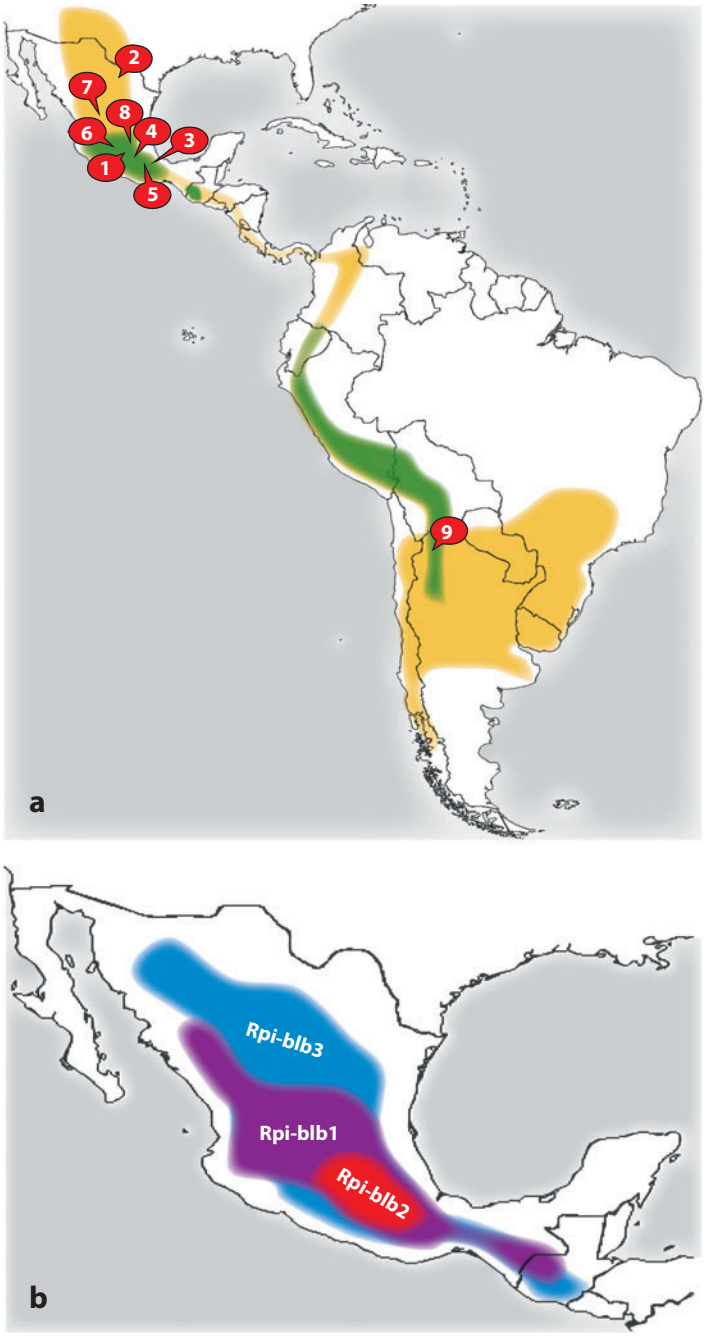

Figure 2

The origin of *R* genes and spread of *Solanum* species. (*a*) *Solanum* section *Petota* species and isolated *R* genes against *Phytophthora infestans* presented on a geographical map, based on 50, 52, 122. Regions that harbor more than five *Solanum* species are yellow, and the two *Solanum* centers of diversity are dark green. Accessions with known *R* genes are depicted on the map: ① *Solanum demissum* (*R1*, *R2*, *R3*, *R4*); ② *Solanum hjertingii* (*Rpi-hjt1*); ③ *Solanum schenckii* (*Rpi-snk1*); ④ *Solanum edinense* (*Rpi-edn1*); ⑤ *Solanum bulbocastanum* (*Rpi-blb1*); ⑥ *Solanum stoloniferum* (*Rpi-sto1*); ⑦ *S. stoloniferum* (*Rpi-pta1*); ⑧ *S. bulbocastanum* (*Rpi-blb2*); ⑨ *Solanum venturii* (*Rpi-vnt1*). (*b*) Geographical spread of *Rpi-blb1*, *Rpi-blb2*, *Rpi-blb3* of *S. bulbocastanum* in central Mexico, adapted from (79).

Table 1 ***Solanum R* genes that confer resistance to *Phytophtora infestans***

R gene	RGH[a]	*Solanum* species[b]	Origin[c]	Chr[d]	Reference
***R1* family**	None				
R1		*demissum*	Mexico	V	(3, 86)
***R2* family**	None				
R2		*demissum*	Mexico	IV	(8, 78, 82)
Rpi-blb3		*bulbocastanum*	Mexico	IV	(78, 95)
Rpi-abpt		Unknown[e]	Mexico	IV	(49, 78, 97)
R2-like		*edinense*	Mexico	IV	(17, 78, 96)
Rpi-edn1.1		*edinense*	Mexico	IV	(17)
Rpi-snk1.1		*schenckii*	Mexico	IV	(17)
Rpi-snk1.2		*schenckii*	Mexico	IV	(17)
Rpi-hjt1.1		*hjertingii*	Mexico	IV	(17)
Rpi-hjt1.2		*hjertingii*	Mexico	IV	(17)
Rpi-hjt1.3		*hjertingii*	Mexico	IV	(17)
Rpi-mcd1		*microdontum*	Argentina	IV	(77)
***R3a* family**	*I2* (94)				
R3a		*demissum*	Mexico	XI	(55, 56)
Rpi-sto2		*stoloniferum*	Mexico	XI	(17)
***R4* family**	Unknown[f]				
R4		*demissum*	Mexico	XI	(136, 142)
***Rpi-blb1* family**	None				
Rpi-blb1, RB		*bulbocastanum*	Mexico	VIII	(123, 134)
Rpi-sto1		*stoloniferum*	Mexico	VIII	(145)
Rpi-pta1		*stoloniferum*[g]	Mexico	VIII	(145)
***Rpi-blb2* family**	*Mi* (114)				
Rpi-blb2		*bulbocastanum*	Mexico	VI	(135)
***Rpi-vnt1* family**	*Tm2*^{2} (72)				
Rpi-vnt1.1		*venturii*	Argentina	IX	(100)
Rpi-vnt1.2		*venturii*	Argentina	IX	(30)
Rpi-vnt1.3		*venturii*	Argentina	IX	(100)

[a]RGH (resistance gene homolog) indicates the closest *R* (resistance) gene homolog from tomato.
[b]Species indicates the donor *Solanum* species from which the *R* gene originated.
[c]Origin indicates the geographic origin of the accession.
[d]Chr indicates the *Solanum* chromosome to which the gene was genetically localized.
[e]*Rpi-abpt* was cloned from breeding material, which was derived from quadruple hybrids ABPT of *Solanum acaule*, *S. bulbocastanum*, *Solanum phureja*, and *Solanum tuberosum*.
[f]Not yet cloned but genetically mapped in vicinity of *N*-like and *Rx*-like sequences.
[g]*Rpi-pta* was originally described to be isolated from *Solanum papita*, which was later renamed to *S. stoloniferum*.

