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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

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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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29

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<sup>N</sup> and PVY<sup>O</sup>) and variants (e.g.  
34 PVY<sup>NTN</sup> and PVY<sup>N-W</sup>). 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 E<sub>419</sub> 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/D<sub>419</sub> mutant. This result opens opportunities for the  
43 future studies on potato/PVY interactions.

44

45 Keywords: PVY<sup>NTN</sup>, 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<sup>NTN</sup> 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<sup>O</sup>, PVY<sup>C</sup>, PVY<sup>N</sup>, PVY<sup>Z</sup> and PVY<sup>E</sup> 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<sup>NTN</sup> and PVY<sup>N-W</sup>) 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<sup>NTN</sup> variant was initially described to group isolates able to  
102 induce PTNRD symptoms under field conditions. Then, molecular characterization of  
103 PVY<sup>NTN</sup> members showed that this variant includes isolates with non recombinant PVY<sup>N</sup>-type  
104 (NA-PVY<sup>NTN</sup>; Boonham et al., 2002; Nie and Singh, 2003) and recombinant PVY<sup>N</sup>-  
105 type/PVY<sup>O</sup>-type (Eu-PVY<sup>NTN</sup>; Boonham et al., 2002; Glais et al., 2002) genomic  
106 organizations. In addition to members of the PVY<sup>NTN</sup> variant, some PVY isolates assigned  
107 into the PVY<sup>N-W</sup> variant, i.e. described for their capacity to induce tobacco vein necrotic  
108 symptoms and for their PVY<sup>O</sup>-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<sup>Z</sup> and PVY<sup>E</sup> 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<sup>N</sup> 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<sup>N</sup>, PVY<sup>NTN</sup>, PVY<sup>N-W</sup>, PVY-NE-11, PVY<sup>Z</sup> and PVY<sup>E</sup>  
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 D<sub>205</sub>, N<sub>339</sub>, K<sub>400</sub>, and E<sub>419</sub> 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<sup>O</sup> (*Ny<sub>ibr</sub>* resistance gene; Cockerham, 1970) or PVY<sup>C</sup> (*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<sup>NTN</sup> 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<sup>N/O</sup> chimeras and mutants

173

174 PVY<sup>N</sup>605 (GenBank accession number X977895; Jakab et al., 1997) and PVY<sup>O</sup>139  
175 (GenBank accession number U09509; Singh and Singh, 1996) isolates were used as  
176 references for PVY<sup>N</sup> and PVY<sup>O</sup> groups, respectively. The genome of PVY<sup>N</sup>605 isolate shares  
177 99.3% nucleotide identity with the non recombinant PVY<sup>NTN</sup>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<sup>N</sup>605 was

