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Pathogen profile

Potato virus Y: a major crop pathogen that has provided major insights into the evolution of viral pathogenicity

JULIE QUENOUILLE[1,2], NIKON VASSILAKOS[3] AND BENOÎT MOURY[1,*]

[1]*INRA, UR407 Pathologie Végétale, Domaine Saint Maurice, CS 60094, F-84143 Montfavet Cedex, France*

[2]*INRA, UR1052, Génétique et Amélioration des Fruits et Légumes, Domaine Saint Maurice, CS 60094, F-84143 Montfavet Cedex, France*

[3]*Laboratory of Virology, Department of Phytopathology, Benaki Phytopathological Institute, S. Delta 8, 145 61 Kifissia, Athens, Greece*

SUMMARY

Taxonomy: *Potato virus Y* (PVY) is the type member of the genus *Potyvirus* in the family *Potyviridae*.

Virion and genome properties: PVY virions have a filamentous, flexuous form, with a length of 730 nm and a diameter of 12 nm. The genomic RNA is single stranded, messenger sense, with a length of 9.7 kb, covalently linked to a viral-encoded protein (VPg) at the 5′ end and to a 3′ polyadenylated tail. The genome is expressed as a polyprotein of approximately 3062 amino acid residues, processed by three virus-specific proteases into 11 mature proteins.

Hosts: PVY is distributed worldwide and has a broad host range, consisting of cultivated solanaceous species and many solanaceous and nonsolanaceous weeds. It is one of the most economically important plant pathogens and causes severe diseases in cultivated hosts, such as potato, tobacco, tomato and pepper, as well as in ornamental plants.

Transmission: PVY is transmitted from plant to plant by more than 40 aphid species in a nonpersistent manner and, in potato, by planting contaminated seed tubers.

Diversity: Five major clades, named C1, C2, Chile, N and O, have been described within the PVY species. In recent decades, a strong increase in prevalence of N × O recombinant isolates has been observed worldwide. A correlation has been observed between PVY phylogeny and certain pathogenicity traits.

Genetic control of PVY: Resistance genes against PVY have been used widely in breeding programmes and deployed in the field. These resistance genes show a large diversity of spectrum of action, durability and genetic determinism. Notably, recessive and dominant major resistance genes show highly contrasting patterns of interaction with PVY populations, displaying rapid co-evolution or stable relationships, respectively.

*Correspondence: Email: moury@avignon.inra.fr

POTATO VIRUS Y, THE TYPE MEMBER OF THE GENUS *POTYVIRUS*

General description

Potato virus Y (PVY) is the type member of the genus *Potyvirus*, which includes some of the most destructive plant viruses (Kerlan, 2006; Scholthof *et al*., 2011), and of the family *Potyviridae*, a group of single-stranded RNA viruses. PVY was first associated with a disease causing potato degeneration in the early 1930s (Smith, 1931) and has a rather large natural host range, comprising nine botanical families (Kerlan, 2006). The most economically important hosts are potato (*Solanum tuberosum*), tobacco (*Nicotiana* spp.), tomato (*S. lycopersicum*) and pepper (*Capsicum* spp.), but PVY also infects other cultivated plants, such as ornamentals (dahlia, petunia), and numerous weeds.

The principal modes of PVY transmission are by the vegetative propagation of infected material, aphid transmission and, to a lesser extent, contact. PVY is transmitted by more than 40 aphid species (Edwardson and Christie, 1997; Kerlan, 2006) (family Aphididae) in a nonpersistent manner, i.e. a few minutes are sufficient for PVY acquisition or inoculation during aphid probing or feeding, and there is no latent period required by the virus to be transmissible after acquisition. *Myzus persicae* is the vector with the highest transmission efficiency and, as a result of its large geographical distribution, it is responsible for a large part of the natural spread of PVY, although many other species, such as cereal aphids, are also involved in epidemics.

PVY is the major viral threat to potato cultivation, affecting both yield and tuber quality, and results in yield losses of up to 80% (De Bokx and Huttinga, 1981; Van der Zaag, 1987). In addition, PVY control in potato production requires costly certification programmes. Symptoms caused by PVY infection on potato depend on the virus isolate, host cultivar, environmental conditions and whether they are produced by aphid-mediated horizontal transmission or vertical transmission through infected tubers (Draper *et al*., 2002). Symptoms on the aerial part of the plant include leaf mosaic, mottle and crinkling, vein necrosis (Fig. 1a) and necrotic spots, stem and petiole necrosis, leaf drop and stunting of the plants in the case of tuber transmission. Some PVY

