



HAL
open science

R-BPMV-mediated resistance to Bean pod mottle virus in *Phaseolus vulgaris* L. is heat-stable but elevated temperatures boost viral infection in susceptible genotypes

Chouaïb Meziadi, Julie Lintz, Masoud Naderpour, Charlotte Gautier, Sophie Blanchet, Alicia Noly, Ariane Gratias-Weill, Valérie Geffroy, Stéphanie Pflieger

► **To cite this version:**

Chouaïb Meziadi, Julie Lintz, Masoud Naderpour, Charlotte Gautier, Sophie Blanchet, et al.. R-BPMV-mediated resistance to Bean pod mottle virus in *Phaseolus vulgaris* L. is heat-stable but elevated temperatures boost viral infection in susceptible genotypes. *Viruses*, 2021, 13 (7), pp.1-24. <10.3390/v13071239>. <hal-03279050>

HAL Id: hal-03279050

<https://hal.science/hal-03279050v1>

Submitted on 6 Jul 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY 4.0 - Attribution - International License

1 Article

2 **R-BPMV-mediated resistance to *Bean pod mottle virus* in *Phaseolus vulgaris* L. is**
3 **heat-stable but elevated temperatures boost viral infection in susceptible geno-**
4 **types**

5 **Chouaïb Meziadi^{1,2}, Julie Lintz^{1,2}, Masoud Naderpour^{1,2,3}, Charlotte Gautier^{1,2}, Sophie Blanchet^{1,2},**
6 **Alicia Noly^{1,2}, Ariane Gratias-Weill^{1,2}, Valérie Geffroy^{1,2} and Stéphanie Pflieger^{1,2*}**

7 ¹ Université Paris-Saclay, CNRS, INRAE, Univ Evry, Institute of Plant Sciences Paris-Saclay (IPS2), F-91405
8 Orsay, France

9 ² Université de Paris, CNRS, INRAE, Institute of Plant Sciences Paris-Saclay (IPS2), F-91405 Orsay, France

10 ³ Seed and Plant Certification and Registration Research Institute, Agricultural Research, Education and Ex-
11 tension Organization, Ministry of Agriculture, Iran

12 * Correspondence: stephanie.pflieger@universite-paris-saclay.fr; Institute of Plant Sciences Paris-Saclay, Rue
13 Noetzelin, CS 80 004, 91192 Gif sur Yvette Cedex, France. Telephone: 33-1-69-15-33-41

14 **Abstract:**

15 In the context of climate change, elevated temperature is a major concern due to the impact on
16 plant-pathogen interactions. Although atmospheric temperature is predicted to increase in the next
17 century, heat waves during summer seasons have already become a current problem. Elevated
18 temperatures strongly influence plant-virus interactions, the most drastic effect being a breakdown
19 of plant viral resistance conferred by some major resistance genes. In this work, we focused on the
20 *R-BPMV* gene, a major resistance gene against *Bean pod mottle virus* in *Phaseolus vulgaris*. We inoc-
21 ulated different BPMV constructs in order to study the behavior of the *R-BPMV*-mediated resis-
22 tance at normal (20°C) and elevated temperatures (constant 25°C, 30°C, and 35°C). Our results
23 show that *R-BPMV* mediates a temperature-dependent phenotype of resistance from hypersensi-
24 tive reaction at 20°C to chlorotic lesions at 35°C in the resistant genotype BAT93. BPMV is detected
25 in inoculated leaves but not in systemic ones suggesting that the resistance remains heat-stable up
26 to 35°C. *R-BPMV* segregates as an incompletely dominant gene in a F2 population. We also inves-
27 tigated the impact of elevated temperature on BPMV infection in susceptible genotypes and our
28 results reveal that elevated temperatures boost BPMV infection both locally and systemically in
29 susceptible genotypes.

Citation: Lastname, F.; Lastname, F.;
Lastname, F. Title. *Viruses* 2021, 13,
x. <https://doi.org/10.3390/xxxxx>

Academic Editor: Firstname Last-
name

Received: date

Accepted: date

Published: date

Publisher's Note: MDPI stays
neutral with regard to jurisdictional
claims in published maps and
institutional affiliations.

30 **Keywords:** *Bean pod mottle virus*; *Phaseolus vulgaris* L.; heat-stable resistance; high temperature

31 **Abbreviations:** Avr factor: avirulence factor; CO₂: carbon dioxide; dpi: day(s) post-inoculation; ETI:
32 effector-triggered immunity; HR: hypersensitive reaction; NLR: nucleotide-binding-leucine rich
repeat domain-containing receptors; ppm : parts per million; R: resistance gene/protein



35
36
37 **Copyright:** © 2021 by the authors.
38 Submitted for possible open access
39 publication under the terms and
40 conditions of the Creative Commons
41 Attribution (CC BY) license
42 (<https://creativecommons.org/licenses/by/4.0/>).
43

35 **1. Introduction**

36 Among all plant diseases, viruses account for about half of all known pathogens [1]
37 and constitute particular entities, non-living organisms but rather obligate and in-
38 tra-cellular parasites that need a live host for replication. Plant viruses are biotic patho-
39 gens that cause serious epidemics in major crops with annual yield losses of more than \$
40 30 billion [2]. No effective pesticide-based control measures are effective against viruses
41 so that the most reliable method of plant protection is by increasing plant genetic re-
42 sistance [2]. Plant resistance relies on different immune responses. First, recognition of
43 viral double-stranded RNAs is a major mechanism in antiviral plant defense [3] that

44 induces both RNA silencing [4] and pattern-triggered immunity [5–7]. Viruses have
45 evolved viral suppressors of these responses in order to promote their own replication
46 [8–11]. Second, plant effector-triggered immunity (ETI) is induced by the recognition of
47 virus effectors by resistance (*R*) proteins mostly represented by nucleo-
48 tide-binding-leucine rich repeat domain-containing receptors (NLRs) [12]. NLR receptors
49 are intracellular and encoded by dominant genes [13]. Many *R* genes against viruses have
50 been identified so far, as well as the corresponding viral-encoded effectors that are sub-
51 sequently referred to as avirulence (*Avr*) factors [14,15]. Other types of resistances are
52 also effective against viruses as those controlled by quantitative trait loci [16] or 'recessive
53 genes', the latter ones encoding mutated or truncated host factors that are hijacked by
54 viruses in their wild type forms for their life cycle [17].

55 Plant resistance is strongly affected by environmental conditions and temperature is
56 undoubtedly one of the key parameters that have a major impact on worldwide plant
57 production. It is well known that elevated temperatures influence plant-virus interac-
58 tions, as well as timing and severity of disease epidemics [18–20] but little is known about
59 the associated molecular mechanisms in plant-pathogen interactions [21]. The more
60 drastic impact of elevated temperatures is the breakdown of plant viral resistance con-
61 ferred by major *R*-genes [18]. Indeed, some *R* genes have been shown to be overcome at
62 high temperatures, usually around 28–30°C, allowing viral infection and spreading at the
63 whole-plant level. This is the case for the *N*-mediated resistance that occurs only at tem-
64 peratures below 28°C in *Nicotiana tabacum* [22], and for the *Tsw* gene mediated resistance
65 that is overcome between 25°C and 30°C in *Capsicum* species [23]. Conversely, for other *R*
66 genes, resistance is still efficient at temperatures above 28–30°C, as for example the
67 *Rx*-mediated resistance that is not compromised against *Potato virus X* (PVX, *Potexvirus*)
68 at temperatures up to 32°C [24]. Alternatively, elevated temperatures may also induce
69 more mild effects on plant resistance conferred by *R* genes. Indeed, the resistance phe-
70 notype can be modified, meaning that local necrotic lesions such as hypersensitive reac-
71 tion (HR, a form of programmed cell death [25]) can be transformed into systemic necro-
72 sis. Also, extreme resistance, that inhibits virus replication without apparent HR, can be
73 shifted to HR or even to systemic necrosis at temperatures above 28–30°C. In the absence
74 of *R* genes, elevated temperature was shown to promote symptom severity, systemic
75 spreading, and replication potential of viruses [26–28]. Other studies reported contra-
76 dictory results, in the sense that elevated temperatures reduced viral symptoms and viral
77 accumulation [29–31], which was hypothesized to be attributable to increased efficiency
78 of the RNA silencing pathway at elevated temperature [32]. Therefore, predicting a gen-
79 eral outcome for all the pathosystems may be difficult.

80 Common bean (*Phaseolus vulgaris* L.) is a major pulse crop of agronomic importance
81 cultivated as a dry grain or fresh vegetable. Indeed, it is the most important grain legume
82 for human consumption worldwide especially in developing countries such as Central
83 and South America and Southeastern Africa [33]. Common bean varieties are grown over
84 a wide range of latitudes but optimal growth conditions need temperatures comprised
85 between 17.5°C and 23°C [34,35]. Indeed, daytime temperatures above 30°C and
86 nighttime temperatures above 22°C lead to yield losses [36,37].

87 In the main production areas of common bean, several viruses including *Potyvirus*
88 *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean yel-*
89 *low mosaic virus* (BYMV), and *Clover yellow vein virus* (CIYVV) affect the quality and
90 quantity of bean productions (reviewed in [38]). The well-known *I* locus, located at the
91 extremity of chromosome 2, confers resistance to a large part of them. In addition to re-
92 sistance against *Potyvirus*, resistance to *Comovirus* has also been positioned in the region
93 of the *I* locus [39,40]. This is the case of the *R-BPMV* gene conferring resistance to *Bean pod*
94 *mottle virus* (BPMV) in *P. vulgaris* genotype BAT93 [41].

95 The aim of the current study was to address several questions concerning the
96 *R-BPMV*-mediated resistance in *P. vulgaris*. Does the *R-BPMV*-mediated resistance to
97 BPMV depend on temperature? If so, at which temperature does the phenotype switch

98 occur ? What is the inheritance of the *R-BPMV* gene? Here, we report that *R-BPMV* in-
99 duces HR lesions at 20°C. Further analysis revealed that at 25°C and 30°C,
100 *R-BPMV*-mediated resistance still induces HR lesions, whereas at 35°C local HR lesions
101 are replaced by chlorotic lesions but resistance is not overcome at the whole-plant level.
102 Finally, we also investigated the impact of elevated temperature on BPMV infection in a
103 compatible context (*i.e.* in susceptible genotypes) and our results highlighted that rising
104 temperature boosts BPMV infection.
105

106 2. Materials and Methods

107 2.1 Common bean material and growing conditions

108 The following genotypes of *Phaseolus vulgaris* were used in this study: BAT93
109 (Mesoamerican breeding line), JaloEEP558, Black Valentine (both Andean landraces) and
110 two Near-Isogenic Lines for the *I* locus [42], Black Turtle 1 (BT-1; *III*, resistant to BCMV
111 and BCMNV) and Black Turtle 2 (BT-2; *ii* susceptible to BCMV and BCMNV). The in-
112 heritance of *R-BPMV* was studied using 60 F2 individuals derived from a cross between
113 BAT93 (*R-BPMV/R-BPMV*, resistant to BPMV) and the Andean landrace JaloEEP558
114 (*r-bpmv/r-bpmv*, susceptible to BPMV).
115

116 Growing conditions from sowing to virus inoculation were followed as described in
117 Pflieger et al. [41] with some modifications. Briefly, seeds were sown in soil instead of
118 vermiculite and grown in a growth chamber at 23°C under a 16h light / 8h dark cycle and
119 75% relative humidity until BPMV-inoculation stage (fully-expanded primary leaf stage,
120 10 days post-sowing in our growth conditions). From sowing to inoculation, seedlings
121 were watered with tap water.
122

123 2.2 Viral material

124 An infectious BPMV cDNA clone derived from isolate IA-Di1 was provided by C.
125 Zhang and S. Whitham (Iowa State University, USA) and described previously [43].
126 Briefly, the infectious clone BPMV-WT contains the WT-RNA1 (on infectious plasmid
127 'pBPMV-IA-R1M') and the WT-RNA2 (infectious plasmid 'pBPMV-IA-V1') of BPMV
128 [43]. We also used the GFP-tagged BPMV (BPMV-GFP, infectious plasmids
129 'pBPMV-IA-R1M'+ 'pBPMV-GFP2') [43] in which a GFP cassette was inserted in frame
130 between the movement protein (MP) and the Large-Coat Protein (L-CP) coding regions
131 and was flanked with protease recognition sites, allowing excision from the RNA2 pol-
132 yprotein.
133

134 2.3 Viral rub-inoculation of *P. vulgaris* plants and high temperature assays

135 Viral rub-inoculation of *P. vulgaris* plants was performed as described previously
136 [41,44]. Briefly, a viral inoculum is prepared by grinding frozen or fresh infected leaves
137 from *P. vulgaris* cv. Black Valentine with a mortar and pestle in presence of mock buffer
138 (potassium phosphate buffer 0.1 M, pH 7) [44]. Mechanical inoculation is then performed
139 on one primary leaf of a healthy plant, using carborundum as an abrasive [44]. Inoculated
140 plants were then placed in a growth chamber either at constant 20°C (control tempera-
141 ture) or at constant 25°C, 30°C or 35°C (high temperature assays) in a growth chamber
142 Aralab (Fitoclima 1.200, Rio de Mouro, Portugal), under 75% constant humidity at 16/8h
143 light/dark condition. After inoculation, plants were watered with nutritive solution. Each
144 experiment was reproduced at least twice.
145

146 2.4 Cell death assays

147 For HR assays, the leaves of 7 days post-inoculation (dpi) plants were stained with
148 trypan blue in lactophenol solution (lactic acid:glycerol:liquid phenol:distilled water
149 (1:1:1:1), 0.067% w/v trypan blue) in universal tubes and heated in a boiling water bath
for 2 min. After cooling, the solution was replaced with chloral hydrate (2.5 g/mL), and

150 samples were shaken until leaves were fully destained. For observations, the chloral hy-
151 drate was replaced with 60% glycerol.

