Review

Elucidation of virus-host interactions to enhance resistance breeding for control of virus diseases in potato

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Potato virus Y (PVY) and *Potato mop-top virus* (PMTV) are viruses whose geographical distribution is expanding and economic losses are increasing, in contrast to most of other viruses infecting potato crops. Most potato cultivars lack broad-spectrum resistance to the new, genetically complex strains of PVY, and no efficient resistance to PMTV is known in potato. Control of the vectors of these viruses is not an efficient or possible strategy to prevent infections. Studies on molecular virus-host interactions can discover plant genes that are important to viral infection or antiviral defence. Both types of genes may be utilized in resistance breeding, which is discussed in this paper. The advanced gene technologies provide means to fortify potato cultivars with effective virus resistance genes or mutated, non-functional host factors that interfere with virus infection.

Key Words: *Solanum tuberosum*, virus resistance, gene-for-gene resistance, recessive resistance, RNA silencing, marker-assisted breeding, gene technology.

Introduction

Resistance breeding plays an important role in the efforts to prevent yield losses caused by virus diseases in potato (*Solanum tuberosum* L.). True resistance prevents virus infection, spread of the virus in the plant, and/or reduces accumulation of the virus in infected cells and tissues. It is different from *tolerance* that lacks any of the aforementioned features, and refers to virus-plant interactions in which the systemically infected plant displays no apparent symptoms (Cooper and Jones 1983). Chemical control of viruses is not possible, in contrast to the potato late blight pathogen or fungal pathogens.

Most viruses infecting potato crops, such as *Potato virus Y* (PVY, genus *Potyvirus*), are transmitted in a non-persistent manner by aphids, mostly by species that do not colonize potato plants (Radcliffe and Ragsdale 2002). PVY acquisition and transmission occur when the winged aphids probe plants briefly. The virus remains infective in the aphid stylet only for minutes or a few hours. This type of virus transmission cannot be prevented using pesticides applied to control aphids, because they do not kill the aphids quickly enough. However, by reducing the populations of potato-colonizing aphids in potato crops pesticides may contribute also to control of PVY. Covering the soil with mulches prior to closure of the canopy reduces landing of aphids on plants and can reduce virus transmission when the numbers of aphids are relatively low (Kirchner *et al.* 2011, Radcliffe and Ragsdale 2002). Mineral oil sprayed on the plants may also reduce PVY transmission, because the oil seems to interfere with probing (Kirchner *et al.* 2014). The efficiency of these practices is variable and not always worth the cost of application. Therefore, growing virus resistant potato cultivars provides the efficient and cost-effective means to control the spread of viruses in potato crops.

Durability of resistance is a general challenge in the control of plant diseases, because pathogens vary genetically and certain mutants may overcome resistance. The strains of the potato late blight pathogen [*Phytophthora infestans* (Mont.) de Bary] overcoming dominant resistance genes in potato provide an example (Akino *et al.* 2014). An important factor contributing to durability of virus resistance is the use of healthy seed potatoes, which is widely applied in the developed countries and increasingly in the developing countries (Gildemacher *et al.* 2011, Kirchner *et al.* 2014). Planting a new potato crop with healthy seed potatoes lowers the overall virus infection pressure in the crop and helps to avoid putative resistance-breaking virus isolates that may have evolved. Consequently, viruses such as the contact-transmitted *Potato virus X* (PVX) and the aphid-transmitted *Potato virus A* (PVA), *Potato virus V*, *Potato virus S*, *Potato virus M* and *Potato leaf roll virus* have become less common in many potato growing areas in the northern hemisphere (Valkonen 2007, Wrobel and Wasik 2014). However, the landrace potato varieties maintained by small farmers or hobby gardeners may act as virus reservoirs (Gildemacher

et al. 2011, Maoka *et al.* 2013). Furthermore, expansion of potato cultivation to warmer climates has brought in new virus problems, such as the highly devastating *Tomato spotted wilt virus* transmitted by thrips (Persley *et al.* 2006). Hence, development of new virus resistant potato cultivars remains an important goal.