Durability: the period of time before the emergence of a pathogen strain that overcomes resistance

to potato, it will be possible to introduce them using cisgenesis, therefore bypassing some of the issues associated with classical transgenic crops.

Resistance to *P. infestans* is characterized by a cell death–associated defense reaction known as the hypersensitive response (HR) (65, 146). At the first stage of infection, *P. infestans* penetrates the plant and translocates effectors inside host cells (6, 149). Specific effectors can act as avirulence (Avr) factors and activate corresponding *R* genes according to the gene-for-gene model (29). Upon recognition of the effector by an R protein, effector-triggered immunity is activated, often resulting in the HR (61). The *R* genes against *P. infestans* (known as *Rpi* genes)

Table 2 ***Phytophthora infestans*** **effectors with avirulence activity**

Effector	Alternative ID	Number of homologs in genome of T30—4 strain	Types of allelic variants in virulent races	Reference
Avr1		1	Unknown	131; Francine Govers, personal communication
Avr2	PexRD11	18	Mutation	17, 78; Paul Birch, personal communication
Avr3a		3	Mutation	(2)
Avr4		1	Pseudogenization, mutation	(137)
Avrblb1	IpiO	1	Mutation, suppression by another effector	(42, 145)
Avrblb2	PexRD39/40	8	Not known	(90)
Avrvnt1		3	Gene silencing	(99)

typically encode immune receptor proteins of the coiled coil, nucleotide binding, leucine rich repeat (CC-NB-LRR) class of intracellular plant proteins (3, 30, 55, 78, 100, 123, 134, 135) (**Table 1**). These *R* genes have been proposed to follow either of two distinct evolutionary patterns, i.e., fast- or slow-evolving, designated type I or type II *R* genes, respectively.

All known *P. infestans Avr* genes that have been identified thus far belong to the RXLR effector class. These genes encode modular, secreted proteins with a RXLR motif for translocation into the host cell, followed by diverse, rapidly evolving C-terminal effector domains (2, 41, 60, 78, 90, 99, 137, 145, 151) (**Table 2**). Typically, *P. infestans Avr* genes (*a*) function inside the host cell, (*b*) reside in gene-sparse, repeat-rich regions that are thought to contribute to genome plasticity (109), and (*c*) are highly upregulated during the early biotrophic phase of infection in potato **(Figure 3)**. These effectors promote pathogen virulence, for example, by suppressing plant immunity (53, 64, 117).

Effectoromics, a high throughput, functional genomics approach that uses effectors to probe plant germplasm for specific recognition by R proteins, has recently emerged as a powerful tool for identification of *Avr* and *R* genes (26, 90, 145). The availability of genome sequence reso urces for *P. infestans* enabled the generation of effector libraries cloned in vectors designed for in planta expression (41, 90). The effectors are transiently expressed in *Solanum* germplasm by agroinfiltration with *Agrobacterium tumefaciens* and/or a virus vector such as *Potato virus X* (PVX) (**Figure 4*a***), and plants are monitored for the occurrence of macroscopic cell death responses to the individual effectors (17, 145) (**Figure 4*b***). Effectors triggering cell death represent candidate *Avr* genes. These are subsequently validated for Avr activity by genetic analyses (**Figure 4*c***). Co-segregation of the cell death response to the effectors correlates with HR-based resistance to *P. infestans* isolates in genetic populations (**Figure 4*c***, top). If the matching *R* gene has been cloned, additional verification of R-AVR pairs can be obtained by coexpression of *R*-gene and *Avr*-gene candidates in leaves of tester plants such as *Nicotiana benthamiana* (**Figure 4*c***, bottom).

A catalog of *R* and *Avr* gene pairs has recently become available for the potato-*P. infestans* pathosystem (**Table 1**, **2**). In this review, we describe these *R-Avr* pairs in more detail. In addition, we provide an update on different aspects of late blight resistance and discuss how we can exploit disease resistance in the age of effectors (92).

Cisgenesis: a marker free recombinant DNA-based gene transfer of natural genes from the crop plant itself or from crossable plant species

Avr: avirulence

***Rpi*:** resistance gene against *Phytophthora infestans*

NB-LRR: nucleotide binding site, leucine-rich repeat

Type I *R* genes: rapidly evolving *R* genes that are characterized by frequent sequence exchanges between paralogs, resulting in obscured allelic relationships

Type II *R* genes: slowly evolving *R* genes that are characterized by infrequent sequence exchange between paralogs, resulting in tractable allelic relationships

R1-AVR1

R1

The first late blight *R* gene to be cloned was *R1*, which originates from *S. demissum* (3)

