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1 Highlights

- 2
- Some PVY isolates are able to induce necrosis on potato tubers
- 3
- We research viral molecular determinants involved in PVY pathogenicity
- 4
- We apply a reverse genetic approach
- 5
- Residue E419 of HC-Pro protein is involved in tuber necrosis
- 6
- We improve our knowledge on potato/PVY interactions
- 7

7 The amino acid 419 in Hc-Pro is involved in the ability of PVY isolate N605 to
8 induce necrotic symptoms on potato tubers.

9

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30

30 **ABSTRACT**

31

32 The ability to induce the potato tuber necrosis ringspot disease (PTNRD) is a property shared
33 by PVY isolates belonging to different groups (e.g. PVY^N and PVY^O) and variants (e.g.
34 PVY^{NTN} and PVY^{N-W}). The identification of viral molecular determinant(s) involved in the
35 expression of PTNRD symptoms is essential for i) an easier detection of tuber necrosis
36 isolates and ii) an improvement of our knowledge on the epidemiology of this potato disease.
37 A reverse genetic approach associated with a biological typing of a collection of PVY
38 chimeras and mutants indicated that residue E₄₁₉ of the HC-Pro protein is linked to the ability
39 of PVY to induce tuber necrosis on four PTNRD-susceptible potato cultivars. Indeed, the
40 substitution of the N-type glutamic acid (E) in O-type aspartic acid (D) at position 419 in the
41 HC-Pro cistron prevents the expression of tuber necrosis on infected tubers without reducing
42 the virulence of the corresponding E/D₄₁₉ mutant. This result opens opportunities for the
43 future studies on potato/PVY interactions.

44

45 Keywords: PVY^{NTN}, potato, PTNRD, HC-Pro, infectious clone

46

46 1. Introduction

47
48 Potato tuber necrosis ringspot disease (PTNRD), one of the most damaging viral diseases for
49 potato industries, is characterized by the presence of purplish bows or rings on the skin of
50 potato tubers. These symptoms mainly located at the heel-end of tubers can i) be observed at
51 harvest or can appear during the first few months of post-harvest tuber storage and ii) reach
52 the total surface of the diseased tubers. While remaining superficial, this necrotic symptom
53 evolves under storage conditions to become protruding and dark brown. Tissues located under
54 the necrotic areas may collapse and lead to the formation of cracks which are possible entries
55 for other potato pathogens such as fungi and bacteria (Beczner et al., 1984; Le Romancer and
56 Kerlan, 1991). PTNRD induces decreases in the quantity and quality of tuber of 24% and 54-
57 56%, respectively (Schiessendoppler, 1992; Dedic and Ptacek, 1996). Moreover, the
58 unmarketability of necrotic tubers can lead to yield losses of up to 100% of the potato
59 production expected from healthy plants. Described for the first time in Hungary in 1982
60 (Beczner et al., 1984), the spread of this disease was favoured by international plant material
61 exchanges. Since its first description, the tuber necrotic symptoms has been reported in
62 numerous European and non European potato growing areas (Weidemann, 1985; Kus, 1990;
63 Le Romancer, 1993; McDonald and Kristjansson, 1993; Ohshima et al., 2000; Boukhris-
64 Bouhachem et al., 2007; Robles-Hernandez et al., 2010; Hutton et al., 2013; Damayanti et al.,
65 2014). The expression of necrotic symptoms on infected tubers depends on several abiotic and
66 biotic parameters. Indeed, it has been showed that a temperature in the range 20-24°C during
67 the potato growing period and/or during the storage of harvested tubers enhances the
68 expression of PTNRD (Weidemann, 1985; Le Romancer and Nedellec, 1997) while storage of
69 tubers at cold temperature limits the occurrence of necrotic symptoms on diseased tubers (Le
70 Romancer and Nedellec, 1997; Dolnicar et al., 2011). The intensity of tuber necrotic
71 symptoms depends also on the PTNRD-susceptibility of the potato genotype. During the last
72 three decades, efforts have been made to describe the ability of cultivars to express tuber
73 necrotic symptoms (Kus, 1992; Le Romancer and Nedellec, 1997; Draper et al., 2002). In
74 several countries including France, field trials are carried out to evaluate the susceptibility of
75 new genotypes before being placed on the market (Elliseche et al., 2004). Since 1996, this
76 procedure has led to rejection by the French potato breeders of numerous PTNRD-susceptible
77 cultivars (Charlet-Ramage and Kerlan, 2005). However, the commercial names of tested
78 genotypes are often omitted from international reports as such information may impact the
79 marketability of the most susceptible cultivars.

80
81 The viral pathogens responsible for PTNRD were initially described as members of the
82 PVY^{NTN} variant of the *Potato virus Y* species (PVY, genus *Potyvirus*, family *Potyviridae*).
83 The PVY genome is constituted by a single-stranded positive sense RNA molecule of
84 approximately 10kb with a VPg protein covalently attached at the 5'-end and a poly-A tail at
85 the 3'-end (Shukla et al., 1994). The viral RNA encodes a polyprotein that is cleaved by three
86 virus-encoded proteases into ten products corresponding from the amino-terminus to the
87 carboxy-terminus to P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP (Dougherty
88 and Carrington, 1988). A short overlapping gene (P3N-PIPO), embedded within the
89 previously described large ORF, was proposed for some potyviruses, including PVY (Chung
90 et al., 2008). PVY infects different solanaceous crops such as potato, tomato, tobacco and
91 pepper. The diversity of biological, serological and molecular characteristics of PVY isolates
92 has led to a complex classification for this viral species. The first level of classification groups
93 isolates into strains according to the host they were collected from (Kerlan and Moury, 2008).
94 In potato, PVY isolates are classified in the PVY^O, PVY^C, PVY^N, PVY^Z and PVY^E strain
95 groups on the basis of i) their capacity to induce necrotic symptoms on *Nicotiana tabacum*, ii)
96 their ability to overcome selected resistance genes introgressed in some *Solanum tuberosum*
97 cultivars and iii) their serological and molecular characteristics (De Bokx and Huttinga, 1981;
98 Singh et al., 2008). Moreover, several variants (e.g. PVY^{NTN} and PVY^{N-W}) that group
99 isolates with original characteristics have been proposed in the literature (Beczner et al., 1984;
100 Chrzanowska, 1991; Lorenzen et al., 2006; Chikh Ali et al., 2007; Karasev et al., 2010;
101 Galvino-Costa et al., 2012). PVY^{NTN} variant was initially described to group isolates able to
102 induce PTNRD symptoms under field conditions. Then, molecular characterization of
103 PVY^{NTN} members showed that this variant includes isolates with non recombinant PVY^N-type
104 (NA-PVY^{NTN}; Boonham et al., 2002; Nie and Singh, 2003) and recombinant PVY^N-
105 type/PVY^O-type (Eu-PVY^{NTN}; Boonham et al., 2002; Glais et al., 2002) genomic
106 organizations. In addition to members of the PVY^{NTN} variant, some PVY isolates assigned
107 into the PVY^{N-W} variant, i.e. described for their capacity to induce tobacco vein necrotic
108 symptoms and for their PVY^O-type serological properties, were reported to be able to induced
109 necrotic symptoms on tubers from Yukon Gold (Piche et al., 2004) and Nadine (Browning et
110 al., 2004) cultivars. However, some of these tuber necrotic symptoms were reported to be
111 atypical as they were roughly circular, sunken necrotic lesions on the surface of the potato
112 tuber instead of the typical external ring pattern of PTNRD symptoms (Piche et al., 2004).
113 The ability to induce tuber necrosis on Yukon Gold was also reported for NE-11, PVY-L26

114 and PVY-MON isolates, the only representatives for PVY-NE11, PVY^Z and PVY^E groups,
115 respectively (Piche et al., 2004; Lorenzen et al., 2008; Kerlan et al., 2011; Galvino-Costa et
116 al., 2012). Actually, it has been proposed that all PVY^N isolates are able, under appropriate
117 growing conditions, to induce necrosis on potato tubers (Kerlan and Tibodet, 1996). These
118 observations suggested that even if PVY^N, PVY^{NTN}, PVY^{N-W}, PVY-NE-11, PVY^Z and PVY^E
119 isolates displayed heterogeneous biological properties and/or molecular characteristics, they
120 all must have genomic determinant(s) required for the induction of necrosis on infected potato
121 tubers. Due to the huge economical impact of PTNRD, the identification of these
122 determinant(s) has become one of the most important challenges for the scientific community
123 working on PVY/potato pathosystem. Studies carried out on the pathogenicity of PVY on
124 different hosts, including potato, allowed to describe some of these determinants. Indeed,
125 using a reverse genetic approach (Tribodet et al., 2005; Faurez et al., 2012) or multiple
126 alignments of PVY genomes (Hu et al., 2009), the D₂₀₅, N₃₃₉, K₄₀₀, and E₄₁₉ amino acids of
127 HC-Pro protein were described to be involved in the expression of veinal necrotic symptom
128 on tobacco. Other parts of the potyvirus genome, including regions corresponding to P1, P3,
129 6K1, CI and CP (Atreya et al., 1992; Chu et al., 1997; Andrejeva et al., 1999; Saenz et al.,
130 2000; Ullah and Groumet, 2002; Jenner et al., 2003; Krause-Sakate et al., 2005; Bukovinszki
131 et al., 2007), have been reported for their involvement in the expression of symptoms on
132 potyvirus-infected hosts. Studies on PVY-potato interactions allowed the identification of
133 PVY avirulence factors. The NIa-Pro gene was identified as elicitor for the Extreme
134 Resistance (ER) conferred against members of the PVY species by the *Ry* gene (Mestre et al.,
135 2000). Regions of the HC-Pro protein corresponding to residues 227 to 327 (Tian and
136 Valkonen, 2013) and to residues 335 to 364 (Moury et al, 2011) were described as avirulence
137 factors for the Hypersensitive Reaction (HR) controlled by *N*-resistance genes (Jones, 1990)
138 toward isolates belonging to the PVY^O (*Ny_{ibr}* resistance gene; Cockerham, 1970) or PVY^C (*Nc*
139 resistance gene; Cockerham, 1970) groups, respectively. Based on data available in literature,
140 there is no evidence that necrosis observed on PVY-infected potato tubers is a HR to specific
141 interaction(s) between avirulence factor(s) encoded by necrotic PVY isolates and potato
142 resistance gene(s). To improve our knowledge on the pathogenicity of PVY species on potato
143 plants, a reverse genetic approach associated with a biological typing of a collection of PVY
144 chimeras and mutants was used. This procedure makes it possible to identify molecular
145 determinants involved in the capacity of PVY to induce necrosis on PTNRD-susceptible
146 potato cultivars.
147

