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Review Article: Molecular Mechanisms of Resistance to *Potato virus X* and *Y* in Potato

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The most important viral pathogens of the cultivated potato are *Potato virus X* (PVX) and *Potato virus Y* (PVY), which can reduce potato production up to 80%. Thus resistance breeding is one of the major goals of plant breeders. Wild potato species are good sources of resistance *(R)* genes. The resistant plants respond to viral infection with hypersensitive reaction (HR) or extreme resistance (ER). HR is accompanied by programmed cell death, while ER localizes the virus at the primary infection site and limits virus replication without visible symptoms. While HR is generally strain-specific, ER can act against a broad spectrum of viral pathogens. This review aims to describe the molecular mechanisms of resistance against PVX and PVY in potato.

Keywords: extreme resistance, hypersensitive response, PVX, PVY, *Solanum tuberosum*.

Potato *(Solanum tuberosum)* is the world's fourth most important food crop, following maize, wheat and rice. Nevertheless, many pathogens like viroids, viruses, bacteria, oomycetes, fungi and nematodes reduce the quantity and quality of potato tubers. In 2013, potato production exceeded 300 million tonnes worldwide and was 443,100 tonnes in Hungary with an average yield of 21.8 tonnes/hectare, which is less than half of the yield in Belgium and the Netherlands (http://faostat.fao.org). One of the major factors adversely affecting potato production in Middle-East European countries, including Hungary, is virus infection.

More than 35 different viruses could infect cultivated potatoes in the field (Salazar, 2003). The most important viral pathogens of the cultivated potato are *Potato virus Y* (PVY) and *Potato virus X* (PVX), which can reduce potato production up to 80% (Roossinck, 2012). PVY belongs to potyviruses, while PVX is a potexvirus. Both groups are very important agriculturally, economically and biologically. PVY is recognised as the fifth most important plant virus regarding its economic importance (Kogovsek and Ravnikar, 2013). Besides PVY, the majority of potato-infecting viruses, e.g., *Potato virus A* (PVA), *Potato virus V* (PVV) and *Tobacco etch virus* (TEV), belong to potyviruses. These viruses are transmitted by aphids, which are most common in temperate zones, East- and Middle-East Europe. The fact that potyviruses are spread by migrating aphids and that each

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potyvirus may be transmitted by many different aphid species and each aphid species may transmit many potyviruses, makes it very difficult to control and prevent potyvirus infections in agriculture (Gibbs et al., 2008). Unlike PVY, PVX is mechanically transmitted. However, it is also easily spread by plant-to-plant contact or by machinery or people moving through the field (Verchot-Lubicz et al., 2007). Since the viruses are intracellular pathogens it is difficult to chemically control or prevent virus infections by the destruction of infected plants or pesticide applications to limit the population of vectors. Nowadays, no efficient control strategy is available to manage potato virus diseases. Thus the need for resistant potato cultivars is urgent.

In wild potato species, several types of natural resistance to viruses can be distinguished, and by their application in the breeding processes, production of potato cultivars resistant to viruses became possible. Resistances are encoded by distinct resistance genes and act against either a specific virus strain or a broad spectrum of strains. Host plants can exhibit incompatible or compatible interaction with the virus. In an incompatible interaction, the plants respond to viral infection with hypersensitive reaction (HR) or an extreme resistance (ER) response (Muthamilarasan and Prasad, 2013). This review aims to describe the molecular mechanisms of resistance against PVY and PVX infection conferred by different resistance genes that act against viruses by limiting their accumulation and/or spread.

Genome organization of PVY and PVX

PVY as a member of potyviruses encode several proteins that are translocated into the nucleus (Mestre et al., 2000). The genome of potyviruses is a positive-sense ssRNA molecule, 9.7 kb in length with its 5' end covalently linked to a virus-encoded protein (VPg) and its 3' end polyadenylated (Dougherty and Carrington, 1988). The virions are 680–900 nm in length and 11–15 nm in width. An important property of potyviruses is that 10 out of 11 proteins that they encode are derived from a single polyprotein precursor, while the 11th, P3N-PIPO, results from a separate translation event (Ivanov et al., 2014). The 10 functional proteins are cleaved by the action of three virally coded proteases, namely Hc-Pro, P1 and NIb (Kerlan, 2006). Following the genetic characteristics, PVY isolates are classified into different groups, which genetic variants called strains. PVY strain groups are based on host response and resistance gene interactions. The different strains interact differently in different potato cultivars, which are indicated by the production of different kinds of disease symptomps (Singh et al., 2008). Among them PVY^{NTN} isolates cause the most devastating disease of potato, i.e., potato tuber necrotic ringspot disease, which has been responsible for severe decreases in the quality and quantity of potato production (Kogovsek and Ravnikar, 2013).