152 2.5 Detection of GFP fluorescence *in planta*

153 GFP fluorescence in whole plants was detected by using a Black Ray long-wave UV
154 lamp (High intensity 100-Watt long-wave UV lamp; UVP, Upland, USA).
155 Higher-magnification fluorescence detection was performed with an epifluorescence
156 microscope for temperature assays at 20°C and 25°C (Leica MZ16F, Leica Microsystems
157 GmbH, Wetzlar, Germany) equipped with a fluorescein isothiocyanate-tetramethyl
158 rhodamine isothiocyanate mutiband filter. GFP fluorescence spectra were checked using a
159 confocal microscope (Zeiss LMS880, Carl Zeiss Microscopy GmbH, Jena, Germany). For
160 temperature assays at 30°C, we used an Axio Zoom V16 fluorescence stereomicroscope
161 (Carl Zeiss Microscopy GmbH, Jena, Germany). Specific GFP fluorescence was detected
162 using short-pass filter (excitation filter, 470/40 nm ; barrier filter, 525/50 nm). To detect all
163 UV-fluorescent cell components including chlorophyll and GFP, a long-pass filter (exci-
164 tation filter, 572/25 nm ; barrier filter, 629/62 nm) was used. Images were obtained using
165 digital video camera (AxioCam 506 mono) coupled to the Axio Zoom microscope.
166
167

168 2.6 RNA isolation and RT-PCR analyses

169 For BPMV RNA detection, inoculated and systemic leaves from BPMV-inoculated
170 plants were sampled at 7 dpi and 14 dpi or 21 dpi, respectively. Total RNA was extracted
171 using the NucleoSpin RNA kit (Macherey-Nagel, Hoerd, France). RNA concentrations
172 were determined by measuring the absorbance at 260 nm on a NanoDrop 8000 (Thermo
173 Fisher Scientific, Waltham, USA) and integrity was checked by electrophoresis on a 1%
174 agarose BET gel. cDNA was synthesized from 1 µg of total RNA using Reverse Tran-
175 scription (RT) ImProm-II™ enzyme (Promega Corp., Madison, USA) and Oligo-dT
176 (Promega Corp., Madison, USA) according to the manufacturer's protocol and finally
177 diluted 2.5 times in Milli-Q H₂O.

178 Semi-quantitative RT-PCR was performed on 1 µL of diluted cDNA using GoTaq G2
179 Flexi (Promega Corp., Madison, USA) and using primers specific to BPMV RNA1, RNA2,
180 *PvUBIQUITIN* (*PvUBI*, reference gene) and *PvINSULIN-DEGRADING ENZYME* (*PvIDE*)
181 (reference gene; primers IDE-F 5'-GCAACCAACCTTTCATCAGC-3' and IDE-R
182 5'-AGAAATGCCTCAACCCTTG-3') as described previously [41]. After either 20 cycles
183 for primers RNA1 and RNA2 or 25 cycles for primers *PvUBI* and *PvIDE*, PCR products
184 were analysed by electrophoresis on a 2% agarose BET gel.

185 BPMV virus titer was estimated using a method based on a quantitative RT-PCR
186 (RT-qPCR) analysis by determining the quantity of BPMV RNA1 and plant *PvIDE*
187 mRNA, in four biological and three technical replicates (unless otherwise stated), in or-
188 der to obtain a ratio of virus RNA to plant RNA. RT-qPCR protocol and analyses were
189 performed as described in Richard et al. [45]. Briefly, RT-qPCR analysis was performed
190 with a LightCycler® 96 instrument in a volume of 15 µL reaction containing 2 µL of di-
191 luted cDNA, each specific primer with a final concentration of 0.1 µM, 7.5 µL of SYBR
192 Green (LightCycler® 480 SYBR Green I Master, Roche) and distilled water. The program
193 used consisted of initial denaturation at 95°C for 5 min, 50 cycles of 15 s of denaturation,
194 15 s of hybridization and 15 s of elongation at 95°C, 60°C, and 72°C, respectively. The
195 results were analysed using LightCycler® 96 software version 1.1.
196

197 3. Results

198 3.1. R-BPMV-mediated resistance is associated with local HR lesions at 20°C where 199 BPMV is able to multiply in a first step

200 In previous work, we have shown that the Mesoamerican genotype BAT93 is re-
201 sistant to BPMV and that resistance is controlled by a major gene, named *R-BPMV* [41]. In
202 order to characterize further BAT93 resistance phenotype, we performed inoculation as-

says using BPMV-WT and BPMV-GFP, a BPMV construct expressing GFP as a mature protein processed from the viral RNA2 polyprotein. We observed that at 7 dpi with BPMV-WT, a large number of macroscopic HR lesions developed in the inoculated leaves of BAT93 at 20°C, whereas mosaic symptoms were visible in JaloEEP558 and Black Valentine, two susceptible genotypes lacking the *R-BPMV* gene (Figure 1A). Trypan blue staining assays at 7 dpi confirmed that HR lesions contained death cells (Figure 1B), thus attesting that these cells have undergone a cell death process induced by the *R-BPMV* gene. In order to study if BPMV can multiply in some individual cells before the establishment of HR lesions, we scanned the upper surface of BPMV-GFP inoculated leaves of BAT93 at 4 dpi using an epifluorescence microscope. JaloEEP558 was used as a susceptible genotype. Interestingly, GFP fluorescence was detected in both BAT93 and JaloEEP558 at 4 dpi in inoculated leaves (Figure 1C). Widespread fluorescence was observed in JaloEEP558 leaves, whereas in BAT93, GFP fluorescence was found concentrated in a few number of discrete areas that we will further call ‘GFP foci’ (Figure 1C). This suggests that resistance conferred by *R-BPMV* in BAT93 allows BPMV multiplication in small cell clusters but finally restricts virus spread, putatively by blocking virus cell-to-cell movement and/or inducing programmed cell death.

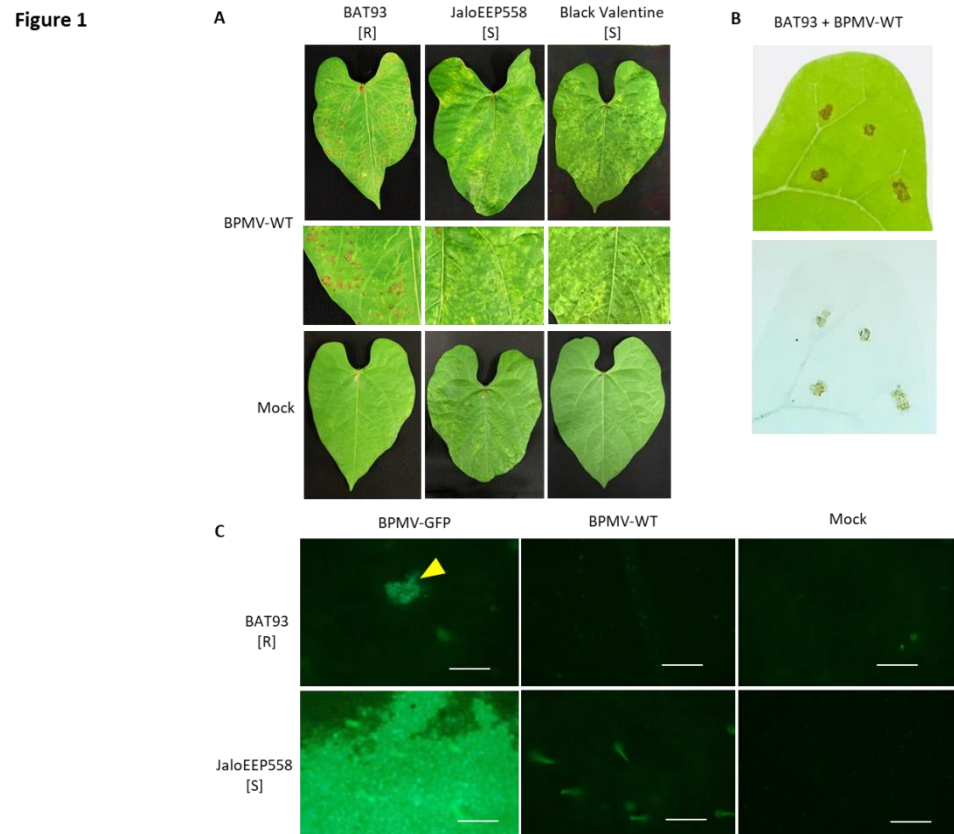


Figure 1: BPMV is able to multiply and move from cell-to-cell in the inoculated leaves of genotype BAT93 at 20°C but infection is finally restricted by the induction of local Hypersensitive-Response (HR) lesions

(A) Representative pictures of local HR lesions on six BAT93-inoculated leaves of six different plants grown at 20°C, 7 days post-inoculation (dpi) with BPMV-WT. JaloEEP558 and Black Valentine were used as susceptible control genotypes on which mosaic/mottling symptoms are visible on the inoculated leaves at 7 dpi with BPMV-WT. Mock was used as control. This experiment was performed at least 3 times with similar results. (B) Local HR lesions (top) and lesions visualized using trypan blue staining (bottom) on BAT93 leaves inoculated with BPMV-WT at 20°C. Pictures were taken at 7 dpi. (C) Microscopic observations of GFP fluorescence in six inoculated leaves of six different plants of either BAT93 or JaloEEP558, both grown at 20°C. Observations were made at 4 dpi. The BPMV-GFP construct expressing GFP as an individual protein processed from the RNA2 polyprotein was used as a reporter of BPMV infection. BPMV-WT and Mock were used as control. Yellow triangle indicates GFP foci. Autofluorescence is visible with BPMV-WT and Mock assays. All infection assays were made at 20°C. Scale bars = 250 µm.

3.2 The *R-BPMV* gene segregates as an incompletely dominant gene

203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220

221

222
223
224

225 To determine the inheritance of *R-BPMV*, we investigated the segregation of resis-
226 stance *versus* susceptibility to BPMV in a F2 population of 60 plants derived from the
227 cross BAT93 (resistant to BPMV) xJaloEEP558 (susceptible to BPMV). Each F2 plant was
228 inoculated with the construct BPMV-GFP and resistant/susceptible phenotypes were
229 scored at 7 to 10 dpi. The observed segregation ratio was 56 resistant : 4 susceptible. The
230 goodness-of-fit test indicated a significant deviation ($\chi^2_{1df}=10.74$, $P=0.001$) from the ex-
231 pected Mendelian ratio (3:1) with a deficit of susceptible phenotypes. Thus, the *R-BPMV*
232 gene segregates as an incompletely dominant gene.

233 3.3. At 25°C and 30°C, R-BPMV induces more expanded local HR lesions

234 In previous work, we showed that *R-BPMV* is closely linked to the *I* gene at one end
235 of chromosome 2 of *P. vulgaris* [41]. It is known that *I*-mediated resistance is associated
236 with a systemic necrosis that may kill the host when infected by either BCMV above 28°C
237 or BCMNV regardless of the temperature [46–48]. Thus, the resistance phenotype con-
238 ferred by the *I* gene in response to BCMV infection is temperature-dependent and
239 switches at 28°C from extreme resistance to systemic necrosis. In order to check if the re-
240 sistance mediated by *R-BPMV* is dependent on temperature, we studied the resistance
241 phenotype at 25°C and 30°C. We first performed inoculation assays using BPMV-WT on
242 the resistant genotype BAT93. We observed that the resistance phenotype is expressed as
243 local HR lesions in the inoculated leaves at all tested temperatures but that HR lesions
244 become more expanded as the temperature increases (Figure 2A). Nevertheless, no vas-
245 cular systemic necrosis appears at 14 dpi in apical parts of the inoculated plants (Figure
246 2A). Moreover, no viral symptoms were visible in these apical parts (Figure 2A) and no
247 viral RNAs were detected in systemic leaves of BAT93-inoculated plants at all tempera-
248 tures (Figure S1). Thus, in our pathosystem, BPMV was blocked in the inoculated leaves
249 and could not spread systemically at all tested temperatures (20°C, 25°C, and 30°C). In
250 conclusion, no switch of phenotype was observed in BAT93 when inoculated with BPMV
251 at either 25°C or 30°C.

