



HAL
open science

Fighting sharka in peach: current limitations and future perspectives

Marco Cirilli, Filippo Geuna, Anna-Rosa Babini, Valentina Bozhkova, Luigi Catalano, Beniamino Cavagna, Sylvie Dallot, Véronique Decroocq, Luca Dondini, Stefano Foschi, et al.

► **To cite this version:**

Marco Cirilli, Filippo Geuna, Anna-Rosa Babini, Valentina Bozhkova, Luigi Catalano, et al.. Fighting sharka in peach: current limitations and future perspectives. *Frontiers in Plant Science*, 2016, 7, 10.3389/fpls.2016.01290 . hal-01512030

HAL Id: hal-01512030

<https://hal.science/hal-01512030v1>

Submitted on 21 Apr 2017

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.

Fighting Sharka in Peach: Current Limitations and Future Perspectives

Marco Cirilli¹, Filippo Geuna¹, Anna Rosa Babini², Valentina Bozhkova³, Luigi Catalano⁴, Beniamino Cavagna⁵, Sylvie Dallot⁶, Veronique Decroocq⁷, Luca Dondini⁸, Stefano Foschi⁹, Vincenza Ilardi¹⁰, Alessandro Liverani¹¹, Bruno Mezzetti¹², Angelantonio Minafra¹³, Marco Pancaldi¹⁴, Tiziana Pandolfini¹⁵, Thierry Pascal¹⁶, Vito Nicola Savino¹⁷, Ralph Scorza¹⁸, Ignazio Verde¹⁹, Daniele Bassi^{1*}

¹Department of Agricultural and Environmental Sciences (DISAA), University of Milan, Italy,

²Phytosanitary Service, Regione Emilia-Romagna, Italy, ³Department of Breeding, Genetic Resources and Biotechnology, Fruit-Growing Institute, Bulgaria, ⁴Centro Interprofessionale per le attività vivaistiche (CIVI-Italia), Italy, ⁵Phytosanitary Service, Regione Lombardia, Italy, ⁶UMR 385 BGPI, INRA, France, ⁷UMR 1332 Biologie du Fruit et Pathologie, INRA, Université de Bordeaux, France,

⁸Dipartimento di Scienze Agrarie, University of Bologna, Italy, ⁹Centro Ricerche Produzioni Vegetali (CRPV), Italy, ¹⁰Centro di Ricerca per la Patologia Vegetale, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Italy, ¹¹Unità di Ricerca per la Frutticoltura di Forlì (FRF), Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Italy, ¹²Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università Politecnica delle Marche, Italy, ¹³Istituto per la Protezione Sostenibile delle Piante (CNR-IPSP), UOS Bari, Italy, ¹⁴Centro Attività Vivaistiche (CAV), Italy,

¹⁵Department of Biotechnology, University of Verona, Italy, ¹⁶UR1052 GAFL, INRA, France, ¹⁷Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (DiSSPA), Università degli Studi di Bari Aldo Moro, Italy, ¹⁸Appalachian Fruit Research Station, USDA-ARS, USA, ¹⁹Centro di Ricerca per la Frutticoltura, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Italy

Submitted to Journal:

Frontiers in Plant Science

Specialty Section:

Crop Science and Horticulture

Article type:

Perspective Article

Manuscript ID:

214991

Received on:

17 Jun 2016

Revised on:

11 Aug 2016

Frontiers website link:

www.frontiersin.org

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

MC: drafted the manuscript and critically revised it;

FG, RS: critically revised the manuscript;

AB, VB, LC, BC, SD, LD, VD, SF, VI, AL, BM, AM, MP, TP, ThP, VS, and IV: critically revised the manuscript for important contents;

DB: conceived the manuscript and critically revised it;

Keywords

Genetic Engineering, Fruit breeding, PPV virus, *Prunus persica* (L.) Batsch, RNAi

Abstract

Word count: 229

Sharka, caused by Plum Pox Virus (PPV), is by far the most important infectious disease of peach [*P. persica* (L.) Batsch] and other *Prunus* species. The progressive spread of the virus in many important growing areas throughout Europe poses serious issues to the economic sustainability of stone fruit crops, peach in particular. The adoption of internationally agreed-upon rules for diagnostic tests, strain-specific monitoring schemes and spatial-temporal modeling of virus spread, are all essential for a more effective sharka containment. The EU regulations on nursery activity should be modified based on the zone delimitation of PPV presence, limiting open-field production of propagation materials only to virus-free areas. Increasing the efficiency of preventive measures should be augmented by the short-term development of resistant cultivars. Putative sources of resistance/tolerance have been recently identified in peach germplasm, although the majority of novel resistant sources to PPV-M have been found in almond. However, the complexity of introgression from related-species imposes the search for alternative strategies. The use of genetic engineering, particularly RNAi-based approaches, appears as one of the most promising perspectives to introduce a durable resistance to PPV in peach germplasm, notwithstanding the well-known difficulties of *in vitro* plant regeneration in this species. In this regard, rootstock transformation to induce RNAi-mediated systemic resistance to PPV would avoid the transformation of numerous commercial cultivars, and may alleviate consumer resistance to the use of GM plants.

Funding statement

The International Workshop was funded by the following Italian subjects: Apofruit, Battistini Vivai, CAV, CIVI Italia, CRPV, Europfruit, Geoplant Vivai, Minguzzi SpA, New Plant, Orogel Fresco, Pempacoror, University of Milan, Vitroplant, Zani Granfrutta, Zanzi F.lli Vivai.

Ethics statement

(Authors are required to state the ethical considerations of their study in the manuscript including for cases where the study was exempt from ethical approval procedures.)

Did the study presented in the manuscript involve human or animal subjects: No

Fighting Sharka in Peach: Current Limitations and Future Perspectives

Marco Cirilli¹, Filippo Geuna¹, Anna Rosa Babini², Valentina Bozhkova³, Luigi Catalano⁴, Beniamino Cavagna⁵, Sylvie Dallot⁶, Véronique Decroocq⁷, Luca Dondini⁸, Stefano Foschi⁹, Vincenza Ilardi¹⁰, Alessandro Liverani¹¹, Bruno Mezzetti¹², Angelantonio Minafra¹³, Marco Pancaldi¹⁴, Tiziana Pandolfini¹⁵, Thierry Pascal¹⁶, Vito Nicola Savino¹⁷, Ralph Scorza¹⁸, Ignazio Verde¹⁹, Daniele Bassi^{1*}

Affiliation

¹Department of Agricultural and Environmental Sciences (DISAA), University of Milan, via Celoria 2, 20133 Milan, Italy

²Phytosanitary Service, Regione Emilia-Romagna, Via di Saliceto 81, 40128 Bologna, Italy