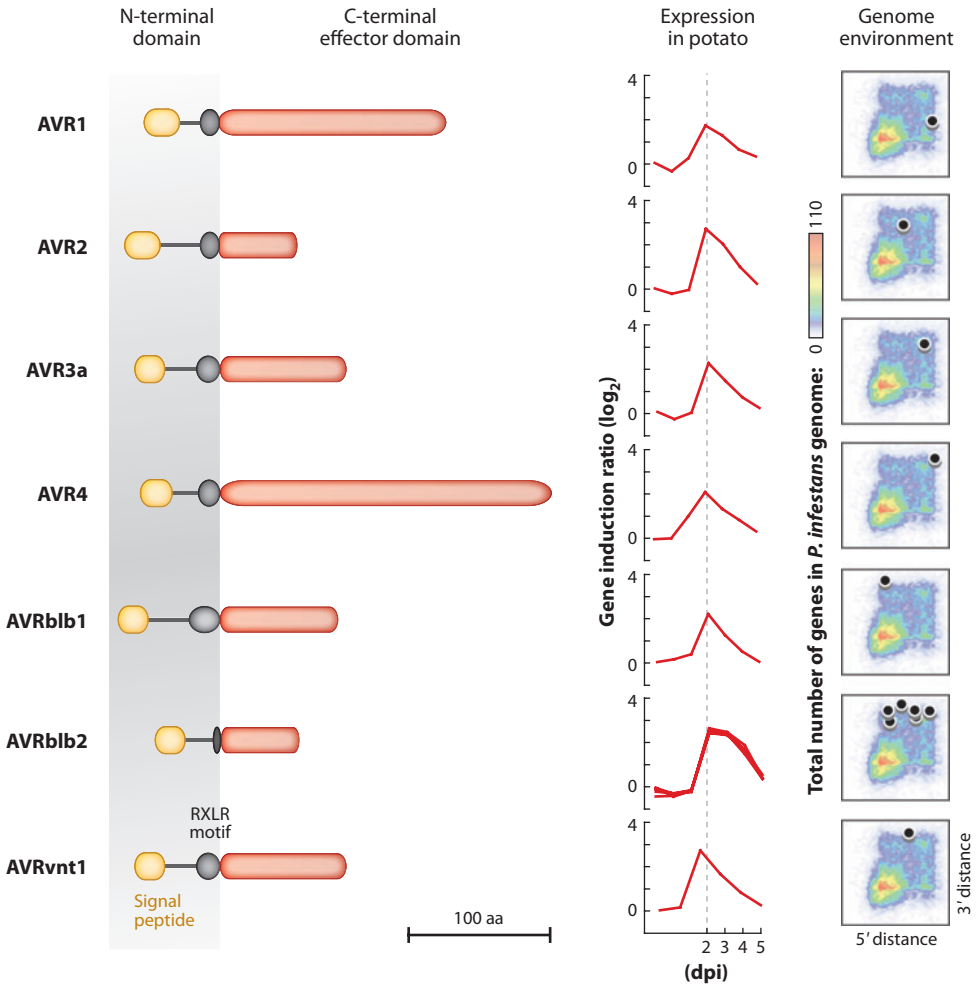

Figure 3

Features of characterized *Phytophthora Avr* gene products. The figure depicts AVR1, AVR2, AVR3a, AVR4, AVRblb1, AVRblb2, and AVRvnt1. The domain structure of *P. infestans* AVR proteins shows a typical RXLR effector modular structure with N-terminal (signal peptide) domain, RXLR motif, and C-terminal effector domain. The N-terminal domain functions in secretion and host translocation whereas the variable C-terminal domain carries the effector biochemical activity. Expression in potato panels illustrate a time course expression pattern of the *Avr* genes during infection of potato [2–5 days post infection (dpi)] with the y-axis showing gene induction (for details see 41). Each of the *Avr* genes is maximally induced at 2 dpi in potato during the early phase of the disease. Genome environment heat maps are two-dimensional plots of 5′ and 3′ intergenic distance for all *P. infestans* genes (for details see 41). The *Avr* genes reside in the gene-sparse regions (upper right corner of the map) with longer distance to their neighboring genes.

RXLR: Single letter amino acid code for arginine, any amino acid, leucine, and arginine; constitutes a motif found in oomycete effector proteins that are translocated into the host.

(**Table 1**, **Figure 2*a***). The *R1* gene was genetically localized on the short arm of chromosome V of potato and isolated by map-based cloning. The gene contains three introns and encodes a CC-NB-LRR protein of 1,293 amino acids (aa). The C-terminal leucine-rich region is unusually short and comprises only approximately 400 aa. Also, the spacing of the leucine residues does not fit the consensus for leucine rich repeats. To our knowledge, there are no known functional homologs of *R1* identified thus far.

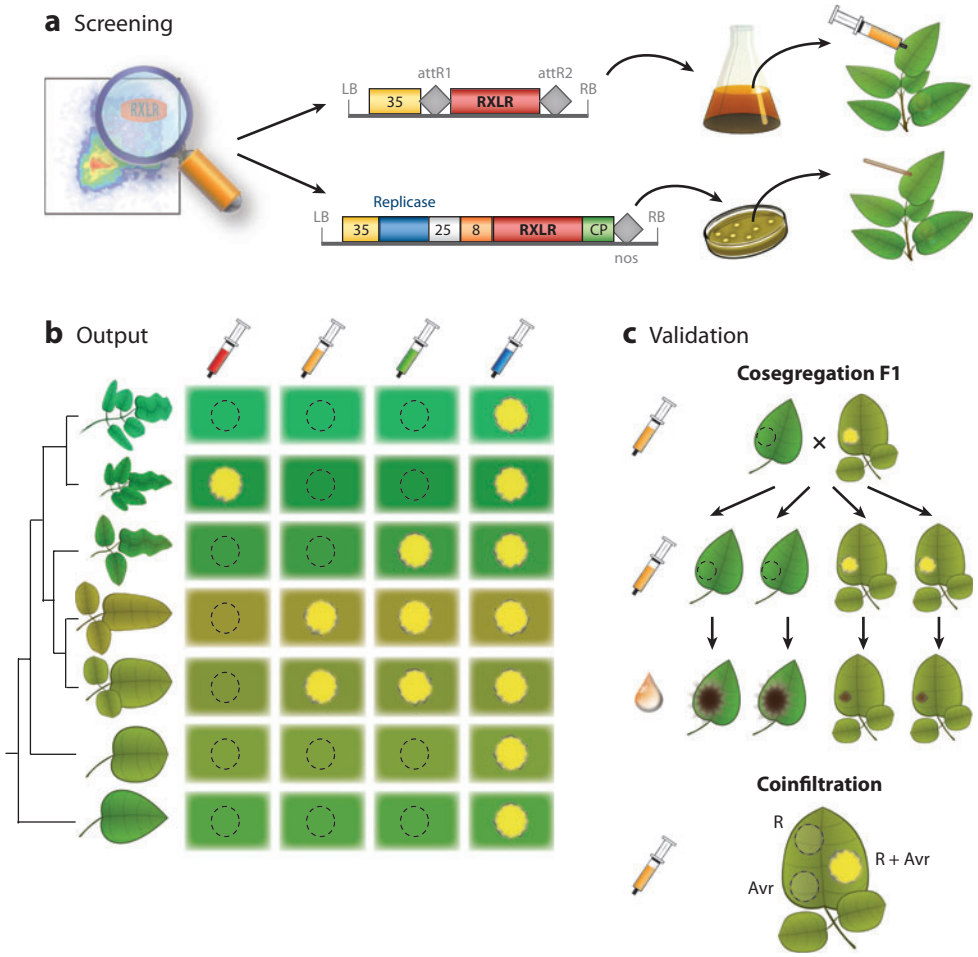

Figure 4

Diagram of effectoromics strategy for identification of corresponding *R* genes and *Avr* genes. (*a*) RXLR effectors are retrieved from *Phytophthora. infestans* genome sequence and cloned into expression vectors. Constructs are introduced in *Agrobacterium tumefaciens* for functional screening in plants by agroinfiltration or *Potato virus X* agroinfection (90, 144, 145). (*b*) Output of screening of *Solanum* germplasm with a range of RXLR effectors depicted in syringes containing different *A. tumefaciens* suspensions (different effectors are illustrated by different liquid colors in the syringes). The different *Solanum* species are shown with different leaf shapes and colors. Columns in the matrix represent patterns of cell death responses that are specific to genotypes, species, or groups, up to nonspecific responses for the *Solanum* section *Petota* (57), as depicted from left to right. Specific responses represent candidate *R-Avr* pairs. (*c*) Validation of *R-Avr* interactions by cosegregation of responses to the effector (syringe) with resistance to *P. infestans* isolates (inoculum droplet) in F1 populations (*top*), and coinfiltration of cultures of *A. tumefaciens* that express *R* and *Avr* gene resulting in specific cell death response (*bottom*).

The *R1* gene is located in an area of the genome where multiple other disease resistance traits have been mapped and resides in a large cluster of paralogous sequences. Substantial structural variation exists among the three *R1* haplotypes from the allohexaploid *S. demissum*. Three groups of independently fast-evolving (type I) *R* genes are represented

Effectoromics: high-throughput approaches to assign activities to computationally predicted effector genes

PVX: *Potato virus X*

aa: amino acids

and characterized by chimeric structures that result from frequent sequence exchange between the paralogs (68).

R1 has been intogressed into many potato cultivars and has been widely deployed in agriculture (62). However, its agricultural value is limited, and it is currently classified as a narrow spectrum resistance gene because the majority of *P. infestans* isolates are virulent on *R1* plants (130).

Avr1

The *Avr1* gene, which encodes the cognate effector of R1, has been isolated using a positional cloning approach (39, 131, 133) (**Table 2)**. However, a full description of AVR1has not yet been published. AVR1 has a RXLR motif in its N-terminal effector domain (131). Similar to other *P. infestans Avr* genes, *Avr1* resides in a repeat-rich and expanded region of the *P. infestans* genome (**Figure 3**). Also, the expression of *Avr1* is highly induced during the biotrophic phase of potato infection (**Figure 3**).