252 In order to assess if larger HR lesions at elevated temperature were correlated with
253 more expanded multiplication area of BPMV, we scanned the upper surface of
254 BPMV-GFP inoculated leaves of BAT93 at 4 dpi using an epifluorescence microscope
255 (25°C) or Axio Zoom microscope (30°C). JaloEEP558 was used as a susceptible genotype
256 [41]. As expected, at 4 dpi, many GFP foci were detected on the upper surface of
257 BPMV-GFP inoculated leaves of BAT93 at 25°C and 30°C (Figure 2B and 2C). Overall, the
258 GFP foci approximately double when shifting from 20°C to 25°C and from 25°C to 30°C.
259 Thus, BPMV is still able to multiply and to move from cell-to-cell in BAT93 at 25°C and
260 30°C. Consequently, the plant defense response mediated by *R-BPMV* seems to become
261 less efficient when temperature increases from 20°C to 30°C as larger GFP foci are ob-
262 served.
263

Figure 2

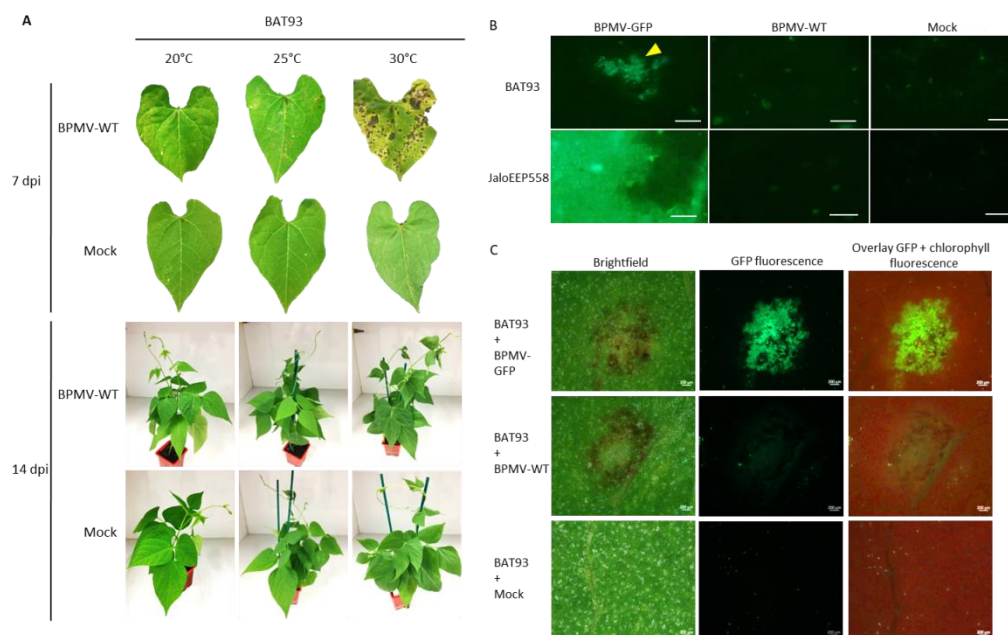


Figure 2: Elevated temperatures promote BPMV multiplication and cell-to-cell movement in the inoculated leaves of cv. BAT93 and larger local Hypersensitive-Response lesions are induced

(A) Representative pictures of local HR lesions on six BAT93-inoculated leaves from six different plants at 20°C, 25°C and 30°C, at 7 days post-inoculation (dpi) with BPMV-WT (upper panel) and six whole plants of BAT93 at 20°C, 25°C and 30°C, 14 dpi with BPMV-WT (lower panel). Mock was used as control. This experiment was performed at least 3 times with similar results. (B) Microscopic observations of BPMV-GFP accumulation in inoculated leaves of BAT93 and JaloEEP558 grown at 25°C. The BPMV-GFP construct expressing GFP as an individual protein processed from the RNA2 polyprotein was used as a reporter of BPMV accumulation. BPMV-WT and Mock were used as control. All infection assays were made at 25°C. Observations of GFP fluorescence (indicated by a yellow triangle) were made at 4 dpi and are representative of six BAT93-inoculated leaves sampled on six different plants. Scale bars = 250 µm. (C) Microscopic observations of BPMV-GFP accumulation in inoculated leaves of BAT93 grown at 30°C. The BPMV-GFP construct expressing GFP as an individual protein processed from the RNA2 polyprotein was used as a reporter of BPMV accumulation. From left to right: brightfield, BPMV-GFP accumulation (green), overlay of chlorophyll fluorescence (red) and BPMV-GFP accumulation (green). BPMV-WT and Mock were used as control. All infection assays were made at 30°C. Observations of GFP fluorescence were made at 4 dpi and are representative of six BAT93-inoculated leaves sampled on six different plants. Scale bars = 200 µm.

Supplementary Figure 1

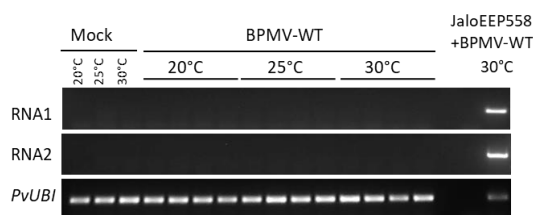


Figure S1: *P. vulgaris* cv. BAT93 is resistant to BPMV systemic infection

Semi-quantitative RT-PCR of BPMV RNA1 and RNA2 in systemic leaves of BAT93. Plants were inoculated with either mock (M) buffer or BPMV-WT at 20°C, 25°C and 30°C. *PvUBI* was used as an internal control. Total RNA was extracted at 21 dpi from a pool of four systemic leaves (third trifoliolate) sampled on four different plants of BAT93. A sample of inoculated leaves of JaloEEP558+BPMV-WT at 30°C was taken as positive control for PCR amplification.

3.4. The resistance mediated by R-BPMV is heat-stable up to 35°C in BAT93 but local HR lesions are replaced by chlorotic lesions

It is known that some dominant R-genes against viruses are overcome at elevated temperatures (e.g. at 28°C for *N*, at 30°C for *Tsw*) [22,23,49] meaning that resistance status of the genotype is broken and systemic infection of upper leaves occurs leading to a

264

265

266

267

268

269

270

271

272

273

274 susceptible state. We found that *R-BPMV*-mediated resistance was still efficient at tem-
 275 peratures up to 30°C but we wanted to test whether higher temperatures (35°C) could
 276 break the resistance conferred by *R-BPMV*. For this purpose, we inoculated BAT93 plants
 277 with BPMV-WT and BPMV-GFP and placed the plants at constant 35°C. As expected, at 7
 278 dpi, HR lesions developed on inoculated leaves of BAT93+BPMV-WT at 30°C and GFP
 279 fluorescence was macroscopically detected on inoculated leaves of BAT93+BPMV-GFP
 280 (Figure 3A). By contrast, at 35°C, only chlorotic lesions (*i.e.* light-green or yellow local le-
 281 sions with no visible cell death/necrosis) were visible at 7 dpi on inoculated leaves of
 282 BAT93+BPMV-WT. This appears to correspond to large infection areas as confirmed by
 283 expanded GFP foci visible in inoculated leaves of BAT93+BPMV-GFP (Figure 3A).
 284 Quantification of virus titer by RT-PCR in the inoculated leaves of BAT93 confirmed a
 285 higher accumulation of viral RNAs at 35°C compared to 30°C and 20°C (Figure 3B and
 286 3C).

287 To ensure that no virus moved systemically, we studied systemic leaves of BAT93
 288 plants grown at 35°C and inoculated with either BPMV-WT or BPMV-GFP. No viral
 289 RNAs were detected in systemic leaves at 7 dpi and no GFP fluorescence was visible
 290 (Figure 3D and 3E), attesting that, albeit local infection is of higher intensity, the systemic
 291 infection is prevented. Furthermore, no systemic necrosis occurred (Figure 3E). In paral-
 292 lel, we used the susceptible genotype Black Valentine as a control to assess if at 35°C,
 293 BPMV-GFP conserved infectiousness and capacity for systemic movement. We observed
 294 that GFP fluorescence was widespread in all systemic leaves of Black Valentine plants at
 295 35°C 7 dpi, including stems, while no GFP was detected in systemic leaves of plants
 296 grown at 20°C (Figure S2). Overall, our results demonstrate that *R-BPMV* is heat-stable
 297 up to 35°C. The *R-BPMV*-mediated phenotype is expressed as chlorotic lesions in the
 298 inoculated leaves and no symptoms are detected in the systemic parts.
 299

Figure 3

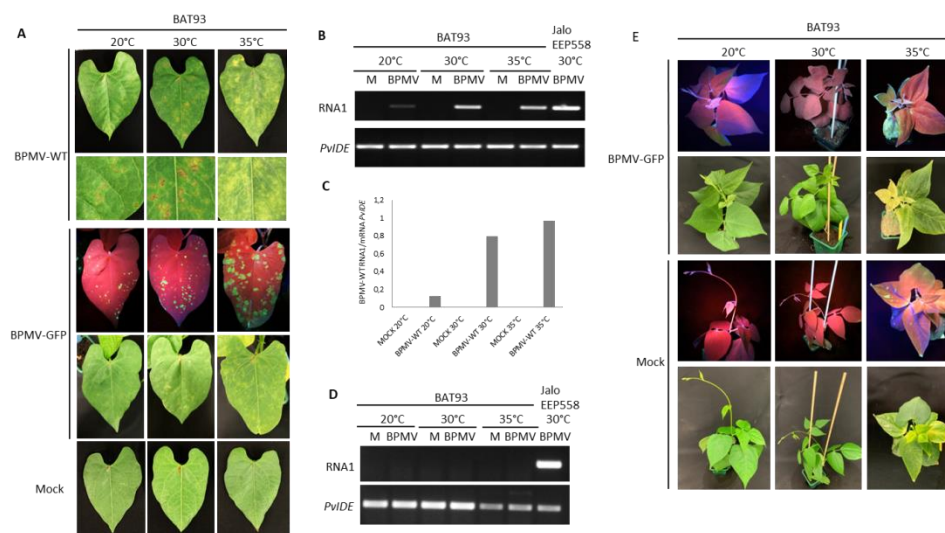


Figure 3: The *R-BPMV* gene of BAT93 is still efficient to confer BPMV resistance at 35°C

(A) Representative pictures of BAT93-inoculated leaves at 20°C, 30°C and 35°C, 7 days post-inoculation (dpi) with BPMV-WT (upper panel; six plants), BPMV-GFP (middle panel; six plants) and Mock (lower panel; six plants). BPMV-GFP was detected under UV light. This experiment was performed at least three times with similar results. (B) Semi-quantitative RT-PCR of BPMV RNA1 (upper panel) in BAT93-inoculated leaves. Plants were inoculated with either mock (M) buffer or BPMV-WT (BPMV) at 20°C, 30°C and 35°C. *Pvldc* was used as an internal control. Total RNA was extracted at 7 dpi from a pool of three BAT93-inoculated leaves sampled on three different plants. A sample of inoculated leaves of JaloEEP558 at 30°C was taken as positive control for PCR amplification. (C) Quantification of BPMV titer at 7 dpi in BPMV-WT-inoculated leaves of BAT93 by calculation of the relative ratio of BPMV RNA1 to plant mRNA of *Pvldc* using a quantitative RT-PCR procedure. Data are mean ratios of pools of three BAT93-inoculated leaves sampled on three different plants. (D) Semi-quantitative RT-PCR of BPMV RNA1 (upper panel) in systemic leaves of BAT93 plants inoculated with either mock (M) buffer or BPMV-WT (BPMV) at 20°C, 30°C and 35°C. *Pvldc* was used as an internal control. Total RNA was extracted at 14 dpi from a pool of two systemic leaves (third and fourth trifoliolate) sampled on two-three different plants of BAT93. A sample of inoculated leaves of JaloEEP558+BPMV-WT at 30°C was taken as positive control for PCR amplification. This experiment was performed twice with similar results. (E) Representative pictures of six whole plants of BAT93 at 20°C, 30°C and 35°C, 11 dpi with either BPMV-GFP (upper panel) or mock buffer (lower panel). BPMV-GFP was detected under UV light.

Supplementary Figure 2



Figure S2: BPMV is still infectious at 35°C and spreads systemically in the susceptible genotype *P. vulgaris* cv. Black Valentine

Representative pictures of four whole plants of Black Valentine respectively grown at 20°C and 35°C, 7 dpi with BPMV-GFP and observed under UV light (upper panel) or natural light (lower panel).

3.5. Elevated temperatures boost BPMV infection in susceptible genotypes

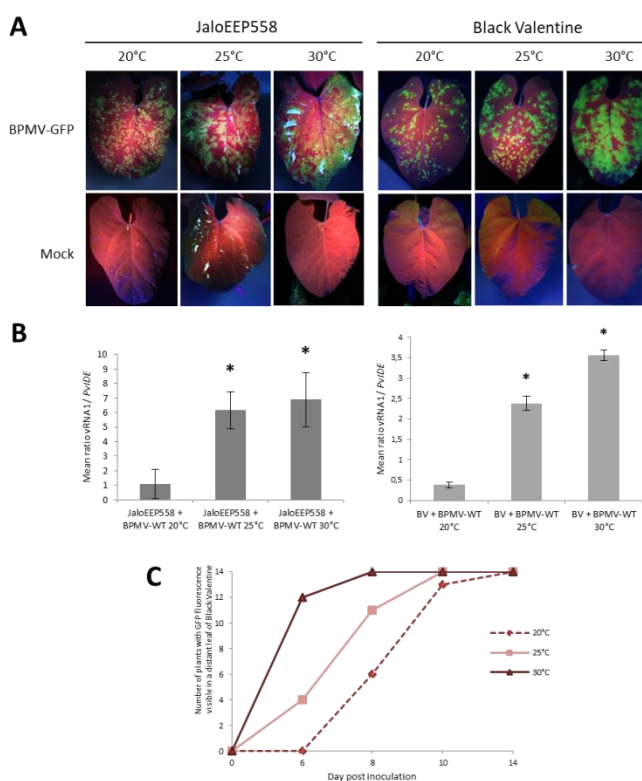
In the resistant genotype BAT93, elevated temperatures (especially 35°C) promoted viral replication and cell-to-cell movement in the inoculated leaves (Figure 3A). Therefore, we investigated the effect of elevated temperatures on BPMV infection in the two susceptible genotypes, JaloEEP558 and Black Valentine. We followed the BPMV-GFP accumulation by visual inspection of plants inoculated with BPMV-GFP grown either at 25°C or 30°C. Compared to control plants at 20°C, GFP foci at 25°C and 30°C on inoculated leaves of JaloEEP558 and Black Valentine at 7 dpi were more expanded and confluent on the upper side of the leaves (Figure 4A). Quantification of BPMV RNA1 in leaves inoculated with BPMV-WT confirmed the higher accumulation of BPMV at 25 and 30°C compared to 20°C (Figure 4B). More precisely, significantly more RNAs were detected at 25°C and 30°C compared to 20°C (~6 fold- and ~7-9-fold increase, respectively) (Figure 4B). Consequently, BPMV replication and cell-to-cell movement is more efficient from 0 to 7 dpi in the susceptible genotypes JaloEEP558 and Black Valentine at elevated temperature (25°C and 30°C) compared to 20°C.