181 considered in this study as a non recombinant PVY<sup>NTN</sup> isolate. To our knowledge and  
182 according to the literature, PVY<sup>O</sup>139 (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<sup>N/O</sup> chimeras resulting from genomic exchanges between  
186 PVY<sup>N</sup>605 and PVY<sup>O</sup>139 sequences. Moreover, three point-mutated versions of the infectious  
187 PVY<sup>N</sup>605 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<sup>NTN</sup> 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<sup>N</sup>605 and PVY<sup>O</sup>139 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<sup>NTN</sup> 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 Na<sub>2</sub>HPO<sub>4</sub>, 12H<sub>2</sub>O;  
221 50mM KH<sub>3</sub>PO<sub>4</sub>; 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<sup>N</sup>605 and PVY<sup>O</sup>139), 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 Na<sub>2</sub>HPO<sub>4</sub>.12H<sub>2</sub>O, 2.7mM KCl, 1.5mM KH<sub>2</sub>PO<sub>4</sub>, 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  $\mu$ l of PVY polyclonal antibody  
250 conjugated to alkaline phosphatase (M. Guillet, FN3PT /INRA, France) were added at a  
251 concentration of 1 $\mu$ 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 $\mu$ 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 OD<sub>405</sub> 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<sup>N</sup>605 and PVY<sup>O</sup>139  
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<sup>N</sup>605 infection, and the green rings (i.e. maritta symptoms;  
301 Kerlan, 2006) on inoculated leaves and systemic vein necrosis induced by PVY<sup>O</sup>139  
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<sup>N/O</sup> 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/R<sub>400</sub> and E/D<sub>419</sub> 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<sup>O</sup>139, the maritta  
315 symptom on inoculated leaves of cv. Béa. Finally, the SwNc\_R3, BsAg, NcBg, N/D<sub>339</sub>, 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<sup>N</sup>605. Consequently, these  
319 constructs and PVY<sup>N</sup>605 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<sup>N</sup>605, DrBs, BsSw, BgTt, SwNc\_R1, SwNc\_R2, SwNc\_R3, N/D<sub>339</sub> and E/D<sub>419</sub>) were  
334 associated to a 100% infection of Béa plants. Members of group B, i.e. the PVY<sup>O</sup>139 isolate  
335 and the constructs K/R<sub>400</sub>, 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<sup>N/O</sup>  
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<sup>N</sup>605 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/R<sub>400</sub>, N/D<sub>339</sub> 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<sup>O</sup>139 isolate (22 tubers) or by the E/D<sub>419</sub> (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/D<sub>419</sub> and Fusion  
365 constructs, systemic PVY infection was detected by DAS-ELISA in inoculated plants (18/18  
366 and 1/18 for E/D<sub>419</sub> and Fusion, respectively; see Table 1). Absorbance values obtained for  
367 E/D<sub>419</sub>-, Fusion-, N605- and O139-infected plants were compared. No significant differences  
368 were observed between data associated to N605- and E/D<sub>419</sub>-infected plants ( $p=0.776$ ) while  
369 O139 infected plants were associated to lower OD<sub>405</sub> values than N605- ( $p=0.014$ ) and  
370 E/D<sub>419</sub>- ( $p=0.01$ ) infected plants. The single plant systemically infected by the Fusion mutant  
371 was associated to higher OD value (OD<sub>405</sub> = 2.23) than the E/D<sub>419</sub> mutant (mean OD<sub>405</sub> = 1.7  
372 +/- 0.5 (N=18)) and the references isolates (mean OD<sub>419</sub> = 1.8 +/- 0.6 (N=18) and mean OD<sub>419</sub>  
373 = 0.9 +/- 0.9 (N=9) for N605 and O139, respectively). Moreover, asymptomatic tubers  
374 produced by E/D<sub>419</sub>- 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<sup>N</sup>605 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/D<sub>419</sub> 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/D<sub>419</sub> 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<sup>N</sup>605, PVY<sup>O</sup>139 and E/D<sub>419</sub> 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<sup>N</sup>605  
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<sup>O</sup>139  
399 isolate were 5.5%, 5.5% and 16.5%, respectively, and the daughter tubers from PVY<sup>O</sup>139-  
400 inoculated plants were all asymptomatic and produced exclusively healthy plants. The  
401 experiment carried out with E/D<sub>419</sub> mutant displayed an infection pattern close to the one  
402 obtained for PVY<sup>N</sup>605 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/D<sub>419</sub>-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/D<sub>419</sub>-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/N<sub>419</sub> mutant produced in Béa to those of the original E/D<sub>419</sub>  
421 construct, the former was inoculated on Bintje, Béa, Hermes, Monalisa and Nicola plants. As  
422 previously denoted for E/D<sub>419</sub>, D/N<sub>419</sub> 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<sup>N605</sup> and D/N<sub>419</sub> isolates contained  
428 a glutamic acid (E<sub>419</sub>) and an asparagine residue (N<sub>419</sub>), respectively (Table 2). The sequence  
429 of E/D<sub>419</sub>-derived progenies present in infected Bintje plants contained the D<sub>419</sub> residue in the  
430 HC-Pro sequence while E/D<sub>419</sub>-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 AA<sub>419</sub> 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 E<sub>419</sub> residue (data not shown).