147 2. Materials and methods

148

149 2.1 Plant materials

150

151 Tobacco and potato plants were used as PVY hosts in the experiments. *Nicotiana tabacum* cv
152 Xanthi and *Nicotiana clevelandii* were produced in a growth chamber at 20°C, relative
153 humidity of 70 % and 12h/12h of light/dark. Thirty days old tobacco plantlets were used as
154 healthy hosts in our inoculation procedures. The five potato cultivars used in the study were
155 selected for their different susceptibility to PTNRD. Cultivar Bintje does not express necrotic
156 symptom on PVY-infected tubers while cvs. Béa, Hermes, Nicola and Monalisa have been
157 described for their ability to produce necrotic tubers (Le Romancer, 1993; Kerlan and
158 Tribodet, 1996) in response to PVY^{NTN} infection. Potato seeds used as initial material in this
159 study correspond to 'super elite' tubers, i.e. seeds sampled from lots with less than 1% of
160 PVY-infected tubers, and have been supplied by the French seed potato growers (FN3PT).
161 After a storage period at 4°C, potato seeds were placed at 20°C in the dark, during 10-15 days
162 allowing the raise of tuber dormancy. When germs reached 0.5 cm in length, tubers were
163 individually planted and grown in an insect-proof greenhouse. Sanitary status of the young
164 potato plants was achieved 10 days after planting using DAS-ELISA assay performed as
165 described below. The few (expected less than 1%) PVY-infected potato plants were removed
166 from the experiments. In order to use potato plants at a homogeneous development stage, all
167 except one emerging stems from each tuber were pulled out at twelve days after planting.
168 Tobacco and potato plants were transferred from a standard insect-proof greenhouse to a
169 regulated insect-proof greenhouse (agreement N°699 for type 2 containment) where PVY-
170 inoculation experiments took place at 20°C ± 2°C with natural light.

171

172 2.2 Virus isolates, PVY^{N/O} chimeras and mutants

173

174 PVY^N605 (GenBank accession number X977895; Jakab et al., 1997) and PVY^O139
175 (GenBank accession number U09509; Singh and Singh, 1996) isolates were used as
176 references for PVY^N and PVY^O groups, respectively. The genome of PVY^N605 isolate shares
177 99.3% nucleotide identity with the non recombinant PVY^{NTN}NZ isolate (GenBank accession
178 number AM268435; Glais et al., 2004) and has been described for its ability to induce tuber
179 necrotic symptoms on Nadine, Nicola and Hermes cultivars under artificial (Browning et al.,
180 2004) and natural (Gugerli, personal communication) conditions. Thus, PVY^N605 was

181 considered in this study as a non recombinant PVY^{NTN} isolate. To our knowledge and
182 according to the literature, PVY^O139 (Singh and Singh, 1996) is not able to induce tuber
183 necrotic symptoms on infected potato cultivars. The biological properties (i.e. systemic
184 infection and symptoms expression) of these reference isolates were compared to those
185 associated with a set of 16 PVY^{N/O} chimeras resulting from genomic exchanges between
186 PVY^N605 and PVY^O139 sequences. Moreover, three point-mutated versions of the infectious
187 PVY^N605 clone were used to test the involvement of the mutated residues on PVY biological
188 properties. A schematic representation of genomic organization of these viral constructs is
189 presented in Figure 1. The different cloning steps used to obtain these chimeras and mutants
190 have been previously described in Tribodet et al. (2005) and in Faurez et al. (2012).

191

192 2.3 PVY^{NTN} isolates sampled from potato fields

193

194 Thirteen necrotic potato tubers, collected from different cultivars produced by the French
195 ware potato industry, were stored in an insect-proof chamber at 20°C ± 2°C in the dark until
196 the first germs emerged from the tuber surface. Then, tubers were individually planted in pots
197 and placed in an insect-proof greenhouse (20°C ± 2°C). One month later, the PVY-infected
198 status of the 13 plants was confirmed using DAS-ELISA procedure as described below. An
199 immunocapture (IC)-RT-PCR procedure (Glais et al., 1998) was applied to PVY-infected
200 leaves from each potato plants in order to make it possible to sequence part of the PVY
201 genomes. The RT-PCR experimental conditions and the sequence of primers used in this
202 procedure are presented in Supplementary Table S1. The sequence data produced by
203 Genoscreen (France) were compared with the PVY^N605 and PVY^O139 sequences retrieved
204 from GenBank. Sequences were analyzed with Geneious Pro 4.7.6 software (Biomatters Ltd.,
205 Auckland, New Zealand) (Drummond et al., 2009). Accession numbers of partial sequence of
206 genome from the 13 PVY^{NTN} field isolates are KP885691, KP885692, KP885693, KP885694,
207 KP885695, KP885696, KP885697, KP885698, KP885699, KP885700, KP885701, KP885702
208 and KP885703.

209

210 2.4 Inoculations of tobacco and potato plants

211

212 Each viral construct (chimeras and mutants) was inoculated to a set of 15 *N. clevelandii* using
213 a particle bombardment procedure and the helios gene gun system [Bio-Rad] as described by
214 Tribodet et al. (2005). Twenty days after the bombardment, *N. Clevelandii* plants were

215 sampled (1-2 non-inoculated leaves/plant) and tested for the presence of virus by a DAS-
216 ELISA assay performed as described below. Then, for each PVY construct a leaf from one
217 PVY-infected *N. clevelandii* was used as viral source to transfer the corresponding viral
218 progeny to *N. tabacum* cv. Xanthi. This inoculation step was carried out by a standard
219 mechanical inoculation procedure as described by Lacroix et al. (2010). Briefly, leaf tissue
220 (+/- 1g) was ground in the presence of 4ml of inoculation buffer (50mM Na₂HPO₄. 12H₂O;
221 50mM KH₃PO₄; 40mM sodium diethyldithiocarbamate, pH 7.2) supplemented with 1.5g of
222 activated charcoal and 2.5g of carborundum. This mixture was rubbed with fingers on two
223 leaves of a young healthy *N. tabacum* (at 4-5 leaves stage). Fifteen days after mechanical
224 inoculation, non inoculated leaves of *N. tabacum* were tested for the presence of PVY using
225 ELISA procedure. Then, non-inoculated leaves (+/- 1g) from infected *N. tabacum* plants were
226 ground in mortar in the presence of 2.5g of carborundum and used as crude inoculum to
227 transfer the virus from tobacco to potato. In this inoculation procedure, both the inoculation
228 buffer and activated charcoal were omitted from the crude sap mixture in order to improve,
229 under our experimental conditions, the efficiency of the mechanical inoculation of PVY on
230 potato. Thus, the crude sap mixture was rubbed on 2-3 true leaves of young potato plants with
231 a stem of 10-20cm. Reference PVY isolates (PVY^N605 and PVY^O139), chimeras and mutants
232 were individually inoculated to 12-18 healthy potato plants of Béa, Hermes, Nicola, Monalisa
233 and Bintje cultivars. Moreover, the used inoculums were also individually inoculated to *N.*
234 *tabacum* cv. Xanthi as positive control for the infectivity of each viral source. The symptoms
235 induced by the PVY constructs on tobacco and potato plants were monitored on both
236 inoculated and non-inoculated leaves at 20 days post inoculation.

237

238 2.5 Detection of PVY in plants

239

240 To test the presence of PVY in inoculated plants, DAS-ELISA assays (Clark and Adams,
241 1977) were performed at 15 days post inoculation (dpi) for tobacco plants and at both 30 dpi
242 and 70 dpi for potato plants. For each plant to be tested, non inoculated leaves (approximately
243 0.5g) were sampled and ground in a plastic bag in the presence of 1ml grinding buffer (PBS
244 (137mM NaCl, 8mM Na₂HPO₄.12H₂O, 2.7mM KCl, 1.5mM KH₂PO₄, pH 7.4), 0.05% Tween
245 20, 2% Polyvinylpyrrolidone 40T). One hundred microlitres of the plant sap extracts were
246 placed into wells of microtiter plates (Nunc Maxisorp) previously coated with 1µg/ml of PVY
247 polyclonal antibody (kindly provided by M. Guillet, FN3PT/INRA, France). The filled plate
248 was maintained at 4°C overnight prior being washed three times with PBS-T buffer (PBS,

249 0.05% Tween 20) and once with deionized water. Ninety μ l of PVY polyclonal antibody
250 conjugated to alkaline phosphatase (M. Guillet, FN3PT /INRA, France) were added at a
251 concentration of 1 μ g/ml in each well. At the end of a 2h-incubation period at 37°C, the plate
252 was washed as described above. Then, 80 μ l of *p*-nitrophenylphosphate (1mg/ml) diluted in
253 substrate buffer (Diethanolamine 1M, pH 9.8) were added in plate wells. The plate was
254 maintained in the dark at room temperature. The colorimetric reaction was scored at 120
255 minutes at 405nm using a microplate reader (Multiskan EX, Thermo). A sample was
256 considered infected when optical density value at 405nm was at least twice the mean value of
257 the negative controls (healthy plants), otherwise plants were considered non-infected. These
258 OD₄₀₅ values were statistically analyzed with XLSTAT® software (Addinsoft, Paris, France)
259 for MS Excel.

260

261 *2.6 Post-harvest procedures*

262

263 Four months after planting, daughter tubers from inoculated potato plants were harvested
264 plant by plant and stored in the dark at 20°C \pm 2°C. The presence of tuber necrotic symptom
265 was checked on washed tubers at harvest and after a two-months period of storage. At two
266 months post harvest, one eye-plug per tuber was sampled, mainly on the sprout rose-end. The
267 remaining part of the tuber was stored at 4°C until used. The eye-plugs were placed into a
268 gibberellic acid solution (4mg/L) for 1h and dried overnight at room temperature for healing
269 the tubers. Then, eye-plugs were planted and grown in greenhouse at 20°C \pm 2°C with natural
270 light. Two months later, potato plantlets were tested for the presence of PVY by DAS-ELISA
271 using the procedure described above. In case of the absence of viral detection in a plantlet
272 produced from a necrotic tuber and/or from a PVY-infected mother plant, a second post-
273 harvest screening procedure was applied to the remaining part of the tuber kept at 4°C since
274 eye-plugging.