There are two main approaches to identify genes for resistance to viruses: *i)* plant populations can be searched for virus resistant and susceptible individuals, which are crossed, and the progeny segregating for virus resistant and susceptible individuals is used to map and identify the host factors (resistance genes) conferring resistance, or *ii)* host factors needed by the virus for infection are identified, and mutated forms of the factors incompatible in the host-virus interaction are utilized as resistance genes. Concerning the first alternative, many dominant genes conferring virus strain-specific resistance or broad-spectrum resistance to all strains of the virus are known and utilized in potato breeding (Ross 1986, Valkonen *et al.* 1996). The second approach for breeding resistance to viruses has remained limited in potato, in contrast to many other solanaceous crops (Truniger and Aranda 2009), because the mutated host factors confer typically recessive resistance, which is more difficult to utilize in the tetraploid potato than other, diploid solanaceous crops such as tomato, pepper and tobacco.

The currently most problematic potato viruses are PVY and *Potato mop-top virus* (PMTV, genus *Pomovirus*). Their prevalence is increasing and their geographical distribution is expanding, in contrast to most of the other 35 potato viruses known to infect potatoes in the field (Valkonen 2007). Therefore, PVY and PMTV will gain particular attention in this paper. There is an urgent need to introduce more durable, broad-spectrum resistance to PVY to potato cultivars and to identify sources of effective resistance to PMTV.

Gene-for-gene resistance to viruses

Jones and Dangl (2006) coined a useful model for dissecting and understanding the layers of pathogen defence and their evolution in plants. According to the model, initially, following infection, plant cells use basal defence to recognize *pathogen-associated molecular patterns* (PAMPs) and elicit responses whose hallmarks are *pathogenesis-related proteins* (PR-proteins) that are pathogen non-specific (Almagro *et al.* 2009). Many PR genes are induced upon virus infection, however, little is known about their effects on viruses. As a counter-defence, pathogens suppress basal defence using specific *virulence proteins*, also called *effectors*. Viruses produce proteins that suppress RNA silencing (RNAi), which is the most important basal antiviral defence system against viruses in plants and also known in other eukaryotic organisms (Incarbone and Dunoyer 2013). These viral proteins hence fulfil the definition of effector. Effectors, in turn, are recognized by the second, target-specific level of host defence provided by R proteins. They function on a "protein-for-protein" basis, as predicted by Flor (1946)

who defined this defence level as "gene-for-gene" resistance using the genetic terms.

R genes elicit a quick and powerful defence response, which can take the form of a hypersensitive resistance response (HR) or extreme resistance (ER). The signalling cascade induced by *R* genes and leading to HR activates a wide range of genes and defence responses. HR prevents virus loading to phloem in the initially infected leaf and translocation to other parts of the plant by an as yet unknown mechanism, but virus replication and initial cell-to-cell movement are not affected. Later, most of the infected cells die, which is noticed as a necrotic local lesion at the site of infection. ER, however, suppresses virus multiplication in the initially infected cells and usually no visible signs of infection can be observed (Barker and Harrison 1984, Ross 1986). Only tiny, necrotic pin-point lesions may be observed and no virus is detected in the inoculated tissue (Cockerham 1970, Ross 1986, Valkonen *et al.* 1996).

R proteins share structural and functional features. The C-proximal leucine-rich repeat (LRR) domain of the protein recognizes the effector, whereas the kinase domains in the central part of the protein are needed for phosphorylation, which activates the defence signalling cascade via the N-proximal Toll and interleukin receptor-like (TIR) or coiled-coil (CC) domain of the protein (Michelmore *et al.* 2013). HR to *Tobacco mosaic virus* (TMV, genus *Tobamovirus*) conferred by the *N* gene in tobacco (*Nicotiana tabacum* L.) is the best characterized example of virus resistance functioning on the gene-for-gene basis. N recognizes the TMV replicase protein (Caplan *et al.* 2008), which is an effector suppressing RNAi (Vogler *et al.* 2007, Wang *et al.* 2012). However, the LRR domain cannot recognize TMV unless the TIR domain interacts with another host protein (NRIP1) (Caplan *et al.* 2008). The currently accepted model supported by experimental evidence indicates that the R protein acts as a "guard" recognizing a specific complex of proteins formed by the pathogen effector protein and its target host protein (Collier and Moffett 2009). The host target of the TMV p126 RNAi suppressor (replicase) is the HEN1 methyltransferase (Vogler *et al.* 2007). HEN1 methylates the viral small interfering RNAs (siRNAs), which are produced by the host Dicer enzymes cleaving double-stranded RNA, e.g., the replicating TMV RNA. Cleavage by Dicer inactivates the viral dsRNA, and methylation stabilizes the virus-derived siRNAs that are used to guide the RNA silencing complexes to the single-stranded copies of the viral genome; they are subsequently cleaved and inactivated (Incarbone and Dunoyer 2013). The TMV p126 protein interferes with HEN1 and siRNA methylation, unstabilizes the siRNA and hence prevents or slows down amplification of the RNAi process and antiviral defence.