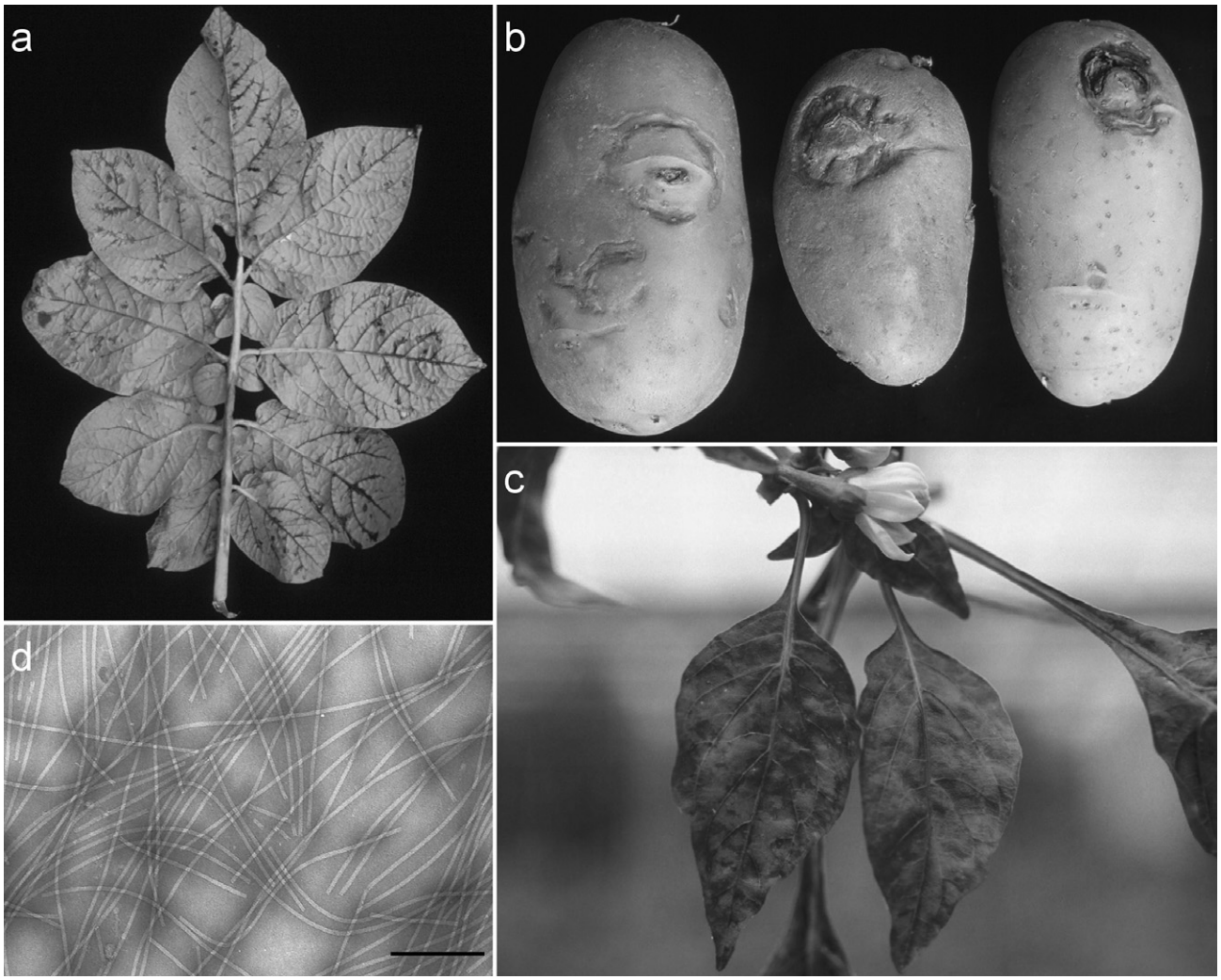


Fig. 1 Symptoms induced by *Potato virus Y* (PVY). (a) Vein necrosis in potato leaf (courtesy of Nikon Vassilakos). (b) Necrotic rings on potato tubers (courtesy of the Laboratory of Virology, Benaki Phytopathological Institute). (c) Leaf mosaic on pepper [courtesy of Alain Palloix, INRA, Provence Alpes Côte d'Azur, Génétique et Amélioration des Fruits et Légumes (GAFL)]. (d) Electron micrograph of PVY virions [courtesy of Isabelle Bornard, INRA, Provence Alpes Côte d'Azur (PACA), Pathologie Végétale]. The scale bar corresponds to 200 nm.

variants also induce potato tuber necrotic ringspot disease, characterized by superficial annular or arch-shaped necroses, protruding from the tuber skin (Beczner *et al*., 1984; Kerlan, 2006) (Fig. 1b).

PVY symptoms in tobacco, tomato and pepper often consist of mild mottling or mosaic (Fig. 1c), although stunting and necrosis may be observed. In addition, in tobacco, PVY modifies the chemical composition of cured leaves, especially the nicotine content (Latorre *et al*., 1984), resulting in high economic losses. In recent decades, tomato and pepper PVY isolates inducing leaf and stem necrosis have been reported in the Mediterranean region (d'Aquino *et al*., 1995; Fanigliulo *et al*., 2005; Gebre Selassie *et al*., 1985; Mascia *et al*., 2010). Mixed infection of PVY and *Cucumber mosaic virus* (CMV) also results in severe diseases in tomato crops.

Control strategies are based on breeding for resistance, the use of certified potato seeds and quarantine regulations to prevent the spread of nonindigenous isolates. The control of aphid vectors is usually of limited effectiveness, because of the nonpersistent mode of transmission. Genetically engineered resistance has been studied extensively, usually by the incorporation of partial or entire sequences of the coat protein (CP), P1 and NIb cistrons into potato or tobacco plants. PVY-resistant genetically engineered cultivars have been registered in the USA (Solomon-Blackburn and Barker, 2001). Most recently, potato plants transformed with a chimeric transgene containing PVY CP sequences have been evaluated in the field for a period of 6 years in Argentina. The plants showed null or negligible infection and preserved their agronomical traits and biochemical characteristics (Bravo-Almonacid *et al*., 2012).

Genome organization and protein functions

PVY virions have a filamentous and flexuous form (Fig. 1d) with a helical symmetry, a length of 730–740 nm and a diameter of 12 nm. The genome consists of a messenger sense, single-stranded RNA of approximately 9.7 kb. It is covalently linked to a single molecule of genome-linked viral protein (VPg) at the 5′ end, and contains a polyadenosine (polyA) tail at the 3′ end. The genome contains a single open reading frame (ORF) flanked by untranslated regions, and is expressed as a polyprotein of approximately 3062 amino acid residues, which is cleaved into 10 mature proteins by three viral proteases (Carrington and Freed, 1990) (Fig. 2). An additional protein is produced from an overlapping ORF after +2 frame shifting of the P3 cistron, as fusion to the amino (N)-terminal part of P3 (P3N-PIPO) (Chung *et al*., 2008).

As a result of the high genome similarities within the genus *Potyvirus*, the functions of PVY proteins have been inferred largely by analogy with other members of the group. Most of them are multifunctional. The first protein coded by the 5′ region of the PVY genome is P1, the most variable protein among potyviruses. It has a protease domain in the carboxy (C)-terminal region and cleaves itself from the adjacent helper-component protease (HC-Pro) (Verchot *et al*., 1991). It has been shown to bind single- and double-stranded RNA (Brantley and Hunt, 1993; Soumounou and Laliberté, 1994), to be involved in genome amplification (Verchot and Carrington, 1995) and to enhance the suppression of plant defences based on RNA silencing (Brigneti *et al*., 1998; Pruss *et al*., 1997).

The next protein, HC-Pro, is involved in aphid transmission (Govier *et al*., 1977), virus multiplication, cell-to-cell (Rojas *et al*., 1997) and systemic (Sáenz *et al*., 2002) movements and symptom