Genomes of potexviruses have monopartite, positive-strand RNA, flexuous and filamentous virions between 470–580 nm in length. There is a methylguanosine cap on their 5' end, while the 3' end has a poly(A) tail (Huang et al., 2004). The RNA genome encodes five open reading frames (ORFs). The first ORF encodes the viral replicase. The central region of the genome encodes three overlapping ORFs, known as the triple-gene block (TGB). These proteins are required for the cell-to-cell movement of the virus. The final

ORF is the viral coat protein (CP), which is required for virion assembly and virus cell-to-cell movement. Many strains of PVX induce inconspicuous interveinal chlorosis in leaves of most potato cultivars (Verchot-Lubicz et al., 2007).

Upon entry into the plant cells, uncoating and replication of the virus is immediately initiated. It has been proposed that the uncoating process involves the bidirectional release of coat protein subunits from around the viral RNA and that these activities may be mediated by cotranslational and coreplicational disassembly mechanisms and completed within 10–20 minutes (Shaw, 1999). At later stages of infection, the viral genome is encapsidated into virions, allowing the virus to survive even in the extracellular environment. Thanks to the aphid's stylet that has a putative receptor, which interacts with the specific amino acid motifs in CP and Hc-Pro of potyviruses, the aphid transmission results in the CP-mediated genome encapsidation (Ivanov and Mäkinen, 2012). The proteins which take part in viral replication are CI, 6K2, NIa, NIb, HC-Pro and P3. Successful infection of host plants by viruses requires cell-to-cell movement through plasmodesmata (Carrington et al., 1996). In the cell-to-cell movement four viral proteins, the CI, VPg, CP and P3N-PIPO, are thought to be involved (Ivanov et al., 2014).

Genes associated with HR type resistance

HR correlates with induction of *N* genes leading to initiation of systemic acquired resistance (SAR). HR in case of PVY is conferred by the *Ny* gene (Szajko et al., 2008). It has been known that some *N* genes that regulate HR type resistance against PVY are derived from different wild *Solanum* species and the grown potato *S. tuberosum*. Ny_{dms} originated from *S. demissum* and Ny_{chc} originated from *S. chacoense* confer HR against the infection of both PVY and PVA (Cockerham, 1970). Ny_{adg} was identified in *S. tuberosum* ssp. *andigena* and protects the potato against the 'ordinary' strain of PVY, PVY^{O} (Valkonen, 1994). Ny_{tbr} originated from *S. tuberosum* and localised on chromosome IV (Jones, 1990; Celebi-Toprak et al., 2005). There are also genes in *S. stoloniferum* that initiate HR type resistance to PVY (Ry_{sto}^{n1}, Ry_{sto}^{n2}) (Cockerham, 1970).

The HR to the infection of PVX in potato is related to two dominant genes, *Nx* and *Nb*. The strains of PVX are separated according to their ability to elicit HR in different potato cultivars carrying the dominant resistance genes *Nb* or *Nx*. *Nx* genes have also different origins. Nx_{chc} originated from *S. chacoense* and Nx_{tbr} originated from *S. tuberosum*. Nx_{phu} from *S. phureja* is located at the distal end of the long arm of chromosome IX (Tommiska et al., 1998). Nb_{tbr} was identified in *S. tuberosum* and has been localised to chromosome V to the same region that contains *Rx2* (De Jong et al., 1997).

Genes associated with ER type resistance

ER against PVY and PVX is initiated by the activation of *R* genes that act against a broad spectrum of virus strains by limiting their accumulation and only a little or no visible symptoms appear. In potato, ER to PVX is controlled by the dominant genes *Rx1* and *Rx2*. *Rx* confers a race-specific resistance to PVX by recognition of the viral coat protein (Bendahmane et al., 1995). The gene *Rx1* is located on chromosome XII and the

gene *Rx2* on chromosome V. On the basis of pedigree analysis, it was assumed that *Rx1* derived from *S. tuberosum* subsp. *andigena* and *Rx2* from *S. acaule* (Ritter et al., 1991). The open reading frames of *Rx1* and *Rx2* show 95% identity at the nucleotide level (Bendahmane et al., 1995). Farnham and Baulcombe (2006) demonstrated that the wild-type potato NBS-LRR protein Rx confers resistance against a single strain of PVX. Rx protein is structurally very similar to products of disease resistance genes conferring HR, but it was proved that Rx-mediated ER against PVX is not associated with a necrotic hypersensitive response at the site of infection. It was verified that *Rx* has a potential to the cell death response induction, but ER is separate and epistatic to necrosis, accordingly cell death and pathogen arrest are separate disease resistance pathways in plants (Bendahmane et al., 1999). Rx was identified as a protein with a conserved nucleotide binding site and leucine-rich repeat (NBS-LRR) belonging to the largest class of plant R proteins, which can mediate both HR and ER responses (Glowacki et al., 2011).