Hypersensitive resistance (HR) to PVY in potato

Wild potato species are rich in genes for HR to potato viruses. Many genes for HR to viruses have been introduced from wild potato species to the cultivated potato gene pool

and used in resistance breeding (Cockerham 1970, Ross 1986, Zimnoch-Guzowska *et al.* 2013). However, virus strain-specificity of most HR genes makes resistance vulnerable to new variants of the virus which are not recognized by the gene and can overcome resistance. This challenge is well known with the genes for HR to PVY in potato. It is important to phenotype the local PVY strains by inoculation to indicator potato cultivars containing specific HR genes, such as *Ny*, *Nc* or *Nz* (Jones 1990), so that cultivars carrying the appropriate HR genes can be selected for cultivation.

PVY strains recognized by *Ny*, *Nc* and *Nz* are placed to strain groups PVY^O (ordinary strains), PVY^C (C strains) and PVY^Z (Z strains), respectively (Singh *et al.* 2008). The most problematic PVY strains are those overcoming all three genes. Most of them are designated to strain group PVY^N because they induce veinal necrosis in tobacco leaves (Singh *et al.* 2008). PVY^N strains used to be mild and many potato cultivars are tolerant of them. However, since 1980s, more severe variants of PVY^N have become prevalent in potato crops in all parts of the world. These variants can cause severe symptoms of mosaic and yellowing in leaves, and necrosis in tubers. Genome sequences reveal that they have evolved via recombination between the PVY^O and PVY^N strains (Singh *et al.* 2008). A large number of different recombinant PVY strains have been described recently, including strains that overcome all the three HR genes and, in contrast to PVY^N, do not induce veinal necrosis in tobacco. They represent a new strain group PVY^E (Galvino-Costa *et al.* 2012, Singh *et al.* 2008).

Molecular methods have enhanced virus diagnostics since 1990s. It was realized that phylogenetic clustering of PVY strains based on the viral coat protein (CP) encoding sequence was well in accordance with the strain grouping based on recognition of PVY strains by the HR genes in potato (van der Vlugt *et al.* 1993). Strain-specific monoclonal antibodies (MAb) to the CPs of characterized PVY^O and PVY^N strains were produced (Boonham and Barker 1998, Nikolaeva *et al.* 2012) and phylogenetic comparison of CP sequences and/or serological testing of PVY isolates using MAbs became a common practice in classification of field isolates of PVY. On the other hand, characterization of PVY strains using the indicator potato cultivars became rare. These changes in PVY strain analysis introduced a risk because the viral proteins recognized by the HR genes were not known. If the resistance gene did not recognize CP but another protein of the virus, recombinant strains of PVY could be misclassified in terms of their ability to overcome resistance. Unfortunately, this risk has materialized.

We know now that *Ny* and *Nc* do not recognize CP, but the HCpro protein, of PVY (Moury *et al.* 2011, Tian and Valkonen 2013). HCpro is a helper component in PVY transmission by aphids and it is also suppressor of RNAi. HCpro is the third mature protein produced from the N-proximal part of the viral polyprotein, whereas CP is the last (tenth) protein produced from the C-terminus of the polyprotein (Adams *et al.* 2012). The new recombinant PVY strains can have a PVY^O-like CP, but they overcome *Ny* because the 5′-proximal part of the genome including the HCpro-encoding sequence is derived from PVY^N (Glais *et al.* 2002). With such strains of PVY serological testing of the CP or analysis of the CP-encoding sequence will fail to provide correct information about the resistance-breaking capacity of the strain. *Ny* recognizes the 3-dimensional conformation of PVY^O HCpro, which differs from PVY^N and is determined by eight residues in the central part of HCpro (amino acids 227 to 327) (Tian and Valkonen 2013, 2015). This knowledge allows more accurate molecular identification of PVY strains in terms of their ability to overcome resistance conferred by *Ny*. The specific residues crucial for recognition of PVY^C HCpro by *Nc* (Moury *et al.* 2011) and the PVY protein recognized by *Nz* remain to be determined.