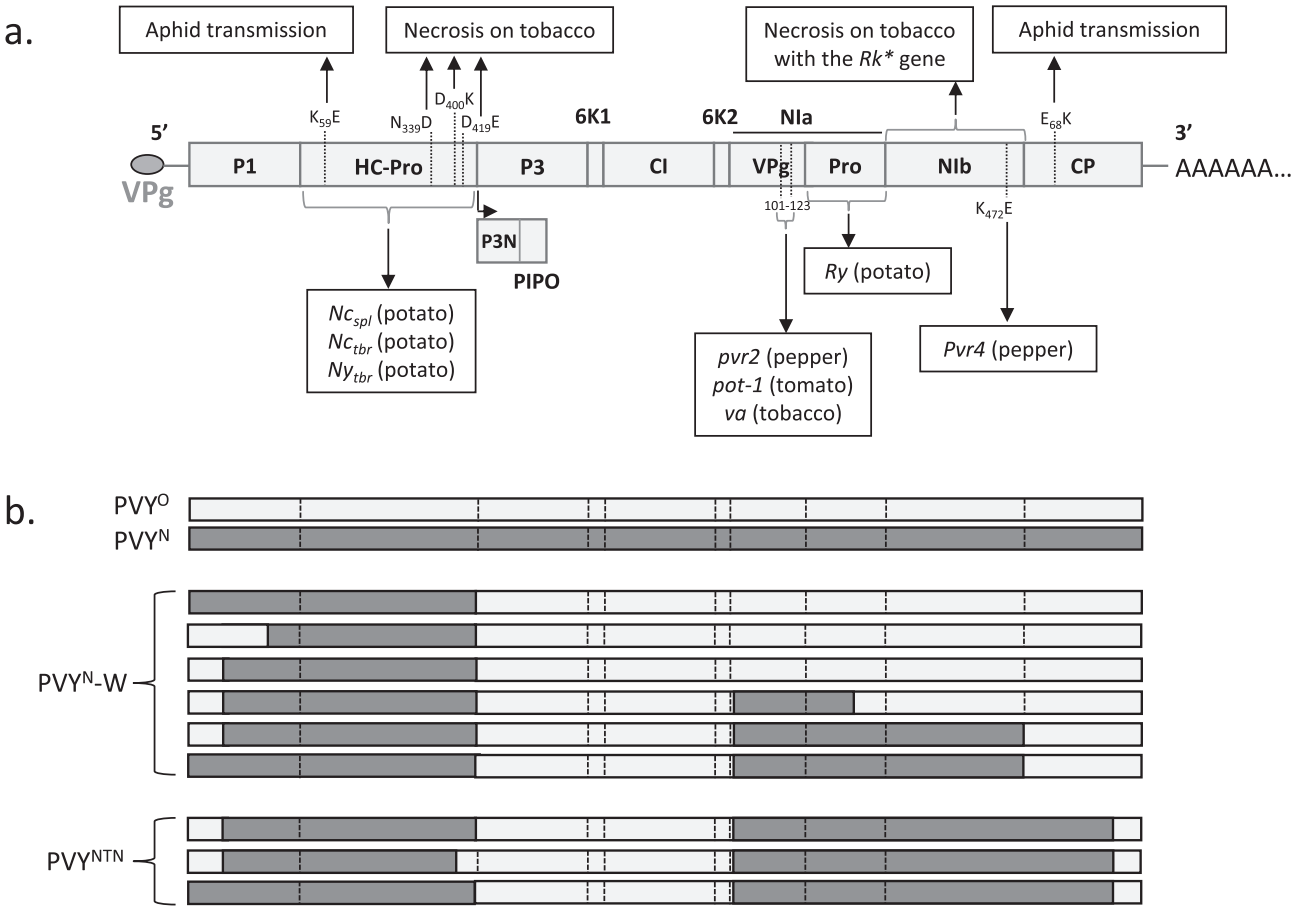


Fig. 2 Determinants of pathogenicity mapped in the *Potato virus Y* (PVY) genome and recombination pattern of PVY^{NTN} and PVY^{N}-W isolates. (a) PVY factors and/or mutations involved in resistance breakdown are indicated below the genome scheme. The dominant genes are specified with a capital letter and the host is indicated n parentheses. Above the genome scheme, PVY determinants of virulence or aphid transmission are noted. (b) Schematic representation of the different recombination patterns between PVY^{O} (light grey) and PVY^{N} (dark grey) clades (modified from Hu *et al*., 2009). *The tobacco *Rk* gene confers a nematode resistance and it (or a very tightly linked gene) induces leaf necrosis on PVY inoculation.

intensity (Redondo *et al*., 2001; Sáenz *et al*., 2002; Shiboleth *et al*., 2007; Torres-Barcelo *et al*., 2008; Yambao *et al*., 2008). Two conserved motifs of HC-Pro, the N-terminal 'KITC' and the C-terminal 'PTK' motifs, are required for aphid transmission, with the first being involved in the interaction with the aphid stylet (Blanc *et al*., 1998) and the latter being a possible binding site for virions (Peng *et al*., 1998). The lysine to glutamic acid (K59E within the KITC motif) mutation observed in some PVY isolates renders HC-Pro unable to interact with aphid stylets and abolishes PVY aphid transmissibility (Blanc *et al*., 1998). It has been suggested that HC-Pro acts as a bridge between the virion CP and an unknown receptor in the aphid stylet (Govier and Kassanis, 1974; Pirone and Blanc, 1996). The C-terminal part of HC-Pro is involved in the suppression of plant defences based on the RNA silencing machinery, by binding to small interfering RNAs (siRNAs) (Brigneti *et al*., 1998; Lakatos *et al*., 2006; Llave *et al*., 2000; Shiboleth *et al*., 2007; Varrelmann *et al*., 2007). This property could account for the general involvement of potyvirus HC-Pro in symptomatology and synergy with co-infecting viruses for symptom severity (Pruss *et al*., 1997), but also for its roles in virus multiplication and systemic movement (Kasschau and Carrington, 2001). Additional plant defence inhibition properties are also suggested by the fact that PVY HC-Pro interacts with subunits of the 20S proteasome, which is related to the antiviral response (Jin *et al*., 2007). A recent study has also shown an interaction between PVY HC-Pro and the eukaryotic initiation factor 4E (eIF4E) and its isoform eIFiso4E of pepper and tobacco, making HC-Pro a putative partner of the translation initiation complex (Ala-Poikela *et al*., 2011).

The protein P3, together with 6K2, are the two potyviral membrane proteins (Eiamtanasate *et al*., 2007; Restrepo-Hartwig and Carrington, 1994). P3 contains two hydrophobic domains located at the N- and C-termini. Several studies have suggested its involvement in virus replication, systemic infection, pathogenicity and movement (Chu *et al*., 1997; Cui *et al*., 2010; Johansen *et al*., 2001; Merits *et al*., 1999). A translational frameshift on the P3 cistron leads to the production of P3N-PIPO (Chung *et al*., 2008), which has been shown to be located to plasmodesmata and to participate in the viral cell-to-cell movement in conjunction with the CI (cylindrical or cytoplasmic inclusion) protein (Wei *et al*., 2010b). The function of the following 6K1 protein is unknown.

The CI protein forms the laminate cytoplasmic inclusion bodies (pinwheel-shaped) (Edwardson, 1992), typical of potyviral infections. Its involvement in cell-to-cell virus movement is well established (Carrington *et al*., 1998; de Cedrón *et al*., 2006; Roberts *et al*., 1998; Wei *et al*., 2010b), whereas the RNA-binding, RNA helicase and ATPase activities strongly suggest an important role in virus replication (Fernández *et al*., 1995, 1997; Merits *et al*., 1998). The C-terminal part of the CI protein has been shown to interact with the plant eIF4E (Tavert-Roudet *et al*., 2012), and the virus VPg and CI could therefore be recruited into the translation initiation complex, which is essential for virus multiplication and/or cell-to-cell movement (Abdul-Razzak *et al*., 2009).

The 6K2 protein is membrane bound (Restrepo-Hartwig and Carrington, 1994) and has been shown to play an essential role in virus replication (Wei and Wang, 2008; Wei *et al*., 2010a) by anchoring the viral replication complex to the endoplasmic reticulum (Schaad *et al*., 1997).

The NIa (first nuclear inclusion) protein possesses two domains: VPg and a protease domain which cleaves most proteins of the precursor polyprotein (Carrington and Dougherty, 1987). VPg is an intrinsically disordered protein and its structural flexibility has been proposed to be the basis of its capacity to interact physically with many viral and plant proteins (Elena and Rodrigo, 2013) and of its functional diversity (Rantalainen *et al*., 2011). Binding of PVY VPg with the host eIF4E is a key component of virus multiplication (translation and/or replication) (Deom *et al*., 1997; Robaglia and Caranta, 2006). VPg is localized in different cellular compartments depending on its combination with the NIa protease and/or the 6K2 protein. The VPg–NIa protease combination is located exclusively in the nucleolus, whereas the 6K2–VPg–NIa protease combination is found within vesicular structures derived from the endoplasmic reticulum (Jiang and Laliberte, 2011). Finally, VPg alone is linked covalently to the 5′ extremity of the viral RNA via a tyrosine residue (Murphy *et al*., 1990, 1991).