275

276 *2.7 Analysis of the viral progenies*

277

278 Sequences of viral progenies present in the infected potato plants were determined and
279 compared to the corresponding initial inoculums. An immunocapture (IC)-RT-PCR procedure
280 (Glais et al., 1998) was applied to PVY-infected potato leaves. The RT-PCR experimental
281 conditions and the sequence of the used primers are described in Supplementary Table S1.
282 PCR fragments were directly sequenced in forward and reverse directions with appropriate

283 primers (Table S1). Sequence data were produced by Genoscreen (France) and analyzed with
284 Geneious Pro 4.7.6 software (Biomatters Ltd., Auckland, New Zealand) (Drummond et al.,
285 2009). This sequencing procedure was applied to viral populations present i) in infected
286 potato plants (two plants/cultivar/isolate) obtained after mechanical inoculations and ii) in
287 infected potato plantlets (two plants/cultivar/isolate) from the post-harvest procedure.

288

289 3. Results

290

291 3.1 Biological properties of PVY chimeras and mutants on Bintje and Béa cultivars

292

293 All the PVY constructs used in the experiment were infectious on *N. tabacum* cv. Xanthi as
294 denoted by the presence of expected symptoms (i.e. mosaic or vein necrosis) and confirmed
295 by PVY detection in systemic leaves by DAS-ELISA procedure (Table 1). The symptoms
296 induced by the different tested constructs on inoculated and systemic leaves of cvs. Bintje and
297 Béa are listed in Table 1. On infected Bintje plants, the reference PVY^N605 and PVY^O139
298 isolates induced no symptoms on inoculated leaves and systemic mottle. However, their
299 biological properties were not similar on cv. Béa as shown by the presence of mild systemic
300 vein necrosis associated to PVY^N605 infection, and the green rings (i.e. maritta symptoms;
301 Kerlan, 2006) on inoculated leaves and systemic vein necrosis induced by PVY^O139
302 infection. The viral constructs tested in the study correspond to genomic associations between
303 N-type and O-type sequences. Their biological properties were expected to be similar to one
304 of the parental genomes. However, data associated to the PVY^{N/O} chimeras and mutants allow
305 the description of different symptoms. In order to simplify the description of the range of
306 symptoms observed in the data set, we decided to assign isolates with similar behavior into
307 groups I to IV. Group I includes PVY constructs (SwNc, SwNc_R2/R3, NrBg and Fusion)
308 associated with asymptomatic systemic leaves. The HC-Pro'O', HC-Pro'O'/aa'N' and AgNr
309 constructs were able to induce local necrotic lesions (NLL) on inoculated leaves of cv. Bintje.
310 Moreover, they were unable, except for inoculation of AgNr on cv. Bintje, to establish
311 systemic symptoms on the tested potato cultivars. These isolates were assigned to group II.
312 The constructs SwNc_R1, SwNc_R1/R3, BgTt, K/R₄₀₀ and E/D₄₁₉ were assigned to group III.
313 Even if these isolates are associated to different types of systemic symptoms (i.e. vein
314 necrosis, mottle and leaf drop), they were able to induce, as does PVY^O139, the maritta
315 symptom on inoculated leaves of cv. Béa. Finally, the SwNc_R3, BsAg, NcBg, N/D₃₃₉, DrBs,
316 BsSw and SwNc_R2 constructs were not associated to symptoms on inoculated leaves but

317 were able to induce systemic vein necrosis on either cv. Bintje or cv. Béa. Such biological
318 property has been considered close to data described for PVY^N605. Consequently, these
319 constructs and PVY^N605 were assigned in group IV.

320

321 *3.2 Infectivity of PVY chimeras on potato plants*

322

323 Serological detections of PVY in non-inoculated potato leaves were performed at 30 and 70
324 days post inoculation. However, results obtained at 70 dpi (Fig. 2) were considered for the
325 following analyses because more plants were described to be infected at this date. The
326 percentages of infected plants on the two potato cultivars were used to perform a hierarchical
327 ascendant classification (HAC) analysis with DARwin software version 6
328 (<http://darwin.cirad.fr/>) by using an Euclidian distance as a measure of dissimilarity. The
329 resulting dendrogram was obtained with UPGMA method. Four groups are identified
330 according to the PVY virulence. The three first groups (A, B and C, Fig. 2) were mainly
331 supported by differences in the percentage of infection observed on cv. Béa as all these PVY
332 isolates were able to infect 90-100% of inoculated Bintje plants. The members of group A
333 (PVY^N605, DrBs, BsSw, BgTt, SwNc_R1, SwNc_R2, SwNc_R3, N/D₃₃₉ and E/D₄₁₉) were
334 associated to a 100% infection of Béa plants. Members of group B, i.e. the PVY^O139 isolate
335 and the constructs K/R₄₀₀, SwNc and SwNc_R2/R3, were detected in 50% to 77% of
336 inoculated Béa plants. Finally, constructs NcBg, BsAg and SwNc_R1/R3, able to infect low
337 proportions of inoculated Béa plants (from 0% to 28%), were assigned to group C. The fourth
338 group (group D, Fig. 2), constituted by AgNr, HC-Pro'O', HC-Pro'O'/aa'N', NrBg and
339 Fusion constructs, displays infection efficiencies in the ranges from 0% to 42% and from 0%
340 to 6% on cv. Bintje and cv. Béa, respectively. To complete the analysis on the ability of the
341 different PVY constructs to systemically infect cvs. Bintje and Béa, a post-harvest control was
342 carried out on plants obtained from the daughter tubers produced by healthy or by PVY-
343 infected potatoes. The results, presented in Supplementary Table S2, showed that PVY^{N/O}
344 constructs able to systemically infect potato plants efficiently spread from inoculated leaves to
345 tubers. However, although AgNr chimera was able to infect 42% of mechanically inoculated
346 potato cv. Bintje plants, none of the post-harvest test was associated with PVY infection.

347

348 *3.3 Symptoms on potato tubers*

349

350 Under our experimental conditions, potato plants produced up to three tubers per plant.
351 Tubers produced by PVY inoculated plants were individually checked for the presence of
352 necrosis at both the harvest and after a storage period of 2 months under optimal conditions
353 for the expression of tuber necrotic symptoms, i.e. at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The proportions of necrotic
354 tubers increased between the two visual inspections. Thus, the data obtained at two months
355 post-harvest was used in the analyses (Fig. 2 and Table S2). As expected, neither the PVY
356 reference isolates nor the different PVY constructs induced necrosis on cv. Bintje tubers. The
357 PTNRD-susceptible Béa cultivar, infected with PVY^N605 isolate or with one of the BsAg,
358 DrBs, BsSw, SwNc, SwNc_R1, SwNc_R2, SwNc_R3, SwNc_R1/R3, SwNc_R2/R3, BgTt,
359 K/R₄₀₀, N/D₃₃₉ PVY constructs, produced from 27% to 100% necrotic tubers whereas no
360 necrosis symptom was observed on tubers harvested from cv. Béa plants infected by
361 PVY^O139 isolate (22 tubers) or by the E/D₄₁₉ (36 tubers) and Fusion (2 tubers) PVY
362 constructs (Fig. 2). For the HC-Pro'O', AgNr, NrBg, HC-Pro'O'/aa'N' and NcBg constructs,
363 the absence of PVY symptoms on tubers can be considered to be a direct consequence of the
364 lack of systemic infection of mechanically-inoculated potatoes. For E/D₄₁₉ and Fusion
365 constructs, systemic PVY infection was detected by DAS-ELISA in inoculated plants (18/18
366 and 1/18 for E/D₄₁₉ and Fusion, respectively; see Table 1). Absorbance values obtained for
367 E/D₄₁₉-, Fusion-, N605- and O139-infected plants were compared. No significant differences
368 were observed between data associated to N605- and E/D₄₁₉-infected plants ($p=0.776$) while
369 O139 infected plants were associated to lower OD₄₀₅ values than N605- ($p=0.014$) and
370 E/D₄₁₉- ($p=0.01$) infected plants. The single plant systemically infected by the Fusion mutant
371 was associated to higher OD value ($\text{OD}_{405} = 2.23$) than the E/D₄₁₉ mutant (mean $\text{OD}_{405} = 1.7$
372 ± 0.5 (N=18)) and the references isolates (mean $\text{OD}_{419} = 1.8 \pm 0.6$ (N=18) and mean OD_{419}
373 $= 0.9 \pm 0.9$ (N=9) for N605 and O139, respectively). Moreover, asymptomatic tubers
374 produced by E/D₄₁₉- and Fusion-infected potato plants lead to the production of infected
375 daughter plants. These results showed that the single mutation of HC-Pro residue 419 in the
376 PVY^N605 genetic background leads to the production of an isolate unable to induce necrosis
377 on infected potato tubers from the PTNRD-susceptible cultivar Béa. The limited number of
378 potato tubers infected by the Fusion construct does not allow to conclude on the role of the
379 P3-6K1 region in the induction of PTNRD symptoms but the weak infectiousness of Fusion
380 (2/12 and 1/18 for cv. Bintje and cv. Béa, respectively; Table 1) is in favor to a role of this
381 PVY genomic region in PVY-potato interactions.

382

383 *3.4 Inoculation of E/D₄₁₉ mutant on PTNRD-susceptible potato cultivars*

384

385 To know whether the HC-Pro residue 419 is involved in the expression of tuber necrosis
386 symptoms for different PTNRD-susceptible potato cultivars, the E/D₄₁₉ construct was
387 inoculated to sets of 12-18 potato plants of cvs. Hermes, Monalisa and Nicola. These three
388 potato cultivars have been described i) to be susceptible to PVY and ii) to express tuber
389 necrosis symptoms in response to PVY infection (Le Romancer, 1993). The virulence of
390 PVY^N605, PVY^O139 and E/D₄₁₉ was monitored by DAS-ELISA performed on leaves
391 sampled from the inoculated plants (at 70 dpi) and from the post-harvest potato plants (Fig.
392 3), and by the observation of necrotic symptoms on tubers. The data associated to PVY^N605
393 isolate highlighted a difference of PVY-susceptibility between cultivars with 95%, 55% and
394 28% systemic infections of Hermes, Monalisa and Nicola plants, respectively. The percentage
395 of necrotic tubers harvested from Hermes, Monalisa and Nicola plants were 37%, 10% and
396 7.7%, respectively. Surprisingly, very low percentage of daughter tubers of cvs. Hermes
397 (3.6%), Monalisa (0%) and Nicola (0%) produced infected potato plantlets. The percentage of
398 systemic infections of Hermes, Monalisa and Nicola obtained for the reference PVY^O139
399 isolate were 5.5%, 5.5% and 16.5%, respectively, and the daughter tubers from PVY^O139-
400 inoculated plants were all asymptomatic and produced exclusively healthy plants. The
401 experiment carried out with E/D₄₁₉ mutant displayed an infection pattern close to the one
402 obtained for PVY^N605 with i) a higher percentage of infection for cv. Hermes (89%) than for
403 cvs. Monalisa (11%) and Nicola (55%) and ii) 4%, 0% and 2% of infections for plants
404 produced from Hermes, Monalisa and Nicola daughter tubers, respectively. However, necrotic
405 symptoms were not observed on potato tubers harvested from PVY-E/D₄₁₉-infected Hermes,
406 Monalisa and Nicola plants.