³Department of Breeding, Genetic Resources and Biotechnology, Fruit-Growing Institute, 12 Ostromila, 4004 Plovdiv, Bulgaria

⁴Centro Interprofessionale per le attività vivaistiche (CIVI-Italia), Corso Vittorio Emanuele II 101, 00186 Roma

⁵Phytosanitary Service, Regione Lombardia, Via Pola 12, 20124 Milano, Italy

⁶INRA, UMR 385 BGPI, Cedex 5, 34398 Montpellier, France

⁷INRA, Université de Bordeaux, UMR 1332 Biologie du Fruit et Pathologie, F-33140, 33882 Villenave d'Ornon, France

⁸Dipartimento di Scienze Agrarie, University of Bologna, Via Fanin 46, 40127, Bologna, Italy

⁹Centro Ricerche Produzioni Vegetali (CRPV), Via dell'Arrigoni 120, 47522 Cesena (FC), Italy

¹⁰Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Centro di Ricerca per la Patologia Vegetale, Via Bertero 22, 00156 Rome, Italy

¹¹Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Unità di Ricerca per la Frutticoltura di Forlì (FRF), via La Canapona 1bis, 47122 Forlì, Italy

¹²Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università Politecnica delle Marche, Via Ranieri 65, 60121 Ancona, Italy

¹³Istituto per la Protezione Sostenibile delle Piante (CNR-IPSP), UOS Bari, Via Amendola 122/D, 70121 Bari, Italy

¹⁴Centro Attività Vivaistiche (CAV), via Tebano 45, 48018 Faenza (RA), Italy

¹⁵Department of Biotechnology, University of Verona, Ca' Vignal 1, Strada Le Grazie 15, 37134 Verona, Italy

¹⁶INRA, UR1052 GAFL, Domaine St Maurice - Allée des Chenes CS 60094, 84143 Montfavet Cedex, France

¹⁷Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (DiSSPA), Università degli Studi di Bari Aldo Moro, Piazza Umberto I, 70121 Bari, Italy

¹⁸USDA-ARS Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, WV, United States

¹⁹Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Centro di Ricerca per la Frutticoltura, Via di Fioranello 52, 00134 Rome, Italy

Correspondence

Daniele Bassi (daniele.bassi@unimi.it)

Abstract

Sharka, caused by *Plum Pox Virus* (PPV), is by far the most important infectious disease of peach [*P. persica* (L.) Batsch] and other *Prunus* species. The progressive spread of the virus in many important growing areas throughout Europe poses serious issues to the economic sustainability of stone fruit crops, peach in particular. The adoption of internationally agreed-upon rules for diagnostic tests, strain-specific monitoring schemes and spatial-temporal modeling of virus spread, are all essential for a more effective sharka containment. The EU regulations on nursery activity should be modified based on the zone delimitation of PPV presence, limiting open-field production of propagation materials only to virus-free areas. Increasing the efficiency of preventive measures should be augmented by the short-term development of resistant cultivars. Putative sources of resistance/tolerance have been recently identified in peach germplasm, although the majority of novel resistant sources to PPV-M have been found in almond. However, the complexity of introgression from related-species imposes the search for alternative strategies. The use of genetic engineering, particularly RNAi-based approaches, appears as one of the most promising perspectives to introduce a durable resistance to PPV in peach germplasm, notwithstanding the well-known difficulties of *in vitro* plant regeneration in this species. In this regard, rootstock transformation to induce RNAi-mediated systemic resistance would avoid the transformation of numerous commercial cultivars, and may alleviate consumer resistance to the use of GM plants.

Keywords

genetic engineering, fruit breeding, PPV virus, *Prunus persica* (L.) Batsch, RNAi.

2 **Introduction**

3 Sharka, caused by *Plum Pox Virus* (PPV), is the most devastating viral disease of peach and other
4 stone fruits, resulting in significant economic losses (Cambra et al., 2006; Sochor et al., 2012). The
5 progressive worldwide spread of this destructive disease requires a coordinated, focused effort to
6 implement effective approaches to control its diffusion. Decades of research experience have made
7 clear the need for transnational coordination involving not only researchers but all who are
8 associated with the fruit industry including the phytosanitary services other than the growers
9 themselves. An International Workshop on sharka disease in peach (Milan and Cesena, Italy, 2016,
10 <https://sites.google.com/site/ppvsymposium2016>), gave the opportunity to review and update the
11 state-of-art with particular attention to the main limitations and short-term perspectives.

12 The aim of this article is to provide a comprehensive picture of the current available
13 possibilities to contrast sharka disease and to implement the resistance in peach, focusing on crucial
14 aspects: the virus spread and management strategies within the EU regulatory framework; old and
15 novel sources of genetic resistance (including the peach-related species almond and *P. davidiana*)
16 and breeding perspectives; the application of novel genomics tools for the introgression of
17 resistance and novel approaches for developing non-host resistance; the opportunity of using
18 genetic engineering techniques, as already experienced in other fruit-tree species.

20 **Spread of sharka disease**

21 Currently, PPV is spreading in many countries, with the occurrence of several strains with
22 different epidemiology and specific infective capabilities. However, precise information and
23 tracking of PPV distribution, correlated with the specific strain(s), is lacking in many peach
24 growing regions. Little is known about the epidemiology of the Eastern Europe strains. Five new
25 strains have been discovered in the last 10 years. The general framework of the PPV strains
26 currently established includes, in addition to the most common PPV-M, -D and -REC, the strains -T
27 (Turkey, Albania), -An (Albania), -EA (Egypt), -W (Canada, Ukraine, Russia, Latvia), -CR
28 (Russia) and -C (Moldavia, Belarus and Russia), the last two generally limited to cherry.
29 Homologous recombination plays an important role in PPV evolution (Garcia et al., 2014). Several
30 features including higher aphid transmission rates, a reduced latency period, faster virus diffusion in
31 the orchards from primary infected plants and broad host range, confer to PPV-M the highest
32 epidemicity in peach (Dallot et al., 2003). Information about the dynamics of host infection as it
33 affects disease spread remains scarce.