439

#### 440 4. Discussion

441

442 PVY isolates assigned to the tuber necrotic variant PVY<sup>NTN</sup> 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<sup>NTN</sup> 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<sup>N</sup>-605/PVY<sup>O</sup>-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<sup>N</sup>/PVY<sup>O</sup> nucleotide exchanges altered the necrotic ability of the  
466 PVY<sup>N</sup>-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/D<sub>419</sub> 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<sup>N</sup>605 isolate. Moreover, post-harvested assays performed on  
481 daughter plants produced using tubers from Fusion- and E/D<sub>419</sub>-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<sup>N</sup>-605 were included in this study. The K/R<sub>400</sub> and  
494 the E/D<sub>419</sub> 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 E<sub>419</sub> is conserved in the sequence of these isolates. However, it has been shown that E<sub>419</sub> is  
500 also present in HC-Pro sequence of PVY<sup>N</sup> and PVY<sup>N</sup>-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 E<sub>419</sub> 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 E<sub>419</sub> in the expression of tuber  
506 necrosis is not specific to Béa cultivar. Indeed, the E/D<sub>419</sub> 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 D<sub>419</sub> residue is systematically mutated to asparagine (i.e.  
512 D419N which corresponds to the G<sub>2269</sub> to A<sub>2269</sub> transition in the PVY sequence). Surprisingly,  
513 this sequence modification is not observed in PVY E/D<sub>419</sub> 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/N<sub>419</sub> 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 D<sub>419</sub> 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 E<sub>419</sub> 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 AA<sub>419</sub> 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 E<sub>419</sub> 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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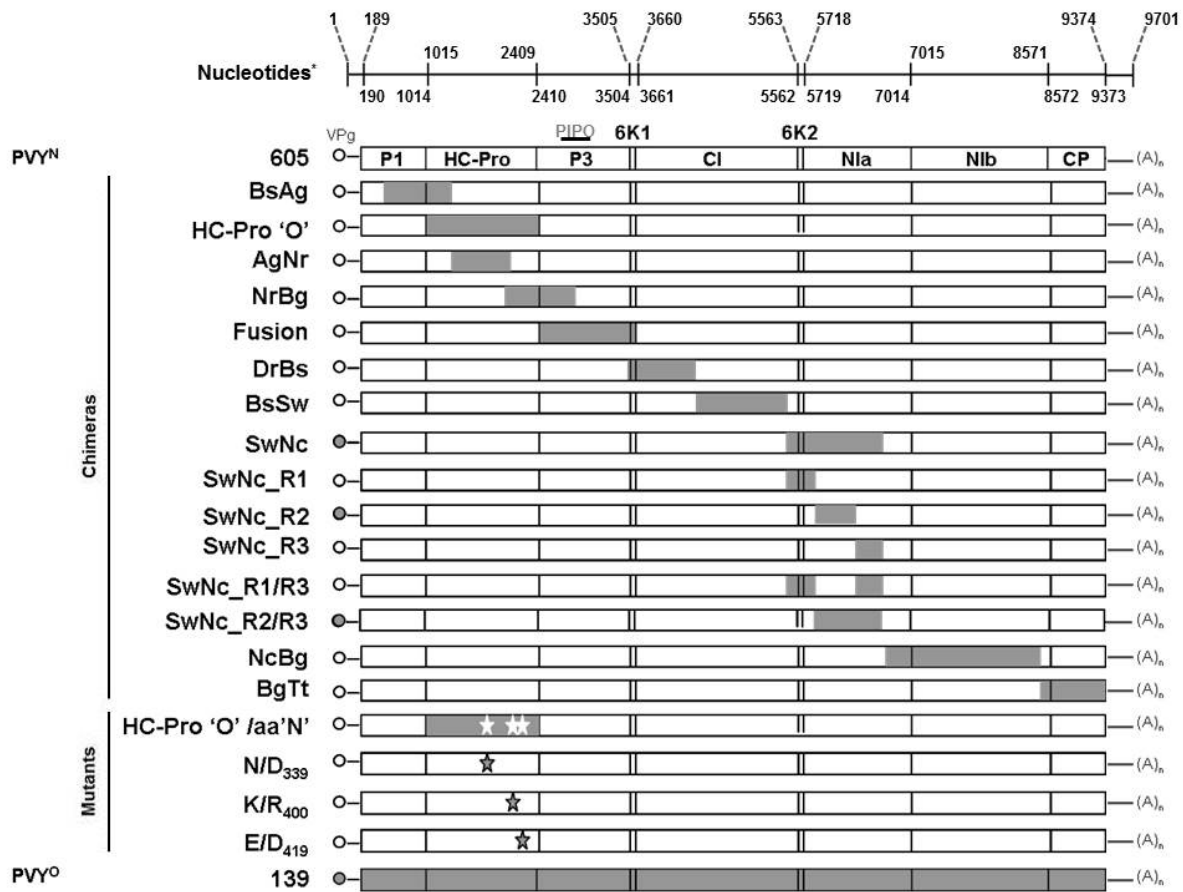
760 **Figure captions**