407

408 *3.5 Molecular analysis of viral progenies*

409

410 Nucleotide sequences of viral progenies were acquired for the different constructs by direct
411 sequencing of IC-RT-PCR products. PVY sequences were obtained from apical infected
412 leaves of post-harvest potato plants. This procedure was applied, for each construct, on two
413 infected Bintje and two infected Béa daughter plants. The comparison of sequences of viral
414 progenies present in post harvested Bintje plants with sequence of corresponding inoculums
415 did not highlight differences. Similar procedure carried out with molecular data produced
416 from infected Béa plants showed only one difference between the viral progenies and the
417 corresponding inoculum. This difference was associated with the codon 419 of the HC-Pro

418 cistron. Indeed, the two E/D₄₁₉-derived viral progenies sequenced from two independent
419 infected Béa plants contained the D419N substitution in the HC-Pro sequence. To compare
420 the biological properties of the D/N₄₁₉ mutant produced in Béa to those of the original E/D₄₁₉
421 construct, the former was inoculated on Bintje, Béa, Hermes, Monalisa and Nicola plants. As
422 previously denoted for E/D₄₁₉, D/N₄₁₉ isolate i) was able to infect these cultivars, ii) was
423 detected in plantlets produced from harvested tubers and iii) did not induce necrotic
424 symptoms on infected potato tubers (data not shown). The identity of the HC-Pro residue at
425 position 419 in genomes of viral progenies present in infected potato plants was determined
426 for each PVY virus/potato cultivar combination and compared to the initial inoculum.
427 Sequence alignments showed that viral progenies of PVY^{N605} and D/N₄₁₉ isolates contained
428 a glutamic acid (E₄₁₉) and an asparagine residue (N₄₁₉), respectively (Table 2). The sequence
429 of E/D₄₁₉-derived progenies present in infected Bintje plants contained the D₄₁₉ residue in the
430 HC-Pro sequence while E/D₄₁₉-derived progenies present in the infected PTNRD-susceptible
431 potato plants, i.e. Béa, Hermes, Monalisa and Nicola, contained the D419N modification
432 (Table 2) suggesting the importance of the HC-Pro residue 419 in virus/host interactions,
433 especially in potato cultivars susceptible to PTNRD.

434 To acquire information about the diversity of HC-Pro AA₄₁₉ in PVY isolates present in
435 necrotic tubers sampled from potato fields, the PVY HC-Pro region of 13 PVY isolates
436 randomly selected from potato tubers showing typical PTNRD symptoms was sequenced. The
437 multiple peptide sequence alignment revealed that these 13 isolates encode a HC-Pro with an
438 E₄₁₉ residue (data not shown).

439

440 4. Discussion

441

442 PVY isolates assigned to the tuber necrotic variant PVY^{NTN} were i) originally described as
443 members of the N-serogroup, ii) considered to be able to induce tobacco vein necrosis
444 symptom on *N. tabacum* and iii) constituted by recombinant genomic organizations.
445 However, some isolates described for their type-O serological properties, their ability to
446 induce mosaic and vein clearing symptoms on tobacco, and/or their non-recombinant genomic
447 organization have been reported for their ability to induce PTNRD. The assignment of isolates
448 in PVY^{NTN} variant is currently based on a multiple-criteria analysis of isolate's characteristics
449 while the accurate identification of PVY isolates able to induce tuber necrotic symptoms still
450 requires i) the inoculation under controlled conditions of a PTNRD-susceptible potato
451 genotype and ii) the monitoring of symptoms on infected daughter tubers (Karasev and Gray,

452 2013). The identification of viral molecular determinant(s) involved in the expression of this
453 biological property constitutes one of the possible ways to simplify detection/characterization
454 of tuber necrosis isolates. Surprisingly, no data have been already published about molecular
455 determinant(s) involved in the induction of tuber necrosis so far. In this paper, PVY chimeras
456 and mutants were biologically characterized in different potato cultivars in order to identify
457 residues of PVY genome responsible of the expression of PTNRD symptoms.

458
459 The pathotyping procedure carried out using a collection of artificial PVY^N-605/PVY^O-139
460 recombinant constructs was expected to show quantitative (percentage of tubers showing
461 necrotic symptoms) and/or qualitative (presence vs. absence of the necrotic symptoms)
462 variations of PTNRD symptoms on infected potato plants. The comparative analysis of data
463 associated to necrotic symptoms and to genomic organization of the different PVY constructs
464 made it possible the identification of viral sequence(s) important for the induction of PTNRD.
465 Actually, only few PVY^N/PVY^O nucleotide exchanges altered the necrotic ability of the
466 PVY^N-605 genetic background. The modifications of N-type sequence in C-ter P1/HC-Pro
467 and in C-ter CI/NiB regions lead to the decrease of the proportion of necrotic tubers produced
468 by PVY-infected plants. However, it is important to note that some of these constructs were
469 also associated to a decrease of the proportion of systemic infection of plants (e.g. Fusion was
470 associated with low rate of systemic infection (1/18)) which suggests a link between virulence
471 and aggressiveness of PVY isolates on potato. Systemic infection is a prerequisite for the
472 induction of tuber necrosis. Indeed, the tuber necrosis can be considered like the physiological
473 expression of the presence of PVY in this potato organ. This assumption is strengthened by
474 the fact that i) none of the virus-free tuber (absence of viral detection in post harvest testing
475 procedure) expressed necrotic symptom and ii) all necrotic tubers were associated to PVY
476 detection in tuber and/or in post-harvest testing procedures. Among the constructs able to
477 systemically infect potato plants, Fusion and E/D₄₁₉ were not able to induce necrosis on
478 infected tubers. However, the virus present in these infected plants seemed, according to the
479 ELISA procedure applied in this work, to accumulate at a level at least similar to the one
480 observed for the necrotic PVY^N605 isolate. Moreover, post-harvested assays performed on
481 daughter plants produced using tubers from Fusion- and E/D₄₁₉-infected potato revealed the
482 infectious status of the used tubers. The systemic infection of potato by PVY-Fusion was
483 limited to a single plant which strongly limits the possible analysis of the data associated to
484 this chimera. Nevertheless, taken into account the fact that i) necrotic symptom was not
485 observed in the two infected daughter tubers produced by the Fusion-infected potato plant, ii)

486 ELISA positive results were obtained in post harvest assay carried out on tubers from this
487 potato plant and iii) the sequence of viral progenies present in the two Bea daughter plants
488 corresponds to the sequence of the Fusion construct, the region corresponding to codons 740
489 to 1157 (P3-6K1 region) of the PVY polyprotein can be considered as a candidate for further
490 investigations on PVY molecular determinant(s) linked to the infectious process that leads to
491 systemic infection and/or the expression of tuber necrotic symptoms.

492
493 Three point-mutated versions of the PVY^N-605 were included in this study. The K/R₄₀₀ and
494 the E/D₄₁₉ mutants are altered in their ability to systemically infect Béa cultivar and to induce
495 tuber necrosis symptom, respectively. The E419D mutation, associated with a light
496 modification in the lateral arm of amino acid, was previously reported for its impact on the
497 expression of tobacco vein necrosis (Tribodet et al., 2005). Sequence comparisons of HC-Pro
498 region from 13 field PVY isolates sampled from necrotic potato tubers shown that residue
499 E₄₁₉ is conserved in the sequence of these isolates. However, it has been shown that E₄₁₉ is
500 also present in HC-Pro sequence of PVY^N and PVY^N-W isolates known to not induce PTNRD
501 (Tribodet et al., 2005). These data are in favor of a crucial, but not sufficient, role of HC-Pro
502 E₄₁₉ in the expression of PTNRD. Thus, the presence of other PTNRD molecular
503 determinant(s) in the PVY genome cannot be ruled out by our results.

504
505 Our results demonstrated that the involvement of the HC-Pro E₄₁₉ in the expression of tuber
506 necrosis is not specific to Béa cultivar. Indeed, the E/D₄₁₉ mutant was shown to be unable to
507 induce tuber necrosis in the PTNRD-susceptible Hermes, Monalisa and Nicola cultivars. This
508 suggests that the process leading to tuber necrosis in these different potato genotypes is at
509 least partly similar. The HC-Pro residue 419, in addition to be crucial for PVY aggressiveness
510 in tubers, seems to be also involved in other virus/host interaction(s). Indeed, in infected
511 PTNRD-susceptible cultivar the D₄₁₉ residue is systematically mutated to asparagine (i.e.
512 D419N which corresponds to the G₂₂₆₉ to A₂₂₆₉ transition in the PVY sequence). Surprisingly,
513 this sequence modification is not observed in PVY E/D₄₁₉ populations sampled from infected
514 potato cultivar non-susceptible to PTNRD (i.e. Bintje) or from infected *N. tabacum* plants.
515 Infection of PTNRD-susceptible potato cultivars with the D/N₄₁₉ mutant did not lead to the
516 expression of necrotic symptom suggesting that the change of residue 419 from D to N has no
517 obvious impact on monitored PVY biological properties. Amino acid comparisons of 85 PVY
518 HC-Pro sequences retrieved from public databases did not show an asparagine (N) at position
519 419 (Faurez et al., 2012). Altogether, these results suggest that i) an O-type D₄₁₉ amino acid in

520 a N-type PVY genetic background is associated to a low fitness in PTNRD-susceptible potato
521 cultivars which leads to the selection of the D419N mutant and ii) that amino acid at position
522 419 in HC-Pro coding region is important, in complement to its role in the induction of
523 PTNRD symptoms, for interaction between the virus and an unknown host factor(s). In
524 tobacco plant, it has been reported that PVY isolate with necrotic genetic background
525 including the HC-Pro E₄₁₉ has a lower fitness (reduced size of the viral progeny) in *Nicotiana*
526 *tabacum* cv. Xanthi and *N. clevelandii* than the non necrotic E/D419 mutant (Rolland et al.,
527 2009). However, these data, collected from experiments carried out on tobacco plants, do not
528 allow to predict the effect(s) of this determinant on the fitness of PVY in another host (e.g. in
529 potato). A recent study has shown that a mutation introduced in PVY CP protein has different
530 effects on PVY fitness according to the host plant. Indeed, in tobacco (*N. tabacum* cv. Xanthi)
531 the accumulation of the CP-mutant was higher than accumulation of the reference wild-type
532 PVY, whereas in potato (cv. Bintje) no difference in PVY accumulation was denoted (Moury
533 and Simon, 2011). Therefore, it would be interesting to define the impact of the modification
534 of the HC-Pro AA₄₁₉ on the PVY fitness in potato cultivars susceptible and non-susceptible to
535 PTNRD, and to determine whether the ability to induce tuber necrosis is correlated with the
536 size of viral progeny produced in infected hosts and/or the characteristics (e.g. dynamics) of
537 PVY systemic infection of potato plant. Such type of information would help to improve our
538 understanding of the increase of the prevalence of tuber necrotic isolates observed during the
539 last two decades in several potato growing areas.