The NIb (second nuclear inclusion) protein is the RNA-dependent RNA polymerase (RdRp) involved in the replication of the viral RNA (Hong and Hunt, 1996). Finally, CP is required for virion assembly, cell-to-cell (Rojas *et al*., 1997) and systemic (Andersen and Johansen, 1998; Dolja *et al*., 1995) movements, and aphid transmission (Atreya *et al*., 1990; Blanc *et al*., 1997). CP is a three-domain protein with variable N- and C-terminal domains exposed on the virion surface and a core region that binds RNA. The highly conserved 'DAG' motif at the N-terminus of CP is essential for aphid transmissibility (Atreya *et al*., 1995). The context of the DAG motif can modulate the efficiency of potyvirus aphid transmission (López-Moya *et al*., 1999). Remarkably, amino acid positions 9, 10 and 11 of the PVY CP, immediately adjacent to the DAG triplet (located at positions 6–8), has been shown to be subjected to positive selection (Moury and Simon, 2011). Hence, variations at these positions could modulate PVY transmission efficiency, as demonstrated for position 9 of *Tobacco etch virus* (TEV) CP (López-Moya *et al*., 1999). In addition, the internal amino acid position 68 of PVY CP has been shown to modify quantitatively its aphid transmission efficiency by *Myzus persicae* or *Aphis gossypii* (Moury and Simon, 2011). The core region of CP has an essential role in virus assembly and cell-to-cell movement, suggesting the involvement of virions in cell-to-cell traffic (Rojas *et al*., 1997). Both the N- and C-terminal regions are not required for assembly, but are involved in systemic movement.

WHAT IS STRUCTURING PVY DIVERSITY?

Where, how and when did PVY emerge?

The species PVY belongs to a large clade of 19 potyvirus species, sometimes referred to as the 'PVY group' or 'PVY clade' (Gibbs and Ohshima, 2010; Li *et al*., 2012; Moury and Verdin, 2012). It represents one of the four potyvirus groups that infect solanaceous crops, together with the TEV/*Potato virus A* clade, the *Pepper veinal mottle virus* (PepVMV)/*Chilli veinal mottle virus* clade and the *Colombian datura virus* (synonymous to *Petunia flower mottle virus*), which does not show any close relationship to other potyviruses. Viruses from the PVY clade are mostly present in America and, more particularly, in South America. Among the 19 species of the clade, 16 are present in America, with 11 being present exclusively on this continent. By contrast, only eight species have been described outside the Americas, indicating a significantly higher richness of viruses belonging to this clade in the American continents ($P = 0.017$; Fisher's exact test). Three species (*Amazon lily mosaic virus*, *Alstroemeria mosaic virus* and *Amaranthus leaf mottle virus*) have been observed only in the Old World, but only sporadically. Moreover, the natural hosts of these three species originate from South America. It is therefore likely that they are (or were) also present in America, but have not been detected because of limited sampling.

There is a significant association between the potyvirus species of the PVY clade that are found outside the American continents and their occurrence in crops propagated by vegetative propagation (Table 1; $P = 0.018$, Fisher's exact test). These data suggest that America (and, more specifically, South America) could be the centre of origin and diversification of the PVY clade, and that a worldwide dispersal of some of its members has involved mostly human activities (trade of potato tubers and bulbs or rhizomes of ornamental plants). The same trend is observed for the PVY species. Among the five major clades within PVY species, four (clade C, which includes the two subclades C1 and C2, and clades N and O; see below) are infectious in potato and show a worldwide distribution. The fifth (Chilean) clade does not seem to infect potato and has been described only in Chile (Moury, 2010; Sudarsono *et al*., 1993). These data are also in accordance with the fact that Central and South America are centres of origin and diversification for most of the hosts of viruses in the PVY clade, including PVY itself, such as species of the families Solanaceae, Asteraceae (Barreda *et al*., 2010) and Amaranthaceae (Segundo *et al*., 2007). These three botanical families also include large numbers of PVY experimental hosts (Edwardson and Christie, 1997; Kerlan, 2006), which could be a 'genetic remnant' of host range and infectivity properties derived from the common ancestor of the PVY clade. Reciprocally, viral species of the PVY clade that are adapted to the Asteraceae are also able to infect species in the family Solanaceae (Dujovny *et al*., 1998; *Bidens mottle virus*; GenBank accession number EF467235).

Recent attempts to place a timeframe on the diversification of PVY have been unsuccessful (Cuevas *et al*., 2012) because of a lack of temporal structure in the PVY sequence dataset available, precluding the estimation of divergence times within the phylogenetic tree. As the initial radiation of the whole *Potyvirus* genus has been estimated to have occurred about 6600 years ago (Gibbs *et al*., 2008), a period which coincided with the development of agriculture, PVY diversification can be considered as very recent and may have been influenced strongly by human activities.

Table 1 A test of association between geographical distribution and host characteristics among the 19 species of the *Potato virus Y* (PVY) group (Gibbs and Ohshima, 2010). Viruses that infect commercialized plants which multiply vegetatively have a greater chance of being present outside the American continents which contain the putative centre of diversification of the group ($P = 0.018$ or $P = 0.0013$ when considering separately the five major PVY clades; Fisher's exact tests). The five PVY clades are shown in bold type.

	Present outside Americas	Present only in Americas
Infectious in commercialized plants with vegetative propagation (tubers, bulbs or rhizomes)	*Alstroemeria mosaic virus* *Amazon lily mosaic virus* *Potato virus V* ***Potato virus Y* (PVY):** **PVY-N** **PVY-O** **PVY-C1** **PVY-C2**	None reported
Not infectious in commercialized plants with vegetative propagation	*Amaranthus leaf mottle virus* *Bidens mottle virus* *Pepper mottle virus* *Sunflower chlorotic mottle virus*	*Alternanthera mild mosaic virus* *Bidens mosaic virus* *Brugmansia suaveolens mottle virus* *Ecuadorian rocoto virus* *Pepper severe mosaic virus* *Pepper yellow mosaic virus* *Peru tomato mosaic virus* *Pfaffia mosaic virus* *Tomato necrotic stunt virus* *Verbena virus Y* *Wild potato mosaic virus* **PVY-Chile**

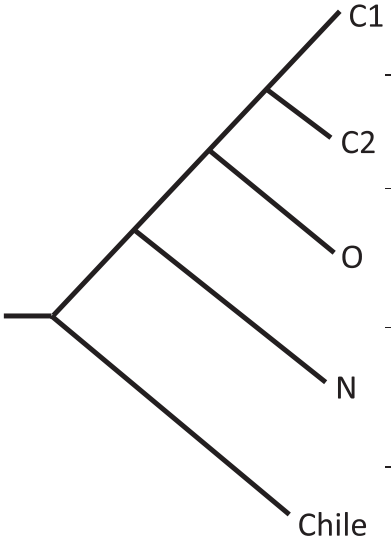
Host and geography as drivers of PVY diversity

PVY has been the focus of interest for many agronomists and virologists, and its biological and genetic diversities have been explored extensively, allowing a rather exhaustive image of its diversity to be drawn. Genome analyses have revealed five major clades within the PVY species (Fig. 3). The most widespread clades are the C1, N and O groups, whereas the C2 and Chilean groups are more restricted, either because of their narrower host range or their limited geographical distribution. Recombinant isolates with different genome parts clustering with distinct clades are also widespread.