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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<sup>N605</sup> and PVY<sup>O139</sup> 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<sup>N605</sup>. 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<sup>N605</sup>**  
 786 **and PVY<sup>O139</sup>) and E/D<sub>419</sub> 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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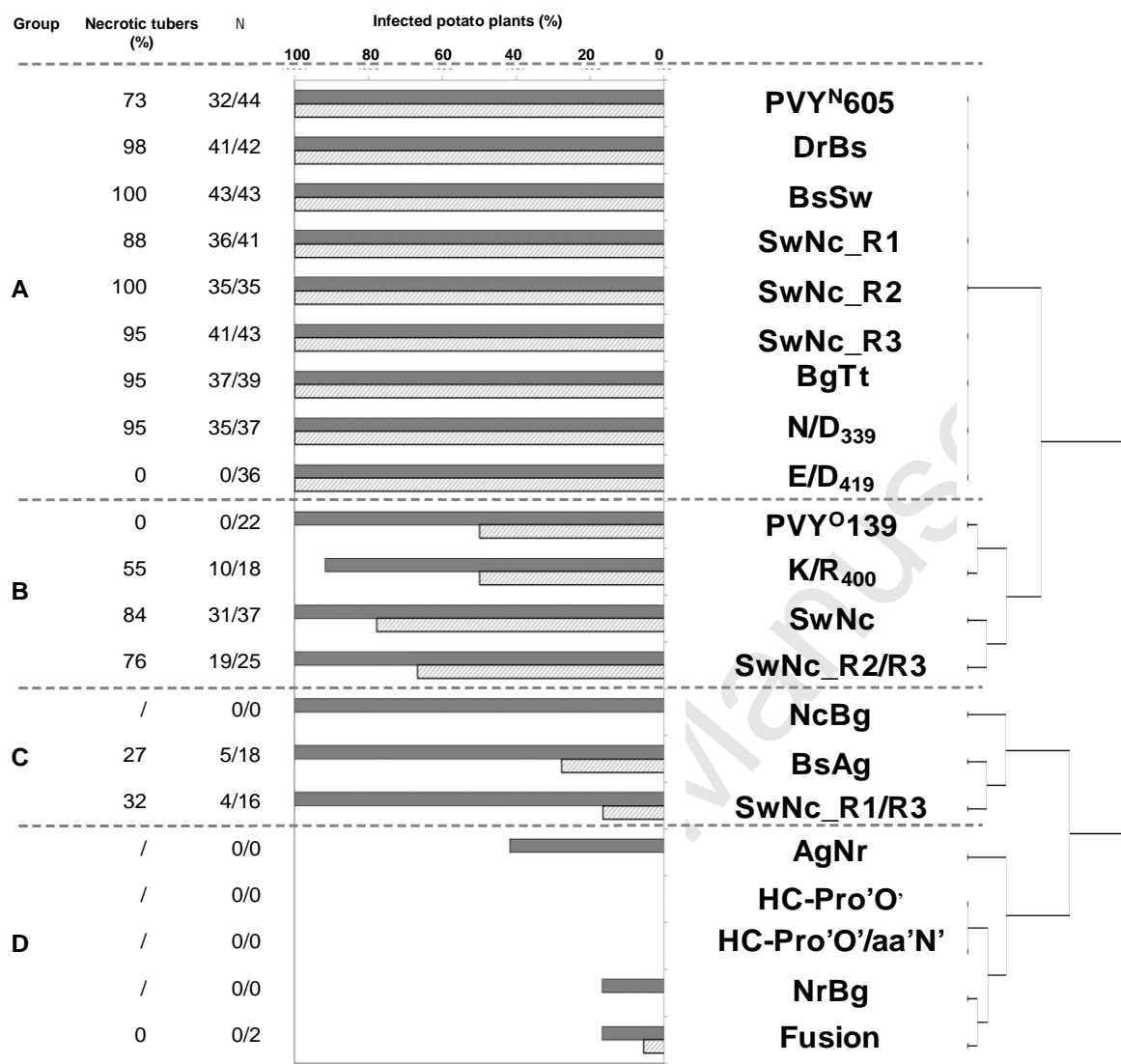
797 Figure 1