R2-AVR2

R2

R2 is a representative of a highly diverse gene family located at a major late blight (MLB) resistance locus on chromosome IV of potato **(Table 1)**. Eleven *R2* orthologs conferring resistance against *P. infestans* have been identified: *R2*, *R2-like*, *Rpi-blb3*, *Rpi-abpt*, *Rpi-mcd1.1*, *Rpi-snk1.1*, *Rpi-snk1.2*, *Rpi-edn1.1*, *Rpi-hjt1.1*, *Rpi-hjt1.2*, and *Rpi-hjt1.3* (8, 17, 58, 76–78, 82, 95–97, 127). *Rpi-blb3* and *Rpi-abpt* were isolated by map-based cloning, and *R2* and *R2-like* by allele mining (78). The others were identified using effectoromics screens of candidate *R2* gene homologs (R2GH) (17). The R2 family proteins have a leucine-zipper (LZ)-NB-LRR structure and homologs that confer resistance to *P. infestans* range in size from 844 to 847 aa. Outside of *Solanums*, R2 homologs share the highest similarity (35% aa) with RPP13 from *Arabidopsis thaliana* (7), which confers resistance to the *Arabidopsis* downy mildew *Hyaloperonospora arabidopsidis*.

The *R2GH* conferring resistance to *P. infestans* originate from diverse *Solanum* species, including *S. demissum*, *Solanum bulbocastanum*, *Solanum hjertingii*, *Solanum edinense*, *Solanum schenckii*, and *Solanum microdontum* (**Figure 2**). Interestingly, recognition of members of the *Avr2* family is geographically restricted to *Solanum* species originating from Mexico (17). Also, multiple *R2/Rpi-blb3* haplotypes have been detected in Mexican *Solanum* species, suggesting that the gene is polymorphic in these plant populations (79) (**Figure 2*b***). In contrast, the *R2* homolog *Rpi-mcd1*, originating from the Argentinean species *S. microdontum*, does not show HR when coagroinfiltrated with *Avr2* family members indicating that it may have evolved to recognize a different *P. infestans* effector (79). This difference could be explained by adaptive evolution of *R* loci driven by local *P. infestans* populations in South versus Central America, which may have led to distinct recognition spectra between late blight *R* genes from the two centers of diversity (77, 111).

R2GH can differ in the degree of resistance that they confer. For example, in contrast to the high levels of resistance conferred by the Mexican *R2* homologs, the Argentinean *Rpi-mcd1* exhibits partial resistance to late blight (14, 15, 75, 116). A potential explanation for the observed difference is discussed in a paragraph below.

In addition to *R2GH* with resistance activity against *P. infestans* isolates, up to 27 other *R2GH* were identified in the potato *R2* breeding lines and wild *Solanum* species (8, 17, 78, 82). Structural analyses of these *R2GHs* revealed clear blocks of sequence exchange between paralogs (78), suggesting that *R2* is a class I fast-evolving *R* gene (17, 79). In wild *Solanum* genotypes, up to four active *R2* homologs are conserved (17), which suggests that *R2* is still useful to sustain resistance to late blight in the natural ecosystem of the host, conceivably in combination with other endemic *R* genes.

R2 has been exploited in agriculture (62) but has been overcome by late blight in the field. However, *R2* still confers resistance to at least part of the local *P. infestans* populations in various geographic regions, including some provinces in China (S. Zhu, personal communication), Russia (43) and in the Netherlands (G. Kessel, unpublished results). Also, *R2* has been shown to delay infection in France (104). It remains possible that a subset of the *R2GH* may confer broader spectrum resistance than the canonical *R2* gene.

Avr2

Avr2 of *P. infestans* (also called *PiAvr2* to distinguish it from homonyms in fungal plant pathogens) is a member of a highly diverse family of 18 RXLR effectors, represented by the *PITG_22870* gene in the genome of *P. infestans* strain T30-4 (17, 78) **(Table 2**, **Figure 3)**. *PITG_22870* was identified as *Avr2* by map-based cloning (E. Gilroy, P. Birch, personal communication); family member *PexRD11* was detected by effectoromics screens, and the other family members by sequence similarity searches of the *P. infestans* genome (41). *PexRD11* and *PITG_22870* share a low level of aa sequence identity sensu stricto, but 63% similarity based on identical, conserved, and semiconserved aa substitutions. From the *PexRD11/PITG_22870* effector family, 13 candidate effectors are nonredundant, and four of these (including PEXRD11 and *PITG_22870*) induce cell death when coexpressed with *R2*, *R2-like*, *Rpi-abpt*, or *Rpi-blb3*. A fifth member of the family is recognized by 6 additional *R2* homologs. The other *PexRD11/PITG_22870* family members are not recognized and correspond to truncated or mutated proteins, or are not expressed during infection. Detailed population and functional studies will reveal whether diversity seen in *R2* family members in central Mexico reflects a coevolutionary arms race between this family and the diverse family of related RXLR effector genes in *P. infestans*.

R3A-AVR3A

R3a

R3a is a well-characterized *R* gene originating from Mexico that occurs at an MLB resistance locus on the short arm of chromosome XI from *S. demissum* (55) **(Table 1**, **Figure 2*a*)**. Originally, *R3*-specific resistance derived from the potato R3 differential (83) was mapped in this locus (24). Fine-mapping studies revealed that the phenotype was conferred by two tightly linked *R* genes, *R3a* and *R3b*, a gene that has a distinct recognition specificity (56). The *R3a* locus is highly expanded in *S. demissum* and harbors 30 to 45 *R3a* homologs per haplotype (31). This MLB resistance locus also contains *R5* through *R11*, making it a hot spot of *Rpi* genes of *S. demissum* origin (13, 25, 54)

Comparative genomic studies with the tomato *I2* locus, which harbors major genes encoding resistance to the fungus *Fusarium oxysporum* (94, 120), enabled the isolation of *R3a*. The *R3a* gene contains a single exon, which belongs to the CC-NB-LRR class, encoding a protein of 1,283 aa with nine imperfect LRR. *R3a* is characterized as a typical type I *R* gene (69) and seems to represent an ancient *R* gene of Mexican origin (17, 54). *R3a* gene homologs (R3aGH) with high sequence conservation were also detected in *Solanum stoloniferum* using functional allele mining with Avr3a. The R3aGH *Rpi-sto2* shares 99.9% aa identity with *R3a* and confers resistance to *P. infestans* with the same specificity as *R3a* (17).

R3a has been widely used since early potato breeding (62), but many *P. infestans* isolates overcome *R3a* in potato growing areas as well as central Mexico (43, 112, 139).