R proteins typically contain NBS and LRR domains (Fig. 1) and belong to the structural class of proteins known as NBS-LRRs (Marone et al., 2013). Within the genome of the sequenced *S. tuberosum* Group Phureja, 704 *NBS-LRR* genes were predicted (Jupe et al., 2013). Many were found to reside in clusters comprising closely related paralogues. In potato, 73% of the mapped *NBS-LRR* genes are grouped into 63 clusters (Jupe et al., 2012). At the N proximal part, the NBS domains are involved in signalling, and they contain several highly conserved and sticky ordered motifs such as P-loop or kinase-2 motifs (Tan and Wu, 2012). At the C proximal part, the LRRs have highly adaptable structural domains that have role in protein–protein interactions and can evolve very different binding specificities. LRRs are under diversifying selection (Ellis et al., 2000; Jones and Dangl, 2006).

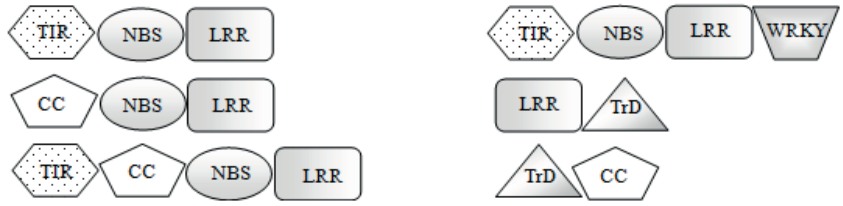

Fig. 1. The major classes of plant resistance genes based on the presence of other domains apart from NBS and LRR. TIR: toll and interleucin receptor; NBS: nucleotide-binding site; LRR: leucine-rich repeat; CC: coiled-coil; WRKY amino acid domain; TrD: transmembrane domain

Rx, as other NBS-LRR proteins, requires the ubiquitin ligase-associated protein SGT1 and the molecular chaperone Hsp90 for its functionality (Peart et al., 2002; Lu et al., 2003). SGT1 is a component of the ubiquitination system and binds specifically to HSP90 and positively regulates disease resistance conferred by many R proteins as well as developmental responses to auxins (Azevedo et al., 2006). During this post-translational regulation pathway ubiquitin is bond to lysine residues of target proteins. Therefore, their degradation is promoted by the 26S/proteosome (Austin et al., 2002). The involvement of SGT1 in defence mechanism is indicated by the mutations in the *CPR30* gene. It was proved in *Arabidopsis thaliana* that CPR30 acted as a negative regulator of

plant defence responses. Many gain-of-resistance mutants have HR-like lesion formation and SAR-like constitutive disease resistance such as the *CPR30* mutant, which is a constitutive expresser of pathogenesis-related genes. The constitutive defence responses are activated by the mutations of these negative regulators. Mutations in *CPR30* cause constitutive resistance to *Pseudomonas syringae*, dramatic induction of defence-response gene expression in the absence of pathogen invasion and dwarf morphology. The growth defect of *cpr30* plants is suppressed by the *eds-1* (enhanced disease susceptibility 1) and *ndr-1* (nonrace-specific disease resistance 1) (Wiermer et al., 2005). In addition to SGT1, Rx interacts with the RanGAP-mediated cellular mechanisms, including nucleocytoplasmic trafficking, for the activation of disease resistance (Tameling and Baulcombe, 2007).

While *Rx*-mediated resistance-breaking isolates of PVX could be identified (Moreira et al., 1980; Querci et al., 1995), no PVY isolates that overcome *Ry* resistance are known (Kogovsek and Ravnikar, 2013). Furthermore, while *Rx*-mediated resistance is race-specific (Cockerham, 1970), the *Ry*-mediated resistance is effective not only against all strains of PVY, including the most aggressive strain, PVY^{NTN}, but also to PVA, PVV and TEV (Cockerham, 1970; Hinrichs et al., 1997).