Furthermore, the effect of temperature on the rate of systemic spreading of BPMV-GFP was investigated by scoring the date of appearance of GFP foci on systemic

323
324
325
326
327
328
329
330
331
332

leaves of Black Valentine plants at 20°C, 25°C, and 30°C between 6 and 14 dpi. From 6 dpi to 10 dpi, the systemic spreading is faster at 30°C compared to 25, and at 25°C compared to 20°C (Figure 4C). At 10 dpi, GFP foci in systemic leaves of plants at 20°C were tiny, whereas GFP fluorescence was widespread at 25°C and 30°C (Figure S3A). At 14 dpi, all plants at 20°C, 25°C, and 30°C were systemically infected by BPMV-GFP (Figure 4C). High levels of fluorescence were visible in all inoculated parts of plants grown at 25°C and 30°C (Figure S3B). These results confirm that BPMV systemic movement becomes faster when temperature increases from 20°C to 30°C, resulting in a higher disease incidence in susceptible genotypes.

Figure 4



333
334

Figure 4: Elevated temperatures promote BPMV infection in the inoculated leaf and BPMV systemic spreading in whole plants of two susceptible genotypes of *P. vulgaris*

(A) Representative pictures of four inoculated leaves from four different plants of either JaloEEP558 or Black Valentine (left and right panel, respectively) at 20°C, 25°C and 30°C, 7 days post-inoculation (dpi) with BPMV-GFP (upper panel) and Mock (lower panel). BPMV-GFP was detected under UV light. This experiment was performed at least three times with similar results. (B) Quantification of BPMV titer at 7 dpi in four BPMV-WT-inoculated leaves of four different plants of JaloEEP558 or Black Valentine (BV) by calculation of the relative ratio of BPMV RNA1 to plant mRNA of *PvIDE* using a quantitative RT-PCR procedure. Asterisks indicate significant differences between the tested temperature and 20°C, the control temperature (t-test, p-value<0,05). No significant difference of viral titer was found between the two elevated temperatures 25°C and 30°. Data are mean ratios ± SD of four biological replicates. (C) BPMV-GFP systemic spreading in whole plants of Black Valentine at 20°C, 25°C, and 30°C. The graph represents the number of plants with GFP fluorescence visible in a distant leaf scored at four dates after inoculation with BPMV-GFP: 0, 6, 8, 10, and 14 dpi on a total of fourteen plants per temperature assay. BPMV-GFP was detected under UV light. This experiment was performed twice with similar results.

335
336

Supplementary Figure 3

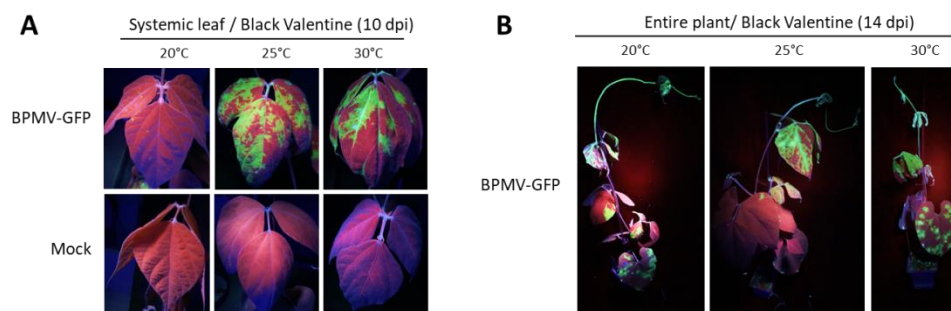


Figure S3: Elevated temperatures promote BPMV spreading in systemic leaves and in whole plants in the susceptible genotype *P. vulgaris* cv. Black Valentine

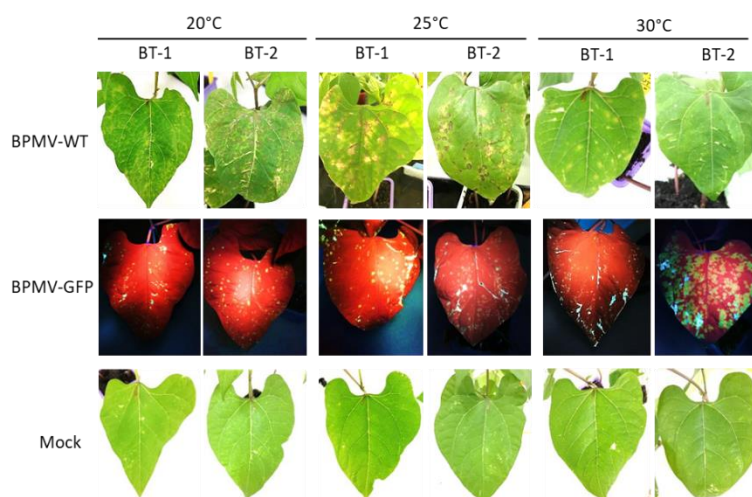
(A) Representative pictures of fourteen systemic leaves of Black Valentine grown respectively at 20°C, 25°C and 30°C, 10 days post-inoculation (dpi) with BPMV-GFP (upper panel) or Mock (lower panel). BPMV-GFP was detected under UV light. (B) Representative pictures of fourteen whole plants of Black Valentine at 20°C, 25°C and 30°C, 14 dpi with BPMV-GFP observed under UV light. Both experiments have been performed at least three times with similar results.

3.6. BT-1 and BT-2, two Near Isogenic Lines for the *I* locus are both resistant to BPMV systemic movement

The *R-BPMV* gene is closely linked to *I*, a famous locus conferring a broad-spectrum resistance to at least ten different *Potyvirus*s (reviewed in [38]) and several *Comovirus*s. In this work, we showed that *R-BPMV* segregates as an incompletely dominant gene. BT-1 (resistant to BCMV, *I/I*) and BT-2 (susceptible to BCMV, *i/i*) are two near isogenic lines created to study the *I* locus [42]. We tested them to decipher the resistance mechanism mediated by the *R-BPMV* gene at the tissue and cell levels as done for the *I* locus [50–52]. We challenged BT-1 and BT-2 for their resistance/susceptibility to BPMV at three temperatures: 20°C, 25°C and 30°C.

At 7 dpi, local HR lesions developed on inoculated leaves of BT-1 and BT-2 at 20°C and 25°C (Figure 5). As in BAT93, these local HR lesions corresponded to infection areas in which BPMV can first multiply since small GFP foci were visible after inoculation with BPMV-GFP in BT-1 and BT-2 (Figure 5). Thus, both BT-1 and BT-2 behave as resistant genotypes to BPMV at 20°C, 25°C and 30°C, and this is strengthened by the observation that no BPMV RNAs were detected in systemic leaves at 21 dpi (Figure S4) as well as no viral symptoms or systemic necrosis (data not shown). In conclusion, BT-1 and BT-2 won't be a useful tool to dissect *R-BPMV*-mediated resistance at the cellular level.

Figure 5



337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356

357

Figure 5: BT-1 and BT-2, two Near Isogenic Lines for the *I* locus are both resistant to BPMV systemic movement

Representative pictures of four BT-1- and four BT-2-inoculated leaves (sampled on four different plants) grown respectively at 20°C, 30°C and 35°C, 7 days post-inoculation (dpi) with BPMV-WT (upper panel), BPMV-GFP (middle panel), and Mock (lower panel). BPMV-GFP was detected under UV light. This experiment was performed twice with similar results.

Supplementary Figure 4

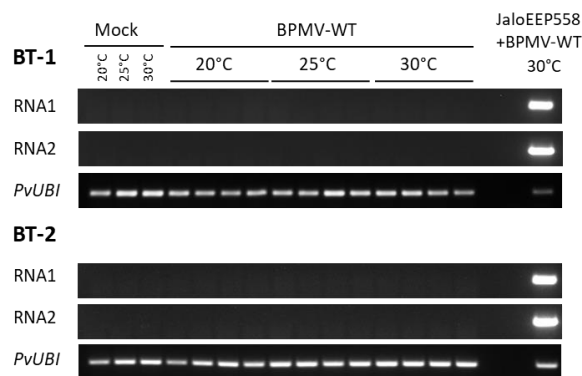


Figure S4: *P. vulgaris* genotypes BT-1 and BT-2, two Near Isogenic Lines for the *I* locus, are both resistant to BPMV systemic infection

Semi-quantitative RT-PCR of BPMV RNA1 and RNA2 in systemic leaves of BT-1 (upper panel) and BT-2 (lower panel). Plants were inoculated with either mock (M) buffer or BPMV-WT at 20°C, 25°C and 30°C. *PvUBI* was used as an internal control. Total RNA was extracted at 21 dpi from a pool of four systemic leaves (third trifoliolate) sampled on four different plants of either BT-1 or BT-2. A sample of inoculated leaves of JaloEEP558+BPMV-WT at 30°C was taken as positive control for PCR amplification. This experiment was performed twice with similar results.

4. Discussion

In this work, we studied the behavior of the *R-BPMV*-mediated resistance to BPMV in *P. vulgaris* at normal (20°C) and elevated temperatures (25°C, 30°C, and 35°C). Strikingly, we found that *R-BPMV*-mediated resistance is heat-stable up to constant 35°C at the whole plant level (Figure 3), in the sense that no systemic infection is observed and that BPMV remains confined to the inoculated leaf.

This is in agreement with results obtained for other plant-virus pathosystems for which heat-stable *R* genes have been described. In pepper, different natural allelic variants from the same gene may have different behaviours as reported for the *L^{1c}* allele which is heat-stable at 30°C whereas other alleles (*L¹*) are not [53]. More recently, the potato NLR *Rx1* gene was reported to confer a temperature-insensitive resistance to PVX in *Nicotiana benthamiana* that is not overcome at temperatures up to 32°C, a temperature at which the virus was no longer infectious [24]. Additional heat-stable *R* gene-mediated resistance has also been reported for other pathogens than viruses. For instance, the tomato *Mi-9* gene confers a heat-stable resistance to root-knot nematodes (*Meloidogyne* sp.) [54,55], although being homologous to the NLR *Mi-1* which is heat sensitive [56]. In pepper, several heat-stable *R* genes against *Meloidogyne* species, named *Me*, have been reported [57,58]. In *Solanum* species, *Ry_{sto}* and *Ry_{chc}* *R* genes present in *S. stoloniferum* and *S. chacoense* respectively, confer extreme resistance to the Tobacco vein necrosis strain of PVY (PVY^N) and are functional at both low (16–20°C) and elevated temperatures (above 24°C) [59,60].

On the other hand, temperature sensitivity has more often been reported in the literature. In tobacco, the NLR *N* is unable to confer resistance to the Tobacco mosaic virus (TMV) above 28°C [22,49]. Accordingly, the *Capsicum* sp. NLR *Tsw* fails to trigger re-

386 sistance to *Tomato spotted wilt virus* (TSWV) at 32°C and above [23,61]. In potato, some *R*
387 genes conferring HR to PVY including *Ny* in *Solanum sparsipilum* and *S. sucrense*, or *Ny-1*
388 in *S. tuberosum* cv. Rywal, confer resistance only at low temperatures (16–20°C) whereas
389 at higher temperatures (24–28°C), resistance is inhibited and PVY infects plants system-
390 ically [60]. Concerning the other kinds of pathogens, temperature sensitivity has been
391 reported for the NLR *Mi-1* in tomato (resistance to *Meloidogyne incognita*, [62]), for the
392 NLR *Bs2* in pepper (resistance to *Xanthomonas axonopodis* pv. *vesicatoria*, [63]), the
393 non-NLR *Cf-4* and *Cf-9* genes (resistance to *Cladosporium fulvum*, [64,65]) as well as for
394 many *R* genes against bacteria in Arabidopsis (*e.g.* *RPS2* and *RPM1* resistance to *Pseu-*
395 *domonas syringae*, [66]; *RPS2* and *ZAR1* resistance to *P. syringae*, [67]; *RPS4/RRS1-R* re-
396 sistance to *Ralstonia solanacearum*, [68] *SNC1* and *RPS4* resistance to *P. syringae* and
397 *Peronospora parasitica*, [69–71]. All these data converge to assess that temperature sensi-
398 tivity of resistance is widespread whereas heat-stable *R* genes are scarce.

399 Mechanisms underlying heat-sensitivity are poorly understood. Zhu et al. [70]
400 demonstrated that the *R* proteins, involved in the recognition of pathogen effectors, can
401 be themselves the causal temperature-sensitive component in defense responses. Indeed,
402 autoimmunity-associated mutations (*e.g.* mutants in which ETI and spontaneous cell
403 death are constitutively activated) in *SNC1*, an Arabidopsis NLR-homolog, cause in-
404 creased *SNC1* nuclear localization at 22°C. On the contrary at 28°C, the reduced nuclear
405 localization is associated with suppression of autoimmune phenotypes. Similarly, nu-
406 clear localization of the *R* protein *N* of tobacco was observed after recognition of the TMV
407 coat protein at 22°C but not at 28°C [70]. When the two *SNC1* mutations were introduced
408 into the *N* gene, resistance was effective at elevated temperature thus suggesting that
409 these mutations may prevent the temperature-sensitive conformational loss of function
410 of the NLR *N* [70] and maintaining its interaction with the TMV coat protein [22,49]. It is
411 not excluded that such differential point mutations could exist between *R-BPMV* and *I*
412 genes and one way to confirm this hypothesis would be to clone their corresponding
413 gene sequences. Other putative mechanisms of temperature sensitivity have been pro-
414 posed such as homeostasis of *R*-interacting chaperons, reduced *R* protein amounts re-
415 sulting in a lower *R* activity, deregulation of nucleo-cytoplasmic localization of *R* pro-
416 teins (reviewed in [18]). More recently, implication of the methionine cycle [72] and de-
417 regulation of the intercellular communication *via* plasmodesmata [73] have also been
418 supposed to influence virus spread within their hosts.