The first descriptions of PVY infections date back to the 1930s (Cockerham, 1943; Salaman and Le Pelley, 1930; Smith, 1931). PVY clades O and C (without distinguishing subclades C1 and C2) were predominant until the 1950s (Rolland *et al*., 2008). PVY C isolates can be distinguished from O isolates by the hypersensitive reactions which they induce in potato cultivars carrying the *Nc* resistance gene. PVY O isolates are a major problem in potato production and induce severe leaf mosaic symptoms and yield losses in potato cultivars lacking major resistance genes. PVY C isolates cause less severe disease in potato, with systemic mosaic or stipple streak symptoms. However, they are still predominant in other crops, such as pepper (mainly the C1 subgroup), where they induce severe mosaic and/or necrotic symptoms and high yield reduction (Gebre Selassie *et al*., 1985).

PVY N isolates induce severe veinal necrosis symptoms in most tobacco cultivars, whereas PVY O and C isolates usually induce mild mosaic symptoms. PVY N isolates have been reported since the 1940s in potato plants in Peru and Bolivia (Nobrega and Silberschmidt, 1944). Such isolates have been described in Europe and the USA since the 1950s (Kahn and Monroe, 1963). Compared with PVY O isolates, they induce less severe symptoms on potato plants, mostly mild mosaic or mottle in leaves. Finally, PVY isolates from the Chilean group were described initially in tobacco plants, where they induced veinal necrosis similar to that induced by N isolates (Sudarsono *et al*., 1993), and later in pepper (*Capsicum baccatum*) (Moury, 2010). Until now, this clade contains only isolates from Chile. According to phylogeny, the Chilean clade was the first to diverge during PVY evolution (Fig. 3).

Before the development of molecular biology, PVY classification was based on serology and on infectivity and symptomatology on different cultivated hosts. A remarkable property of PVY diversity is the correlation between the phylogenetic classification and some host range properties (Fig. 3). Examples include the infectivity in pepper and potato (isolates of clades C2, N and O are not or are poorly infectious in pepper, whereas isolates of clades C1 and Chile are not or are poorly infectious in potato; Fereres *et al*.,



Potato (*Solanum tuberosum*)	Pepper (*Capsicum* spp.)	Tobacco (*Nicotiana tabacum*)	Tomato (*Solanum lycopersicum*)	Eggplant (*Solanum melongena*)
Rare (Dullemans *et al*., 2011; Kehoe and Jones, 2011)	Frequent worldwide	Frequent worldwide	Frequent worldwide	Reported (Sadeghi *et al.,* 2008)
Rare (Schubert *et al*., 2007; Kehoe and Jones, 2011)	Not reported; not infectious in the laboratory (Blanco-Urgoiti *et al.,* 1998)	Not reported but infectious in the laboratory	Not reported but infectious in the laboratory	Not reported
Frequent worldwide	Reported (Ibaba and Gubba, 2011); poorly infectious in the laboratory (Moury, 2010)	Frequent worldwide	Reported (Ibaba and Gubba, 2011; Kehoe and Jones, 2011)	Reported (Bhat *et al*., 1999)
Frequent worldwide	Reported (Margaritopoulos *et al.,* 2010); poorly infectious in the laboratory (Moury 2010)	Frequent worldwide	Reported (Aramburu *et al.,* 2006)	Not reported
Not reported; not infectious in the laboratory (Moury, 2010)	Reported on *Capsicum baccatum* in Chile (Moury' 2010)	Reported in Chile (Sudarsono *et al*., 1993)	Not reported; infectious in the laboratory (Moury, 2010)	Not reported

Fig. 3 Simplified *Potato virus Y* (PVY) phylogeny (excluding the recombinant isolates) and infectivity of PVY isolates from the five major clades in crop plants of the family Solanaceae.

1993; Gebre Selassie *et al*., 1985; Moury, 2010; Romero *et al*., 2001) and the interaction with potato resistance genes *Nc* (specific for PVY C isolates) and *Ny* (specific for PVY O isolates). This suggests that relatively few host jumps and few changes in pathogenicity have occurred during PVY history. Moreover, it indicates that only few genetic exchanges have occurred between these major clades, as they would have obscured these correlations. Some of the pathogenicity traits, however, do not match with the phylogenetic clustering of PVY isolates, such as infectivity towards pepper or tobacco genotypes carrying recessive resistance, interaction with the resistance of the potato cultivar Maris Bard and symptomatology in tobacco varieties carrying the *Nk* nematode resistance gene.

Phylogenetic analysis has also revealed an effect of the continental origin on the genetic structuring of PVY populations, mainly for Europe, Japan, South Africa and North America (Cuevas *et al*., 2012). More particularly, the PVY N group splits into European and North American subclades and, although few isolates have been characterized, the Chilean group seems to be restricted to South America. For potato PVY isolates, the geographical structuring is probably hindered by the international trade of seed tubers and repeated introductions of PVY-infected material (for example, at least five independent introductions have been postulated in Australia; Kehoe and Jones, 2011). Evidence of geographical structure could therefore reflect a smaller amount of potato material exchange or more efficient quarantine procedure or sanitary control policies.

The emergence of PVY recombinants

Since the end of the 1970s, PVY isolates inducing necrotic ringspot in potato tubers have been described and, later, were shown to be recombinants, with parts of their genome clustering with PVY O isolates and others with PVY N isolates (Le Romancer *et al*., 1994). Isolates showing this recombination pattern (grouped as PVY^{NTN}; Fig. 2b) showed a dramatic increase in frequency in European potato crops in the 1990s and 2000s, representing more than 50% of isolates in France and Germany (Rolland *et al*., 2008). Another recombinant group (PVY^{N}-W; Fig. 2b) also showed an increase in prevalence in Europe and northern America during the early 2000s (Rolland *et al*., 2008). By contrast, PVY C isolates were no longer detected in European or American potato crops, which could be a result of the wide distribution of the *Nc* resistance gene in potato cultivars (Cockerham, 1943). The reasons for these spectacular increases in frequency are still unknown. These recombinant isolates possess a clear fitness advantage over nonrecombinants, as these increases in prevalence occurred independently in different geographical regions worldwide. They have a clear advantage over PVY O isolates, because they can infect potato cultivars carrying the *Ny* gene, which is present in many cultivars. However, why they outcompete isolates of the N clade remains unexplained. It has been shown that the molecular determinants of veinal necrosis in tobacco (cultivar Xanthi) present among PVY N, but not O, isolates confer a fitness cost in terms of the accumulation and competitiveness in Xanthi (Rolland *et al*., 2009). The recombination events that lead to the PVY^{NTN} group might compensate for this fitness penalty. There is probably no direct link between the recombinant nature of PVY^{NTN} isolates and the tuber necrosis that they induce. Indeed, similar symptoms can be induced by nonrecombinant isolates of the N clade (Nie and Singh, 2003; Singh *et al*., 2008).