Potyviruses encode several proteins that are translocated into the nucleus. One of them is the small nuclear inclusion protein, NIa. Despite of its involvement in viral replication on the surface of the cytoplasmic membranes, this protein accumulates predominantly in the nucleus of infected cells (Ivanov et al., 2014). The elicitor of *Ry*-mediated resistance is presumably NIa, a proteinase, which interact directly or indirectly with the Ry protein and initiate a primary response leading to ER by preventing virus accumulation at an early stage of infection. Three mechanisms whereby the NIa could act were proposed: [1] the structure of the NIa might have elicitor activity, [2] the elicitor might be produced by degradation of a host-encoded protein by NIa, [3] the NIa might degrade a negative regulator of the resistance (Mestre et al., 2000). The role of callose deposition in inhibition of viral cell-to-cell movement by *R*-mediated ER has also been reported in soybean-SMV pathosystem (Seo et al., 2014).

Up to the present, three *Ry* genes have been mapped on potato chromosomes. One of them is originated from the wild species *S. stoloniferum* (gene Ry_{sto}) and mapped to chromosome XII (Flis et al., 2005; Song et al., 2005; Cernák et al., 2008). Although the Ry_{sto} gene has not been identified yet, it is very probable that, as the majority of characterised viral resistance genes from plants, it belongs to the *NBS-LRR* class of resistance genes (Gururani et al., 2012). The idea is supported by similarities between *Ry*-mediated resistance and ER conferred by the thoroughly studied Rx NBS-LRR-PVX interaction in potato (Moffett, 2009). Another *Ry* gene, Ry_{adg}, is derived from *S. tuberosum* ssp. *andigena* and mapped on chromosome XI (Hamalainen et al., 1997), while the third one, derived from *S. chacoense* (Ry_{chc}), is located on chromosome IX (Hosaka et al., 2001).

Global expression analysis of *NBS-LRR*-encoding and related genes in *Arabidopsis* suggests that most of their transcripts are present at low levels (Tan et al., 2007). Nevertheless, significant induction of *R* gene expression during the plant defence response also occurs in certain cases (Yoshimura et al., 1998; Halterman et al., 2003; Levy et al., 2004; Gu et al., 2005; Cao et al., 2007; Mohr et al., 2010). There are examples of increasing the expression of an *R* gene converts HR to ER suggesting that the level of R protein regulates

the strength of resistance to a virus. One of the examples is the overexpression of *RCY1* responding to *Cucumber mosaic virus Y* (CMVY) in *Arabidopsis* (Sekine et al., 2008). It was also demonstrated that while the wild-type potato NBS-LRR protein Rx confers resistance against a single strain of PVX, LRR mutants protect against both a second PVX strain and the distantly related *Poplar mosaic virus* (PopMV) (Farnham and Baulcombe, 2006). Furthermore, it was shown that Rx mutants, retaining broad-spectrum resistance against PVX strains and PopMV without HR, can be obtained by artificial evolution (Harris et al., 2013). It is hypothesized that similar mutations can be generated by natural evolution resulting in a gene conferring resistance to different viruses. Ry_{sto} might be an example for this kind of natural evolution since the Ry_{sto}-mediated resistance extends from PVY to PVA, PVV and TEV (Cockerham, 1970; Hinrichs et al., 1997).

Effects of viruses on potato gene expression

Several publications point out that salicylic acid (SA) is a key component in the manifestation of HR (Lewsey et al., 2009). To evaluate the role of SA in *Nb*-mediated HR to PVX in *S. tuberosum*, Sánchez et al. (2010) constructed SA-deficient transgenic potato plant lines by overexpressing the bacterial enzyme salicylate hydroxylase (NahG), which degrades SA, and found that basal levels of SA correlated with HR to PVX. It was also demonstrated that SA can improve the immunity by delaying the onset of viral replication and disease development, through mediation of the expression of a cohort of defence-related genes that induce a defence-like response (Baebler et al., 2011). In response to PVY infection, SA is synthesised *de novo*. The whole transcriptome analysis confirmed the central role of SA in orchestrating *Ny-1*-mediated responses and showed that the absence of SA leads to significant changes at the transcriptome level, including a delay in activation of expression of genes known to participate in defence responses (Baebler et al., 2014).

Still very limited information is available on the downstream signalling pathway involved in ER. One way to explore the underlying mechanism is to study changes at the transcriptome level, however, only a few experiments followed whole transcriptome changes in ER elicited by viral infection so far. Experiments were also performed at proteome level. These experiments showed that the potyvirus-induced changes are not as pronounced at the proteome level as they are at the transcriptome level. It was concluded that the potyvirus infection induces selective changes in the host gene expression rather than a complete shut-off of protein synthesis (Ivanov et al., 2014).