419
420 Our results show that the major gene *R-BPMV* in *P. vulgaris* genotype BAT93 confers
421 resistance to BPMV by inducing local HR lesions at 20°C where BPMV is able to multiply
422 in a first step (Figure 1). HR lesions have also been observed at 25°C and 30°C 7 dpi
423 (Figure 2). Interestingly, at 7 dpi we observed larger HR lesions at 25°C and 30°C com-
424 pared to 20°C, and this observation was correlated with i) more expanded multiplication
425 areas of BPMV visualised as green fluorescence areas in BPMV-GFP infected leaves at 4
426 dpi (Figure 2), and ii) a rising virus titer in the inoculated leaves at 7 dpi (Figure 3B and
427 3C). Consequently, we propose that the higher levels of BPMV accumulation and
428 cell-to-cell spreading in the primary sites of infection could be attributed to a delayed
429 defense response when temperature increases from 20°C to 30°C. As biotrophic patho-
430 gens, viruses mainly induce defense responses regulated by salicylic acid (SA) signaling
431 [74] and SA was shown to be a key component that orchestrates the events restricting
432 viral spread in HR [75,76]. Moreover, HR-mediated resistance against *Turnip crinkle virus*
433 (TCV) was impaired in Arabidopsis *eds5* and *sid2* SA-deficient mutants, without affecting
434 HR cell death [77]. Lukan et al. [78] showed that *Potato virus Y* (PVY, *Potyvirus*) spread is
435 even faster in SA-depleted plants (*NahG*-potato transgenic plants) with rapid lesion ex-
436 pansion. Interestingly, the expression of SA-dependent responses were reduced at ele-
437 vated temperature in tobacco and potato plants infected with *Cucumber mosaic virus*
438 (CMV) and PVY, respectively [79,80]. Whether the SA accumulation or other defenses are
439 inhibited or downregulated at elevated temperature needs further work in our patho-

440 system. Moreover, it has been shown that virus resistance is uncoupled from cell death
441 and that these two events are likely independent [77,78,81–87]. For example, in potato
442 plants bearing the temperature-sensitive *Ny-1 R* gene against PVY, temperature shift as-
443 says from 22°C to 28°C induced the detection of PVY in infected cells outside the cell
444 death zone [78]. Nevertheless, the molecular mechanisms restricting both virus spread
445 and cell death remain unknown [88].

446 Interestingly, at 35°C, HR lesions that developed on BPMV-inoculated leaves of
447 BAT93 were replaced by chlorotic lesions, while the *R-BPMV*-mediated resistance re-
448 mains functional at the whole plant level (Figure 3). We confirmed that BPMV is still in-
449fectious and capable of systemic movement at 35°C (Figure S2). Thus, it seems that cell
450 death is completely abolished at 35°C whereas the local defense response is still efficient
451 to block BPMV systemic infection (Figure 3E). The reduction of cell death at elevated
452 temperature has already been reported in soybean (*Glycine max*) infected by strains G1
453 and G7 of *Soybean mosaic virus* (SMV) [89]. Indeed, a stem-tip necrosis (STN) develops in
454 several soybean cultivars carrying the *R* genes *Rsv1* and *Rsv1-n*. The STN in genotype
455 V262 induced by strain G1 and in genotype V94-3971 by strain G7 developed at 10°C,
456 15°C, 20°C, 25°C, and 30°C, on 44, 41, 14, 8 and 8 days after inoculation, respectively. By
457 contrast, at 35°C, no STN reaction and consequently no cell death, was induced by any
458 strain. Interestingly, cell death reduction at elevated temperature has also been reported
459 for plant-bacteria interactions. *AvrRpt2*-mediated cell death in *Dex-AvrRpt2* plants was
460 significantly reduced at 28°C and was almost completely abolished at 32°C [66]. Inter-
461 estingly, the the expression of the *WORKY46* transcription factor acting as an upstream
462 regulator of SA metabolism was also temperature-sensitive with a peak at 16°C and a
463 decrease at temperatures above 28°C. In the same way, *AvrRpm1*- and *AvrB*-mediated
464 cell death was significantly attenuated at elevated temperatures (32°C) [66]. Thus, the
465 data indicate that elevated temperature could suppress ETI signaling in *R* gene-mediated
466 response.

467
468 We previously showed that *R-BPMV* is present in genotype BAT93 of *P. vulgaris* and
469 that this gene is genetically linked to the *I* locus on chromosome 2 [41,90]. The *I* locus is a
470 broad-spectrum resistance locus that confers resistance to BCMV, BCMNV [91,92], and to
471 nine other potyviral species (*Watermelon mosaic virus-2* [93,94], *Cowpea aphid-borne mosaic*
472 *virus* [93,95], *Soybean mosaic virus* [96,97], *Peanut mottle virus* [98,99], *Zucchini yellow mosaic*
473 *virus*, *Thailand passiflora mosaic virus* and *Passionfruit woodiness virus-K* [100], BYMV and
474 CIYVV [101,102]). Thus, we hypothesized that *R-BPMV* and *I* could correspond to the
475 same gene with pleiotropic effects and if so, one important question would be: do
476 *R-BPMV*- and *I*-mediated resistance have the same behavior regarding temperature?

477 *I* was reported to be temperature-sensitive for BCMV resistance [47,48]: an extreme
478 resistance or micro-HR phenotypes are observed on inoculated leaves below 28°C and
479 BCMV replication was effective [51], but above 28°C, the ER evolves into a systemic ne-
480crosis [48,50,52] (Figure 6). However, for BCMNV, a local HR in inoculated leaves
481 evolves into a systemic necrosis regardless of temperature [46–48] (Figure 6). With
482 BPMV, we observed local HR lesions in inoculated leaves of BAT93 at 20°C, 25°C and
483 30°C at 7 dpi in which BPMV is able to multiply in a first step (Figure 1 and 2). By con-
484 trast, at 35°C, chlorotic lesions develop in inoculated leaves with no BPMV detected in
485 the systemic leaves (Figure 3A), albeit BPMV is still infectious at 35°C in the susceptible
486 genotype BV (Figure S2) with a higher rate of systemic spread at 25°C, 30°C and 35°C
487 compared to 20°C (Figure S2 and S3). Thus, our results highlight a switch in the resis-
488 tance phenotype mediated by *R-BPMV* that occurs between 30°C and 35°C (Figure 6).
489 Compared to the *I* gene, this is a significant difference of behaviour of the two genetically
490 linked-resistance genes, since *I* induces local HR and systemic necrosis from 28°C with
491 BCMV detected in the inoculated leaves and systemic leaves [50,52] (Figure 6).

492 Overall, we show here that *R-BPMV*- and *I*-mediated resistance phenotypes do not
493 behave in the same way at elevated temperature. Thus, although genetically tight-

ly-linked, *BPMV* and *I* could correspond to two different genes. Another hypothesis is that resistance to *BPMV* and *BCMV* are controlled by the same gene but that the downstream signaling cascades differ, explaining the two contrasting kinds of resistance phenotypes. Neither *I* nor *R-BPMV* have been cloned but both were hypothesized to encode NLR receptors [90,91,103]. Consistent with this hypothesis, the resistance phenotypes are expressed for *I* as either extreme resistance or systemic necrosis or for *R-BPMV* as local HR that are hallmarks of ETI mediated by NLR receptors. Moreover, Vallejos et al. [91] reported an over-expression of several NLR genes of the *I* region after *BCMV* or *BCMN* inoculations suggesting a role of NLR genes in the response to *BCMV/BCMN*.

Finally, it is worth noting that both *R-BPMV* (our study) and *I* segregate as incompletely dominant genes [50,51,91]. Indeed, when investigating the inheritance of *R-BPMV* in a F2 population of 60 plants, we found a deficit of susceptible phenotypes (56 resistant : 4 susceptible) and thus, a significant deviation from the expected Mendelian ratio (3:1). A similar segregation deviation was reported for the *I* gene in a F2 population obtained from the cross Calima (susceptible Andean genotype) × Jamapa (resistant Mesoamerican genotype) [91]. Vallejos et al. [91] suggested that this phenomenon was a consequence of the partial compatibility of the Andean and Mesoamerican genomes interacting in this type of crosses [91].

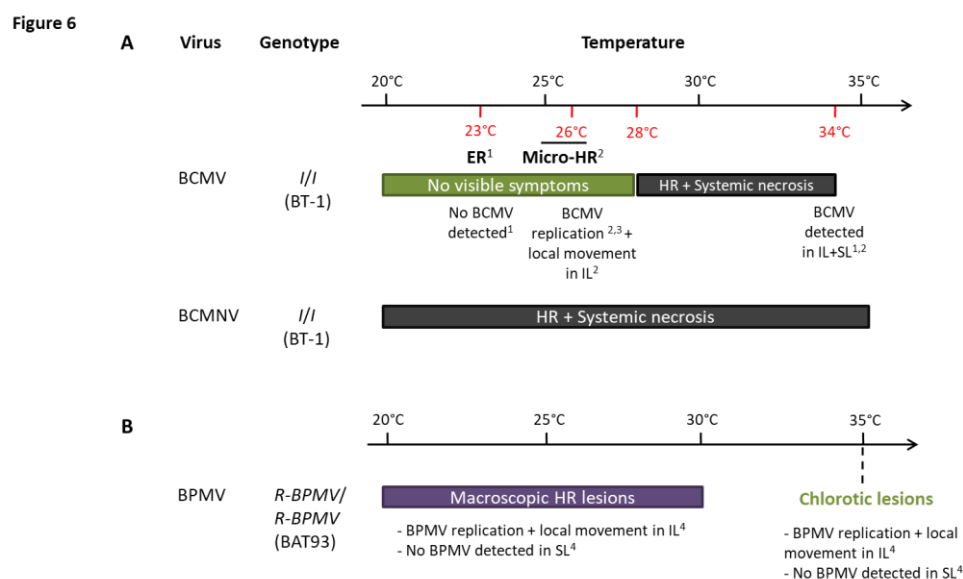


Figure 6: Resistance phenotypes of *R-BPMV*- and *I*-derived resistances do not share the same features regarding temperature

(A) Potyviruses *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMN)

(B) Comovirus *Bean pod mottle virus* (BPMV)

Abbreviations: ER: Extreme resistance, HR: Hypersensitive Reaction, IL: inoculated leaf, Micro-HR: microscopic Hypersensitive Reaction, SL: systemic leaf. References: 1: Collmer et al. 2000 (whole-plant study); 2: Cadle-Davidson and Jahn 2006 (whole-plant study); 3: Cadle-Davidson and Jahn 2005 (BCMV transfection in protoplasts); 4: this study

To study the effect of elevated temperatures in a *P. vulgaris*-BPMV compatible interaction, we performed experiments on two BPMV-susceptible genotypes *P. vulgaris* cv. Black Valentine and cv. JaloEEP558. We reported that in both genotypes, the area of primary infection sites at 7 dpi was larger on BPMV-GFP-inoculated leaves at 25°C and 30°C compared to 20°C and these results were correlated with a higher virus titer in these leaves (Figure 4A and 4B). Moreover, in Black Valentine, the virus spreads systemically even more rapidly when the temperature increases (Figure 4C). Overall, our results show a positive effect of temperature elevation on the level of susceptibility in two susceptible genotypes. Our results are in agreement with several other studies on different plant-virus interactions in compatible context. For example, Chung et al. [29] investigated

526 the effects of different temperature regimes on the speed of systemic spread after inocu-
527 lation of *Turnip mosaic virus* (TuMV) in Chinese cabbage. It took 48 days for systemic in-
528 fection to occur at 13°C but only 6 days at 22–33°C. Likewise, in *Solanum tuberosum*, plants
529 became 100% systemically infected at 24°C and 28°C while at 20°C only 20% of the plants
530 were systemically infected [26,27]. Nancarrow et al. [27] studied the effects of elevated
531 (10–21°C, night/ day) or ambient (5–16°C, night/day) temperature winter growing season
532 regimes on wheat plants infected with *Barley yellow dwarf virus* (BYDV, *Luteovirus*). In-
533 fected plants grown under elevated temperature developed virus symptoms earlier, and
534 had higher virus titers than plants grown at ambient temperature. In potato, susceptibil-
535 ity to PVY was dramatically increased in systemic infected leaves at higher temperatures
536 [80]. In cassava, geminiviruses responsible for cassava mosaic disease cause more
537 symptoms and have higher viral titer at 25°C compared to 30°C [32]. Nevertheless, other
538 studies have shown opposite results showing a negative effect on the level of suscepti-
539 bility in susceptible genotypes. In *N. benthamiana*, plants infected with the potyviruses
540 PVY and *Potato virus A* had fewer symptoms and reduced CP protein accumulation at
541 20°C compared to 30°C [104]. In the same way, Del Toro et al. [30] worked in *N. ben-*
542 *thamiana* infected with PVY and PVX and showed attenuated symptoms for PVY and no
543 symptoms for PVX at 30°C compared to 25°C. Aguilar et al. [105] described that elevated
544 temperatures decrease both virulence and virus titers in the synergistic infection PVX /
545 *Plum pox virus* (PPV) in *N. benthamiana*. Importantly, the RNA silencing machinery was
546 shown to be more active at elevated temperatures in several plants including cassava and
547 *N. benthamiana* [32,106], suggesting that more efficient RNA silencing is responsible for
548 impaired viral replication (or the viral RNA suppressor is less active). To conclude, all
549 these studies demonstrate that depending on virus and plant host, elevated temperature
550 may either increase or decrease virus susceptibility. To investigate this differential im-
551 pact, further studies are needed on other pathosystems in the future. Moreover, molecu-
552 lar mechanisms underlying the differential susceptibility to viruses must be investigated
553 in more depth, especially concerning the implication of the RNA silencing pathway in
554 pathosystems for which viral infection is boosted at elevated temperature like *P. vulga-*
555 *ris*-BPMV.