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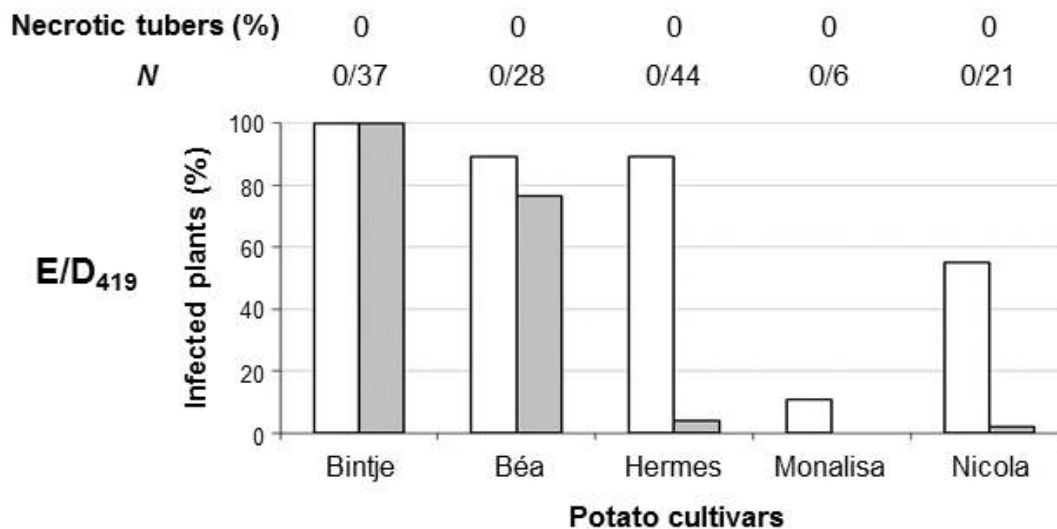
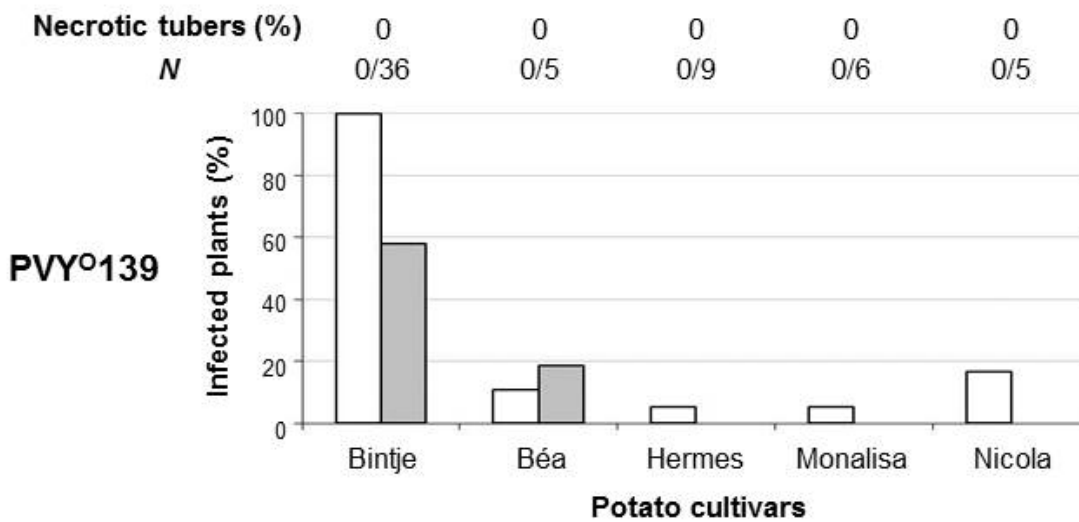
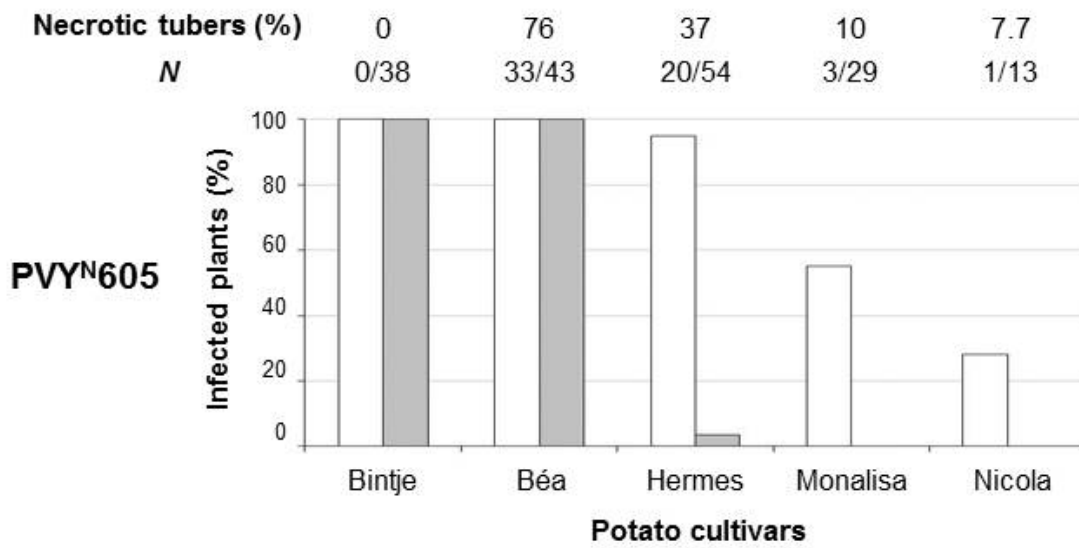
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Figure 3