Using transcriptomics to study soybean-*Soybean mosaic virus* (SMV) pathosystem Seo et al. (2014) identified a 2C protein phosphatase type gene *(PP2C)* as a key regulator of ER, confirming transcriptomics as a valuable approach. Baebler et al. (2009) detected differences in expression of hundreds of genes as soon as half an hour after inoculation of potato leaves with PVY^{NTN}. Signalling and defence responses specific to cv. Santé bearing the Ry_{sto} gene included brassinosteroid-, polyamine-, and secondary metabolite synthesis and the increased expression of genes coding for proteinase inhibitors and pathogenesis-related proteins. Later, a next generation sequencing-based transcirptome analysis was performed to study the early responses in cv. White Lady to PVX, PVY^{NTN} and the oomycota, *Phytophthora infestans* causing late blight of potato. Results showed that triple

infection with these pathogens induces up- and down-regulation of a large set of genes in the potato cv. White Lady, a Hungarian cultivar carrying the Ry_{sto} and *Rx2* genes conferring ER to PVY and PVX, respectively (Ahmadvand, 2013). Nevertheless, the effect of each pathogen alone was not analysed in that study.

Conclusion

Molecular analyses in *S. tuberosum* have discovered *N* and *R* type of resistance genes originated from different *Solanum* species conferring HR and ER, respectively. Many advances in understanding the molecular basis of PVX and PVY resistance in potato have focused on *NBS-LRR* type genes. Future research targets are likely to understand the molecular mechanisms underlying these types of resistances and facilitate thereby the breeding of potato cultivars with broad spectrum durable virus resistance.

Literature

Ahmadvand, R. (2013): Analysis of resistance genes in potato with special attention to expressional approaches. PhD dissertation. http://konyvtar.uni-pannon.hu/doktori/2013/Rahim_Ahmadvand_theses_en.pdf

Austin, M. J., Muskett, P., Kahn, K., Feys, B. J., Jones, J. D. and Parker, J. E. (2002): Regulatory role of SGT1 in early *R* gene-mediated plant defenses. Science 295, 2077–2080.

Azevedo, C., Betsuyaku, S., Peart, J., Takahashi, A., Noël, L., Sadanandom, A., Casais, C., Parker, J. and Shirasu, K. (2006): Role of SGT1 in resistance protein accumulation in plant immunity. EMBO J. 25, 2007–2016.

Baebler, Š., Krečič-Stres, H., Rotter, A., Kogovsek, P., Cankar, K., Kok, E. J., Gruden, K., Kovac, M., Zel, J., Pompe-Novak, M. and Ravnikar, M. (2009): PVY^{NTN} elicits a diverse gene expression response in different potato genotypes in the first 12 h after inoculation. Mol. Plant Pathol. 10, 263–275.

Baebler, Š., Stare, K., Kovač, M., Blejec, A., Prezelj, N., Stare, T., Kogovšek, P., Pompe-Novak, M., Rosahl, S., Ravnikar, M. and Gruden, K. (2011): Dynamics of responses in compatible potato–Potato virus Y interaction are modulated by salicylic acid. PLoS ONE 6, e29009.

Baebler, Š., Witek, K., Petek, M., Stare, K., Tušek-Žnidarič, M., Pompe-Novak, M., Renaut, J., Szajko, K., Strzelczyk-Żyta, D., Marczewski, W., Morgiewicz, K., Gruden, K. and Hennig, J. (2014): Salicylic acid is an indispensable component of the *Ny-1* resistance-gene-mediated response against Potato virus Y infection in potato. J. Exp. Bot. 65, 1095–1109.

Bendahmane, A., Kohn, B. A., Dedi, C. and Baulcombe, D. C. (1995): The coat protein of potato virus X is a strain-specific elicitor of Rx1-mediated virus resistance in potato. Plant J. 8, 933–941.

Bendahmane, A., Kanyuka, K. and Baulcombe, D. C. (1999): The *Rx* gene from potato controls separate virus resistance and cell death responses. Plant Cell 11, 781–791.

Cao, Y., Ding, X., Cai, M., Zhao, J., Lin, Y., Li, X., Xu, C. and Wang, S. (2007): The expression pattern of a rice disease resistance gene *Xa3/Xa26* is differentially regulated by the genetic backgrounds and developmental stages that influence its function. Genetics 177, 523–533.

Carrington, J. C., Kasschau, K. D., Mahajan, S. K. and Schaad, M. C. (1996): Cell-to-cell and long-distance transport of viruses in plants. Plant Cell 8, 1669–1681.