557 Global warming leads to rising temperatures, which alters plant-virus interactions
558 [73], potentially inducing yield losses and decreasing quality of crop productions [107]. It
559 is therefore timely to study the effect of elevated temperatures on plant responses to virus
560 infections. Importantly, future experiments on the effect of temperature on plant-virus
561 interactions will need to consider the dynamic nature of field conditions, with diurnal
562 fluctuating temperature cycles and heat waves such as those experienced by crops in the
563 field. As performed in Nancarrow et al., [27] growing chambers will need to more closely
564 resemble the dynamic conditions to which plants and pathogens are subjected in nature
565 so that field trials can be mimicked. After studying the effect of a single environmental
566 factor in order to have a specific response, it will be important to test the effect of several
567 combined environmental factors (elevated temperature + high CO₂ for example) to
568 analyse whether their effects are additive or antagonistic. In C3 plants like *P. vulgaris*,
569 high CO₂ improves photosynthesis, activates hormonal pathways, whereas high tempe-
570 ratures decrease the efficiency of Rubisco and the solubility of CO₂. Consequently, com-
571 bined high CO₂ and elevated temperatures may induce antagonistic effects. At the in-
572 ternational level, little work has been published on the combined effects of elevated
573 temperatures and high CO₂ on plant-virus interactions (reviewed in [20]). Del Toro et al.
574 [108] focused on combined climate conditions (CCC, elevated temperature (30°C *versus*
575 25°C) and high CO₂ level (970 ppm *versus* 405 ppm)) by studying CMV, PVY and PVX in
576 the model plant *N. benthamiana*. They showed that viral titers in systemic leaves under
577 CCC showed no significant differences at 7 and 12 dpi for CMV. Conversely, viral titers
578 of PVY and PVX were significantly lower under CCC compared to standard condition at
579 both 7 and 12 dpi. In the case of PVY, viral titer was lower at 12 dpi compared to 7 dpi

580 under CCC and the contrary was observed for PVX [108]. In potato (*S. tuberosum*), Chung
581 et al. [109] demonstrated that *Potato leaf roll virus* (PLRV) RNA accumulated at higher
582 levels and larger numbers of potato plants were infected by PLRV under combined high
583 CO₂ levels (940 ppm) and elevated temperature (30°C). More recently, Aguilar et al. [110]
584 studied a multifactorial system combining biotic (virus and bacteria) and abiotic
585 (drought) stresses in *A. thaliana* (compatible context) and *N. benthamiana* plants (incom-
586 compatible context). They demonstrated that infection by the PVX / PPV virus combination
587 induced resistance to the bacteria *P. syringae* pv. *tomato* and to drought in both compatible
588 and incompatible host-bacteria interactions. However, combined high CO₂ levels (970
589 ppm) and elevated temperature (30°C) negatively affected resistance to *Pst* and to
590 drought induced by virus infection, and this correlated with diminished H₂O₂ produc-
591 tion, decreased expression of defence genes and a drop in virus titers.

592 5. Conclusions

593 To conclude, our study gives a first insight on the impact of elevated temperature on
594 the level of resistance/susceptibility to viruses in common bean. Indeed, natural genetic
595 resistance is often the most effective and environmentally friendly way of controlling
596 plant diseases in particular against virus where no chemicals alternative is available. This
597 research needs to be continued in order to decipher the underlying cellular and molecu-
598 lar mechanisms of heat-tolerance and to engineer robust thermostable resistances in the
599 context of global warming.

600
601 **Supplementary Materials:** Figure S1, Semi-quantitative RT-PCR showing that *P. vulgaris* cv.
602 BAT93 is resistant to BPMV systemic infection ; Figure S2, BPMV is still infectious at 35°C and
603 spreads systemically in the susceptible genotype *P. vulgaris* cv. Black Valentine ; Figure S3, Ele-
604 vated temperatures promote BPMV spreading in the systemic leaves and in whole plants in the
605 susceptible genotype *P. vulgaris* cv. Black Valentine ; Figure S4, Semi-quantitative RT-PCR showing
606 that *P. vulgaris* cv. BT-1 and BT-2, two Near Isogenic Lines for the *I* locus, are both resistant to
607 BPMV systemic infection.

608 **Author contributions:** Conceptualization, CM and SP; methodology, CM and SP; formal analysis,
609 CM, JL, AG, SP; investigation, CM, JL, CG, MN, SB, AN and SP; writing, CM, JL, SB, MN and SP;
610 manuscript editing, CM, JL, MN, SB, AG, VG, and SP; supervision and project administration, SP;
611 funding acquisition, VG and SP. All authors have read and agreed to the published version of the
612 manuscript.

613 **Funding:** CM and JL were supported by fellowships from the French Research Ministry and from
614 the « Ecole Universitaire de Recherche Saclay Plant Sciences (EUR SPS) Graduate School of
615 Research », respectively. MN was supported by an individual 'Invited-researcher' fellowship from
616 Université de Paris in 2019 and 2021. This work was supported by fundings from the Plant Biology
617 and Breeding (BAP) department of French National Research Institute for Agriculture, Food and
618 Environment (INRAE) (HiT-VIRUSRESIST project), from the French National Research Agency
619 (HiPATH project, Grant Agreement No. ANR-17-CE20-0025) and from the European Union's Ho-
620 rizon 2020 research and innovation program (BRESOV project, Grant Agreement No. 774244).
621 IPS2 benefits from the support of Saclay Plant Sciences-SPS (ANR-17-EUR-0007).

622 **Acknowledgments:** We thank Séverine Domenichini and Gautier Bernal from IPS2 for their im-
623 plication in the microscope work. We also thank Chunquan Zhang and Steven Whitham at Iowa
624 State University, USA for sharing the set of BPMV vectors and for providing the seeds of *P. vulgaris*
625 cv. Black Valentine, Juan Camilo Alvarez Diaz and Etienne Delannoy for advices with RT-qPCR
626 analysis and quantification of BPMV virus titer, Manon Richard for managing the construction of
627 the F2 population, Holger Ornstrup and all the greenhouse team of IPS2 for precious help with
628 plant growing, and Benoît Moury and Cécile Desbiez of INRAE Avignon, France for helpful dis-
629 cussions.

630 **Conflicts of Interest:** The authors declare no conflicts of interest.

632 **References**

- 633 1. Anderson, P.K.; Cunningham, A.A.; Patel, N.G.; Morales, F.J.; Epstein, P.R.; Daszak, P. Emerging infectious diseases of plants : pathogen
634 pollution , climate change and agrotechnology drivers. *Trends Ecol. Evol.* **2004**, *19*, 535–544, doi:10.1016/j.tree.2004.07.021.
- 635 2. Sastry, K.S.; Zitter, T.A. *Plant Virus and Viroid Diseases in the Tropics. Volume 2: Epidemiology and Management*; Springer, Dordrecht, The
636 Netherlands, 2014;
- 637 3. Amari, K.; Niehl, A. Nucleic acid-mediated PAMP-triggered immunity in plants. *Curr. Opin. Virol.* **2020**, *42*, 32–39,
638 doi:10.1016/j.coviro.2020.04.003.
- 639 4. Ratcliff, F.; Harrison, B.D.; Baulcombe, D.C. A similarity between viral defense and gene silencing in plants. *Science (80-.)*. **1997**, *276*, 1558–
640 1560, doi:10.1126/science.276.5318.1558.
- 641 5. KØrner, C.; Klauser, D.; Niehl, A.; Domínguez-Ferreras, A.; Chinchilla, D.; Boller, T.; Heinlein, M.; Hann, D.R. The immunity regulator
642 BAK1 contributes to resistance against diverse RNA viruses. *Mol. Plant-Microbe Interact.* **2013**, *26*, 1271–1280,
643 doi:10.1094/MPMI-06-13-0179-R.
- 644 6. Lee, B.; Park, Y.S.; Lee, S.; Song, G.C.; Ryu, C.M. Bacterial RNAs activate innate immunity in Arabidopsis. *New Phytol.* **2016**, *209*, 785–797,
645 doi:10.1111/nph.13717.
- 646 7. Niehl, A.; Wyrsh, I.; Boller, T.; Heinlein, M. Double-stranded RNAs induce a pattern-triggered immune signaling pathway in plants. *New*
647 *Phytol.* **2016**, *1*–12.
- 648 8. Csorba, T.; Kontra, L.; Burgyán, J. Viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. *Virology* **2015**, *479*–480,
649 85–103, doi:10.1016/j.virol.2015.02.028.
- 650 9. Zvereva, A.S.; Golyaev, V.; Turco, S.; Gubaeva, E.G.; Rajeswaran, R.; Schepetilnikov, M. V.; Srour, O.; Ryabova, L.A.; Boller, T.; Pooggin,
651 M.M. Viral protein suppresses oxidative burst and salicylic acid-dependent autophagy and facilitates bacterial growth on virus-infected
652 plants. *New Phytol.* **2016**, *211*, 1020–1034, doi:10.1111/nph.13967.
- 653 10. Nicaise, V.; Candresse, T. Plum pox virus capsid protein suppresses plant pathogen-associated molecular pattern (PAMP)-triggered
654 immunity. *Mol. Plant Pathol.* **2017**, *18*, 878–886, doi:10.1111/mpp.12447.
- 655 11. Kong, J.; Wei, M.; Li, G.; Lei, R.; Qiu, Y.; Wang, C.; Li, Z.H.; Zhu, S. The cucumber mosaic virus movement protein suppresses
656 PAMP-triggered immune responses in Arabidopsis and tobacco. *Biochem. Biophys. Res. Commun.* **2018**, *498*, 395–401,
657 doi:10.1016/j.bbrc.2018.01.072.
- 658 12. Palukaitis, P.; Yoon, J.Y. R gene mediated defense against viruses. *Curr. Opin. Virol.* **2020**, *45*, 1–7, doi:10.1016/j.coviro.2020.04.001.
- 659 13. Flor, H.H. Current statut of the gene-for-gene concept. *Annu. Rev. Phytopathol.* **1955**, *1971*, 275–296.
- 660 14. Gouveia, B.C.; Calil, I.P.; Machado, J.P.B.; Santos, A.A.; Fontes, E.P.B. Immune Receptors and Co-receptors in Antiviral Innate Immunity in
661 Plants. *Front. Microbiol.* **2017**, *7*, 2139, doi:10.3389/fmicb.2016.02139.
- 662 15. de Ronde, D.; Butterbach, P.; Kormelink, R. Dominant resistance against plant viruses. *Front. Plant Sci.* **2014**, *5*, 307,
663 doi:10.3389/fpls.2014.00307.
- 664 16. Kou, Y.; Wang, S. Broad-spectrum and durability: understanding of quantitative disease resistance. *Curr. Opin. Plant Biol.* **2010**, *13*, 181–185,
665 doi:10.1016/j.pbi.2009.12.010.
- 666 17. Schmitt-Keichinger, C. Manipulating cellular factors to combat viruses: A case study from the plant eukaryotic translation initiation factors
667 eIF4. *Front. Microbiol.* **2019**, *10*, 1–8, doi:10.3389/fmicb.2019.00017.
- 668 18. Venkatesh, J.; Kang, B.C. Current views on temperature-modulated R gene-mediated plant defense responses and tradeoffs between plant
669 growth and immunity. *Curr. Opin. Plant Biol.* **2019**, *50*, 9–17, doi:10.1016/j.pbi.2019.02.002.
- 670 19. Trebicki, P. Climate change and plant virus epidemiology. *Virus Res.* **2020**, *286*, 198059, doi:10.1016/j.virusres.2020.198059.
- 671 20. Tenllado, F.; Canto, T. Effects of a changing environment on the defenses of plants to viruses. *Curr. Opin. Virol.* **2020**, *42*, 40–46,
672 doi:10.1016/j.coviro.2020.04.007.
- 673 21. Desaint, H.; Aoun, N.; Deslandes, L.; Vailleau, F.; Roux, F.; Berthomé, R. Fight hard or die trying: when plants face pathogens under heat