34 Several strategies have been deployed to manage sharka disease, depending on the epidemic
35 context. Eradication, based on orchard surveillance and removal of infected plants has been widely
36 adopted in Western Europe and North America, whereas tolerant cultivars have been used in

37 Eastern Europe (particularly for plum). In the former case, it becomes imperative to organize and
38 carry out efficient strain-specific monitoring schemes implementing spatial-temporal modeling of
39 disease spread. In France, surveillance intensity varies according to disease risk and control
40 activities of the Plant Health Services are supported by skilled private professional organizations.
41 The heterogeneity of PPV diagnostic tests is one of the main limitations for disease management.
42 The test method(s) should be established by specific internationally agreed-upon rules. More rapid
43 and accurate techniques for PPV diagnosis on candidate plants entering into the certification system
44 are required, along with the ability to detect virus infection during the latency period. Novel
45 techniques, such as Digital Droplet PCR (ddPCR) (Gutiérrez-Aguirre et al., 2015) with the potential
46 to detect up to one copy of viral RNA and isothermal amplification by reverse transcription-
47 recombinase polymerase (Zhang et al., 2014), applicable directly on-field, are quite promising. To
48 develop the next generation of novel diagnostics, close cooperation among all the interested actors,
49 including entomologists will be important. For more information on PPV management strategies,
50 see Rimbaud et al., (2015).

51 **Regulatory framework**

52 Considering the spread of sharka in Europe, the disease can no longer be controlled through
53 quarantine legislation only, but the latter must be combined with the regulation of the Plant
54 Propagating Material Quality. Evidence of such changes in the EU policies are found within
55 documents in preparation which are now discussed in the context of the Harmonization of the
56 Certification Scheme for Fruit Plants, particularly the Annex to the Commission Implementing
57 Directive amending Annex IV Part A Section II of Directive 2000/29/EC on protective measures
58 against the introduction in the Community of organisms harmful to plants or plant products and
59 against their spread within the Community. Concerning PPV, many of the directives contained in
60 the documents are not compatible with the epidemiology of the disease and its danger. For example,
61 the time intervals provided for diagnostic tests are too long for an effective management. Another
62 controversial and unexplained aspect is the criterion allowing the possibility to maintain mother
63 plants under field condition in endemic areas.

64 The control of nursery plant material and trading pathways is critical. Insufficient controls
65 on hundreds of new peach varieties offered to nurseries and growers every year, and the sporadic
66 presence of PPV infected material in nurseries, along with the trade and/or exchange of
67 uncontrolled materials for grafting by fruit growers, set up ideal conditions for national and
68 international virus spread. Moreover, the release of the European Plant Passport according to the
69 EU regulations and upon Phytosanitary Service control does not guarantee preventative testing of
70 trade materials. In this regard the inspection and control on breeding and nursery materials, the
71 utilization of virus-free (VF) certified plants, the restriction of nursery activities to pest-free areas

72 or under screenhouse, and the safeguarding of pest-free areas are all imperative. For the
73 management of sharka disease it is essential that:

74 i) the effective and adequate application of a mandatory certification system for propagation
75 material be used for all EU members;

76 ii) the zone delimitation of European fruit growing areas based on PPV presence should
77 limit the nursery production in open field only in virus-free areas (alternatively, in secure
78 screenhouses);

79 iii) PPV isolates detected in new foci be characterized molecularly and serologically for risk
80 assessment evaluation;

81 iv) PPV infection status of any new cultivar released and its behavior toward PPV infection
82 be evaluated at least for the most common PPV strains (M, D and Rec).

83 In conclusion, EU legislation must consider all these aspects since PPV should be regarded
84 as a quarantine pest and the level of alert be enforced and not reduced.

85

86 **Source of resistance to sharka and breeding perspectives**

87 The results of two decades of research confirm the absence of immune or resistant cultivars
88 in peach germplasm, although a general low susceptibility to PPV-D strain has been reported
89 (Rubio et al., 2012). The introgression of resistance from peach-related species, such as *Prunus*
90 *davidiana* (Carrière) (Pascal et al., 1998) and *Prunus dulcis* (Webb) (Pascal et al., 2002; Martínez-
91 Gómez et al., 2004), has been unsuccessful so far, mainly due to the difficulty in combining PPV
92 resistance with traits suitable for the peach marketing (Moing et al., 2003). Most of the limitations
93 come from the low rates of resistant individuals produced by crossbreeding and by the many
94 unfavorable traits expressed by F₁ hybrids, requiring at least several rounds of backcrossing to
95 recover ‘commercial’ fruit traits. The absence of molecular markers associated with resistance,
96 which would facilitate the selection of resistant seedlings, adds to the complexity of breeding PPV
97 resistant cultivars. The introduction of resistance from *P. davidiana* has been recently suspended by
98 French breeding programs in favor of the introgression from almond. As a result of the
99 collaboration between INRA-Avignon (France) and CEBAS-CSIC (Spain) several almond cultivars
100 resistant to PPV-M were identified, including ‘Del Cid’, and have been chosen by INRA as resistant
101 parents for building hybrid populations. Nevertheless, the experience within the Italian PPVCON
102 project suggests that the use of almond as a resistance donor could suffer from the same limitations
103 observed in resistant peach selections coming from UCD hybrids [(‘Padre’(almond) x ‘54P455’
104 (peach)) x ‘Hesse’ (peach) x self], characterized by poor fruit quality (Liverani et al., 2011).

105 Among other approaches attempted to confer sharka resistance, the use of aphid-resistant
106 peach selections proved to be ineffective, since the trait was unable to ensure a protection against
107 virus transmission in endemic areas (Liverani et al., 2015).

108 The extensive evaluation of more than 300 peach cultivars from core collections and
109 recently released varieties showed the presence of a small number of accessions highly tolerant to
110 the PPV-M strain (e.g. infectable by the virus but asymptomatic or developing only mild symptoms,
111 particularly on fruit) (Liverani et al., 2011). A promising resistant selection (e.g. ‘Spasena’) derived
112 from the resistant parent ‘Dupnsika’ (Gabova et al., 1994) is currently under evaluation at the Fruit
113 Growing Institute of Plovdiv (Bulgaria). Notably, the selection process occurring in open-field
114 conditions within endemic sites, allows an increased reliability of resistance evaluation results.
115 Indeed, as observed from field trials in Italian endemic areas, PPV-M was able to infect in just a
116 few years about 70% of advanced selections, found resistant after several years of greenhouse
117 ‘heavy test’ evaluations, i.e. grafting on already infected GF305 (Liverani et al., 2015). In
118 perspective, the outdoor trials in endemic areas appear as a more practical, cost-effective and
119 reliable solution for the screening of promising selections.

120 An interesting research field is the use of resistant rootstocks, such as the almond cultivar
121 ‘Garrigues’, to induce resistance in the scion (Rubio et al., 2013). The postulated mechanism
122 responsible for the prevention or recovery from infection is the systemic transmission by graft
123 (almond) to scion (peach) of a silencing signal. Apart from the repeatability of the experiment in
124 peach cultivars other than the already tested ‘GF305’, some other questions are raised about its
125 durability in time and in the field. Most important, considering the general tolerance of peach
126 species to PPV-D strain, further studies should ascertain the stability of this mechanism against the
127 most virulent PPV-M strain.