Avr3a

Avr3a encodes an RXLR cytoplasmic effector (5, 88) that was cloned based on the interaction between candidate *P. infestans* extracellular proteins (*Pex*) (128) and potato genotypes carrying *S. demissum R* genes (2) (**Table 2**, **Figure 3**). Homologs of *Avr3a* have

been found in *Phytophthora sojae* and *Phytophthora capsici* (10), and the genomic region containing *Avr3a* shows collinearity with the *ATR1* locus in *Hyaloperonospora parasitica* (2, 110). In *P. infestans* populations, two alleles of *Avr3a* have been identified that encode secreted proteins AVR3a$^{K80/I103}$ (AVR3a^{KI}) and AVR3a$^{E80/M103}$ (AVR3a^{EM}), which differ in two aa in their effector domains (2) **(Table 2)**. This difference profoundly affects host response: AVR3a^{KI} but not AVR3a^{EM} triggers effector-triggered immunity by activating the potato resistance protein R3a (2, 47, 55). Both forms of AVR3a can suppress the cell death response induced in potato by the *P. infestans*-secreted protein INF1, although AVR3a^{KI} is stronger (10, 11). Recently, AVR3a was found to suppress immunity by binding and stabilizing the host E3 ubiquitin ligase CMPG1 (9). Interestingly, these different activities of AVR3a can be uncoupled as mutants that are deficient in either R3a activation or suppression of cell death have been recovered (reviewed in 91). Armstrong et al. (2) proposed that *Avr3a^{EM}* arose from an *Avr* allele after gene duplication and positive selection. It was recently shown that *Avr3a* is essential for full virulence of *P. infestans* (9), which suggests that this effector can be an important target for durable resistance breeding if *R* genes that target all the allelic forms can be identified (see below).

When *R3a* was bred into potato cultivars in the 1950s, virulent races of *P. infestans* rapidly emerged to overcome the resistance in multiple regions of the world (32). It was assumed that the *Avr3a* gene must be highly mutable and that mutated *Avr3a* must arise very rapidly in *P. infestans* populations. The cloning of *Avr3a* revealed that, surprisingly, this gene has little allelic diversity and that only two major haplotypes occurr (*Avr3a^{KI}* and *Avr3a^{EM}*) (2). It appears that the great majority of avirulent races of *P. infestans*, including the US1 clonal lineage that dominated pathogen populations in the 1950s, are heterozygous for the *Avr3a* gene (2). Therefore, virulence on *R3a* potatoes did not evolve by repeated independent mutation of *Avr3a* but by allele reassortment in sexual populations. In asexual populations, strains homozygous for *Avr3a^{EM}* probably evolved from heterozygous strains through mitotic gene conversion, which is known to occur at relatively high frequency in *Phytophthora* (16).

R4-AVR4

R4

The fourth resistance gene from *S. demissum*, *R4* **(Figure 2*a*)** has not yet been cloned; however, it has been placed on the genetic map. The *R4* gene is linked to a molecular genetic marker with sequence homology to the *Rx* gene, which confers resistance to PVX (4, 136). In another study, response to AVR4 was localized on the long arm of chromosome XI (142). Indeed, this chromosomal region contains *Rx*-like sequences in the *Solanum phureja* genome sequence (101, 143). This suggests that *R4* resides in an *R* gene cluster containing *Rx*-like sequences on chromosome XI.

Similar to *R1*, *R2*, and *R3*, *R4* is classified as a narrow spectrum *R* gene, which was introgressed into potato cultivars in the mid-twentieth century (62). It remains of limited agricultural value because of the high frequency of virulent pathogen races (43, 139).

Avr4

Avr4 of *P. infestans* (also called *PiAvr4*) was cloned using a combination of map-based cloning (133, 150) and cDNA-AFLP (amplified fragment length polymorphism)-based transcriptional profiling (40, 137). *Avr4* encodes a typical RXLR effector of 287 aa. It is a single-copy gene residing in an approxmimately 100-kb expanded repeat-rich region of the *P. infestans* genome **(Table 2**, **Figure 3)** (41, 138). Homologs that are related to *Avr4* exist in *P. sojae* and *Phytophthora ramorum* (137, 138). Unlike *Avr3a*, *Avr4* is highly polymorphic in *P. infestans*. Allelic variants in virulent races show deletions causing a premature stop codon, resulting in truncated, probably nonfunctional, proteins. Strains that carry a full-length copy of

Avr4 are always avirulent on plants carrying *R4*. Interestingly, nonsense or premature termination mutations of *Avr4* do not seem to penalize fitness of *P. infestans*, which explains why virulent races evolved repeatedly and at a somewhat high frequency (137). Therefore, *Avr4* is an example of a dispensable effector that evades resistance through pseudogenization of the full-length gene. R proteins recognizing such dispensable effectors cannot provide durable resistance and are not considered as useful targets in resistance breeding programs.

RPI-BLB1-AVRBLB1

Rpi-blb1

Rpi-blb1, also known as *RB*, was isolated from the Mexican wild potato species *S. bulbocastanum* using a map-based cloning approach in combination with a long-range polymerase chain reaction strategy (**Table 1**, **Figure 2*a***). *Rpi-blb1* encodes a CC-NB-LRR protein of 970 aa. Functional homologs of *Rpi-blb1* were also detected in *S. stoloniferum* following effectoromics screens and resulted in the rapid isolation of *Rpi-sto1* and *Rpi-pta1* (145). *Rpi-blb1* was originally described as a broad spectrum *R* gene conferring race-nonspecific resistance to all tested *P. infestans* isolates. However, virulent races on *Rpi-blb1* potatoes have recently been identified in Mexico (18). Nonetheless, virulent races appear to be rare, particularly in Europe and North America, and thus *Rpi-blb1* appears to resist a broader spectrum of *P. infestans* isolates than most of the other exploited *R* genes.

Rpi-blb1 locates to a cluster of four CC-NB-LRR paralogs on chromosome VIII. *Rpi-bt1*, another *S. bulbocastanum R* gene that confers resistance to late blight, also localizes on chromosome VIII and is 78% similar at the aa level to *Rpi-blb1* (93). *Rpi-blb1* is described as an ancient *R* gene and has an evolutionary pattern similar to Type II *R* genes, which are predicted to evolve slowly and show significant orthologous relationships in diverse species (69, 123, 134). Allele mining and gene-specific marker studies in diverse *Solanum* germplasm demonstrated that *Rpi-blb1* is geographically restricted to Mexico **(Figure 2*b*)** and is limited to only a few *Solanum* species (79, 125, 147).

S. bulbocastanum cannot directly be crossed with cultivated potato *S. tuberosum*, and complicated techniques that require several years to complete, such as bridge crosses or somatic hybridizations, are required to introgress *Rpi-blb1* into cultivated potato (48, 49). For these reasons, *Rpi-blb1* is not yet prevalent in potato cultivars. A more rapid introgression became recently feasible by the identification of the functional homolog *Rpi-sto1* from *S. stoloniferum*, which can be directly crossed with *S. tuberosum* cultivars (145, 147).

Besides classical breeding, the cloning of *Rpi-blb1/RB* has opened the possibility of producing resistant potatoes with increased resistance to late blight by direct transfer of the gene using recombinant DNA techniques (70). However, the exploitation of *Rpi-blb1* may be complicated by the observation that when introduced into cultivated potato, this gene confers only partial resistance to aggressive isolates. In a collection of independent *Rpi-blb1* transgenic lines, the level of resistance was positively correlated to the amount of *Rpi-blb1* transcript and negatively correlated with the aggressiveness of the applied *P. infestans* isolate (12, 67). In detached leaf assays, highly aggressive isolates can infect most transgenic potatoes, but not the wild *S. bulbocastanum*, which showed the highest endogenous *Rpi-blb1* transcript levels as well as a dramatic transcriptional increase after inoculation (12, 18, 67). These studies suggest that expression of *Rpi-blb1* alone may not be sufficient to provide satisfactory resistance for potatoes in the field and that stacking with other *R* genes may be necessary (see below).