- 674 stress. *New Phytol.* **2021**, *229*, 712–734, doi:10.1111/nph.16965.
- 675 22. Weststeijn, E. Lesion growth and virus localization in leaves of *Nicotiana tabacum* cv. Xanthi nc. after inoculation with tobacco mosaic
676 virus and incubation alternately at 22°C and 32°C. *Physiol Plant Pathol.* **1981**, 357–368, 357–368.
- 677 23. Chung, B.N.; Lee, J.H.; Kang, B.C.; Koh, S.W.; Joa, J.H.; Choi, K.S.; Ahn, J.J. HR-mediated defense response is overcome at high
678 temperatures in capsicum species. *Plant Pathol. J.* **2018**, *34*, 71–77, doi:10.5423/PPJ.NT.06.2017.0120.
- 679 24. Richard, M.M.S.; Knip, M.; Aalders, T.; Beijaert, M.S.; Takken, F.L.W. Unlike Many Disease Resistances, Rx1-Mediated Immunity to Potato
680 Virus X Is Not Compromised at Elevated Temperatures. *Front. Genet.* **2020**, *11*, 1–8, doi:10.3389/fgene.2020.00417.
- 681 25. Kabbage, M.; Kessens, R.; Bartholomay, L.C.; Williams, B. The Life and Death of a Plant Cell. *Annu. Rev. Plant Biol.* **2017**, *68*, 375–404,
682 doi:10.1146/annurev-arplant-043015-111655.
- 683 26. Choi, K.S.; del Toro, F.; Tenllado, F.; Canto, T.; Chung, B.N. A model to explain temperature dependent systemic infection of potato plants
684 by Potato virus Y. *Plant Pathol. J.* **2017**, *33*, 206–211, doi:10.5423/PPJ.NT.06.2016.0144.
- 685 27. Nancarrow, N.; Constable, F.E.; Finlay, K.J.; Freeman, A.J.; Rodoni, B.C.; Trebicki, P.; Vassiliadis, S.; Yen, A.L.; Luck, J.E. The effect of
686 elevated temperature on barley yellow dwarf virus-PAV in wheat. *Virus Res.* **2014**, *186*, 97–103, doi:10.1016/j.virusres.2013.12.023.
- 687 28. Kido, K.; Tanaka, C.; Mochizuki, T.; Kubota, K.; Ohki, T.; Ohnishi, J.; Knight, L.M.; Tsuda, S. High temperatures activate local viral
688 multiplication and cell-to-cell movement of Melon necrotic spot virus but restrict expression of systemic symptoms. *Phytopathology* **2008**, *98*,
689 181–186, doi:10.1094/PHYTO-98-2-0181.
- 690 29. Chung, B.N.; Choi, K.S.; Ahn, J.J.; Joa, J.H.; Do, K.S.; Park, K.S. Effects of temperature on systemic infection and symptom expression of
691 turnip mosaic virus in chinese cabbage (*Brassica campestris*). *Plant Pathol. J.* **2015**, *31*, 363–370, doi:10.5423/PPJ.NT.06.2015.0107.
- 692 30. Del Toro, F.J.; Aguilar, E.; Hernández-Walias, F.J.; Tenllado, F.; Chung, B.N.; Canto, T. High temperature, high ambient CO₂ affect the
693 interactions between three positive-sense RNA viruses and a compatible host differentially, but not their silencing suppression efficiencies.
694 *PLoS One* **2015**, *10*, 1–18, doi:10.1371/journal.pone.0136062.
- 695 31. Dahal, G.; Hughes, J.D.; Thottappilly, G.; Lockhart, B.E.L. Effect of temperature on symptom expression and reliability of banana streak
696 badnavirus detection in naturally infected plantain and banana (*Musa* spp.). *Plant Dis.* **1998**, *82*, 16–21, doi:10.1094/PDIS.1998.82.1.16.
- 697 32. Chellappan, P.; Vanitharani, R.; Ogbe, F.; Fauquet, C.M. Effect of temperature on geminivirus-induced RNA silencing in plants. *Plant*
698 *Physiol.* **2005**, *138*, 1828–1841, doi:10.1104/pp.105.066563.
- 699 33. Broughton, W.J.; Hernandez, G.; Blair, M.; Beebe, S.; Gepts, P.; Vanderleyden, J. Beans (*Phaseolus* spp.)—model food legumes. *Plant Soil*
700 **2003**, *252*, 55–128.
- 701 34. Beebe, S.; Ramirez, J.; Jarvis, A.; Rao, I.M.; Mosquera, G.; Bueno, J.M.; Blair, M.W. Genetic Improvement of Common Beans and the
702 Challenges of Climate Change. *Crop Adapt. to Clim. Chang.* **2011**, 356–369, doi:10.1002/9780470960929.ch25.
- 703 35. Ramirez, J. <https://pt.slideshare.net/ciatdapa/julian-r-climate-change-and-bean-production/2>.
- 704 36. Omae, H.; Kumar, A.; Kashiwaba, K.; Shono, M. Adaptation to high temperature and water deficit in the common bean (*Phaseolus*
705 *vulgaris* L.) during the reproductive period. *J. Bot.* **2012**, <https://doi.org/10.1155/2012/803413>.
- 706 37. Suarez, J.C.; Polania, J.A.; Contreras, A.T.; Rodriguez, L.; Machado, L.; Ordonez, C.; Beebe, S.; Rao, I. Adaptation of common bean lines to
707 high temperature conditions : genotypic differences in phenological and agronomic performance Adaptation of common bean lines to high
708 temperature conditions: genotypic differences in phenological and agronomic performan. *Euphytica* **2020**, *216*, 28,
709 doi:10.1007/s10681-020-2565-4.
- 710 38. Meziadi, C.; Blanchet, S.; Geffroy, V.; Pflieger, S. Genetic resistance against viruses in *Phaseolus vulgaris* L.: State of the art and future
711 prospects. *Plant Sci.* **2017**, *265*, doi:10.1016/j.plantsci.2017.08.009.
- 712 39. Morales, F.J.; Castano, M. Increased disease severity induced by some comoviruses in bean genotypes possessing monogenic dominant
713 resistance to Bean common mosaic potyvirus. *Plant Dis.* **1992**, *76*, 570–573.
- 714 40. Morales, F.J.; Singh, S.P. Inheritance of the mosaic and necroses reactions induced by bean severe mosaic comoviruses in *Phaseolus*
715 *vulgaris* L. *Euphytica* **1997**, *93*, 223–226, doi:10.1023/A:1002902207395.

- 716 41. Pflieger, S.; Blanchet, S.; Meziadi, C.; Richard, M.M.S.; Thareau, V.; Mary, F.; Mazoyer, C.; Geffroy, V. The “ one-step ” Bean pod mottle
717 virus (BPMV) - derived vector is a functional genomics tool for efficient overexpression of heterologous protein , virus-induced gene
718 silencing and genetic mapping of BPMV R-gene in common bean (Phaseolus vulgaris L .). *BMC Plant Biol.* **2014**, *14*, 1–16,
719 doi:10.1186/s12870-014-0232-4.
- 720 42. Provvidenti, R. Two useful selections of the bean cultivar Black Turtle Soup for viral identification. *Bean Improv. Coop. Annu. Rep.* **1983**, *26*,
721 73–75.
- 722 43. Zhang, C.; Bradshaw, J.D.; Whitham, S.A.; Hill, J.H. The Development of an Efficient Multipurpose Bean Pod Mottle Virus Viral Vector Set
723 for Foreign Gene Expression and RNA silencing. *Plant Physiol.* **2010**, *153*, 52–65, doi:10.1104/pp.109.151639.
- 724 44. Pflieger, S.; Blanchet, S.; Meziadi, C.; Richard, M.M.S.; Geffroy, V. Bean pod mottle virus (BPMV) viral inoculation procedure in common
725 bean (*Phaseolus vulgaris* L.). *Bio-protocol* **2015**, *5*, e1524.
- 726 45. Richard, M.M.S.; Gratias, A.; Alvarez Diaz, J.C.; Thareau, V.; Pflieger, S.; Meziadi, C.; Blanchet, S.; Marande, W.; Bitocchi, E.; Papa, R.; et al.
727 A common bean truncated CRINKLY4 kinase controls gene-for-gene resistance to the fungus *Colletotrichum lindemuthianum*. *J. Exp. Bot.*
728 **2021**, *72*, 3569–3581, doi:10.1093/jxb/erab082.
- 729 46. Ali, M.A. Genetics of resistance to the common Bean mosaic virus in the bean (*Phaseolus vulgaris* L). *Phytopathology* **1950**, *40*, 69–79.
- 730 47. Bos, L. Bean common mosaic virus. *C. Descr. Plant Viruses* **1971**, *73*.
- 731 48. Drijfhout, E. Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and
732 breeding for resistance. *Agric. Res. Rep. Cent. Agric. Publ. Doc. Wageningen, Netherlands* **1978**, *872*, 1–98.
- 733 49. Whitham, S.; McCormick, S.; Baker, B. The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. *Proc. Natl.*
734 *Acad. Sci. U. S. A.* **1996**, *93*, 8776–8781, doi:10.1073/pnas.93.16.8776.
- 735 50. Collmer, C.W.; Marston, M.F.; Taylor, J.C.; Jahn, M. The I gene of bean: a dosage-dependent allele conferring extreme resistance,
736 hypersensitive resistance, or spreading vascular necrosis in response to the potyvirus Bean common mosaic virus. *Mol. Plant-Microbe*
737 *Interact.* **2000**, *13*, 1266–1270.
- 738 51. Cadle-Davidson, M.M.; Jahn, M.M. Resistance conferred against bean common mosaic virus by the incompletely dominant I locus of
739 *Phaseolus vulgaris* is active at the single cell level. *Arch. Virol.* **2005**, *150*, 2601–2608, doi:10.1007/s00705-005-0592-z.
- 740 52. Cadle-Davidson, M.M.; Jahn, M.M. Patterns of accumulation of Bean common mosaic virus in *Phaseolus vulgaris* genotypes nearly
741 isogenic for the I locus. *Ann. Appl. Biol.* **2006**, *148*, 179–185, doi:10.1111/j.1744-7348.2006.00059.x.
- 742 53. Tomita, R.; Sekine, K.-T.; Mizumoto, H.; Sakamoto, M.; Murai, J.; Kiba, A.; Hikichi, Y.; Suzuki, K.; Kobayashi, K. Genetic Basis for the
743 Hierarchical Interaction Between Tobamovirus spp. and L Resistance Gene Alleles from Different Pepper Species. *Mol. Plant-Microbe*
744 *Interact.* **2011**, *24*, 108–117.
- 745 54. Veremis, J.C.; Van Heusden, A.W.; Roberts, P.A. Mapping a novel heat-stable resistance to *Meloidogyne* in *Lycopersicon peruvianum*.
746 *Theor. Appl. Genet.* **1999**, *98*, 274–280, doi:10.1007/s001220051068.
- 747 55. Ammiraju, J.S.S.; Veremis, J.C.; Huang, X.; Roberts, P.A.; Kaloshian, I. The heat-stable root-knot nematode resistance gene Mi-9 from
748 *Lycopersicon peruvianum* is localized on the short arm of chromosome 6. *Theor. Appl. Genet.* **2003**, *106*, 478–484,
749 doi:10.1007/s00122-002-1106-y.
- 750 56. Jablonska, B.; Ammiraju, J.S.S.; Bhattarai, K.K.; Mantelin, S.; De Ilarduya, O.M.; Roberts, P.A.; Kaloshian, I. The Mi-9 gene from *Solanum*
751 *arcanum* conferring heat-stable resistance to root-knot nematodes is a homolog of Mi-1. *Plant Physiol.* **2007**, *143*, 1044–1054,
752 doi:10.1104/pp.106.089615.
- 753 57. Djian-Caporalino, C.; Pijarowski, L.; Januel, A.; Lefebvre, V.; Daubèze, A.; Palloix, A.; Dalmasso, A.; Abad, P. Spectrum of resistance to
754 root-knot nematodes and inheritance of heat-stable resistance in in pepper (*Capsicum annuum* L.). *Theor. Appl. Genet.* **1999**, *99*, 496–502,
755 doi:10.1007/s001220051262.
- 756 58. Djian-Caporalino, C.; Pijarowski, L.; Fazari, A.; Samson, M.; Gaveau, L.; O’Byrne, C.; Lefebvre, V.; Caranta, C.; Palloix, A.; Abad, P.
757 High-resolution genetic mapping of the pepper (*Capsicum annuum* L.) resistance loci Me3 and Me4 conferring heat-stable resistance to

