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LETTER TO THE EDITOR

Suitability of different plant species for experimental agroinfection with Plum pox virus-based expression vector for potential production of edible vaccines

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Summary. – Nine herbaceous plant species were tested for susceptibility to Plum pox virus (PPV) by *Agrobacterium*-mediated delivery of its infectious cDNA clone. Two of them became infected, namely spinach (local infection) and oilseed poppy (systemic infection). As a control, PPV infection was successfully established in plum seedlings following agroinfiltration, thus providing the first report of agroinfection in *Prunus* species. According to our results, oilseed poppy can be considered as a candidate host for the production of edible vaccines by a PPV-derived expression vector.

Keywords: agroinfiltration; virus host; poppy; spinach

Natural host range of Plum pox virus (PPV, the family *Potyviridae*) includes stone fruit trees (*Prunus* sp.). However, replication of PPV has been also observed in several annual and perennial herbaceous plant species, in both natural and experimental conditions (*1*). In the past, we engineered PPV genome for the heterologous gene expression in plants (*2*). Our pilot experiments focused mainly on *Nicotiana benthamiana*, where several polypeptides were produced including non-structural PB1-F2 protein of Influenza A virus (*3* and unpublished data). *N. benthamiana* is widely used model plant species in phytopathology. Its numerous advantages include easy cultivation of homogenous lots, fast growth and high susceptibility to a wide variety of plant pathogens without developing unwanted defensive response (*4*). This allows a high virus accumulation in systemically infected plant tissues. Although these features make *N. benthamiana* an ideal plant model for basic virus research, application of this plant for the production of biopharmaceuticals is limited due to its high alkaloid content (*5*). Therefore, in order to prepare safe plant-derived recombinant proteins such as edible vaccines, we tested a variety of potential PPV host species for susceptibility to PPV infection.

The expression vector pAD-agro was created by combination of the full-length cDNA clone of PPV bearing a cloning linker between the viral replicase (NIb) and capsid protein (CP) coding regions (*2*) and shortened pCambia 1304 vector (Abcam). Experimental plants were transfected by *Agrobacterium tumefaciens*-mediated gene delivery (agroinfiltration) of pAD-agro. At different times after inoculation, both inoculated and non-inoculated leaves were analyzed for the presence of PPV CP by Western blotting using polyclonal anti-PPV antibody (*6*). Total RNA was isolated from leaves by Nucleospin RNA Plant Kit (Macherey-Nagel) and analyzed by RT-PCR using primers NCuniFor/NCuniRev spanning the NIb/CP border (*7*) with subsequent sequencing of amplification products. To verify the PPV-specificity of the observed symptoms,

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Abbreviations: PPV = Plum pox virus

Table 1. Susceptibility of tested plant species to PPV infection

Species tested	Number of plants infected/ inoculated	References detection method about susceptibility to PPV
Prunus domestica	18/20 [a]	many (natural host)
Nicotiana benthamiana	20/20 [b]	many (main experimental host)
Papaver somniferum	18/18 [b]	Sutic (1977) / biological test
Spinacia oleracea	15/16 [a]	-
Rumex acetosa	0/8	-
Trifolium pratense	0/10	Manachini *et al.* (2007) / RT-PCR
Trifolium incarnatum	0/9	-
Arctium lappa	0/8	-
Erigeron annuus	0/10	Kil *et al.* (2021) / RT-PCR
Lactuca sativa	0/10	-
Taraxacum officinale	0/10	Kil *et al.* (2021) / RT-PCR

[a]Local or [b]systemic infection.

plants infiltrated by agrobacteria without pAD-agro were included in the analysis.

Although several hosts species are commonly used in biological experiments with PPV (mainly *Nicotiana* sp.), knowledge about its natural herbaceous hosts is insufficient due to the lack of relevant studies. It is possible that advances in high-throughput sequencing will shed light on this matter in the near future. Here, we tested the infectivity of PPV in several commonly spread weeds and cultural crop plants (Table 1). Some of them have already been demonstrated to be susceptible to PPV infection (*Papaver somniferum, Taraxacum officinale, Trifolium pratense*, *Erigeron anuus*) (*8, 9, 10*), while no information about the susceptibility of other tested species was available. Three-week-old plum seedlings (*Prunus domestica*, var. Toptaste) and *N. benthamiana* plants were included in the test as known PPV hosts.

In our experiments, infection of most tested species was unsuccessful, likely due to incompatibility with either PPV or agrobacteria. On the other hand, besides *N. benthamiana* a successful establishment of PPV infection was observed in plum (*P. domestica*, *Rosaceae*), oilseed poppy (*Papaver somniferum*, *Papaveraceae*) and spinach (*Spinacia oleracea*, *Amaranthaceae*).