Other recombinant PVY isolates, mostly issued from recombination between PVY O and N isolates, have been described, but show more limited geographical ranges. Although many different recombination patterns have been observed among PVY isolates, they seem to involve a limited number of independent recombination events. For example, PVY^{NTN} and PVY^{N}-W isolates share three recombination breakpoints and may therefore have common origins (Schubert *et al*., 2007; Fig. 2).

PVY AND ITS HOSTS: MODELS OF PATHOGENICITY EVOLUTION IN PLANT VIRUSES

eIF4E-mediated resistance and PVY: evidence of recent and rapid co-evolution

The first cloned natural recessive gene conferring pathogen resistance in plants was the *pvr2* gene of pepper (*Capsicum* sp.) which provides PVY resistance (Ruffel *et al*., 2002). It was also the first cloned PVY resistance gene (a list of the identified PVY resistance genes is provided in Table 2). *pvr2* encodes an eIF4E, which is involved in the initiation of translation of mRNAs in eukaryotes. eIF4E binds the cap attached to the 5′ extremity of mRNAs and initiates the recruitment of a scaffold of plant proteins, ending up with the translation process (Robaglia and Caranta, 2006). In plants, eIF4E belongs to a small gene family (Patrick and Browning, 2012). There are three copies of eIF4E and one isoform (eIFiso4E) in the genome of *Arabidopsis thaliana*. Pepper and tomato possess one copy of eIFiso4E and at least two copies of eIF4E (named eIF4E1 and eIF4E2). It has been suggested that, among these copies, one is constitutively expressed and possesses ubiquitous translation functions, whereas others are more specialized and may also possess functions unrelated to translation (Hernández and Vazquez-Pianzola, 2005). Potyviruses have no caps at the 5′ end of their genomic RNAs, but a viral protein: VPg. Potyvirus VPgs are able to interact physically with one or other eIF4E isoform, which suggests that they have hijacked the translation machinery of eukaryotic cells for their own multiplication. Indeed, it has been shown that virus VPg and plant mRNAs compete for binding to eIF4E forms (Léonard *et al*., 2000). In addition, direct binding between eIF4E and potyvirus VPg has been demonstrated with yeast two-hybrid experiments (Wittmann

Table 2 Genes conferring resistance to *Potato virus Y* (PVY).

Plant species	Resistance gene	Chromosome location	Nature of resistance	Spectrum of resistance	PVY pathogenicity factor	RB mutation and cost associated	References
Pepper	*pvr2*^{1}	P4	Recessive	Medium; some RB isolates	VPg	$N_{121}H$, no cost	Charron *et al*. (2008); Fabre *et al*. (2012); Kyle and Palloix (1997); Montarry *et al*. (2011); Moury *et al*. (2004); Ruffel *et al*. (2002)
	pvr2^{2}	P4	Recessive	Large; very few RB isolates	VPg	$N_{119}D + N_{121}H$, costly	
	pvr2^{3}	P4	Recessive	Narrow; many RB isolates	VPg	Many single-nucleotide mutations, no cost	
	pvr2^{4} to *pvr2*^{9}	P4	Recessive	Not reported	Not reported	Not reported	
	Pvr4	P10	Dominant (HR/ER)	All clades	NIb	$K_{472}E$, costly	Dogimont *et al*. (1996); Janzac *et al*. (2010)
	QTLs	P1, P6, P9, P10	Polygenic	All clades	Not reported	Not reported	Caranta *et al*., 1997
Potato	*Nc*$_{spl}$	IV	Dominant (HR)	Clade C	HC-Pro	Not reported	Moury *et al*. (2011)
	Nc$_{tbr}$	Not reported	Dominant (HR)	Clade C	HC-Pro	Not reported	Cockerham (1970); Jones (1990); Moury *et al*. (2011)
	Ny$_{tbr}$	IV	Dominant (HR)	Clade O	HC-Pro	Not reported	Celebi-Toprak *et al*. (2002); Cockerham (1970); Jones (1990); Moury *et al*. (2011)
	Ry$_{adg}$	XI	Dominant (ER)	All clades	Not reported	Not reported	Hämäläinen *et al*. (1997); Ross (1986)
	Ry$_{sto}$	XII	Dominant (ER)	All clades	Nla-Pro	Not reported	Cockerham (1943); Mestre *et al*. (2000); Song *et al*. (2005)
Tobacco	*va*^{0} to *va*^{2}	E	Recessive	All clades	VPg	Not reported	Masuta *et al*. (1999); Lacroix *et al*. (2011)
	va2	Not reported	Recessive	Not reported	Not reported	Not reported	Acosta-Leal and Xiong (2008)
Tomato	*pot-1*	T3	Recessive	All clades	VPg	($R_{119}H$)	Moury *et al*. (2004); Parrella *et al*. (2002)

ER, extreme resistance; HC-Pro, helper-component proteinase; HR, hypersensitive response; Nla, first nuclear inclusion; Nlb, second nuclear inclusion; QTL, quantitative trait locus; RB, resistance breaking; VPg, genome-linked viral protein.

et al., 1997), and has been shown to be essential for virus infectivity. Indeed, plants with eIF4E or eIFiso4E knockout mutations or encoding eIF4E containing amino acid substitutions that abolish interaction with VPg are resistant to potyvirus infection (Duprat *et al*., 2002; Lellis *et al*., 2002; Ruffel *et al*., 2002). In turn, viruses are able to counter-adapt through VPg mutations that restore interaction with the mutated eIF4E (Charron *et al*., 2008) or, in some cases, possibly through the use of an eIF4E-independent pathway for their infectivity (Gallois *et al*., 2010). The PVY VPg has been shown to be the resistance-breaking (RB) factor towards recessive resistance in pepper (Moury *et al*., 2004), the tomato relative *Solanum habrochaites* (Moury *et al*., 2004) and tobacco (Lacroix *et al*., 2011; Masuta *et al*., 1999). These recessive resistance traits are conferred by resistance alleles at the *pvr2* locus, the *pot-1* orthologue of *pvr2* (Ruffel *et al*., 2005) and alleles at the *va* locus, respectively.