- 758 root-knot nematodes (*Meloidogyne* spp.). *Theor. Appl. Genet.* **2001**, *103*, 592–600, doi:10.1007/PL00002914.
- 759 59. Bradshaw, J.; G, R. Utilisation of the Commonwealth Potato Collection in potato breeding. *Euphytica* **2005**, *146*, 9–19.
- 760 60. Solomon-Blackburn, R.; Bradshaw, J. Resistance to Potato virus Y in a Multitrait Potato Breeding Scheme without Direct Selection in Each
761 Generation. *Potato Res.* **2007**, *50*, 87–95.
- 762 61. Moury, B.; Selassie, K.G.; Marchoux, G.; Daub, A.; Palloix, A. High temperature effects on hypersensitive resistance to Tomato Spotted
763 Wilt Tospovirus (TSWV) in pepper (*Capsicum chinense* Jacq.). *Eur. J. Plant Pathol.* **1998**, *104*, 489–498.
- 764 62. de Carvalho, L.; Benda, N.; Vaughan, M.; Cabrera, A.; Hung, K.; Cox, T.; Abdo, Z.; Allen, L.; Teal, P. Mi-1-Mediated Nematode Resistance
765 in Tomatoes is Broken by Short-Term Heat Stress but Recovers Over Time. *J. Nematol.* **2015**, *47*, 133–140.
- 766 63. Romero, A.M.; Kousik, C.S.; Ritchie, D.F. Temperature Sensitivity of the Hypersensitive Response of Bell Pepper to *Xanthomonas*
767 *axonopodis* pv. *vesicatoria*. *Phytopathology* **2002**, *92*, 197–203.
- 768 64. Cai, X.; Takken, F.; Joosten, M.; De Wit, P. Specific recognition of AVR4 and AVR9 results in distinct patterns of hypersensitive cell death
769 in tomato, but similar patterns of defence-related gene expression. *Mol. Plant Pathol.* **2001**, *2*, 77–86.
- 770 65. Jong, C.F. De; Takken, F.L.W.; Cai, X.; Wit, P.J.G.M. De; Joosten, M.H.A.J. Attenuation of Cf-Mediated Defense Responses at Elevated
771 Temperatures Correlates With a Decrease in Elicitor-Binding Sites. *Mol. Plant-Microbe Interact.* **2002**, *15*, 1040–1049.
- 772 66. Cheng, C.; Gao, X.; Feng, B.; Sheen, J.; Shan, L.; He, P. Differential temperature operation of plant immune responses. *Nat. Commun.* **2013**, *4*,
773 2530, doi:10.1038/ncomms3530.Differential.
- 774 67. Menna, A.; Nguyen, D.; Guttman, D.S.; Desveaux, D. Elevated temperature differentially influences effector-triggered immunity outputs
775 in *Arabidopsis*. *Front. Plant Sci.* **2015**, *6*, 1–7, doi:10.3389/fpls.2015.00995.
- 776 68. Aoun, N.; Tauleigne, L.; Lonjon, F.; Deslandes, L.; Vailleau, F.; Roux, F.; Berthomé, R. Quantitative disease resistance under elevated
777 temperature: Genetic basis of new resistance mechanisms to *Ralstonia solanacearum*. *Front. Plant Sci.* **2017**, *8*, 1387,
778 doi:10.3389/fpls.2017.01387.
- 779 69. Yang, S.; Hua, J. A haplotype-specific Resistance gene regulated by BONZAI1 mediates temperature-dependent growth control in
780 *Arabidopsis*. *Plant Cell* **2004**, *16*, 1060–1071, doi:10.1105/tpc.020479.
- 781 70. Zhu, Y.; Qian, W.; Hua, J. Temperature modulates plant defense responses through NB-LRR proteins. *PLoS Pathog.* **2010**, *6*, 1–12,
782 doi:10.1371/journal.ppat.1000844.
- 783 71. Mang, H.G.; Qian, W.; Zhu, Y.; Qian, J.; Kang, H.G.; Klessig, D.F.; Hua, J. Abscisic acid deficiency antagonizes high-temperature inhibition
784 of disease resistance through enhancing nuclear accumulation of resistance proteins SNC1 and RPS4 in *Arabidopsis*. *Plant Cell* **2012**, *24*,
785 1271–1284, doi:10.1105/tpc.112.096198.
- 786 72. Fesenko, I.; Spechenkova, N.; Mamaeva, A.; Makhotenko, A. V.; Love, A.J.; Kalinina, N.O.; Taliany, M. Role of the methionine cycle in
787 the temperature-sensitive responses of potato plants to potato virus Y. *Mol. Plant Pathol.* **2021**, *22*, 77–91, doi:10.1111/mpp.13009.
- 788 73. Amari, K.; Huang, C.; Heinlein, M. Potential Impact of Global Warming on Virus Propagation in Infected Plants and Agricultural
789 Productivity. *Front. Plant Sci.* **2021**, *12*, 649768, doi:10.3389/fpls.2021.649768.
- 790 74. Whitham, S.A.; Yang, C.; Goodin, M.M. Global impact: Elucidating plant responses to viral infection. *Mol. Plant-Microbe Interact.* **2006**, *19*,
791 1207–1215, doi:10.1094/MPMI-19-1207.
- 792 75. Lewsey, M.; Palukaitis, P.; Carr, J. Plant–Virus Interactions: Defence and Counter-Defence. In *Annual Plant Reviews Volume 34: Molecular*
793 *Aspects of Plant Disease Resistance*; 2009; pp. 134–176.
- 794 76. Baebler, Š.; Witek, K.; Petek, M.; Stare, K.; Tušek-Žnidarič, M.; Pompe-Novak, M.; Renaut, J.; Szajko, K.; Strzelczyk-Zyta, D.; Marczewski,
795 W.; et al. Salicylic acid is an indispensable component of the Ny-1 resistance-gene-mediated response against Potato virus y infection in
796 potato. *J. Exp. Bot.* **2014**, *65*, 1095–1109, doi:10.1093/jxb/ert447.
- 797 77. Chandra-Shekhara, A.; Navarre, D.; Kachroo, A.; Kang, H.; Klessig, D.; Kachroo, P. Signaling requirements and role of salicylic acid in HRT
798 - and rrt -mediated resistance to turnip crinkle virus in *Arabidopsis*. *Plant J.* **2004**, *40*, 647–659, doi:10.1111/j.1365-313X.2004.02241.x.
- 799 78. Lukan, T.; Baebler, S.; Pompe-Novak, M.; Gucek, K.; Zagorščak, M.; Coll, A.; Gruden, K. Cell Death Is Not Sufficient for the Restriction of

- Potato Virus Y Spread in Hypersensitive Response-Conferred Resistance in Potato. *Front. Plant Sci.* **2018**, *9*, 1–12, doi:10.3389/fpls.2018.00168.
79. Zhao, F.; Yanan, L.; Lijuan, C.; Lisha, Z.; Han, R.; Honghui, L.; Dehui, X. Temperature dependent defence of *Nicotiana tabacum* against Cucumber mosaic virus and recovery occurs with the formation of dark green islands. *J. Plant Biol.* **2016**, *59*, 293–301.
80. Makarova, S.; Makhotenko, A.; Spechenkova, N.; Love, A.J.; Kalinina, N.O.; Taliansky, M. Interactive Responses of Potato (*Solanum tuberosum* L.) Plants to Heat Stress and Infection With Potato Virus Y. *Front. Microbiol.* **2018**, *9*, 1–14, doi:10.3389/fmicb.2018.02582.
81. Chu, M.; Desvoyes, B.; Turina, M.; Noad, R.; Scholthof, H.B. Genetic dissection of tomato bushy stunt virus p19-protein-mediated host-dependent symptom induction and systemic invasion. *Virology* **2000**, *266*, 79–87, doi:10.1006/viro.1999.0071.
82. Cawly, J.; Cole, A.B.; Király, L.; Qiu, W.; Schoelz, J.E. The plant gene CCD1 selectively blocks cell death during the hypersensitive response to cauliflower mosaic virus infection. *Mol. Plant-Microbe Interact.* **2005**, *18*, 212–219, doi:10.1094/MPMI-18-0212.
83. Ishibashi, K.; Masuda, K.; Naito, S.; Meshi, T.; Ishikawa, M. An inhibitor of viral RNA replication is encoded by a plant resistance gene. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 13833–13838, doi:10.1073/pnas.0703203104.
84. Komatsu, K.; Hashimoto, M.; Ozeki, J.; Yamaji, Y.; Maejima, K.; Senshu, H.; Himeno, M.; Okano, Y.; Kagiwada, S.; Namba, S. Viral-Induced systemic necrosis in plants involves both programmed cell death and the inhibition of viral multiplication, which are regulated by independent pathways. *Mol. Plant-Microbe Interact.* **2010**, *23*, 283–293, doi:10.1094/MPMI-23-3-0283.
85. Liu, P.P.; Bhattacharjee, S.; Klessig, D.F.; Moffett, P. Systemic acquired resistance is induced by R gene-mediated responses independent of cell death. *Mol. Plant Pathol.* **2010**, *11*, 155–160, doi:10.1111/j.1364-3703.2009.00564.x.
86. Hafez, Y.M.; Bacsó, R.; Király, Z.; Künstler, A.; Király, L. Up-regulation of antioxidants in tobacco by low concentrations of H₂O₂ suppresses necrotic disease symptoms. *Phytopathology* **2012**, *102*, 848–856, doi:10.1094/PHYTO-01-12-0012-R.
87. Ando, S.; Obinata, A.; Takahashi, H. WRKY70 interacting with RCY1 disease resistance protein is required for resistance to Cucumber mosaic virus in *Arabidopsis thaliana*. *Physiol. Mol. Plant Pathol.* **2014**, *85*, 8–14, doi:10.1016/j.pmp.2013.11.001.
88. Harris, J.M.; Balint-kurti, P.; Bede, J.C.; Day, B.; Gold, S.; Goss, E.M.; Grenville-briggs, L.J.; Jones, K.M.; Wang, A.; Wang, Y.; et al. What are the Top 10 Unanswered Questions in Molecular Plant-Microbe Interactions? *Mol. Plant-Microbe Interact.* **2020**, *X*, 1–12.
89. Zheng, C.; Chen, P.; Gergerich, R. Characterization of Resistance to Soybean mosaic virus in Diverse Soybean Germplasm. *Crop Sci.* **2005**, *45*, 2503–2509.
90. Meziadi, C. Personal communication, 2021. *IPS2, Orsay, Fr.*
91. Vallejos, C.E.; Astua-monge, G.; Jones, V.; Plyler, T.R.; Sakiyama, N.S.; Mackenzie, S.A. Genetic and Molecular Characterization of the I Locus of *Phaseolus vulgaris*. *Genetics* **2006**, *172*, 1229–1242, doi:10.1534/genetics.105.050815.
92. Nodari, R.O.; Tsai, S.M.; Gilbertson, R.L.; Gepts, P. Towards an integrated linkage map of common bean. 2. Development of an RFLP-based linkage map. *Theor. Appl. Genet.* **1993**, *85*, 513–520.
93. Kyle, M.M.; Provvidenti, R. Inheritance of resistance to potato y viruses in *Phaseolus vulgaris* L. 1. Two independent genes for resistance to watermelon mosaic virus-2. *Theor. Appl. Genet.* **1987**, *74*, 595–600.
94. Provvidenti, R. Inheritance of resistance to watermelon mosaic virus 2 in *Phaseolus vulgaris*. *Phytopathology* **1974**, *64*, 1448–1450.
95. Provvidenti, R.; Gonsalves, D.; Taiwo, M.A. Inheritance of resistance to Blackeye cowpea mosaic and Cowpea aphid-borne mosaic viruses in *Phaseolus vulgaris*. *J. Hered.* **1983**, *74*, 60–61.
96. Provvidenti, R.; Gonsalves, D.; Ranalli, P. Inheritance of resistance to soybean mosaic virus in *Phaseolus vulgaris*. *J. Hered.* **1982**, *73*, 302–303.
97. Kyle, M.M.; Provvidenti, R. Inheritance of resistance to potyviruses in *Phaseolus vulgaris* L. 2. Linkage relations and utility of a dominant gene for lethal systemic necrosis to Soybean mosaic virus. *Theor. Appl. Genet.* **1993**, *86*, 189–196.
98. Provvidenti, R.; Chirco, E.M. Inheritance of resistance to peanut mottle virus in *Phaseolus vulgaris*. *J. Hered.* **1987**, *78*, 402–403.
99. Li, R.H.; Zettler, F.W.; Elliott, M.S.; Petersen, M.A.; Still, P.E.; Baker, C.A.; Mink, G.I. A strain of Peanut Mottle Virus Seedborne in Bambarra Groundnut. *Plant Dis.* **1991**, *75*, 130–133.

- 842 100. Fisher, M.L.; Kyle, M.M. Inheritance of resistance to potyviruses in *Phaseolus vulgaris* L. III. Cosegregation of phenotypically similar
843 dominant responses to nine potyviruses. *Theor. Appl. Genet.* **1994**, *89*, 818–823.
- 844 101. Hart, J.P.; Griffiths, P.D. Genotyping-by-Sequencing Enabled Mapping and Marker Development for the By-2 Potyvirus Resistance Allele
845 in Common Bean. *Plant Genome* **2015**, *8*, 1–14, doi:10.3835/plantgenome2014.09.0058.
- 846 102. Dickson, M.H.; Natti, J.J. Inheritance of resistance of *Phaseolus vulgaris* to bean yellow mosaic virus. *Phytopathology* **1968**, *58*, 1450.
- 847 103. Bello, M.H.; Moghaddam, S.M.; Massoudi, M.; Mclean, P.E.; Cregan, P.B.; Miklas, P.N. Application of in silico bulked segregant analysis
848 for rapid development of markers linked to Bean common mosaic virus resistance in common bean. *BMC Genomics* **2014**, *15*, 1–13.
- 849 104. Chung, B.N.; Canto, T.; Tenllado, F.; Choi, K.S.; Joa, J.H.; Ahn, J.J.; Kim, C.H.; Do, K.S. The effects of high temperature on infection by
850 Potato virus Y, potato virus A, and Potato leafroll virus. *Plant Pathol. J.* **2016**, *32*, 321–328, doi:10.5423/PPJ.OA.12.2015.0259.
- 851 105. Aguilar, E.; Allende, L.; Del Toro, F.J.; Chung, B.N.; Canto, T.; Tenllado, F. Effects of elevated CO₂ and temperature on pathogenicity
852 determinants and virulence of potato virus X/Potyvirus-Associated synergism. *Mol. Plant-Microbe Interact.* **2015**, *28*, 1364–1373,
853 doi:10.1094/MPMI-08-15-0178-R.
- 854 106. Szittyá, G.; Silhavy, D.; Molnar, A.; Havelda, Z.; Lovas, A.; Lakatos, L.; Banfaldi, Z.; Burgyan, J. Low temperature inhibits RNA
855 silencing-mediated defence by the control of siRNA generation. *EMBO J.* **2003**, *22*, 633–640.
- 856 107. Raza, A.; Razzaq, A.; Mehmood, S.S.; Zou, X.; Zhang, X.; Lv, Y.; Xu, J. Impact of Climate Change on Crops Adaptation and Strategies to
857 Tackle Its Outcome: A Review. *Plants* **2019**, *8*, 1–29, doi:10.3390/plants8020034.
- 858 108. del Toro, F.; Rakhshandehroo, F.; Larruy, B.; Aguilar, E.; Tenllado, F.; Canto, T. Effects of simultaneously elevated temperature and CO₂
859 levels on *Nicotiana benthamiana* and its infection by different positive-sense RNA viruses are cumulative and virus type-specific.
860 *Virology* **2017**, *511*, 184–192, doi:10.1016/j.virol.2017.08.015.
- 861 109. Chung, B.N.; Koh, S.W.; Choi, K.S.; Joa, J.H.; Kim, C.H.; Selvakumar, G. Temperature and CO₂ Level Influence Potato leafroll virus
862 Infection in *Solanum tuberosum*. *Plant Pathol. J.* **2017**, *33*, 522–527.
- 863 110. Aguilar, E.; del Toro, F.J.; Figueira-Galán, D.; Hou, W.; Canto, T.; Tenllado, F. Virus infection induces resistance to *Pseudomonas syringae*
864 and to drought in both compatible and incompatible bacteria – host interactions, which are compromised under conditions of elevated
865 temperature and CO₂ levels. *J. Gen. Virol.* **2020**, *101*, 122–135, doi:10.1099/jgv.0.001353.
- 866
- 867