540
541 To our knowledge, the link between potato tuber necrotic symptoms and a hypersensitive
542 response to PVY infection has not been demonstrated yet. However, because i) tuber necrotic
543 symptom is not reported from all PVY-susceptible potato cultivars, ii) a viral genotype could
544 fail in the induction of necrotic symptom on a PTNRD-susceptible potato cultivar and iii)
545 necrotic symptoms observed on tubers and on leaves of PVY-infected potato are
546 phenotypically similar to each other, the expression of PTNRD symptoms depends on specific
547 virus-potato interactions. The data produced in the presented study strongly suggest that the
548 PVY HC-Pro E₄₁₉ residue is involved in a PVY/host interaction that lead in PTNRD-
549 susceptible potato genotypes to the expression of tuber necrotic symptoms. This HC-Pro
550 residue is, then, the first molecular determinant of PTNRD described so far. Further study on
551 PVY/potato interactions should now be carried out to identify the plant product(s) interacting
552 with the E419 of the HC-Pro protein.

553

554

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556

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561

561 **References**

- 562
- 563 Andrejeva, J., Puurand, U., Merits, A., Rabenstein, F., Jarvekulg, L., Valkonen, J.P.T., 1999.
- 564 Potyvirus helper component-proteinase and coat protein (CP) have coordinated
- 565 functions in virus-host interactions and the same CP motif affects virus transmission
- 566 and accumulation. *J. Gen. Virol.* 80, 1133-1139.
- 567 Atreya, C.D., Atreya, P.L., Thornbury, D.W., Pirone, T.P., 1992. Site-directed mutations in
- 568 the Potyvirus HC-Pro gene affect helper component activity, virus accumulation, and
- 569 symptom expression in infected tobacco plants. *Virology* 191, 106-111.
- 570 Balme-Sinibaldi, V., Tribodet, M., Croizat, F., Lefeuvre, P., Kerlan, C., Jacquot, E., 2006.
- 571 Improvement of *Potato virus Y* (PVY) detection and quantitation using PVY^N- and
- 572 PVY^O-specific real-time RT-PCR assays. *J. Virol. Methods* 134, 261-266.
- 573 Beczner, L., Horvath, J., Romhanyi, I., Forster, H., 1984. Studies on the etiology of tuber
- 574 necrotic ringspot disease in potato. *Potato Res.* 27, 339-352.
- 575 Boonham, N., Walsh, K., Hims, M., Preston, S., North, J., Barker, I., 2002. Biological and
- 576 sequence comparisons of *Potato virus Y* isolates associated with potato tuber necrotic
- 577 ringspot disease. *Plant Pathol.* 51, 117-126.
- 578 Boukhris-Bouhachem, S., Hullé, M., Rouze-Jouan, J., Glais, L., Kerlan, C., 2007. *Solanum*
- 579 *elaeagnifolium*, a potential source of *Potato virus Y* (PVY) propagation. *OEPP*, 37, 125-
- 580 8.
- 581 Browning, I., Charlet, K., Chrzanowska, M., Dedic, P., Kerlan, C., Kryszczuk, A., Schubert,
- 582 J., Varveri, C., Werkman, A., Wolf, I., 2004. What is PVY^{NTN}? The reaction of potato
- 583 cultivars to inoculation with a range of PVY isolates. In: Proceedings of the virology
- 584 section meeting of the European Association for Potato Research. Rennes, France, pp.
- 585 51-53.
- 586 Bukovinszki, A., Gotz, R., Johansen, E., Maiss, E., Balazs, E., 2007. The role of the coat
- 587 protein region in symptom formation on *Physalis floridana* varies between PVY strains.
- 588 *Virus Res.* 127, 122-125.
- 589 Charlet-Ramage, K., Kerlan, C., 2005. Outil de sélection évaluant la sensibilité variétale à la
- 590 maladie des nécroses annulaires des tubercules causée par le virus Y de la pomme de
- 591 terre. *Le Cahier des Techniques de l'INRA* 203-208.
- 592 Chikh Ali, M., Maoka, T., Natsuaki, K.T., 2007. The occurrence and characterization of new
- 593 recombinant isolates of PVY displaying shared properties of PVY^{NW} and PVY^{NTN}. *J.*
- 594 *Phytopathol.* 155, 409-415.

- 595 Chrzanowska, M., 1991. New isolates of the necrotic strain of *Potato virus Y* (PVY^N) found
596 recently in Poland. *Potato Res.* 34, 179-182.
- 597 Chu, M.H., Lopez-Moya, J.J., Llave-Correas, C., Pirone, T.P., 1997. Two separate regions in
598 the genome of the *Tobacco etch virus* contain determinants of the wilting response of
599 Tabasco pepper. *Mol. Plant-Microbe Interact.* 10, 472-480.
- 600 Chung, B.Y.W., Miller, W.A., Atkins, J.F., Firth, A.E., 2008. An overlapping essential gene
601 in the Potyviridae. *PNAS* 105, 5897-902.
- 602 Clark, M.F., Adams, A.N., 1977. Characteristics of the microplate method of enzyme-linked
603 immunosorbent assay. *J. Gen. Virol.* 34, 475-483.
- 604 Cockerham, G., 1970. Genetical studies on resistance to *Potato viruses X* and *Y*. *Heredity* 25,
605 309-348.
- 606 Damayanti, T.A., Alabi, O.J., Hidayat, S.H., Crosslin, J.M., Naidu, R.A., 2014. First report of
607 *Potato virus Y* in potato in West Java, Indonesia. *Plant Dis.* 98, 287.
- 608 De Bokx, J.A., Huttinga, H., 1981. *Potato virus Y*. AAB/CMI Description of the Plant
609 Viruses, vol 242 (No. 37 revised). Commonw Mycol Inst/Assoc Appl Biol, Kew,
610 Surrey, UK.
- 611 Dedic, P., Ptacek, J., 1996. The reaction of czech potato cultivars to PVY^{NTN}. In: Proceedings
612 of the virology section meeting of the European Association for Potato Research.
613 Veldhoven, The Netherlands, pp. 298-299.
- 614 Dolnicar, P., Mavric-Plesko, I., Meglic, V., 2011. Long-term cold storage suppress the
615 development of tuber necrosis caused by PVY^{NTN}. *Am. J. Pot. Res.* 88, 318-323.
- 616 Dougherty, W.G., Carrington, J.C., 1988. Expression and function of potyviral gene products.
617 *Ann. Rev. Phytopathol.* 26, 123-143.
- 618 Draper, M.D., Pasche, J.S., Gudmestad, N.C., 2002. Factors influencing PVY development
619 and disease expression in three potato cultivars. *Am. J. Pot. Res.* 79, 155-165.
- 620 Drummond, A., Ashton, B., Cheung, M., Heled, J., Kears, M., Moir, R., Stones-Havas, S.,
621 Thierer, T., Wilson, A., 2009. Geneious v4.7. Available at <http://www.geneious.com/>
622 [accessed on April 4, 2012].
- 623 Elliseche, D., Bozec, M., Dantec, J-P., Pellé, R., Charlet-Ramage, K., Kerlan, C., 2004. Field
624 assessment of the resistance of potato genotypes to PVY^{NTN}. In: Proceedings of the
625 virology section meeting of the European Association for Potato Research. Rennes,
626 France, p. 97.