Among solanaceous plants, most eIF4E-encoding resistance genes have been identified in pepper and some in tomato relatives; in tobacco, the *va* resistance can be related to eIF4E, given the breakdown mechanisms, but this has not yet been demonstrated. No eIF4E-mediated resistance has been identified in potato (*S. tuberosum*), which is probably a result of the tetraploid nature of this plant species, or in potato relatives. In pepper, 10 different *pvr2* alleles have been described from a collection of 25 inbred lines (Charron *et al*., 2008), with the most widespread corresponding to the susceptibility allele *pvr2*$^{+}$. In spite of this relatively high allelic richness, overall, there is very limited variability in the *pvr2* sequences. The other nine alleles differ from *pvr2*$^{+}$ by only one to four amino acid substitutions in the encoded eIF4Es. Almost only nonsynonymous substitutions have been observed in the sequence of *pvr2* and most polymorphic positions show signatures of positive selection (Cavatorta *et al*., 2008). Polymorphism is concentrated in three eIF4E domains, two coinciding with the catalytic site (mRNA cap-binding site). Remarkably, all pepper genotypes carrying alleles that differ from *pvr2*$^{+}$ show resistance to PVY. In spite of their high sequence similarity, these resistance alleles show a contrasting resistance spectrum and durability. Among the nine *pvr2* alleles conferring PVY resistance, three (*pvr2*^{1}, *pvr2*^{2} and *pvr2*^{7}) also confer resistance to PepVMV, when combined with the *pvr6*-eIFiso4E gene (Rubio *et al*., 2009), and one (*pvr2*^{2}) confers resistance to TEV (Charron *et al*., 2008). These three species belong to three distinct clades in the genus *Potyvirus*, showing a large spectrum of action of eIF4E-mediated resistance and that the infection strategies of many potyviruses rely on similar patterns of interaction with eIF4E. The *pvr2* alleles also differ largely in durability. *pvr2*^{1} and *pvr2*^{2} have been deployed in pepper crops worldwide for more than 50 years. Almost no natural PVY isolates able to infect plants with *pvr2*^{2} have been described so far (Moury and Verdin, 2012). *pvr2*^{1}-breaking isolates are more frequent in natural conditions, but tend to be less prevalent than wild-type (i.e. non-RB) isolates

(Luis-Arteaga and Gil-Ortega, 1986). The *pvr2*^{3} allele is not largely deployed, except in traditional cultivars. However, *pvr2*^{3}-breaking isolates are highly prevalent in pepper crops (Ben Khalifa *et al*., 2012) and can be selected easily in laboratory conditions (Ayme *et al*., 2006; Montarry *et al*., 2011). *pvr2*^{3} is therefore expected to have a very low durability potential. The differences in durability between *pvr2*^{1}, *pvr2*^{2} and *pvr2*^{3} have been linked to the number and complexity of mutational pathways in the VPg coding region conferring RB capacity to PVY (Moury and Verdin, 2012) and to the pleiotropic fitness costs caused by these mutations (Ben Khalifa *et al*., 2009; Fabre *et al*., 2012).

The pattern of variation of VPg of pepper PVY isolates shows striking similarities to that of its *pvr2*-encoded eIF4E ligand among pepper cultivars. Most of VPg is highly constrained, shows very limited amino acid variation and evolves under significant negative selection (B. Moury, unpublished data). By contrast, a small central region (amino acid positions 101–123) shows extensive amino acid variation, associated with significant positive selection (Moury *et al*., 2004). The functional relevance of these amino acid substitutions is further highlighted by the fact that almost all substitutions involved in the breakdown of the *pvr2* alleles of pepper during laboratory experimental evolution coincide with these positively selected positions (Ayme *et al*., 2006). This correlation also implies that the RB events observed in the laboratory are representative of those which occur in natural epidemiological conditions, and that experimental evolution can be used, to a certain extent, to evaluate the durability potential of resistance. The eIF4E-VPg pattern of diversity has been suggested to be emblematic of the matching allele model of plant–pathogen interactions (by contrast with the gene-for-gene model of interaction; Ben Khalifa *et al*., 2012; Sacristán and García-Arenal, 2008), where each pathogen genotype is adapted to only one (or a very limited number of) resistance allele(s) in the host population, and where the RB capacity incurs no fitness cost to the pathogen.

Taken together, (i) the high variability and positive selection observed at a very small number of amino acid positions of VPg and eIF4E; the facts that (ii) the remainder of these two proteins are highly conserved and/or constrained and that (iii) almost all amino acid variability is linked to gains of function (resistance of the plant or pathogenicity of the virus); and (iv) the recent emergence of potyviruses provide rare evidence of a recent and rapid co-evolution in plant-pathogen interactions.

Steady relationships between PVY and dominant resistance

Dominant monogenic resistance to PVY has been characterized in potato and pepper. The pattern of interaction between these resistance genes and PVY isolates shows a remarkable stability, which contrasts sharply with the rapid diversification observed for recessive resistance alleles and PVY VPg ligands. Two kinds of phenotypic reaction have been distinguished among dominant resistance: extreme resistance (ER) and hypersensitive response (HR). The former includes the Ry_{sto} and Ry_{adg} genes from the wild potato relatives *S. stoloniferum* and *S. tuberosum* ssp. *andigena*, respectively, and the *Pvr4* gene from pepper. No PVY accumulation can be detected in inoculated organs of plants carrying these ER genes (Janzac *et al*., 2009; Jones, 1990) and no necrotic lesions typical of a HR can be observed. The ability of Ry_{sto} to reduce PVY accumulation at the within-cell level has been demonstrated by protoplast experiments (Barker and Harrison, 1984). Several lines of experimental evidence support links between ER and HR. For the *Pvr4* resistance in pepper, some PVY isolates are able to induce HR-like necrotic lesions in inoculated cotyledons, and PVY can be detected in these organs (Janzac *et al*., 2009). However, none of the isolates was able to induce necrotic lesions or to be detected by enzyme-linked immunosorbent assay (ELISA) or reverse transcription-polymerase chain reaction (RT-PCR) in *Pvr4* pepper leaves. HR-like reactions also occur in *Pvr4* pepper under high inoculation pressures, such as in graft inoculations, in which the infected rootstock continuously provides virus to the scion (Janzac *et al*., 2009), and in Ry_{sto} potato plants by *Agrobacterium*-mediated expression of the resistance elicitor (Mestre *et al*., 2000). Therefore, ER and HR seem to be manifestations of the same resistance mechanisms, ER possibly being an HR which is expressed more rapidly and/or with a higher intensity by the plant, which can circumvent more efficiently the virus infection (Bendahmane *et al*., 1999). Remarkably, *Pvr4* and *Ry* show a large spectrum of action: *Ry* genes are efficient against all PVY isolates and *Pvr4* against all PVY isolates and five additional potyvirus species. Both are also highly durable as no RB by PVY has been observed in field conditions (for more than 20 years for *Pvr4*). Graft inoculation of *Pvr4* scions enabled the selection of RB PVY isolates that contained a single-nucleotide RB substitution in the NIb coding region. This nonsynonymous mutation changes a lysine residue at position 472 in NIb to glutamic acid and induces a high competitiveness cost to PVY in a susceptible pepper genotype. Although this mutation did not seem to modify the three-dimensional structure of the protein, the high cost it induced to the virus could be linked to the overall high evolutionary constraint that is exerted on NIb (Janzac *et al*., 2010).