Celebi-Toprak, F., Watanabe, J. A. and Watanabe, K. N. (2005): Molecular markers in identification of genotypic variation. In: M. K. Razdan and A. K. Mattoo (eds): Genetic Improvement of Solanaceous Crop. Volume I: Potato, Science Publisher Inc., Enfied, NH, USA, pp. 115–141.

Cernák, I., Decsi, K., Nagy, S., Wolf, I., Polgár, Z., Gulyás, G., Hirata, Y. and Taller, J. (2008): Development of a locus-specific marker and localization of the Ry_{sto} gene based on linkage to a catalase gene on chromosome XII in the tetraploid potato genome. Breeding Sci. 58, 309–314.

Cockerham, G. (1970): Genetical studies on resistance to Potato virus X and Y. Heredity 25, 309–348.

De Jong, W., Forsyth, A., Leister, D., Gebhardt, C. and Baulcombe, D. C. (1997): A potato hypersensitive resistance gene against potato virus X maps to a resistance gene cluster on chromosome 5. Theor. Appl. Genet. 95, 246–252.

Dougherty, W. G. and Carrington, J. C. (1988): Expression and function of potyviral gene products. Annu. Rev. Phytopathol. 26, 123–143.

Ellis, J., Dodds, P. and Pryor, T. (2000): Structure, function and evolution of plant disease resistance genes. Curr. Opin. Plant Biol. 3, 278–284.

Farnham, G. and Baulcombe, D. C. (2006): Artificial evolution extends the spectrum of viruses that are targeted by a disease-resistance gene from potato. Proc. Natl Acad. Sci. USA 103, 18828–18833.

Flis, B., Hennig, J., Zyta, D. S., Gebhardt, C. and Marczewski, W. (2005): The *Ry*-$_{fsto}$ gene from *Solanum stoloniferum* for extreme resistant to Potato virus Y maps to potato chromosome XII and is diagnosed by PCR marker GP122 $_{718}$ in PVY resistant potato cultivars. Mol. Breeding 15, 95–101.

Gibbs, A. J., Ohshima, K., Phillips, M. J. and Gibbs, M. J. (2008): The prehistory of potyviruses: their initial radiation was during the dawn of agriculture. PLoS ONE 3, e2523.

Glowacki, S., Macioszek, V. K. and Kononowicz, A. K. (2011): R proteins as fundamentals of plant innate immunity. Cell. Mol. Biol. Lett. 16, 1–24.

Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., Yang, F., Chu, Z., Wang, G-L., White, F. F. and Yin, Z. (2005): *R* gene expression induced by a type-III effector triggers disease resistance in rice. Nature 435, 1122–1125.

Gururani, M. A., Venkatesh, J., Upadhyaya, C. P., Nookaraju, A., Pandey, S. K. and Park, S. W. (2012): Plant disease resistance genes: Current status and future directions. Physiol. Mol. Plant Pathol. 78, 51–65.

Halterman, D. A., Wei, F. S. and Wise, R. P. (2003): Powdery mildew-induced *Mla* mRNAs are alternatively spliced and contain multiple upstream open reading frames. Plant Physiol. 131, 558–567.

Hamalainen, J. H., Watanabe, K. N., Valkonen, J. P. T., Arihara, A., Plaisted, R. L., Pehu, E., Miller, L. and Slack, S. A. (1997): Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. Theor. Appl. Genet. 94, 192–197.

Harris, C. J., Slootweg, E. J., Goverse, A. and Baulcombe D. C. (2013): Stepwise artificial evolution of a plant disease resistance gene. Proc. Natl Acad. Sci. USA 110, 21189–21194.

Hinrichs, J., Berger, S. and Buchenauer, H. (1997): Susceptibility of potato cultivars to the N and O strain of potato virus Y (PVY) and other potyviruses. Zeitschrift für Pflanzenkrankheiten und Pfanzenschutz 104, 193–199.

Hosaka, K., Hosaka, Y., Mori, M., Maida, T. and Matsunaga, H. (2001): Detection of a simplex RAPD marker linked to resistance to potato virus Y in a tetraploid potato. Am. J. Pot. Res. 78, 191–196.

Huang, Y. L., Han, Y. T., Chang, Y. T., Hsu, Y. H. and Meng, M. (2004): Critical residues for GTP methylation and formation of the covalent m7GMP-enzyme intermediate in the capping enzyme domain of bamboo mosaic virus. J. Virol. 78, 1271–1280.

Ivanov, K. I. and Mäkinen, K. (2012): Coat proteins, host factors and plant viral replication. Curr. Opin. Virol. 2, 712–718.