- 627 Faurez, F., Baldwin, T., Tribodet, M., Jacquot, E., 2012. Identification of new *Potato virus Y*
628 (PVY) molecular determinants for the induction of vein necrosis in tobacco. *Mol. Plant*
629 *Pathol.* 13, 948-959.
- 630 Galvino-Costa, S.B.F., dos Reis Figueira, A., Camargos, V.V., Geraldino, P.S., Hu, X-J.,
631 Nikolaeva, O.V., Kerlan, C., Karasev, A.V., 2012. A novel type of *Potato virus Y*
632 recombinant genome, determined for the genetic strain PVY^E. *Plant Pathol.* 61, 388-398.
- 633 Glais, L., Tribodet, M., Gauthier, J-P., Astier-Manifacier, S., Robaglia, C., Kerlan, C., 1998.
634 RFLP mapping of the whole genome of ten viral isolates representative of different
635 biological groups of *Potato virus Y*. *Arch. Virol.* 143, 2077-2091.
- 636 Glais, L., Tribodet, M., Kerlan, C., 2002. Genomic variability in Potato potyvirus Y (PVY):
637 evidence that PVY^{NW} and PVY^{NTN} variants are single to multiple recombinants
638 between PVY^O and PVY^N isolates. *Arch. Virol.* 147, 363-378.
- 639 Glais, L., Colombel, A.S., Tribodet, M., Kerlan, C., 2004. PVY^N-605, the reference PVY^N
640 isolate displays a PVY^{NTN} non-recombinant genome. In: Proceedings of the virology
641 section meeting of the European Association for Potato Research. Rennes, France, p.50.
- 642 Glais, L., Jacquot, E., 2015. Detection and characterization of viral species/sub-species using
643 isothermal Recombinase Polymerase Amplification (RPA) assays. In C. Lacomme
644 (Ed.), *Methods in Molecular Biology, Plant Pathology Techniques and Protocols:*
645 *Humana Press*, in press.
- 646 Hu, X.J., Meacham, T., Ewing, L., Gray, S.M., Karasev, A.V., 2009. A novel recombinant
647 strain of *Potato virus Y* suggests a new viral genetic determinant of vein necrosis in
648 tobacco. *Virus Res.* 143, 68-76.
- 649 Hutton, F., Kildea, S., Griffin, D., Spink, J., Doherty, G., Hunter, A., 2013. First report of
650 potato tuber necrotic ringspot disease associated with PVY recombinant strains in
651 Ireland. *New Dis. Rep.* 28, 12.
- 652 Jacquot, E., Tribodet, M., Croizat, F., Balme-Sinibaldi, V., Kerlan, C., 2005. A single
653 nucleotide polymorphism-based technique for specific characterization of Y^O and Y^N
654 isolates of *Potato virus Y* (PVY). *J. Virol. Methods* 125, 83-93.
- 655 Jakab, G., Droz, E., Brigneti, G., Baulcombe, D., Malnoe, P., 1997. Infectious *in vivo* and *in*
656 *vitro* transcripts from a full-length cDNA clone of PVY-N605, a Swiss necrotic isolate
657 of *Potato virus Y*. *J. Gen. Virol.* 78, 3141-3145.
- 658 Jenner, C.E., Wang, X.W., Tomimura, K., Ohshima, K., Ponz, F., Walsh, J.A., 2003. The dual
659 role of the potyvirus P3 protein of *Turnip mosaic virus* as a symptom and avirulence
660 determinant in brassicas. *Mol. Plant-Microbe Interact.* 16, 777-784.

- 661 Jones , R.A.C. 1990. Strain group specific and virus specific hypersensitive reactions to
662 infection with potyviruses in potato cultivars. *Ann. Appl. Biol.* 117, 93-105.
- 663 Karasev, A.V., Nikolaeva, O.V., Hu, X.J., Sielaff, Z., Whitworth, J., Lorenzen, J.H., Gray,
664 S.M., 2010. Serological properties of ordinary and necrotic isolates of *Potato virus Y*: A
665 case study of PVY^N misidentification. *Am. J. Potato Res.* 87, 1-9.
- 666 Karasev, A.V., Gray, S.M., 2013. Continuous and emerging challenges of *Potato virus Y* in
667 potato. *Ann. Rev. Phytopathol.* 51, 571-586.
- 668 Kerlan, C., Tribodet, M., 1996. Are all PVY^N isolates able to induce potato tuber necrosis
669 ringspot disease ? In: Proceedings of the 13th Triennial Conference of the European
670 Association for Potato Research. Veldhoven, The Netherlands, pp. 65-66.
- 671 Kerlan, C., Tribodet, M., Glais, L., Guillet, M., 1999. Variability of *Potato virus Y* in potato
672 crops in France. *J. Phytopathol.* 147, 643-51.
- 673 Kerlan C., 2006. *Potato virus Y*. In AAB (Ed.), Descriptions of plant viruses, pp. 31.
- 674 Kerlan, C., Moury, B., 2008. *Potato Virus Y*. *Encyclopedia of Virology*, third ed., pp. 287-
675 296.
- 676 Kerlan, C., Nikolaeva, O.V., Hu, X., Meacham, T., Gray, S.M., Karasev, A.V., 2011.
677 Identification of the molecular make-up of the *Potato virus Y* strain PVY^Z: genetic
678 typing of PVY^Z-NTN. *Virology* 101, 1052-1060.
- 679 Krause-Sakate, R., Redondo, E., Richard-Forget, F., Jadao, A.S., Houvenaghel, M.C.,
680 German-Retana, S., Pavan, M.A., Candresse, T., Zerbini, F.M., Le Gall, O., 2005.
681 Molecular mapping of the viral determinants of systemic wilting induced by a *Lettuce*
682 *mosaic virus* (LMV) isolate in some lettuce cultivars. *Virus Res.* 109, 175-180.
- 683 Kus, M., 1992. Potato tuber necrotic ringspot disease. Varietal differences in appearance of
684 ringspot necrosis symptoms on tubers. In: Proceedings of the virology section meeting
685 of the European Association for Potato Research. Vitoria-Gasteiz, Spain, pp. 81-83.
- 686 Lacroix, C., Glais L., Kerlan C., Verrier J.L., Jacquot E., 2010. Biological characterization of
687 French *Potato virus Y* (PVY) isolates collected from susceptible or PVY-resistant
688 tobacco plants possessing the recessive resistance gene “*va*”. *Plant Pathol.* 59, 1133-
689 1143.
- 690 Le Romancer, M., Kerlan, C., 1991. La maladie des nécroses annulaires superficielles des
691 tubercules : une affection de la pomme de terre, due au virus Y. *Agronomie* 11, 889-
692 900.

- 693 Le Romancer, M., 1993. La maladie des nécroses annulaires superficielles des tubercules de
 694 pomme de terre. Identification et caractérisation de l'agent pathogène : le variant Y^{NTN}
 695 du virus Y de la pomme de terre. France, University of Rennes. Doctorate thesis.
- 696 Le Romancer, M., Nedellec, M., 1997. Effect of plant genotype, virus isolate and temperature
 697 on the expression of the potato tuber necrotic ringspot disease (PTNRD). *Plant Pathol.*
 698 46, 104-111.
- 699 Lorenzen, J.H., Meacham, T., Berger, P.H., Shiel, P.J., Crosslin, J.M., Hamm, P.B., Kopp, H.,
 700 2006. Whole genome characterization of *Potato virus Y* isolates collected in the western
 701 USA and their comparison to isolates from Europe and Canada. *Arch. Virol.* 151, 1055-
 702 1074.
- 703 Lorenzen, J., Nolte, P., Martin, D., Pasche, J.S., Gudmestad, N.C., 2008. NE-11 represents a
 704 new strain variant class of *Potato virus Y*. *Arch. Virol.* 153, 517-525.
- 705 McDonald, J.G., Kristjansson, G.T., 1993. Properties of strains of *Potato virus Y*^N in North
 706 America. *Plant Dis.* 77, 87-9.
- 707 Mestre, P., Brigneti, G., Baulcombe D.C., 2000. An *Ry*-mediated resistance response in potato
 708 requires the intact active site of the NIa proteinase from *Potato virus Y*. *Plant J.* 23, 653-
 709 661.
- 710 Moury, B., Simon, V., 2011. dN/dS-based methods detect positive selection linked to trade-
 711 offs between different fitness traits in the coat protein of *Potato virus Y*. *Mol. Biol. Evol.*
 712 28, 2707-2717.
- 713 Moury, B., Caromel, B., Johansen, E., Simon, V., Chauvin, L., Jacquot, E., Kerlan, C.,
 714 Lefebvre, V., 2011. The helper component proteinase cistron of *Potato virus Y* Induces
 715 hypersensitivity and resistance in potato genotypes carrying dominant resistance genes
 716 on chromosome IV. *Mol. Plant-Microbe Interact.* 24, 787-797.
- 717 Nie, X., Singh, R.P., 2003. Evolution of North American PVY^{NTN} strain Tu660 from local
 718 PVY^N by mutation rather than recombination. *Virus Gene* 26, 39-47.
- 719 Ohshima, K., Sako, K., Hiraishi, C., 2000. Potato tuber necrotic ringspot disease occurring in
 720 Japan: its association with *Potato virus Y* necrotic strain. *Plant Dis.* 84, 1109-1115.
- 721 Piche, L.M., Singh, R.P., Nie, X., Gudmestad, N.C., 2004. Diversity among *Potato virus Y*
 722 isolates obtained from potatoes grown in the United States. *Virology* 94, 1368-1375.
- 723 Robles-Hernandez, L., Gonzalez-Franco, A.C., Hernandez-Huerta, J., Meacham, T.M.,
 724 Nikolaeva, O.V., Karasev, A.V., 2010. First identification of an unusual recombinant
 725 *Potato virus Y* strain in potato in Mexico. *Plant Dis.* 94, 1262.

- 726 Rolland, M., Glais, L., Kerlan, C., Jacquot, E., 2008. A multiple single nucleotide
727 polymorphisms interrogation assay for reliable *Potato virus Y* group and variant
728 characterization. *J. Virol. Methods* 147, 108-117.
- 729 Rolland, M., Kerlan, C., Jacquot, E., 2009. The acquisition of molecular determinants
730 involved in *Potato virus Y* necrosis capacity leads to fitness reduction in tobacco plants.
731 *J. Gen. Virol.* 90, 244-252.
- 732 Saenz, P., Cervera, M.T., Dallot, S., Quiot, L., Quiot, J.B., Riechmann, J.L., Garcia, J.A.,
733 2000. Identification of a pathogenicity determinant of *Plum pox virus* in the sequence
734 encoding the C-terminal region of protein P3+6K(1). *J. Gen. Virol.* 81, 557-566.
- 735 Schiessendoppler, E., 1992. Potato tuber necrotic ringspot disease: Studies on transmission
736 rate, symptom expression and yield reduction. In: Proceedings of the virology section
737 meeting of the European Association for Potato Research. Vitoria-Gasteiz, Spain, pp.
738 85-90.
- 739 Shukla, D.D., Ward, C.W., Burnt, A.A., 1994. *The Potyviridae*. Cambridge University Press,
740 Cambridge.
- 741 Singh, M., Singh, R.P., 1996. Nucleotide sequence and genome organization of a Canadian
742 isolate of the common strain of *Potato virus Y* (PVY^O). *Can. J. Plant Pathol.* 18, 209-
743 224.
- 744 Singh, R.P., Valkonen, J.P.T., Gray, S.M., Boonham, N., Jones, R.A.C., Kerlan, C., Schubert
745 J. 2008. Discussion paper: The naming of *Potato virus Y* strains infecting potato. *Arch.*
746 *Virol.* 153, 1-13.
- 747 Tian, Y-P., Valkonen, J.P.T., 2013. Genetic determinants of *Potato virus Y* required to
748 overcome or trigger hypersensitive resistance to PVY strain group O controlled by the
749 gene *Ny* in potato. *Mol. Plant-Microbe Interact.* 26, 297-305.
- 750 Tribodet, M., Glais, L., Kerlan, C., Jacquot, E., 2005. Characterization of *Potato virus Y*
751 (PVY) molecular determinants involved in the vein necrosis symptom induced by PVY^N
752 isolates in infected *Nicotiana tabacum* cv. Xanthi. *J. Gen. Virol.* 86, 2101-2105.
- 753 Ullah, Z., Groumet, R., 2002. Localization of *Zucchini yellow mosaic virus* to the veinal
754 regions and role of viral coat protein in veinal chlorosis conditioned by the zym
755 potyvirus resistance locus in cucumber. *Physiol. Mol. Plant Pathol.* 60, 79-89.
- 756 Urcuqui-Inchima, S., Haenni, A.L., Bernardi, F., 2001. Potyvirus proteins: a wealth of
757 functions. *Virus Res.* 74, 157-75.
- 758 Weidemann, H.L., 1985. Ringsymptome an kartoffelknollen: kartoffelvirus Y als vermutliche
759 ursache. *Der kartoffelbau*, 36, 356-357.