As no RB isolate was available, the viral factor corresponding to Ry_{sto} was identified through transient expression of the PVY proteins in potato leaves. Only the NIa protease was able to induce necrotic HR-like reactions. It is also not excluded that the elicitor of Ry_{sto} is a host factor derived from the protease activity of NIa, and not the NIa protease directly. These reactions were specific of plants carrying Ry_{sto} in a segregating population of potato plants (Mestre *et al*., 2000). The capacity of the NIa protease to induce the HR-like response was further shown to overlap largely with the amino acid positions involved in the protease activity to the protein (Mestre *et al*., 2003). This suggests that putative RB

mutations in PVY NIa protease would abolish or decrease the protease activity, would alter the maturation of most viral proteins and hence would be extremely costly for the virus. Overall, the high durability of the *Ry* and *Pvr4* ER is most probably a result of the fitness cost, rather than the mutational pathways associated with RB.

Dominant PVY resistance genes conferring HR have been described only in potato or potato relatives in the genus *Solanum*. The Nc_{tbr} and Nc_{spl} genes, from *S. tuberosum* and *S. sparsipilum*, respectively, confer HR to PVY C (C1 and C2) isolates only, and the Ny_{tbr} gene from *S. tuberosum* confers HR resistance to PVY O isolates only. Nc_{spl} and Ny_{tbr} map to the same region of chromosome IV in the potato genome and could therefore be allelic or belong to the same gene cluster (Moury *et al*., 2011). PVY HC-Pro is the viral factor that corresponds to Nc_{tbr}, Nc_{spl} and Ny_{tbr} (Moury *et al*., 2011), and the HR-eliciting regions of Nc_{spl} and Ny_{tbr} seem to be contiguous in the C-terminal end of HC-Pro (Moury *et al*., 2011; Tian and Valkonen, 2013). The fact that the specificity of the HR of potato correlates largely with the clustering of PVY clades suggests few, if any, resistance breakdowns by the accumulation of point mutations along PVY evolution. Instead, breakdown of Ny_{tbr} by recombination was suggested for PVY^{NTN} and PVY^{N}-W (Moury *et al*., 2011).

Consequently, in contrast with recessive resistance genes, dominant resistance genes show rather stable patterns of interaction with PVY diversity, as they reveal: (i) no (or very little) evidence of breakdown; (ii) little allelic variability; and (iii) no evidence of positive selection in the corresponding viral factors (HC-Pro, NIa protease and NIb) (Moury *et al*., 2002, 2006).

Insights into the durability of oligo- and polygenic resistance

Combining different resistance genes against the same pathogen in the same plant genotype (gene pyramiding strategy) has been proposed to improve resistance durability. PVY has provided experimental evidence of this concept and insights about the mechanisms involved in this enhanced durability. Acosta-Leal and Xiong (2008) studied the tobacco cultivars 'VAM' and 'NC745', both carrying the *va* recessive resistance gene, and observed a rapid selection of RB variants in NC745, but not in VAM, for which no RB variants were obtained. The *va* gene confers resistance to PVY cell-to-cell movement. In VAM, a second unlinked recessive gene, named *va2*, also conferred resistance to within-cell PVY accumulation, as observed in transfected protoplasts. Therefore, the higher durability of VAM resistance was caused by a combination of resistance genes that acted at two different steps of the virus infection cycle.

A similar situation was observed in pepper, where the durability of the $pvr2^3$ gene could be enhanced strongly by the combination with quantitative trait loci (QTLs) that increased the resistance efficiency (Palloix *et al*., 2009). Several mechanisms seemed to contribute to the higher durability of the polygenic resistance ($pvr2^3$ + QTLs), including higher efficiency, more complex mutational pathways leading to RB and slower selection of RB variants (Quenouille *et al*., in press). Interestingly, by themselves, the QTLs had only a small resistance effect, decreasing PVY accumulation slightly at the systemic level and delaying symptom onset and severity. Moreover, the QTLs were not durable by themselves, as eight serial passages in a pepper genotype carrying these QTLs showed a drastic increase in virus accumulation (Montarry *et al*., 2012). These results suggest that the increase in durability of the polygenic resistance composed of $pvr2^3$ and QTLs was caused by more-than-additive epistatic effects of the genetic components.

PVY infectivity, virulence and fitness: correlated evolution of pathogenicity traits

Beyond their interaction with particular resistance genes, PVY isolates more generally show large differences in virulence, such as the quantitative level of damage in infected hosts, and in infectivity in different host species. Variation of these traits has, for a long time, been the basis of PVY classification (Singh *et al*., 2008).

With regard to virulence, the most obvious differences reside in the capacity of some PVY isolates to induce necrotic (versus mosaic) reactions in their hosts, mainly in tobacco, pepper and potato. Usually, genetic variation for the necrotic phenotype also exists in the host. As mentioned above, PVY N isolates induce veinal necrosis in most tobacco cultivars, such as Xanthi. The necrotic phenotype is mostly a result of the combination of three amino acid substitutions in HC-Pro (at positions 339, 400 and 419) (Faurez *et al*., 2012; Tribodet *et al*., 2005). The same three substitutions are present in isolates from the Chilean group, which also induce veinal necrosis in tobacco (Moury, 2010). As the Chilean group was the first to diverge in PVY history, this suggests that tobacco necrosis is an ancestral PVY trait. There is also genetic variation among potato and PVY genotypes for the induction of tuber necrosis, but the genetic bases of this phenotype have not been determined, in either PVY or the potato host.

In addition, major differences in host species adaptation are observed for potato and pepper, whereas few differences in infectivity are observed in tobacco and tomato (both susceptible to most PVY isolates) or *S. melongena* eggplant (resistant to most PVY isolates) (Fig. 3).

Remarkably, several pathogenicity traits are not independently distributed among PVY isolates, but are strongly associated, suggesting both common evolutionary history and genetic correlation (by linkage or pleiotropy). For example, among the five major PVY clades, a negative correlation exists between the capacities to infect pepper and potato plants. This suggests very limited numbers of host jumps during PVY evolution, followed by

secondary specialization onto the new host (Moury, 2010). Similarly, all PVY isolates able to induce potato tuber necrosis belong to group N or N × O recombinant groups that are also able to induce veinal necrosis in Xanthi tobacco. Because the genetic determinism of most of these traits in PVY is still unknown, we do not know whether these correlations are caused by linkage or by pleiotropic effects of mutations. By contrast, pleiotropic effects of PVY mutations involved in fitness and/or pathogenicity traits have already been evidenced. Mutations involved in tobacco necrosis at positions 400 and 419 of HC-Pro confer simultaneously a fitness cost in terms of competitiveness and accumulation in Xanthi (Rolland *et al*., 2009). Similarly, in the CP coding region of PVY N isolates, positive selection has been detected at amino acid position 25, indicating that variation at this position might have played an important role in PVY adaptation. Confirming this assumption, the mutation of this position has been shown to increase significantly PVY competitiveness in tobacco, but to decrease competitiveness in potato (Moury and Simon, 2011).

To conclude, the fact that distinct clades within PVY species show limited genetic exchanges and are correlated with adaptation to different host species and/or genotypes are indicative of a speciation in progress. The genetic differentiation into separate clades could be reinforced by host barriers, similar to those observed between pepper and potato, so that PVY could eventually evolve to form distinct viral species.

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