128 In conclusion, a divergent strategy for incoming breeding programs appears evident among
129 some Italian and French research groups: the former is aiming at (short-term) development of
130 cultivars highly tolerant to PPV-M (no symptoms, at least on fruit) cultivars suitable for the
131 preservation of a peach industry in endemic areas; the latter attempts to introduce durable resistance
132 (no virus replication in the tree) from almond (as PPV-M resistant parent or as a resistance-inducing
133 rootstock). The introduction of tolerant plants is a matter of debate, since epidemiologists are
134 concerned about the possible development of more aggressive PPV strains raising by recombination
135 from mixed PPV infections and also for increasing the difficulties of implementing containment
136 strategies on asymptomatic plants, since a PPV inoculum reservoir still persists in the area.

137

138 **Molecular and functional genomics**

139 As demonstrated by several studies, the resistance to sharka conferred by the *P. davidiana*
140 clone 'P1908' is of a quantitative nature, regulated by several QTLs with small effects, often
141 variable across years (Decroocq et al., 2005). Linkage mapping experiments performed on
142 progenies derived from different cross combinations of 'P1908' and/or SD hybrids ('P1908' x
143 'Summergrand') also demonstrated that QTL numbers and positions are affected by the genetic
144 background of the peach parents (Marandel et al., 2009; Rubio et al., 2010). Currently, candidate
145 gene(s) or molecular markers associated with PPV resistance have not been identified in *P.*
146 *davidiana* or derived peach hybrids, hindering the short-term launching of marker-assisted selection
147 (MAS) programs. The availability of the Peach Genome reference sequence and a 9K SNP array
148 platform (Verde et al., 2012), in addition to the availability of novel, powerful 'omics' tools may
149 accelerate the identification of the genetic basis of PPV resistance in peach related species. For
150 example, a Genotyping-By-Sequencing approach has been recently adopted to develop high-density
151 genome markers for the introgression of resistance from almond (French FruitSELGEN project).
152 While this approach is undoubtedly promising at the scientific level, concerns still persist about the
153 real possibility of developing valuable cultivars from peach-almond hybrid(s) in the short-medium
154 term.

155 The main bottlenecks for the identification of the genetic bases of PPV resistance, are still
156 the cost, complexity and time-consuming phenotyping procedures, given the well-known
157 unresolved issues:

158 i) The lack of standardized phenotyping methods among research groups, i.e 'heavy test'
159 (see above) vs. 'standard test' (by inoculating buds from infected plants onto the accessions to be
160 challenged), restricting the cross-validation of results.

161 ii) A subjective and non-uniform interpretation of visual symptoms and discrepancies in
162 terminology, particularly for 'resistance' and 'tolerance'. An alternative scoring system to
163 overcome the strong subjectivity of symptoms evaluation is still lacking. A proposal for a
164 standardized terminology is shown in Table 1.

165 iii) The long-term requirement of evaluation trials for a reliable assessment of resistance. No
166 quick protocol for resistance evaluation has been implemented so far and is not recommended, since
167 the alternance of resting and growing periods is obviously important for the reliability of the trial.

168 iv) The reliability of 'in-field' resistance evaluation vs. 'screenhouse' testing.

169 Knowledge of the mechanism of PPV-peach interaction at physiological, proteomic,
170 metabolic and gene expression level are still scarce, although it may have practical implication for
171 disease management (reviewed by Clemente-Moreno et al., 2015) or for the development of
172 biomarkers to detect early PPV infection, especially for nursery mother trees. Recently, proteome
173 analysis of PPV-infected peach plant showed that infection affected the abundance of proteins

174 related to photosynthesis, carbohydrate and amino acid metabolism (Clemente-Moreno et al., 2013).
175 RNA-seq of peach leaf transcriptome after PPV-D infection demonstrated the complexity of plant
176 response and the critical role of early responsive genes as a reaction against virus replication and
177 translocation, before symptoms development (Rubio et al., 2015). Unfortunately, the experiments
178 above were not performed on PPV resistant accessions, thus there are not information about
179 candidate genes involved in sharka resistance in peach so far.

180 Developing non-host resistance in peach is a new frontier of research. Viruses encode a
181 limited number of essential proteins, since recruit plant proteins (host factors) for every stage of the
182 infection cycle (Garcia et al., 2015; van Schie and Takken, 2014). Gene(s) coding for such factors
183 can be considered as susceptibility (S) genes and their mutation or loss of function limit the ability
184 of the pathogen to cause disease. For example, recessive resistance to some *Potyvirus*es, PPV
185 included, is associated with mutations of genes belonging to the eukaryotic translation initiation
186 factors (*eIF*) family in several plant species, thus representing an excellent candidate to also confer
187 non-host resistance in peach. Different tools have been deployed to explore the possibility of
188 developing a non-host resistance in peach:

189 i) EcoTILLING approach, searching for peach variants in which susceptibility gene(s) are
190 deleted or non-functional. Nevertheless, as a consequence of reduced genetic diversity, peach shows
191 the lowest number of variants in comparison with other *Prunus* species. All promising individuals
192 are hybrids (peach crossed to almond or peach wild related species);

193 ii) TILLING approach, by creating *de novo* non-functional host genes through chemical
194 mutagenesis (EMS). Three thousand individuals derived from the susceptible peach rootstock
195 ‘GF305’ are currently under screening by whole-genome re-sequencing to evaluate the mutation
196 rate after EMS treatment and the mutation rate in susceptibility genes.

197 iii) S gene(s) silencing and/or Genome Editing (discussed in the next section).

198 The application of novel ‘omics’ approaches holds great promise for unraveling the
199 complexity of the genetic mechanisms regulating sharka resistance. This knowledge is essential for
200 developing useful molecular tools in breeding PPV resistant varieties as well for implementing
201 more efficient disease management strategies. The development of non-host resistance in peach is
202 an ambitious target, that is now moving its first steps.

203

204 **Introducing resistance in peach by genetic engineering**

205 In light of the complexity of breeding PPV-resistant peach cultivars by conventional
206 strategies, as determined by the absence of resistant cultivars, by the low genetic diversity in peach
207 germplasm, and by the ineffectiveness of introgression from related species, genetic engineering

208 (GE) approaches appear an alternative strategy at the current status of knowledge (Ilardi and
209 Tavazza, 2015).