Ivanov, K. I., Eskelin, K., Lohmus, A. and Makinen, K. (2014): Molecular and cellular mechanisms underlying potyvirus infection. J. Gen. Virol. 95, 1415–1429.

Jones, R. A. C. (1990): Strain group specific and virus specific hypersensitive reactions to infection with potyvirus in potato cultivars. Ann. Appl. Biol. 117, 93–105.

Jones, J. D. and Dangl, J. L. (2006): The plant immune system. Nature 444, 323–329.

Jupe, F., Pritchard, L., Etherington, G. J., MacKenzie, K., Cock, P. J. A., Wright, F., Sharma, S. K., Bolser, D., Bryan, G. J., Jones, J. D. G. and Hein, I. (2012): Identification and localisation of the *NB-LRR* gene family within the potato genome. BMC Genomics 13, 75.

Jupe, F., Witek, K., Verweij, W., Sliwka, J., Pritchard, L., Etherington, G. J., Maclean, D., Cock, P. J., Leggett, R. M., Bryan, G. J., Cardle, L., Hein, I. and Jones, J. D. G. (2013): Resistance gene enrichment sequencing (RenSeq) enables reannotation of the *NB-LRR* gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. Plant J. 76, 530–544.

Kerlan, C. (2006): Potato Virus Y. Descriptions of Plant Viruses #414. Commonwealth Agricultural Bureau/ Association of Applied Biologists. http://www.dpvweb.net/dpv/showdpv.php?dpvno=414

Kogovsek, P. and Ravnikar, M. (2013): Physiology of the potato–*Potato virus Y* interaction. In: U. Lüttge, W. Beyschlag, D. Francis and J. Cushman (eds): Progress in Botany. Springer-Verlag Berlin Heidelberg, pp. 101–131.

Levy, M., Edelbaum, O. and Sela, H. (2004): Tobacco mosaic virus regulates the expression of its own resistance gene *N*. Plant Physiol. 135, 2392–2397.

Lewsey, M., Palukaitis, P. and Carr, J. P. (2009): Plant–virus interactions: defence and counter-defence. In: J. Parker (ed.): Molecular Aspects of Plant Disease Resistance. Wiley-Blackwell, Oxford. pp. 134–176.

Lu, R., Malcuit, I., Moffett, P., Ruiz, M. T., Peart, J., Wu, A. J., Rathjen, J. P., Bendahmane, A., Day, L. and Baulcombe, D. C. (2003): High throughput virus-induced gene silencing implicates heat shock protein 90 in plant disease resistance. EMBO J. 22, 5690–5699.

Marone, D., Russo, M. A., Laidò, G., Leonardis, A. M. and Mastrangelo, A. M. (2013): Plant nucleotide binding site-leucine-rich repeat (*NBS-LRR*) genes: active guardians in host defense responses. Int. J. Mol. Sci. 14, 7302–7326.

Mestre, P., Brigneti, G. and Baulcombe, D. C. (2000): An *Ry*-mediated resistance response in potato requires the intact active site of the NIa proteinase from potato virus Y. Plant J. 23, 653–661.

Moffett P. (2009): Mechanisms of recognition in dominant R gene mediated resistance. Adv. Virus Res. 75, 1–33.

Mohr, T. J., Mammarella, N. D., Woffenden, T. H. B. J., Jelesko, J. G. and McDowell, J. M. (2010): The *Arabidopsis* downy mildew resistance gene *RPP8* is induced by pathogens and salicylic acid and is regulated by W box *cis* elements. Mol. Plant-Microbe Interact. 23, 1303–1315.

Moreira, A., Jones, R. A. C. and Fribourg, C. E. (1980): Properties of a resistance-breaking strain of potato virus X. Ann. Appl. Biol. 95, 93–103.

Muthamilarasan, M. and Prasad, M. (2013): Plant innate immunity: an updated insight into defense mechanism. J. Biosci. 38, 433–449.

Peart, J. R., Lu, R., Sadanandom, A., Malcuit, I., Moffett, P., Brice, D. C., Schauser, L., Jaggard, D. A., Xiao, S., Coleman, M. J., Dow, M., Jones, J. D., Shirasu, K. and Baulcombe, D. C. (2002): Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. Proc. Natl Acad. Sci. USA 99, 10865–10869.

Querci, M., Baulcombe, D. C., Goldbach, R. W. and Salazar, L. F. (1995): Analysis of the resistance-breaking determinants of potato virus X (PVX) strain HB on different potato genotypes expressing extreme resistance to PVX. Phytopathology 85, 1003-1010.