Avrblb1

Avrblb1 was identified following effectoromics screens of a collection of late blight resistant *Solanum* with candidate RXLR effectors (145) **(Figure 4)**. *Avr-blb1* turned out to be identical to the in planta induced gene *ipiO*, which was initially postulated to be involved

in pathogenicity based on its expression profile (102, 103, 141) (**Table 2**, **Figure 3)**. In the genome of particular *P. infestans* strains, *ipiO* is present in two copies in inverted orientation (41, 103). The *ipiO* gene family is highly diverse among a series of *P. infestans* isolates that were collected worldwide (18, 42). The *ipiO* variants are grouped into three classes, I, II, and III, from which class I contains most variants, including the well-characterized *ipiO*1 and *ipiO*2 (18). Absence of class I *ipiO* was correlated with virulence on *Rpi-blb1* plants. In line with this, class I and II, but not class III *ipiO*, induce cell death when coexpressed with *Rpi-blb1*. Interestingly, class III *ipiO* can suppress *Rpi-blb1*-mediated cell death, which could explain virulence phenotypes on *Rpi-blb1* plants (42). The observation that class I *ipiO* occurs in most *P. infestans* isolates collected worldwide explains why *Rpi-blb1* is functional against the majority of *P. infestans* isolates (18). Therefore, *Rpi-blb1* is a useful *R* gene to deploy in agriculture.

RPI-BLB2-AVRBLB2

Rpi-blb2

Rpi-blb2 was isolated by positional cloning and originates from *S. bulbocastanum* (135) (**Table 1)**. The deduced open reading frame encodes a predicted polypeptide of 1,267 aa, and belongs to the CC-NB-LRR proteins. *Rpi-blb2* is located on the short arm of chromosome VI, in a hot spot of NB-LRR genes. In tomato, this same region harbors the *Mi-1* gene, which confers resistance to nematodes, aphids, and white flies (87, 89, 114), and Mi-1 shares 82% of the aa sequence identity with Rpi-blb2.

The origin of *Rpi-blb2* lies in central Mexico, and its occurrence is confined to accessions from that region **(Figure 2)**. Sequences similar to *Rpi-blb2* occur at low frequency in *Solanum*, and the lack of allelic diversity among *S. bulbocastanum* accessions suggests that this *R* gene evolved recently (79).

Rpi-blb2 confers effective resistance against the *P. infestans* strains tested so far, making it a desired gene for breeding (135). Only on rare occasions has infection been found on *Rpi-blb2*-containing *Solanum* species in the Netherlands (G. Kessel, unpublished results). Introgression of *Rpi-blb2* has resulted in the resistant potato varieties Bionica and Toluca after 46 years of strenuous breeding efforts that utilized bridge-crosses and successive backcrosses to diminish linkage drag (44, 132). The broad spectrum activity associated with *Rpi-blb2* has also motivated the use of genetic engineering to produce plants carrying a combination of *Rpi-blb1* and *Rpi-blb2* by companies and governmental programs (44). A genetically-engineered potato variety with the trade name Fortuna that carries both *Rpi-blb1* and *Rpi-blb2* genes has entered the commercialization pipeline in Europe but will require a few more years before final approval for cultivation (145).

Avrblb2

Avrblb2 was identified after allele mining and functional screening of candidate RXLR effector genes (90). *Avrblb2* belongs to a multi-gene family with at least seven duplicated copies in the genome of *P. infestans* strain T30–4 (41) (**Table 2**, **Figure 3**). The gene encodes a secreted protein of 100 aa that is highly polymorphic and exhibits high rates of nonsynonymous substitutions. Among 13 variable residues in the mature protein, Oh and colleagues (90) found a key residue at position 69 that compromises activation of Rpi-blb2. When this residue was mutated from Ala, Ile, or Val to Phe_{69} in the *Avrblb2* background, activation was lost. Interestingly, natural $Avrblb2^{Pbe69}$ variants coexist with avirulence copies of the gene in the genome of several isolates of *P. infestans* (90; R. Oliva & S. Kamoun, unpublished results). This suggests a potential benefit for *P. infestans* in maintaining duplicated copies of *Avrblb2*. Under these conditions, it is less likely that consecutive point mutations or deletions would enable gain of virulence in the short term. Whether other mechanisms such as epigenetic gene silencing or suppression may play a more predominant role in the attenuation of avirulence is unpredictable.

RPI-VNT1-AVRVNT1

Rpi-vnt1

Rpi-vnt1 was the first late blight *R* gene to be cloned from a plant of South American origin **(Figure 2*a*)**. Three highly similar allelic variants, *Rpi-vnt1.1*, *Rpi-vnt1.2*, and *Rpi-vnt1.3*, were isolated from three different accessions of *Solanum venturii* (30, 100) **(Table 1)**. *Rpi-vnt1* contains a single exon encoding a 891 aa CC-NB-LRR protein with a C-terminal leucine rich region that is relatively short (377 aa) and loosely fits the consensus for LRR. *Rpi-vnt1* shares 75% aa identity with the mosaic virus resistance protein $Tm2^2$ from tomato (72).

Rpi-vnt1.1, *Rpi-vnt1.2*, and *Rpi-vnt1.3* are located on the long arm of chromosome IX. From *Solanum mochiquense*, *Rpi-mcq1* (formerly *Rpi-moc1)* was mapped to a similar genetic location (121) and was found to be homologous to $Tm2^2$ and *Rpi-vnt1* (30). Spectrum analyses with *P. infestans* isolates and functional assays using effectors suggest that *Rpi-mcq1* and *Rpi-vnt1* have distinct recognition specificities (H. Rietman, unpublished results).

Genomic analysis revealed the presence of many related sequences in the genome, of which only one copy is located at the *Rpi-vnt1* locus. Pel et al. (99) hypothesized that allelic variants of *Rpi-vnt1* evolved through illegitimate recombination, and the conservation of functionally equivalent allelic variants suggests that *Rpi-vnt1* is a type II *R* gene (69). The low distribution of *Rpi-vnt1* allelic variants through the section *Petota* and the confinement of this gene to a geographic area in the Andes, suggests that *Rpi-vnt1* emerged recently.

Rpi-vnt1 has not widely been used in agriculture, even though it confers resistance to a broad spectrum of isolates from potato-growing areas in Europe (100). A three year field trail with a genetically engineered potato based on the popular variety Desirée expressing *Rpi-vnt1* started in 2010 in Norfolk, United Kingdom (66) and will determine whether *Rpi-vnt1*-based resistance to late blight is realized under field conditions in Europe.