210 Long-term evaluations, lasting over 20 years, in both greenhouse and endemic areas
211 throughout Eastern Europe, have demonstrated the effectiveness of GE approaches to confer stable
212 and durable sharka resistance in plum (Scorza et al., 2016). PPV resistance in transgenic
213 ‘HoneySweet’ plum is based on RNA interference (RNAi), triggered by a complex multi copy
214 insertion of an inverted repeat/hairpin configuration of the PPV coat protein (PPV-CP) transgene,
215 activating the production of 25-26nt class siRNA and ultimately leading to viral RNA degradation
216 (Kundu et al., 2008). A more detailed knowledge of PTGS mechanisms has led to the development
217 of more effective tools for RNAi-mediated engineered resistance. In particular, intron hairpin RNA
218 (ihpRNA) constructs have proven to be highly effective inducers of local and systemic resistance
219 against PPV in both model species *N. benthamiana* (Pandolfini et al., 2003; Di Nicola-Negri et al.,
220 2005) and *P. domestica* (Hily et al., 2007; Monticelli et al., 2012). In addition, PPV-derived
221 ihpRNA constructs were able to induce high resistance to a wide range of PPV strains (Di Nicola-
222 Negri et al., 2010; Ravelonandro et al., 2014) also under variable abiotic and biotic conditions that
223 are known to have a negative impact on gene silencing in plants (Di Nicola et al., 2014).
224 Advantages of ihpRNA constructs include the high flexibility (it is possible to target multiple
225 sequences from different PPV genomic regions or different PPV strains, Wang et al., 2013), high
226 stability of siRNAs production, no documented interference with the endogenous RNA silencing
227 machinery, robust and durable antiviral resistance and absence of transgene-derived proteins. Some
228 questions related to undesirable consequences of RNAi still remain, such as off-target effects
229 leading to changes in the host transcriptome; trade-off between defense and growth/development
230 processes; and virus escape from silencing (Fuentes et al., 2016). However, the risk of such off-
231 target effects can be identified by a well-developed science based risk assessment and reduced by a
232 continuous monitoring as defined in a proper post-monitoring program to be applied after the
233 release of the new events.

234 However, the application of genetic transformation techniques in peach has been limited by
235 the difficulties in developing efficient regeneration and transformation protocols. Nevertheless, a
236 protocol for *in vitro* regeneration via organogenesis, has been already developed for one of the most
237 widely used peach rootstocks ‘GF677’ (Sabbadini et al., 2015). The protocol may be further
238 improved by using different selection markers since ‘GF677’ appears to be naturally rather resistant
239 to the antibiotic kanamycin. At present, the availability and the already demonstrated capabilities of
240 many viral-derived constructs to induce RNAi against PPV, jointly with a high probability of
241 transforming ‘GF677’, make the hypothesis of rootstock engineering one of the most feasible and
242 promising for peach cultivars (MIUR - PRIN VIREs project). Root-to-scion (and scion-to-root)

243 siRNAs transfer has been already demonstrated in several annual model plants (reviewed in Pyott
244 and Molnar, 2015). Rootstock transformation would be appealing, for several reasons: the scion
245 would maintain its genetic background; no gene flow, because pollen and seeds would not be
246 allowed to be produced by the genetically modified rootstock; the same transgenic rootstock can be
247 used for many cultivars, avoiding the transformation of each single accession; and it may simplify
248 many aspects related to the opinion of the consumers on GM plants (Lemgo et al., 2013). Recently,
249 this approach has been successfully applied in a cherry rootstock to induce RNAi-mediated
250 systemic resistance to Prunus Necrotic Ringspot Virus (Song et al., 2013; Zhao and Song, 2014),
251 although the stability and durability of this approach requires further evaluation. On the contrary,
252 transmission of RNA silencing was not observed in non-transgenic scions in apple (Flachowsky et
253 al., 2012), and thus, additional research is required to set-up the appropriate strategies for an
254 efficient silencing through grafting (Gohlke and Mosher, 2015).

255 While the ability of PPV-resistant ‘HoneySweet’ plum to transfer the silencing signal has
256 not been demonstrated, the idea to use it directly as a rootstock in European peach growing areas is
257 attractive. The main limitation arises from the implementation of experimental field trials, which
258 require the approval of competent authorities. ‘HoneySweet’ and other reported GE and
259 conventional PPV resistant *Prunus* should be field tested as rootstocks (and as scions) in PPV
260 endemic areas. This work requires that competent authorities evaluate GE field tests on scientific
261 merit and realistic biosafety issues, and that local authorities guarantee the safety of these plantings
262 against destruction by radical GE opponents.

263 In spite of the success obtained through GE approaches to induce virus resistance in several
264 crops, public concerns over the potential ecological impact of GE organisms and/or products
265 strongly limit their use in Europe. In this sense, the approval of ‘HoneySweet’ by US authorities is a
266 paradigm shift for fruit trees in terms of the application of GE technology (Scorza et al., 2013). Risk
267 assessment is an integral part of GE plant production, and the time and costs that it requires should
268 be added to those of plant transformation, selection and evaluation. In addition to the molecular
269 analysis of plants, the biochemical characterization of fruits and the evaluation of resistance, further
270 information may be required from authorities and consumers, including plant-virus and plant-insect
271 interactions, gene flow and the potential risk of off-target gene silencing (All of which were
272 addressed for ‘HoneySweet’ in the U.S. regulatory dossiers). Excluding marker proteins, the risks
273 associated with newly expressed proteins (i.e. allergenicity or toxicity) would not be meaningful for
274 RNAi-based engineering, due to the lack of viral proteins produced by RNAi plants.

275 Genome editing is a novel and promising type of GE, based on the use of engineered
276 nucleases for the introduction of mutations at target genomic sequences. Despite the growing
277 positive opinion about its application from authorities and consumers, it is still hardly applicable in

278 peach. In order to maintain clonal stability in vegetative propagated plants, the application of
279 genome editing techniques requires the development of effective protocols of plant regeneration
280 from protoplast culture, unavailable in peach. Lacking the possibility of transient expression, stable
281 transformation with the Cas9 system will be necessary (Jia and Wang, 2014; Xu, 2013), which will
282 then require a round of backcrossing or self-pollination for its removal after editing procedures and
283 regeneration. The mandatory germline transition generates a novel cultivar that must then be
284 evaluated before market introduction. In addition, some concerns still persist about how this
285 procedure of developing non transgenic plants using GE techniques would be classified by the
286 authorities. Despite this, the resistance conferred by the editing of susceptibility genes (S) to confer
287 PPV resistance is an interesting perspective. The *eIF4E(s)*, *cPGK*, *DBP1* and/or *PpDDXL* genes
288 recently functionally characterized in peach represent good candidates for the application of
289 Genome Editing (Castelló et al., 2011; Poque et al., 2015; Huang et al., 2010). However, because S
290 genes have a function other than as a compatibility factor for the pathogen, the side effects caused
291 by their mutation demand a one-by-one assessment of their usefulness for application. Plant
292 regeneration from protoplast cell lines has never been demonstrated in peach and this remains the
293 major issue for the application of genome editing in heterozygous vegetatively propagated plants.
294 Therefore, current knowledge of RNAi approaches seem more suitable to yield positive results.