Agroinfiltration is a simple and effective method that allows rapid transfection of a large number of experimental plants. Our results confirmed for the first time the applicability of this method to stone fruit trees of the genus *Prunus*. Typical PPV symptoms (diffuse chlorotic spots and vein clearing) were observed in transfected plum seedlings approximately from day 30 post infection mostly on inoculated leaves, occasionally also on the adjacent non-inoculated leaves (Suppl. Fig. 1). PPV infection in symptomatic tissues was confirmed by both Western blot and RT-PCR analysis/sequencing. Neither further systemic spread of the virus, nor its successful reinoculation to new plants by chip budding has been recorded, probably due to high level of resistance of the plum variety used (*11*). The infection remained localized close to the inoculation sites and later caused tissue necrosis, resulting in the death of affected leaves.

Infiltrated leaves of *S. oleracea* showed occasional chloroses while no systemic symptoms were observed. Western blotting and RT-PCR also proved the PPV presence exclusively in inoculated leaves (Suppl. Fig. 2). This was the first observation of PPV replication in spinach tissues, although without systemic spread. It is noteworthy that *Chenopodium foetidum,* used as hypersensitive (non-systemic) indicator host for PPV, belongs to the same family as spinach (*Amaranthaceae*) (*1*). Spinach leaves are commonly consumed raw, however, potential use of inoculated leaves as edible vaccine is limited by possible adverse effect of agrobacteria themselves as *Agrobacterium* spp. has been shown to be able to cause opportunistic infection in immunocompromised individuals (*12*).

Concerning *P. somniferum*, previous research has reported oilseed poppy infection by some other potyviruses including Turnip mosaic virus, Bean yellow mosaic virus or Beet mosaic virus (*13, 14*). The proof of PPV infection, however, relied yet exclusively on biological experiments based on indicator plant species. The oilseed poppy plants showed moderate leaf mottling and impaired plant growth in the past (8). Identical symptoms were observed in our experiments in non-inoculated leaves of transfected poppy plants at 3–4 weeks post inoculation (Suppl. Fig. 1). Both Western blotting and RT-PCR analysis reliably and reproducibly confirmed virus presence in systemically infected leaves (Suppl. Fig. 2). Thus, our

results provide the first immunochemical and molecular evidence of oilseed poppy infection by PPV. In order to prepare edible vaccines, targeting the accumulation of antigen into poppy seeds would be promising. Although the vertical transmission of PPV has not yet been reliably described, current knowledge is restricted to the genus *Prunus* (*15*). Hence, further research has to be conducted to analyze potential PPV translocation to seeds in herbaceous hosts, especially in oilseed poppy plants.

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Supplementary information is available in the online version of the paper.

References

1. Llácer G, EPPO Bull. 36, 227–228, 2006. https://doi.org/10.1111/j.1365-2338.2006.00978.x
2. Kamencayová M, Šubr Z, Acta Fytotech. Zootech. 15, special number, 24-26, 2012.
3. Kamencayová M, Košík I, Hunková J, Šubr ZW, Acta Virol. 58, 274–277, 2014. https://doi.org/10.4149/av_2014_03_274
4. Goodin MM, Zaitlin D, Naidu RA, Lommel SA, MPPI 21, 1015–1026, 2008. https://doi.org/10.1094/MPMI-21-8-1015
5. Saitoh F, Noma M, Kawashima N, Phytochemistry 24, 477–480, 1985. https://doi.org/10.1016/S0031-9422(00)80751-7
6. Šubr Z, Matisová J, Acta Virol. 43, 255–257, 1999.
7. Šubr ZW, Nagyová A, Glasa M, Julius Kühn Archiv 427, 339–341, 2010.
8. Sutic, D, Comptes Rendus des Seances de l'Academie d'Agriculture de France 63, 440–443, 1977.
9. Manachini B, Casati P, Cinanni L, Bianco P, J. Econ. Entomol. 100, 1047-1052, 2007.
10. Kil EJ, Ho PT, Fadhila C, Lal A, Vo TTB, Kim M, Lee S, J. Plant Dis. Prot. 128, 1091–1099, 2021.
11. Molnár ÁM, Ladányi M, Kovács S, Acta Univ. Agric. Silvic. Mendel. Brun. 6, 109–114, 2016
12. Adnan M, Khan S, Patel M, Al-Shammaria E, Ashankyty IMA, Rev. Med. Micro. 24, 94–97, 2013. https://doi.org/10.1097/MRM.0b013e3283642449
13. Kubelková D, Špak J, Plant Prot. Sci. 35, 33–36, 1999. https://doi.org/10.17221/9671-PPS
14. Glasa M, Šoltys K, Predajňa L, Sihelská N, Nováková S, Šubr Z, Kraic J, Mihálik D, Viruses 10, 430, 2018. https://doi.org/10.3390/v10080430
15. Glasa M, Candresse T, Encyclopedia of Virology, Fourth Edition, Vol. 3, Academic Press, San Diego, pp. 586–593, 2021. https://doi.org/10.1016/B978-0-12-809633-8.21242-9