Avrvnt1

Avrvnt1 was recently identified using effectoromics (99) (**Figure 4**). A genome-wide set of expressed RXLR effectors of *P. infestans* was functionally screened in a resistant S. *venturii* containing *Rpi-vnt1*, and few candidate *Avr* genes were found to induce defense responses. One effector, designated *Avrvnt1*, specifically induced cell death in the resistant offspring of a population that segregated for *Rpi-vnt1*. In the reference genome of *P. infestans* T30-4, *Avrvnt1* locates in a gene-sparse region at a single locus (41) (**Figure 3)**. Only three homologous RXLR effectors were detected in the T30-4 genome, pointing to a small gene family (**Table 2)**. The genetic diversity of *Avrvnt1* among a broader set of *P. infestans* isolates was limited to four variants. In *P. infestans* strains that are avirulent on *Rpi-vnt1* plants, *Avrvnt1* is upregulated during the early biotrophic phases of the infection (**Figure 3)**. In virulent strains of the South American EC1 lineage however, *Avrvnt1* coding sequence was intact but its transcript was not detected. This suggests that *Avrvnt1* evades *R* gene recognition by reduced expression, and conserved sequence between the avirulent and virulent alleles points to epiallelic variation (99).

Broad spectrum: effective against a wide range of pathogen isolates

DIVERSITY OF LATE BLIGHT *R* GENES IS HIGHEST IN CENTRAL MEXICO

The antagonistic interplay between the pathogen and host is driven by *R* and *Avr* genes that are expected to be the direct targets of coevolutionary forces (20, 88). Therefore, one would expect diversity of late blight *R* genes to be high in regions where coevolution between *P. infestans* and *Solanum* has occurred over a long period of time. In contrast, host plants from geographical regions that have been only relatively recently invaded by the pathogen are expected to display reduced levels of late blight *R* gene diversity. Many of the Mexican *R* genes that confer high levels of

QTL: quantitative trait loci

race-specific resistance, including *R1* through *R4* of *S. demissum*, appear to represent products of ancient and extensive coevolution (122) (**Table 1**, **Figure 2*a***). In addition, virulent alleles of the corresponding Avr effectors have been detected in Mexican populations of *P. infestans*. These observations are consistent with the hypothesis that central Mexico, not South America, is the primary center of origin of *P. infestans* (34, 37, 38). This said, several distinct lineages of *P. infestans* occur in South America and matching *R* genes have been detected in South American *Solanum* species (1, 36).

R GENES THAT CONFER PARTIAL RESISTANCE

Some *R* genes appear to confer partial resistance to *P. infestans*. These *R* genes often segregate as quantitative trait loci (QTL) in genetic mapping populations and breeders tend to wrongly assume that their mode of action differs from classical gene-for-gene interactions (reviewed in 35, 46, 106, 111). In these cases, coevolution between pathogen and host may be less advanced than for genes conferring full resistance. An example is *Rpi-mcd1* (77), which was originally described as a QTL from an Argentinean *S. microdontum* (127). Initially, this gene was assumed to be similar to other QTL identified in South America genotypes, but it was later shown to be a classical *R* gene of the *R2* family (78). An example from Mexico is *Rpi-blb1*, which confers partial resistance in transgenic or introgressed potatoes (12, 18, 22, 67). Perhaps this slowly evolving (Type II) *R* gene has not fully unleashed its evolutionary potential and the partial level of resistance may reflect a weaker recognition of the *Avrblb1* effector. Consistent with this proposal, the *Rpi-blb1* donor species, *S. bulbocastanum* and *S. stoloniferum*, typically occur in more arid habitats that are less conducive to *P. infestans* infections, compared with *S. demissum* (18, 54).

PARTIAL EVASION OF R PROTEIN RECOGNITION BY *P. INFESTANS*

As described above, there are many examples of *P. infestans* races that have evolved to overcome *R* gene–mediated resistance. In all cases reported so far, the virulent races are equally aggressive on genotypes that differentially carry/lack the cognate *R* gene (**Figure 5*a***). This is explained by the *Avr* effector gene being usually mutated or pseudogenized to an inactive form. The impact of such mutations on pathogen virulence is negligible, possibly due to functional redundancy in the effector repertoire (5).

Recently, we have noted that some isolates of *P. infestans* are partially virulent on *R* gene–containing plants (G. Kessel, R, Oliva, J. Vossen, V. Vleeshouwers & S. Kamoun, unpublished results). In these cases, although the virulent race of the pathogen causes expanding lesions on the *R* gene–containing genotype, these lesions are smaller and expand less rapidly than on the *R* gene–lacking genotype (**Figure 5*b***). This indicates that the *R* gene confers partial resistance against the virulent race of *P. infestans*. Therefore, when assaying virulent races of *P. infestans*, it is critical to perform side-by-side assays with *R* gene differential plant genotypes to discriminate between fully and partially virulent strains as described in **Figure 5**.

What is the molecular basis of partial evasion of *R* gene mediated resistance by *P. infestans*? The observation that the *R* gene retains a residual effect against the partially virulent *P. infestans* indicates that the virulent strain carries a functional *Avr* effector gene. One explanation is that the *Avr* gene has not pseudogenized but may have mutated to an allele that partially evades activation of the *R* gene. An alternative is that the timing and level of expression of the *Avr* effector may be altered enough to delay or reduce the intensity of recognition by the *R* gene. A third possibility is that the partially virulent race evolved a suppressor gene

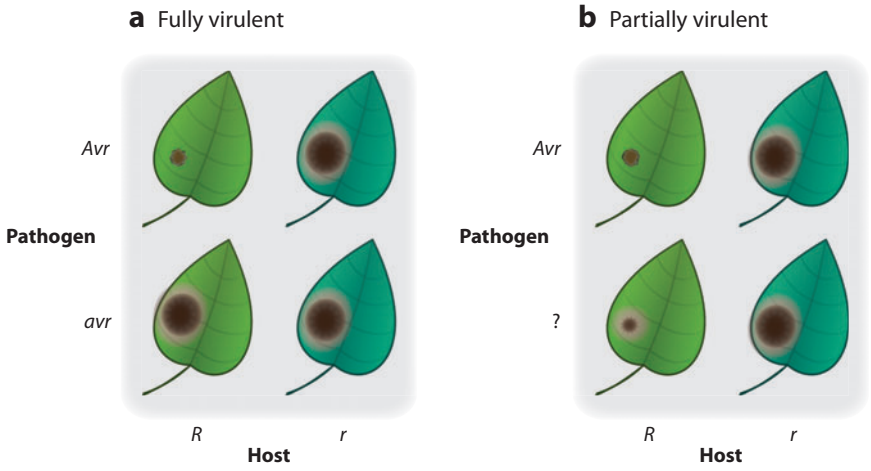

Figure 5

Phenotypic distinctions between fully versus partially virulent strains of *Phytophthora infestans*. (*a*) Fully virulent *P. infestans* strains grow at the same rate irrespective of *R* gene presence. (*b*) For partially virulent strains, the presence of the *R* gene has an impact on the overall performance of the strain. In the scheme, small circles represent typical nonexpanding hypersensitive response lesions, big circles represent expanding lesions with sporulation of *P. infestans*, and the intermediate-size circle represents a slowly expanding lesion. A difference in the degree of disease caused by the virulent race between *R* versus *r* plants indicates that the *R* gene still confers partial resistance.

of the *R* gene response. This situation is more likely to arise in cases where the effector is essential for full virulence of *P. infestans* (73).