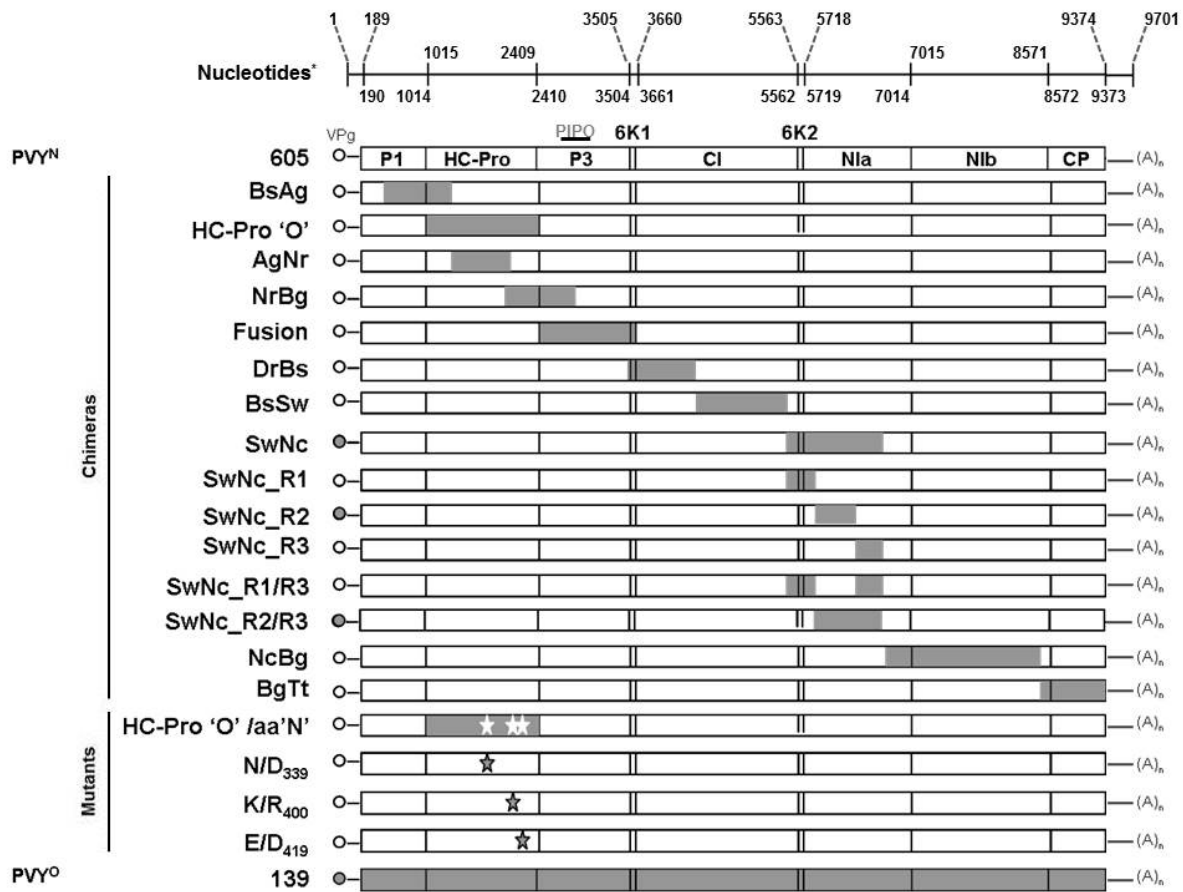
760 **Figure captions**

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 762 **Figure 1. Schematic representation of genomic organizations of *Potato virus Y* isolates,**
 763 **chimeras and mutants.** White and grey colour code, used for boxes, circles and stars, assign
 764 the corresponding data to PVY^{N605} and PVY^{O139} reference isolates, respectively. The viral
 765 genome-linked protein (VPg) is illustrated by circle at the 5'-end of genomes. The poly-A tail
 766 is presented at the 3'-end of PVY genomes. The large open reading frame (ORF) encoded by
 767 the viral genome is presented with the names of corresponding proteins (P1, HC-Pro, P3,
 768 6K1, CI, 6K2, NIa, NIb and CP, for a review see Urcuqui-Inchima et al., 2001). The recently
 769 described P3N-PIPO ORF (Chung et al., 2008), overlapping the P3 protein, is presented
 770 above genomic organization of PVY^{N605}. The nucleotide scale, according to Jakab et al.,
 771 1997, locates the 5'- and 3'-ends of sequences encoding PVY proteins.

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 773 **Figure 2. Characterization of biological properties of PVY isolates, chimeras and**
 774 **mutants, and schematic representation of hierarchical ascendant classification (HAC)**
 775 **analysis.** The percentages of infected potato associated to each PVY were calculated using
 776 serological data produced 70 days after mechanical inoculation. Results are illustrated using
 777 dark and light grey bars for experiments carried out with potato cv. Bintje and cv. Béa,
 778 respectively. *N*: number of necrotic tubers/total number of tubers harvested from infected Béa
 779 plants. The percentages of Béa necrotic tubers correspond to data associated to visual
 780 observations carried out after a two-months period of storage at 20°C on tubers from
 781 systemically infected plants. As none of the cv. Bintje tubers expressed necrotic symptoms,
 782 this data was not illustrated. The four groups (A to D) were defined according to a HAC
 783 analysis based on the virulence of tested viruses.

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 785 **Figure 3. Characterization of biological properties of reference PVY isolates (PVY^{N605}**
 786 **and PVY^{O139}) and E/D₄₁₉ mutant on five potato cultivars.** The sanitary status of tested
 787 plants was determined using serological detection performed 70 days post mechanical
 788 inoculation of plantlet or 60 days post planting of harvested tubers. The percentages of
 789 infected potato plants associated to mechanical inoculations and to post-harvest testing
 790 procedure are presented by white and grey bars, respectively. The percentages of necrotic
 791 potato tubers observed after a two-month period of storage at 20°C are presented for each
 792 isolate/cultivar combination. *N*: number of necrotic tubers/total number of tubers from
 793 infected potato plants of each cultivar.

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797 Figure 1

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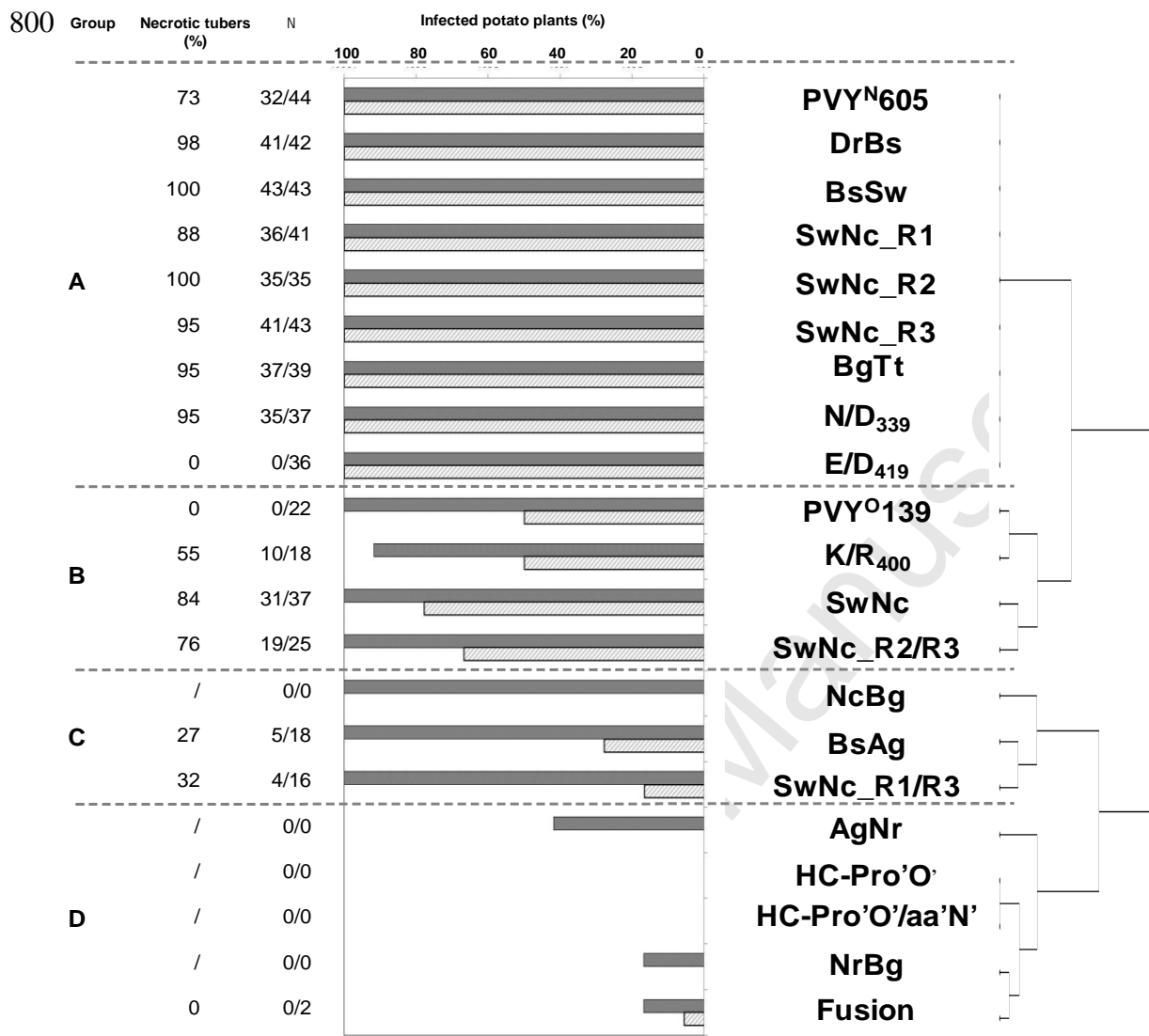
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Figure 2