295 A relatively recent technology for rapid cycle breeding ('FastTrack') developed in other
296 species (apple, plum, etc.) is a breeding system that uses a GE tree expressing a flowering pathway
297 gene, such as *FT* gene orthologs, to obtain fruiting trees in one year (or even less) (Flachowsky et
298 al, 2011; Srinivasan et al, 2012). Shortening the juvenile stage in peach would accelerate the
299 conventional breeding procedures for the introgression of sharka resistance from peach-related
300 species. Also, the final resulting trees would not be GE unless the PPV resistance gene used is a
301 transgene.

302

303 **Conclusions**

The containment of sharka disease spread is one of the most important priorities of the European peach industry. The complexity of this phytosanitary issue does not allow simple, rapid solutions. The application of preventive measure with the maximum alert level and, possibly, their reinforcement through the implementation of more effective management strategies, are of utmost importance. The adoption of internationally agreed-upon rules for diagnostic tests, strain-specific monitoring schemes and spatial-temporal modeling of virus spread, are all essential for a more effective sharka containment. The EU regulations on nursery activity should be modified based on the zone delimitation of PPV presence, limiting open-field production of propagation materials only to virus-free areas. Prevention should be combined with the introduction of genetic resistance in a

reasonable time, but not at the expense of high fruit quality productions. A part from still to be verified existence of intraspecific sources of PPV resistance in peach, the complexity of introgression from related-species imposes the search for alternative strategies. Currently, the use of genetic engineering, particularly RNAi-based approaches, appears as one of the most promising perspectives, notwithstanding the well-known difficulties of *in vitro* plant regeneration in this species. Rootstock transformation to induce RNAi-mediated systemic resistance to PPV would avoid the transformation of numerous commercial cultivars, and may alleviate consumer concerns to the use of GM plants. In this regard, the use of genetic engineering approaches represent a fundamental opportunity, and as such it should be supported not only from a technical-scientific view point, but also in a broader socio-political context.

304

305 **Conflict of Interest**

306 The authors declare no conflict of interest.

307

308 **Acknowledgments**

309 The International Workshop was funded by the following Italian subjects: Apofruit, BattistiniVivai,
310 CAV, CIVI Italia, CRPV, Europfruit, GeoplantVivai, MinguzziSpA, New Plant, Orogel Fresco,
311 Pempacorner, University of Milan, Vitroplant, ZaniGranfrutta, ZanziF.lliVivai. Special thanks to
312 Michele Ardizzone (GMO Department, European Food Safety Authority) for his participation and
313 active discussion in the workshop ‘Sharka (PPV) Disease in Peach’.

314

315 **Author contributions**

MC: drafted the manuscript and critically revised it;

FG, RS: critically revised the manuscript;

316 AB, VB, LC, BC, SD, LD, VD, SF, VI, AL, BM, AM, MP, TP, ThP, VS, and IV: critically revised
317 the manuscript for important contents;

318 DB: conceived the manuscript and critically revised it;

319 All authors: read and approved the final manuscript

320

321 **References**

322 Cambra, M., Capote, N., Myrta, A., and Llácer, G. (2006) Plum pox virus and the estimated costs
323 associated with sharka disease. *Bulletin OEPP/EPPO* 36, 202-204.

324 Castelló, M., Carrasco, J.L., Navarrete-Gómez, M., Daniel, J., Granot, D., and Vera, P. (2011) A
325 plant small polypeptide is a novel component of DNA-binding protein phosphatase 1-mediated

- 326 resistance to plum pox virus in Arabidopsis. *Plant Physiol.* 157(4), 2206-15.
327 doi:10.1104/pp.111.188953
- 328 Clemente-Moreno, M.J., Diaz-Vivancos, P., Rubio, M., Fernandez, N., Hernández, J.A. (2013)
329 Chloroplast protection in Plum Pox Virus-infected peach plants by 1-2-oxo-4-thiazolidine-
330 carboxylic acid treatments: effect in the proteome. *Plant Cell Environ.*36, 640-654.doi:
331 10.1111/pce.12003.
- 332 Clemente-Moreno, M.J., Hernández, J.A., Díaz-Vivancos, P. (2015) Sharka: how do plants respond
333 to Plum pox virus infection? *J. Exp. Bot.* 66, 25-35. doi:10.1093/jxb/eru428
- 334 Dallot, S., Gottwald, T., Labonne, G. and Quiot, J.B. (2003) Spatial pattern analysis of sharka
335 disease (Plum pox virus strain M) in peach orchards of southern France. *Phytopathology*, 93, 1543–
336 1552. doi:10.1094/PHYTO.2003.93.12.1543.
- 337 Decroocq, V., Foulongne, M., Lambert, P., Le Gall, O., Mantin, C., Pascal, T., et al. (2005)
338 Analogues of virus resistance genes map to QTLs for resistance to sharka disease in *Prunus*
339 *davidiana*. *Mol. Gen. Genomics* 272, 680-689. doi:10.1007/s00438-004-1099-0
- 340 Di Nicola-Negri, E., Brunetti, A., Tavazza, M., and Ilardi, V. (2005) Hairpin RNA-mediated
341 silencing of Plum pox virus P1 and HC-Pro genes for efficient and predictable resistance to the
342 virus. *Transgenic Res.* 14(6), 989-994. doi:10.1007/s11248-005-1773-y
- 343 Di Nicola-Negri, E., Tavazza, M., Salandri, L., and Ilardi, V. (2010) Silencing of Plum pox virus 5'
344 UTR/P1 sequence confers resistance to a wide range of PPV strains. *Plant Cell Rep.* 29(12), 1435-
345 1444. doi:10.1007/s00299-010-0933-6
- 346 Di Nicola, E., Tavazza, M., Lucioli, A., Salandri, L., and Ilardi, V. (2014) Robust RNA silencing-
347 mediated resistance to Plum pox virus under variable abiotic and biotic conditions. *Mol. Plant*
348 *Pathol.* 15(8), 841-847. doi:10.1111/mpp.12132
- 349 Flachowsky, H., Le Roux, P.M., Peil, A., Patocchi, A., Richter, K., Hanke, M.V. (2011)
350 Application of a high-speed breeding technology to apple (*Malus x domestica*) based on transgenic
351 early flowering plants and marker-assisted selection. *New Phytol.* 192, 364-377.
352 doi:10.1111/j.1469-8137.2011.03813.x
- 353 Flachowsky, H., Tränkner, C., Szankowski, I., Waidmann, S., Hanke, M., Treutter, D. et al. (2012)
354 RNA-mediated gene silencing signals are not graft transmissible from the rootstock to the scion in
355 greenhouse-grown apple plants *Malus* sp. *Int. J. Mol. Sci.* 13(8), 9992-10009.
356 doi: 10.3390/ijms13089992
- 357 Fuentes, A., Carlos, N., Ruíz, Y., Callard, D., Sánchez, Y., Ochagavía, M.E., et al. (2016) Field trial
358 and molecular characterization of RNAi-transgenic tomato plants that exhibit resistance to tomato
359 yellow leaf curl geminivirus. *Mol. Plant Microbe Interact.* 29(3), 197-209. doi:10.1094/MPMI-08-
360 15-0181-R