Importantly, *R* genes that have been only partially defeated remain useful for deployment in agriculture, especially when stacked with other *R* genes. Targeting a critical pathogen effector (Achilles heel hypothesis), as for instance in the tomato–*Fusarium oxysporum* system (*I* and *Avr1* interaction) (126), might be beneficial for maximizing resistance durability in the field. But even under such Achilles heel conditions, *P. infestans* appears able to evolve into a somewhat virulent race in line with its reputation as a pathogen with a high evolutionary potential (84). Nevertheless, this theoretical extension of the gene-for-gene model provides a first insight to explain the partially resistant phenotypes often observed in the potato–*P. infestans* pathosystem.

EXPLOITING EFFECTORS IN RESISTANCE BREEDING

The emerging field of pathogen effector biology has valuable implications for breeding and deployment of disease resistance (26, 90, 145). Here, we present four ways by which the knowledge on pathogen effector diversity and mode of action can be utilized to improve the use and deployment of late blight disease resistance.

First, the cloning of *R* genes is accelerated when matching *Avr* genes are available. *R* gene activity can efficiently be assessed by rapid functional assays that consist of coexpressing the *Avr* gene in planta with candidate *R* genes, e.g., by agroinfection or agroinfiltration (**Figure 4**) (90, 145). This means that the slow and tedious process of generating stable transformants for complementation studies in potato is no longer a limiting step.

Second, functional allele mining with *Avr* genes in large collections of germplasm can quickly lead to identification of functional resistance gene homolog (*RGH*) in a variety of species. This can accelerate resistance breeding as sexually more compatible species with a particular resistance specificity can be identified and used for introgression (145). In addition, redundant breeding or cloning efforts can be avoided by classifying germplasm or *R* genes

RGH: resistance gene homolog

based on their responses to effectors. This is particularly important for *R* genes with broad spectrum effects for which diagnostic pathogen races are not available.

Third, expanding the effector recognition specificity of a given *R* gene to new virulent alleles is another strategy to increase the pool of genes available for breeding. Artificial evolution by random mutagenesis is a potential tool to accomplish this goal, as previously demonstrated for the PVX resistance gene *Rx* (4, 28). Expanded recognition specificity by a new *R* gene variant could be due to a single aa change in the R protein as we recently discovered for *R3a* (M.E. Segretin & S. Kamoun, unpublished results). In this scenario and whenever the original *R* gene is present in the crop species, targeted mutagenesis (genome editing) by new technologies, such as zinc finger nuclease–based approaches (119, 129), can be implemented. This would potentially provide a nontransgenic resistant variety that does not carry extraneous pieces of DNA (81). A key requirement for the success of this strategy is to have basic knowledge of the pathogen effectors. The right choice of the *R-Avr* pair will influence the probabilities of success in terms of durability once the new *R* gene is deployed in the field. Also, knowledge related to the effector protein is needed in order to design an efficient screening system to identify the mutated *R* gene candidates. Moreover, if homologs of the targeted *Avr* gene are present in other species, the R protein recognition specificity could be potentially expanded to confer resistance to related pathogens.

EXPLOITING EFFECTORS IN DEPLOYMENT OF RESISTANCE

A fourth aspect of exploiting pathogen effectors is to improve the spatio-temporal deployment of *R* gene–based disease resistance. The geographical structure and dynamics of *P. infestans* populations impact the effectiveness of *R* gene–based late blight management. For instance, sub-Saharan Africa is dominated by few locally adapted *P. infestans* asexual lineages (85, 107), whereas more dynamic or complex populations tend to occur in Europe or North America (33). Effector assessment can assist with evaluating the potential of a given *R* gene by informing about the distribution of virulence alleles in local *P. infestans* populations. Such analysis would detect emerging virulent races of *P. infestans* before they reach epidemic proportions. Along with genetic adaptation and selection, emergence of highly aggressive clones is accelerated because primary inoculum will be increased in the following season. In Europe for instance, genotype_13 appeared in 2004, and increased in frequency from 12% to 71% of populations in the United Kingdom over three seasons (19, 33). One alternative to manage these types of epidemics is to buffer the occurrence of the clone by choosing appropriate cultivars. With the availability of the *P. infestans* genome sequence (41) and next-generation sequencing technology, rapid profiling of the effector repertoires of emerging genotypes of *P. infestans* is possible. Within a couple of months it is possible to generate a list of *Avr* gene targets for a given isolate (or genotype) of *P. infestans*. This can assist decision making in the next potato growing season. In season monitoring for virulence frequencies of *Avr* genes is also possible, and in an experiment in 2010 a strong reduction of fungicide input on field plots with *R* gene–carrying potatoes was achieved through effector monitoring (G. Kessel, unpublished results). Given that important efforts to understand and characterize Avr effector function and variability are underway (18, 42, 90, 145), we will soon be in a position to monitor potential changes in pathogen populations.

CONCLUSION

The most sustainable strategy to manage late blight is to breed broad-spectrum disease resistance into potato. However, traditional disease resistance breeding approaches are slow, inefficient, and have taken little advantage of emerging knowledge of pathogen mechanisms. Late blight resistance genes have been

identified, bred, and deployed in agriculture in a 'blind' fashion without detailed knowledge of the effectors they are recognizing. It remains problematic to predict the extent to which an *R* gene will be durable until it is actually deployed in agriculture. Nonetheless, it is relatively easy to point to non-durable *R* genes, i.e. *R* genes such as *R4* that target dispensable effectors. Therefore, the first step in a breeding program should be to avoid wasting efforts on such ineffective *R* genes to maximize the potential for resistance durability. This is already a step beyond traditional "pathogen-blind" resistance breeding approach.

SUMMARY POINTS

1. Map-based cloning, allele mining, effectoromics, and sequencing of the potato genome led to isolation of 21 *R* genes conferring resistance to late blight.
2. Late blight *R* gene families occur in several different genomic locations. *R* genes differ in resistance spectrum, geographic origin, evolution rate, and in the degree of resistance they confer.
3. Most *R* genes identified thus far confer high levels of race-specific resistance and were retrieved from *Solanum* species originating from Central America, the center of origin of *P. infestans*.
4. *Avr* effector genes reside in gene-sparse regions of the *P. infestans* genome, belong to diverse gene families that vary in size between isolates and are typically upregulated at early stages of the biotrophic interaction. They encode modular proteins with a signal peptide, an RXLR motif, and a C-terminal effector domain.
5. *Avr* genes avoid recognition by diverse mechanisms, e.g., mutation, gene loss, suppression, and gene silencing.
6. Exploiting effectors in breeding has led to accelerated cloning of *R* genes and enables accurate profiling of *R* gene specificities.
7. Effectors can be exploited in deployment of resistance by monitoring pathogen populations for effector allelic diversity.
8. Eliminating ineffective *R* genes that target dispensable *Avr* effectors maximizes the potential for resistance durability in the field and is a step beyond traditional "pathogen-blind" approaches.

FUTURE ISSUES

1. Next generation sequencing and new approaches are expected to further accelerate *R* gene cloning.
2. Next generation resistance breeding will enhance resistance spectrum and durability by incorporating engineered, synthetic *R* genes with expanded pathogen recognition specificities.
3. Investigating the molecular mechanisms of partial resistance and partial virulence will contribute to understanding observed phenotypes and lead to educated deployment of available resistance genes and QTL.

4. Exploiting another layer of defense, based on pathogen molecular pattern recognition by recognition receptors could contribute to producing a defense response that is more durable and broad spectrum (71).

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