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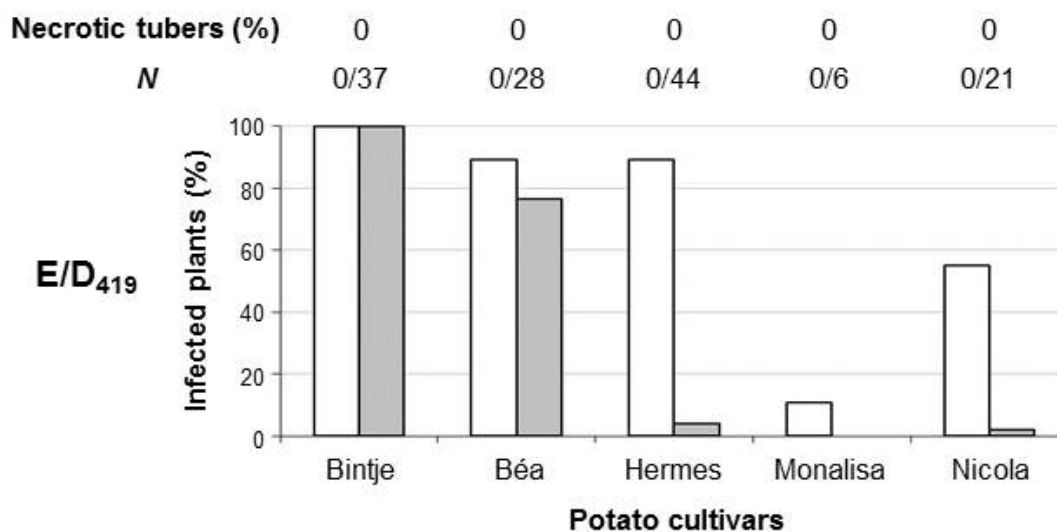
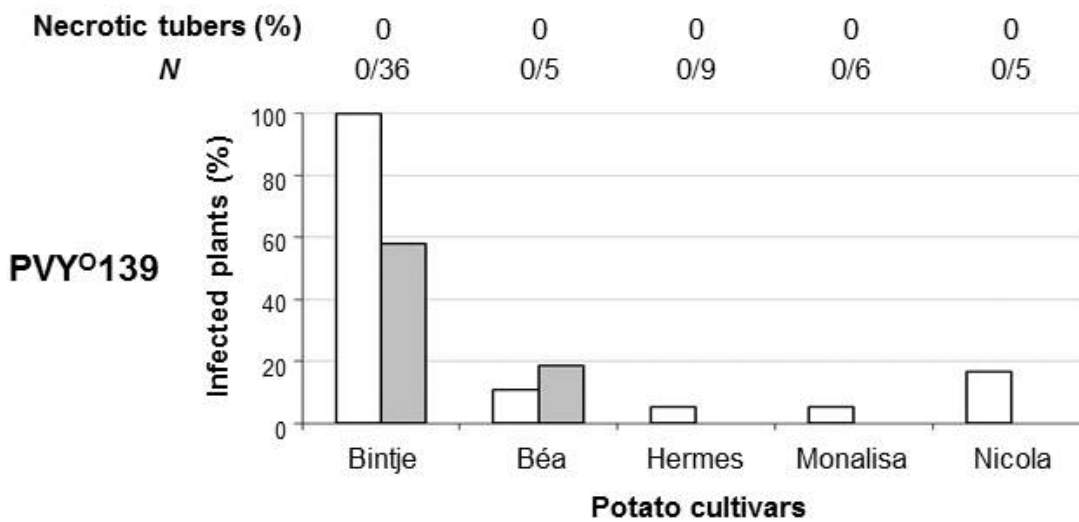
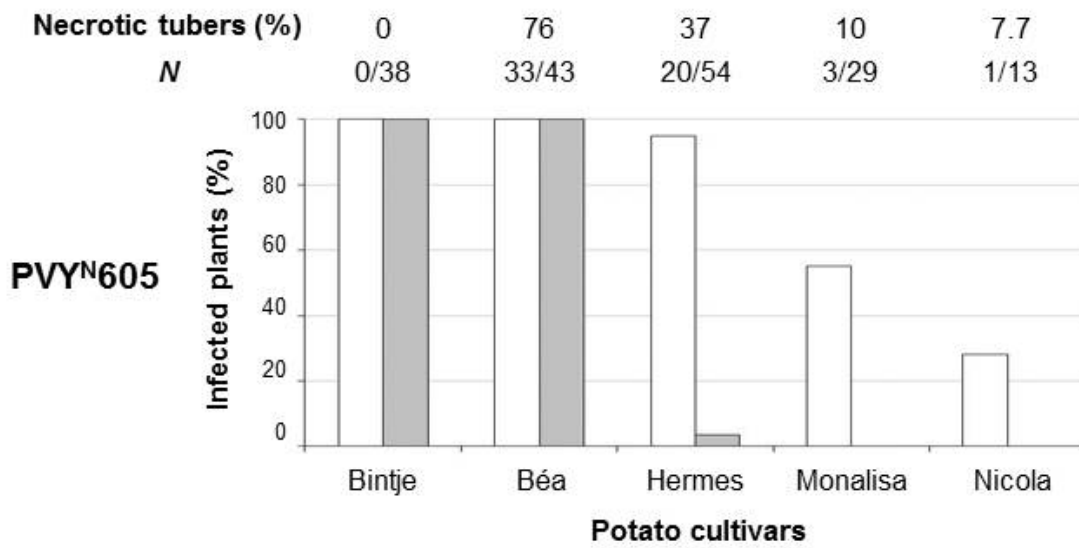
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Figure 3

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809 **Table 1. Symptoms induced by PVY isolates, chimeras and mutants on tobacco and**
810 **potato plants.**

Isolates / Constructs	<i>N. tabacum</i> cv. Xanthi			<i>S. tuberosum</i>						Group
	ELISA ^a	Symptoms ^b	N	cv. Bintje		cv. Béa				
				ELISA ^c	Symptoms ^d	N	ELISA ^c	Symptoms ^d		
Chimeras and mutants	PVY ^N 605	+	VN	12/12	2.6	ns/Mo	18/18	1.7	ns/mild VN	IV
	SwNc_R3	+	VN	12/12	2	ns/VN	18/18	1.6	ns/ns	
	BsAg	+	VN	12/12	1.2	ns/VN	5/18	0.4	ns/ns	
	NcBg	+	VN	12/12	1.8	ns/VN	0/18	0	ns/LD	
	N/D ₃₃₉	+	Mos	12/12	1.6	ns/ns	18/18	1	ns/VN	
	DrBs	+	VN	12/12	2.9	ns/ns	18/18	1.1	ns/VN	
	BsSw	+	VN	12/12	2.4	ns/ns	18/18	2.2	ns/VN	
	SwNc_R2	+	VN	12/12	2.7	ns/ns	18/18	1.7	ns/VN	
	SwNc	+	Mos	12/12	2	ns/ns	14/18	0.5	ns/ns	I
	SwNc_R2/R3	+	VN	12/12	1.3	ns/ns	12/18	0.9	ns/ns	
	NrBg	+	Mos	2/12	1.7	ns/ns	0/18	0	ns/ns	
	Fusion	+	VN	2/12	0.8	ns/ns	1/18	2.2	ns/ns	
	HC-Pro O	+	Mos	0/12	0	NLL/ns	0/18	0	ns/ns	II
	HC-Pro'O'/aa'N'	+	VN	0/12	0	NLL/ns	0/18	0	ns/ns	
	AgNr	+	Mos	5/12	0.5	NLL/VN	0/18	0	ns/ns	
	SwNc_R1	+	VN	12/12	2.3	ns/ns	18/18	1.7	Ma/VN	III
	SwNc_R1/R3	+	Mos	12/12	1.8	ns/ns	3/18	0.2	Ma/ns	
	BgTt	+	VN	12/12	1.1	ns/VN	18/18	1.5	Ma/VN	
	K/R ₄₀₀	+	Mos	11/12	1.3	ns/VN	9/18	0.8	Ma/LD	
E/D ₄₁₉	+	Mos	12/12	1.9	ns/Mo	18/18	1.7	Ma/LD		
PVY ^O 139	+	Mos	12/12	1.2	ns/Mo	9/18	0.9	Ma/VN		

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814 ^a: ability of PVY to infect ('+') or not ('-') inoculated tobacco plants. Data obtained using
815 ELISA test carried out on non-inoculated leaves of inoculated plants at 15 days post
816 inoculation (dpi).817 ^b: symptoms observed on systemic tobacco leaves at 20 dpi (VN: vein necrosis; Mos: mosaic).818 *N*: number of infected potato plants/total number of inoculated plants.819 ^c: Absorbance at 405nm (OD₄₀₅) values from DAS-ELISA. Presented values correspond to the
820 mean OD₄₀₅ calculated using data from infected plants.821 ^d: symptoms observed on inoculated/systemic potato leaves. Inoculated and systemic leaves
822 were monitored at 20 and 60 dpi, respectively (ns: no symptoms; Mo: mottle; NLL: necrotic
823 local lesions; Ma: maritta; VN: vein necrosis; LD: leaf drop).

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Table 2. Identity of HC-Pro amino acid 419 in genome of PVY isolates and mutants progenies present in infected potato plants.

Inoculum		Infected host ^a				
PVY	AA ₄₁₉ ^b	PTNRD -	PTNRD +			
		Bintje	Béa	Monalisa	Hermes	Nicola
N605	E	E	E	E	E	E
E/D₄₁₉	D	D	N	N	N	N
D/N₄₁₉	N	N	N	N	N	N

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^a: viral progeny obtained in potato cultivars susceptible (PTNRD +) or not (PTNRD -) to tuber necrosis.

^b: HC-Pro amino acid at position 419 according to Jakab et al. (1997).

E: glutamic acid; D: aspartic acid; N: asparagine.

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