Extreme resistance (ER) to PVY in potato

The dominant virus-specific *R* genes for ER inhibit virus multiplication and protect potato plants against all strains of the virus. The genes designated as *Ry* confer ER to PVY (Ross 1986), which is the desired type of resistance to control the currently widely spread severe, recombinant PVY strains. The genes *Ry$_{adg}$*, *Ry$_{sto}$* and *Ry$_{chc}$* derived from *S. tuberosum* subsp. *andigena* Hawkes, *S. stoloniferum* Schlechtd. et Bché. and *S. chacoense* Bitt., respectively, are used in potato breeding program (Cockerham 1970, Gebhardt *et al.* 2006, Mori *et al.* 2012, Muñoz *et al.* 1975, Ross 1986, Sato *et al.* 2006).

R genes occur as clusters of highly similar genes in plant chromosomes. An *R* gene cluster can contain gene homologs conferring either ER or HR to the same virus, to different strains of the virus, to different viruses, and also to other pathogens (Bakker *et al.* 2011, Gebhardt and Valkonen 2001, Hämäläinen *et al.* 1998, 2000). Therefore, gene-specific markers are needed for efficient selection of the desired resistance genes or gene alleles in the breeding program. Many PCR markers are available and used for marker-assisted selection of *Ry* genes from various sources (e.g., Gebhardt *et al.* 2006, Hosaka *et al.* 2001, Kasai *et al.* 2000, Lopez-Pardo *et al.* 2013, Ortega and Lopez-Vizcon 2012, Sorri *et al.* 1999, Szajko *et al.* 2014, Valkonen *et al.* 2008, Whitworth *et al.* 2009, Witek *et al.* 2006).

ER to PVY is epistatic to HR (Valkonen *et al.* 1994). Hence, a potato genotype carrying *Ry* and *Ny* expresses only ER when inoculated with PVY^O; no necrotic symptoms are observed. These observations indicate that the genes for ER act earlier and more efficiently than the genes for HR, which was shown in comparative studies on the potato gene *Rx* (ER to PVX) and gene *N* of tobacco. TMV was engineered to express PVX CP, the trigger of *Rx*. On the other hand, a tobacco cultivar that carries the gene *N* for HR to TMV was transformed with *Rx*. When the TMV chimera expressing PVX CP was inoculated to the transgenic tobacco plants carrying both *N* and *Rx*, no HR (necrotic response) expected from recognition of TMV by *N* was observed and no TMV was detected in the plants. These results indicate that the

Rx-mediated resistance inhibited TMV replication before the virus titres reached levels needed to trigger the gene *N* and HR to TMV (Bendahmane *et al.* 1999).

Challenges with the use of *R* gene-mediated resistance

Strain-specificity of the HR genes to viruses is a challenge to potato breeding, and another problem is the temperature-sensitivity of some HR genes. Higher temperatures may render the resistance less efficient, which results in systemic spread of the virus and severe, even lethal necrotic symptoms in the plant (Adams *et al.* 1986, Valkonen 1997, Valkonen *et al.* 1998). Hence, the resistance response is converted to a suicide mechanism, which may be meaningful in a wild plant population of genetically different individuals, but is devastating in a genetically uniform potato crop. For example, HR to PVY^O in potato cv. Pito is effective at the temperature of 16/18°C, but at higher temperatures (19/24°C) PVY^O spreads out from the inoculated leaves and causes leaf-drop and mosaic symptoms in upper parts of the plant (Valkonen 1997, Valkonen *et al.* 1998). The HR genes *Ny-1* and *Ny-2* protect potato cultivars at 20°C against systemic infection with the "Wilga-type" recombinant strain of PVY (Chrzanowska 1991, Glais *et al.* 2002), but lose their effectiveness at 28°C (Szajko *et al.* 2014). The wild tuber-bearing potato species *S. sparsipilum* (Bitt.) Juz et Buk. and *S. sucrense* Hawkes express HR to PVY^N at a low temperature, but also here resistance is overcome at high temperatures (Valkonen 1997).

The mechanism by which temperature influences the outcome of HR remains to be elucidated. However, allelic variation of additional genes involved in virus recognition besides the *R* gene or downstream signalling for defence responses may cause genotype-dependent phenotypic changes in the outcome of resistance responses triggered by the *R* genes (Valkonen *et al.* 1998). Besides epistasis discussed above, allelic variation in the genes involved in signalling for defence may also explain why the progeny of crosses involving *S. stoloniferum* as the source of *Ry$_{sto}$* may include genotypes that express HR rather than ER to PVY (Ross 1986, Valkonen *et al.* 2008).