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

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

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<sup>a</sup>: ability of PVY to infect ('+') or not ('-') inoculated tobacco plants. Data obtained using ELISA test carried out on non-inoculated leaves of inoculated plants at 15 days post inoculation (dpi).

<sup>b</sup>: symptoms observed on systemic tobacco leaves at 20 dpi (VN: vein necrosis; Mos: mosaic).

*N*: number of infected potato plants/total number of inoculated plants.

<sup>c</sup>: Absorbance at 405nm (OD<sub>405</sub>) values from DAS-ELISA. Presented values correspond to the mean OD<sub>405</sub> calculated using data from infected plants.

<sup>d</sup>: symptoms observed on inoculated/systemic potato leaves. Inoculated and systemic leaves were monitored at 20 and 60 dpi, respectively (ns: no symptoms; Mo: mottle; NLL: necrotic 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 <sup>a</sup>				
PVY	AA <sub>419</sub> <sup>b</sup>	PTNRD -	PTNRD +			
		Bintje	Béa	Monalisa	Hermes	Nicola
<b>N605</b>	E	E	E	E	E	E
<b>E/D<sub>419</sub></b>	D	D	N	N	N	N
<b>D/N<sub>419</sub></b>	N	N	N	N	N	N

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831 <sup>a</sup>: viral progeny obtained in potato cultivars susceptible (PTNRD +) or not (PTNRD -) to tuber  
832 necrosis.

833 <sup>b</sup>: HC-Pro amino acid at position 419 according to Jakab et al. (1997).

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

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