Ritter, E., Debener, T., Barone, A., Salamini, F. and Gebhardt, C. (1991): RFLP mapping on potato chromosomes of two genes controlling extreme resistance to potato virus X (PVX). Mol. Gen. Genet. 227, 81–85.

Roossinck, M. J. (2012): Plant virus metagenomics: biodiversity and ecology. Annu. Rev. Genet. 46, 359–369.

Salazar, L. (2003): Potato viruses after the XXth century: effects, dissemination and their control. Seminar transcript. http://www.crawfordfund.org/wp-content/uploads/2014/03/Salazar-Potato-Viruses.pdf

Sánchez, G., Gerhardt, N., Siciliano, F., Vojnov, A., Malcuit, I. and Marano, M. R. (2010): Salicylic acid is involved in the *Nb*-mediated defense responses to Potato virus X in *Solanum tuberosum*. Mol. Plant-Microbe Interact. 23, 394–405.

Sekine, K. T., Kawakami, S., Hase, S., Kubota, M., Ichinose, Y., Shah, J., Kang, H. G., Klessig, D. F. and Takahashi, H. (2008): High level expression of a virus resistance gene, *RCY1*, confers extreme resistance to Cucumber mosaic virus in *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. 21, 1398–1407.

Seo, J-K., Kwon, S-J., Cho, W. K., Choi, H-S. and Kim, K-H. (2014): Type 2C protein phosphatase is a key regulator of antiviral extreme resistance limiting virus spread. Sci. Rep. 4, 5905.

Shaw J. G. (1999): Tobacco mosaic virus and the study of early events in virus infections. Phil. Transact. Royal Soc. B: Biol. Sci. 354, 603–611.

Singh, R. P., Valkonen, J. P., Gray, S. M., Boonham, N., Jones, R. A., Kerlan, C. and Schubert, J. (2008): Discussion paper: the naming of Potato virus Y strains infecting potato. Arch. Virol. 153, 1–13.

Song, Y-S., Hepting, L., Schweizer, G., Hartl, L., Wenzel, G. and Schwarzfischer, A. (2005): Mapping of extreme resistance to PVY ($Ry_{(sto)}$) on chromosome XII using anther-culture-derived primary dihaploid potato lines. Theor. Appl. Genet. 111, 879–887.

Szajko, K., Chrzanowska, M., Witek, K. Strzelczyk-Zyta, D., Zagórska, H., Gebhardt, C., Hennig, J. and Marczewski, W. (2008): The novel gene *Ny-1* on potato chromosome IX confers hypersensitive resistance to Potato virus Y and is an alternative to *Ry* genes in potato breeding for PVY resistance. Theor. Appl. Genet. 116, 297–303.

Tameling, W. I. and Baulcombe, D. C. (2007): Physical association of the NB-LRR resistance protein Rx with a Ran GTPase-activating protein is required for extreme resistance to Potato virus X. Plant Cell 19, 1682–1694.

Tan, S. and Wu, S. (2012): Genome wide analysis of nucleotide-binding site disease resistance genes in *Brachypodium distachyon*. Comp. Funct. Genomics 2012, 418208.

Tan, X. P., Meyers, B. C., Kozik, A., West, M., Morgante, M., St Clair, D. A., Bent, A. F. and Michelmore, R. W. (2007): Global expression analysis of nucleotide binding site-leucine rich repeat-encoding and related genes in Arabidopsis. BMC Plant Biol. 7, 56.

Tommiska, T. J., Hämäläinen, J. H., Watanabe, K. N. and Valkonen, J. P. T. (1998): Mapping of the gene Nx_{phu} that controls hypersensitive resistance to potato virus X in *Solanum phureja* IvP35. Theor. Appl. Genet. 96, 840–843.

Yoshimura, S., Yamanouchi, U., Katayose, Y., Toki, S., Wang, Z. X., Kono, I., Kurata, N., Yano, M., Iwata, N. and Sasaki, T. (1998): Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc. Natl Acad. Sci. USA 95, 1663–1668.

Valkonen, J. P. T. (1994): Natural genes and mechanisms for resistance to viruses in cultivated and wild potato species (*Solanum* spp.). Plant Breeding 112, 1–16.

Verchot-Lubicz, J., Ye, C. M. and Bamunusinghe, D. (2007): Molecular biology of potexviruses: recent advances. J. Gen. Virol. 88, 1643–1655.

Wiermer, M., Feys, B. J. and Parker, J. E. (2005): Plant immunity: The EDS1 regulatory node. Curr. Opin. Plant Biol. 8, 383–389.