Potential to expand the use of *R* gene-mediated resistance

Despite of the challenges introduced by the strain-specificity and temperature-sensitivity, it is important to emphasize that after all, it seems that PVY cannot very easily acquire mutations overcoming HR without compromising infectivity (Tian and Valkonen 2015) and most HR genes are not sensitive to the range of temperatures normally encountered in potato fields. Furthermore, wild potato species provide a rich, but underutilized source of useful virus resistance genes. For example, testing only a single, randomly picked genotype from eight tuber-bearing wild potato species stud-

ied revealed that all these eight genotypes expressed HR to at least one potyvirus. Four genotypes expressed HR to PVY^N, which is rare in cultivated potatoes. One genotype combined genes for HR to PVY^N, PVY^O and three other potyviruses (Valkonen 1997). Crossing barriers can be overcome and resistance genes transferred from wild potatoes to cultivated potato gene pool with help of various methods, such as haploid induction, embryo rescue, rescue pollination or somatic hybridization (Valkonen *et al.* 1995, Valkonen and Rokka 1998, Valkonen and Watanabe 1999, Watanabe *et al.* 1995). Characterization of wild potato accessions for resistance to viruses, genetic studies on inheritance and linkage of the resistance genes, development of markers for marker-assisted selection, and eventual isolation of the most useful resistance genes and their introduction to potato cultivars by the methods of gene technology (Yau and Stewart 2013) should be high in the agenda of resistance breeders.

Whole genome sequencing of plants and gene mining have become affordable and accessible owing to the efficient DNA sequence technology and improved bioinformatics tools. *Rx* is so far the only effective virus resistance gene isolated and characterised from potato (Bendahmane *et al.* 1999). Studies on *Rx* have demonstrated that the target specificity of *R* genes can be expanded by introducing amino acid substitutions to the LRR region. Indeed, an *Rx* gene with an engineered LLR region can recognize another potexvirus, *Poplar mosaic virus* (Farnham and Baulcombe 2006). Gene *Y-1* was isolated from *S. tuberosum* ssp. *andigena* and found to be structurally most similar to gene *N* (Vidal *et al.* 2002). *Y-1* recognises PVY and induces cell death but does not prevent systemic spread of PVY in potato plants (Vidal *et al.* 2002). However, *Y-1* might be developed to a useful resistance gene by engineering the LRR or other regions of the gene. *Y-1* resides in potato chromosome XI in an *R* gene cluster containing also the gene *Na* for HR to PVA and the gene *Ry$_{adg}$* conferring ER to PVY (Hämäläinen *et al.* 1997, 1998, 2000). More recently, gene *G-Ry* that seems to be a homolog of *Y-1* was isolated and reported to enhance resistance to PVY (Lee *et al.* 2010).

Converting host susceptibility factors to genes for resistance to PVY and PMTV

Breeding for recessive resistance is a well-adopted concept, especially in control of potyviruses (Truniger and Aranda 2009). The viral protein VPg linked to the 5′-end of viral RNA in potyviruses interacts with the cellular translation initiation factor eIF4E and its isoform eIF(iso)4E (referred to as 4E further on) (Wittman *et al.* 1997). Disruption of the interaction by mutation of VPg or 4E inhibits virus infection (Leonard *et al.* 2000). It has been realized that many recessive resistance genes used in breeding for resistance to potyviruses in tomato, pepper and tobacco are variants of *4E* genes (Robaglia and Caranta 2006, Truniger and Aranda 2009). However, the mechanism by which mutations in 4E

confer resistance has remained elusive. Phenotypically, the resistance can take many forms, including inhibited virus replication in the initially infected cells, or restricted cell-to-cell or long-distance (vascular) movement of the virus, which has been difficult to explain.

Recently, it was found that that also HCpro interacts with the 4Es (Ala-Poikela *et al.* 2011). The interaction is mediated by a specific 4E-binding motif that is conserved in potyviruses. The motif is similar to the 4E-binding motif of the cellular scaffold protein eIF4G that binds 4E to initiate cap-dependent translation of cellular messenger RNAs. When the conserved amino acids in the 4E-binding motif of HCpro were mutated in PVA, only very low virus titres accumulated in the inoculated plants (Ala-Poikela *et al.* 2011). Further study of the details of the interaction will reveal which mutations in eIF4G and 4E could confer broad-spectrum resistance to many potyviruses simultaneously.