- 361 Gabova, R.(1994). Evaluation of peach and nectarine cultivars in Bulgaria for their resistance to
362 Plum pox potyvirus. *Bulletin EPPO* 24(3), 755-760.
- 363 Garcia, J.A., Glasa, M., Cambra, M., and Candresse, T. (2014) Plum pox virus and sharka: a model
364 potyvirus and a major disease. *Mol. Plant Pathol.* 15(3), 226-241. doi:10.1111/mpp.12083
- 365 García, J.A. and Pallás, V. (2015). Viral factors involved in plant pathogenesis. *Curr. Opin. Virol.*
366 11, 21-30. doi:10.1016/j.coviro.2015.01.001
- 367 Gohlke, J., and Mosher, R.A. (2015) Exploiting mobile RNA silencing for crop improvement. *Am.*
368 *J. Bot.* 102 (9), 1399-1400.
- 369 Gutiérrez-Aguirre, I., Rački, N., Dreo, T., and Ravnikar, M. (2015) Droplet digital PCR for absolute
370 quantification of pathogens. *Methods Mol. Biol.* 1302, 331-47. doi:10.1007/978-1-4939-2620-6_24
- 371 Hily, J.M., Ravelonandro, M., Damsteegt, V., Bassett, C., Petri, C., Liu, Z., et al. (2007) Plum pox
372 virus coat protein gene Intron-hairpin-RNA (ihpRNA) constructs provide resistance to plum pox
373 virus in *Nicotiana benthamiana* and *Prunus domestica*. *J. Am. Soc. Hortic. Sci.* 132(6), 850-858.
- 374 Huang, T.S., Wei, T., Laliberte, J.F., and Wang, A. (2010) A host RNA helicase-like protein,
375 AtRH8, interacts with the Potyviral genome-linked protein, VPg, associates with the virus
376 accumulation complex, and is essential for infection. *Plant Physiol.* 152, 255-266.
377 doi:10.1104/pp.109.147983
- 378 Ilardi, V., and Tavazza, M. (2015) Biotechnological strategies and tools for Plum pox virus
379 resistance: trans-, intra-, cis-genesis, and beyond. *Front. Plant Sci.* 6, 379.
380 doi:10.3389/fpls.2015.00379
- 381 Jia, H., and Wang, N. (2014) Targeted genome editing of sweet orange using Cas9/sgRNA. *PLoS*
382 *One* 9(4): e93806.
- 383 Kundu, J.K., Briard, P., Hily, J.M., Ravelonandro, M., and Scorza, R. (2008) Role of the 25–26
384 ntsiRNA in the resistance of transgenic *Prunus domestica* graft inoculated with plum pox virus.
385 *Virus Genes.*36(1), 215-220. doi: 10.1007/s11262-007-0176-y
- 386 Lemgo, G.N., Sabbadini, S., Pandolfini, T., and Mezzetti, B. (2013) Biosafety considerations of
387 RNAi-mediated virus resistance in fruit-tree cultivars and in rootstock. *Transgenic Res.* 22(6),
388 1073-1088. doi:10.1007/s11248-013-9728-1
- 389 Liverani, A., Babini, A.R., Bassi, D., Brandi, F., Ciarmiello, L.F., Conte, L., et al. (2011) Il
390 miglioramento genetico per la resistenza a sharka in pesco: risultati del progetto italiano PPVCON.
391 *ItalusHortus* 18: 35-44 (Italian)
- 392 Liverani, A., Brandi, F., Sirri, S., Giovannini, D., Baroni, G., and Lonardi, F. (2015) Preliminary
393 Evaluation on Susceptibility to natural infections of putative resistant peach breeding selections in a
394 PPV endemic site. *ActaHortic.* 1084, 585-590. doi:10.17660/ActaHortic.2015.1084.79

- 395 Marandel, G., Pascal, T., Candresse, T., and Decroocq, V. (2009) Quantitative resistance to Plum
396 pox virus in *Prunus davidiana* P1908 linked to components of the eukaryotic translation initiation
397 complex. *Plant Pathol.* 58, 425-435. doi:10.1111/j.1365-3059.2008.02012.x
- 398 Martínez-Gómez, P., Rubio, M., Dicenta, F., and Gradziel, T.M. (2004) Resistance to Plum Pox
399 Virus (RB3.30 isolate) in a group of California almonds and transfer of resistance to peach. *J. Am.*
400 *Soc. Hortic. Sci.* 129 (4), 544-548.
- 401 Moing, A., Poessel, J.L., Svanella-Dumas, L., Loonis, M., and Kervella, J. (2003) Biochemical
402 basis of low fruit quality of *Prunus davidiana*, a pest and disease resistance donor for peach
403 breeding. *J. Am. Soc. Hortic. Sci.* 128, 55-62.
- 404 Monticelli, S., Di Nicola-Negri, D., Gentile, A., Damiano, C., and Ilardi, V. (2012) Production and
405 in vitro assessment of transgenic plums for resistance to Plum pox virus: a feasible, environmental
406 risk-free, cost-effective approach. *Ann. Appl. Biol.* 161(3), 293-301. doi:10.1111/j.1744-
407 7348.2012.00573.x
- 408 Pandolfini, T., Molesini, B., Avesani, L., Spena, A., and Polverari, A. (2003) Expression of self-
409 complementary hairpin RNA under the control of the rolC promoter confers systemic disease
410 resistance to plum pox virus without preventing local infection. *BMC Biotechnol.* 3(1), 7.
411 doi:10.1186/1472-6750-3-7
- 412 Pascal, T., Kervella, J., Pfeiffer, F., Sauge, M.H., and Esmenjaud, D. (1998) Evaluation of
413 interspecific progeny *Prunus persica* cv. Summergrand × *Prunus davidiana* for resistance and some
414 agronomic traits. *ActaHortic.* 465, 185-192. doi:10.17660/ActaHortic.1998.465.21
- 415 Poque, S., Pagny, G., Ouibrahim, L., Chague, A., Eyquard, J.P., Caballero, M., et al. (2015) Allelic
416 variation at the rpv1 locus controls partial resistance to Plum pox virus infection in *Arabidopsis*
417 *thaliana*. *BMC Plant Biol.* 15, 159. doi:10.1186/s12870-015-0559-5
- 418 Pyott, D.E., and Molnar, A. (2015) Going mobile: non-cell-autonomous small RNAs shape the
419 genetic landscape of plants. *Plant Biotechnol. J.* 13, 306-318.
- 420 Ravelonandro, M., Scorza, R., Michel, H. J., and Briard, P. (2014) The efficiency of RNA
421 interference for conferring stable resistance to Plum pox virus. *Plant Cell Tiss. Org.* 118(2), 347-
422 356. doi:10.1007/s11240-014-0487-3
- 423 Rimbaud, L., Dallot, S., Gottwald, T., Decroocq, V., Jacquot, E., Soubeyrand S., et al. (2015)
424 Sharka epidemiology and worldwide management strategies: Learning lessons to optimize disease
425 control in perennial plants. *Annu. Rev. Phytopathol.* 53:357-378. doi:10.1146/annurev-phyto-
426 080614-120140
- 427 Rubio, M., Martínez-Gómez, P., García, J.A., and Dicenta, F. (2013) Interspecific transfer of
428 resistance to Plum pox virus from almond to peach by grafting. *Ann. Appl. Biol.* 163, 466-474.
429 doi:10.1111/aab.12069