PMTV is an RNA virus unrelated to PVY, but as with the new strains of PVY, the economic losses caused by PMTV are increasing while its geographical distribution is expanding. The situation with PMTV is more difficult than with PVY, because no effective resistance to PMTV is known in potato cultivars. Only few potato breeding lines and wild potato species have been found to show a low incidence of PMTV infection and/or contain low virus titres in tubers (Sandgren *et al.* 2002, Santala *et al.* 2010). Therefore, converting host factors needed for infection by PMTV to resistance factors is one of the approaches that may be used to enhance breeding of PMTV resistant potato cultivars.

PMTV is transmitted by the soil micro-organism *Spongospora subterranea* (Wallr.) Lagerh. f. sp. *subterranea* J.A. Toml. which itself is a potato pathogen causing powdery scab on tubers. The geographical distribution of PMTV is expanding, most likely due to dissemination of the virus in infected seed potatoes or in PMTV-containing resting spores of *S. subterranea* adhering to the surface of seed tubers. It is believed that PMTV was introduced from South America to other continents (Gil *et al.* 2011, Hinostroza and French 1972). In Europe it was detected first in North Ireland and Scotland (Calvert and Harrison 1966). In Japan PMTV was reported in early 1980s (Imoto *et al.* 1981). In the beginning of 1990s the virus had been found in many countries of northern Europe (Torrance *et al.* 1993). Ten years later its occurrence was confirmed in Canada and U.S.A. (Xu *et al.* 2004). PMTV is currently wide-spread in the potato growing areas of Scotland (Carnegie *et al.* 2012), Nordic countries (Beuch *et al.* 2014, Santala *et al.* 2010) and Japan (Nakayama *et al.* 2010). Continued dispersal of PMTV to new areas is evidenced by its recent detection in Poland (Budziszewska *et al.* 2010), several potato-growing states of U.S.A. (Crosslin 2011, David *et al.* 2010, Whitworth and Crosslin 2013), China (Hu *et al.* 2013) and Pakistan (Arif *et al.* 2013).

S. subtrerranea transmits PMTV inside the zoospores released from infected roots and resting spores (sporangia) (Jones and Harrison 1969). The resting spores may remain viable in soil at least for 15 years (Santala *et al.* 2010) and maintain a reservoir of infectious virus particles in soil. Treatment of soil with chemicals does not kill the resting spores but is potentially very harmful to the beneficial soil organisms and environment and is not allowed in many countries. Studies show that potato cultivars highly resistant to *S. subterranea* are not protected against infection with PMTV (unpublished information of H. Kirk; cited in Santala *et al.* 2010). Hence, resistance of potato cultivars to PMTV remains as the only option for control of PMTV.

It is important to screen cultivated potato germplasm in gene banks more widely and comprehensively for resistance to PMTV. In the meanwhile, approaches similar to those described above with recessive resistance to PVY might provide alternative solutions. An infectious cDNA clone of PMTV is available (Savenkov *et al.* 2003) for critical studies aiming to identify host factors needed for infection by PMTV. Recent studies have revealed that TGBp3, one of the cell-to-cell movement proteins of PMTV, undergoes tyrosine phosphorylation, which is novel for plant virus proteins (Samuilova *et al.* 2013). Proteins of mammalian viruses, such as vaccinia, variola and monkeypox virus, are phosphorylated by tyrosine kinases. Kinase inhibitors can be used to increase the survival of the hosts infected by these viruses, because lack of tyrosine phosphorylation of the viral proteins reduces viral load and dissemination of the virus to distal tissues (Keating and Striker 2012). These findings suggest that inhibition of certain tyrosine kinases might also help to combat infections with PMTV.

Conclusions

Studies on molecular virus-host interactions have potential to discover novel genes that are important to viral infection or antiviral defence. Both types of genes may be utilized in resistance breeding, as discussed above. The available potato genome sequence (Potato Genome Sequencing Consortium 2011) and the currently affordable costs of whole-genome sequencing of potato genotypes will enhance the efforts. Isolation of *R* genes offers an opportunity to speed up the resistance breeding process by introducing the required *R* genes to cultivars by genetic transformation. Furthermore, it is possible to engineer *R* genes and expand their target specificity to cover a wider range of virus strains, viruses or other pathogens. The latest technologies allow removal of the selectable marker genes used in plant transformation, which will reduce the concerns related to health and environmental issues and the need for governmental regulations (Yau and Stewart 2013). Hence the advanced gene technologies allow creating or complementing virus resistance in potato cultivars, either based on the *R* genes or other host factors that can be mutated to interfere with virus infection.

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