- 430 Rubio, M., Pascal, T., Bachellez, A., and Lambert, P. (2010) Quantitative trait loci analysis of PPV
431 resistance in *P. davidiana*: new insights on the organization of genomic resistance regions. *Tree*
432 *Genet. Genomes* 6, 291-304. doi:10.1007/s11295-009-0249-2
- 433 Rubio, M., Martínez-Gómez, P., García-Brunton, J., Pascal, T., García-Ibarra, A. and Dicenta, F.
434 (2012) Sensitivity of peach cultivars against a Dideron isolate of Plum pox virus. *Sci. Hortic.* 144,
435 81-86.
- 436 Rubio, M., Rodríguez-Moreno, L., Ballester, A.R., Moura, M.C., Bonghi, C., Candresse, T. et al.
437 (2015) Analysis of gene expression changes in peach leaves in response to Plum pox virus infection
438 using RNA-Seq. *Mol. Plant Pathol.* 16, 164–176. doi:10.1111/mpp.12169
- 439 Sabbadini, S., Pandolfini, T., Girolomini, L., Molesini, B., and Navacchi, O. (2015) Peach (*Prunus*
440 *persica* L.). *Methods Mol. Biol.* 1224, 205-15. doi:10.1007/978-1-4939-1658-0_17.
- 441 Scorza, R., Callahan, A., Dardick, C., Ravelonandro, M., Polak J., Malinowski, T., et al. (2013)
442 Genetic engineering of Plum pox virus resistance: ‘HoneySweet’ plum—from concept to product.
443 *Plant Cell Tiss. Org.* 115, 1-12. doi:10.1007/s11240-013-0339-6
- 444 Scorza, R., Ravelonandro, M., Callahan, A., Zagrai, I., Polak J., Malinowski, T., et al. (2016)
445 ‘HoneySweet’ (C5), the first genetically engineered Plum pox virus-resistant plum (*Prunus*
446 *domestica* L.) cultivar. *HortScience* 51:601-603.
- 447 Sochor, J., Babula, P., Adam, V., Krska, B. and Kizek, R. (2012) Sharka: the past, the present and
448 the future. *Viruses* 4, 2853-2901.
- 449 Song, G.Q., Sink, K.C., Walworth, A.E., Cook, M.A., Allison, R.F., and Lang, G.A. (2013)
450 Engineering cherry rootstocks with resistance to *Prunus* necrotic ring spot virus through RNAi-
451 mediated silencing. *Plant Biotechnol. J.* 11, 702-708. doi:10.1111/pbi.12060
- 452 Srinivasan, C., Dardick, C., Callahan, A., and Scorza, R. (2012) Plum (*Prunus domestica*) trees
453 transformed with poplar FT1 result in altered architecture, dormancy requirement, and continuous
454 flowering. *PLoS One* 7,7 e40715.
- 455 van Schie, C.C., and Takken, F.L. (2014) Susceptibility genes 101: how to be a good host. *Annu.*
456 *Rev. Phytopathol.* 52, 551–581. doi:10.1146/annurev-phyto-102313-045854
- 457 Verde, I., Bassil, N., Scalabrin, S., Gilmore, B., Lawley, CT., Gasic, K., et al. (2012) Development
458 and evaluation of a 9K SNP array for peach by internationally coordinated SNP detection and
459 validation in breeding germplasm. *PLoS One* 7. doi: 10.1371/journal.pone.0035668
- 460 Xu, K. (2013) The next generation biotechnology for apple improvement and beyond: The
461 CRISP/cas9 story. *New York Fruit Quarterly* 21 (4), 19-22.
- 462 Wang, X., Kohalmi, S.E., Svircev, A., Wang, A., Sanfaçon, H., and Tian, L. (2013) Silencing of the
463 host factor eIF(iso)4E gene confers plum pox virus resistance in plum. *PLoS One* 8, e50627.

464 Zhang, S., Ravelonandro, M., Russell, P., McOwen, N., Briard, P., Bohannon, S., et al. (2014)
 465 Rapid diagnostic detection of plum pox virus in *Prunus* plants by isothermal AmplifyRP® using
 466 reverse transcription-recombinase polymerase amplification. *J. Virol. Meth.* 207, 114-120.
 467 doi:10.1016/j.jviromet.2014.06.026

468 Zhao, D., and Song, G. (2014) Rootstock-to-scion transfer of transgene-derived small interfering
 469 RNAs and their effect on virus resistance in nontransgenic sweet cherry. *Plant Biotechnol. J.* 12(9),
 470 1319-28. doi:10.1111/pbi.12243.

471
 472 Table 1. Proposal for a standardized terminology. *Immunity: non-host resistance (plant cannot be
 473 infected); Resistant: negative ELISA and RT-PCR assay, no visible symptoms; Susceptible:
 474 positive or negative ELISA, positive RT-PCR assay, symptoms on plant organs from absence
 475 (tolerant) to severe (sensitive) (mandatory specification of organ/tissue). ‘Recovery reaction’,
 476 characterized by an initial low infection (RT-PCR positive only) later followed by no detectable
 477 infection (RT-PCR negative).

478

Classification	Visual Symphoms	ELISA	RT-PCR
Non-host			
Immune	-	-	-
Host			
Resistant	absent	-	-
Susceptible	absent (tolerant) to severe (sensitive)	+/-	+
Recovery	absent	-	+ to -

479