Acta virologica **62**: 109 – 111, 2018 doi:10.4149/av_2018_114

LETTER TO THE EDITOR

In vitro micrografting of different *Prunus* species by cherry-adapted Plum pox virus isolate

Z. VOZÁROVÁ[1], A. NAGYOVÁ[1,2], S. NOVÁKOVÁ[1*]

[1]Institute of Virology, Biomedical Research Center Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic; [2]NWT, a. s. tř. Tomáše Bati, 760 01 Zlín, Czech Republic

Received November 10, 2017; accepted January 8, 2018

Keywords: PPV; in vitro; micrografting; host specificity

Sharka is serious disease of stone fruits caused by *Plum pox virus* (PPV), having worldwide spread and great economic impact due to the decrease of yield and quality of *Prunus* fruit production. Nowadays, at least 9 PPV strains differing at the molecular level are recognized (*1*). Although PPV is able to infect naturally almost all members of *Prunus* species and experimentally a range of herbaceous hosts, particular PPV strains show apparently more or less stringent host preference (*2*). Especially two strains, i.e. PPV-C and PPV-CR, are unique in their ability to naturally infect cherry trees (*3, 4*). To study this unusual property, various approaches were used, resulting in identification of specific mutations likely associated with particular adaptation to different non-*Prunus* species. These mutations were observed in the regions that span viral proteins P1, P3, 6K1, CI and CP (*1, 5*). Biological experiments with PPV are often performed in artificial herbaceous host systems, however, most relevant data concerning the virus-host interactions should be obtained from the natural woody host species. To overcome the slow and irregular growth of woody plants under field and greenhouse conditions, micropropagation constitutes the most efficient plant multiplication way. It is generally used for preservation and multiplication of healthy cultivars, however, in combination with micrografting, applications for early virus detection (*6*) as well as for biological research in different fields (for instance, studies of substance transport, long-distance signaling in plants) (*7*) have been reported, too.

Here we report an optimised protocol for *in vitro* multiplication of two *Prunus* species. Briefly, green sprouts of PPV-negative sour cherry (*P. cerasus,* wild seedling) and plum (*P. domestica cv. Saint Julien*) were washed under running tap water for 15 min and surface-sterilized by gentle agitation for 2 min in 70% ethanol, followed by immersion in 0.1% $HgCl_2$ for 5–10 min. Finally, explants were washed three times with sterile distilled water and transferred aseptically into the cultivation basic MS medium (*8*) containing 3.0% (w/v) sucrose, 0.8% (w/v) agar, 0.06% (w/v) MES (2-(*N*-morpholino) ethanesulfonic acid). Medium was supplemented with BAP (6-Benzylaminopurine, 1 mg/l), IBA (Indole-3-butyric acid, 0.1 mg/l) before autoclaving and GA3 (Gibberellic acid, 0.1 mg/l) (*9*). The pH was adjusted to 5.8 before adding agar. Prior each passage to a fresh medium, the explants were cultured for 4–6 weeks at 25 ± 1°C and 14/10 h (light/dark) photoperiod, photon flux density 40 ± 5 $\mu mol.m^{-2}.s^{-1}$. The same protocol has been applied for *in vitro* maintained *P. cerasus*, naturally infected by PPV-CR (RU-63sc isolate (*4*)) as scion culture. Regenerated shoots (~ 2–3 cm long) from both (scion and rootstock) cultures compatible in diameter were used as material for wedge type of *in vitro* micrografting (*10*). The plants were aseptically cultured on the same medium and under conditions reported above. The success of micrografting was evaluated 21–28 days post micrografting (dpm), when emerged rootstock shoots were tested for the viral spread from the infected scion into the PPV-free

*Corresponding author. E-mail: viruslno@savba.sk; phone: + 421-2-59302-447.

Abbrevations: DAS-ELISA = double antibody sandwich ELISA; dpm = days post micrografting; PPV = Plum pox virus

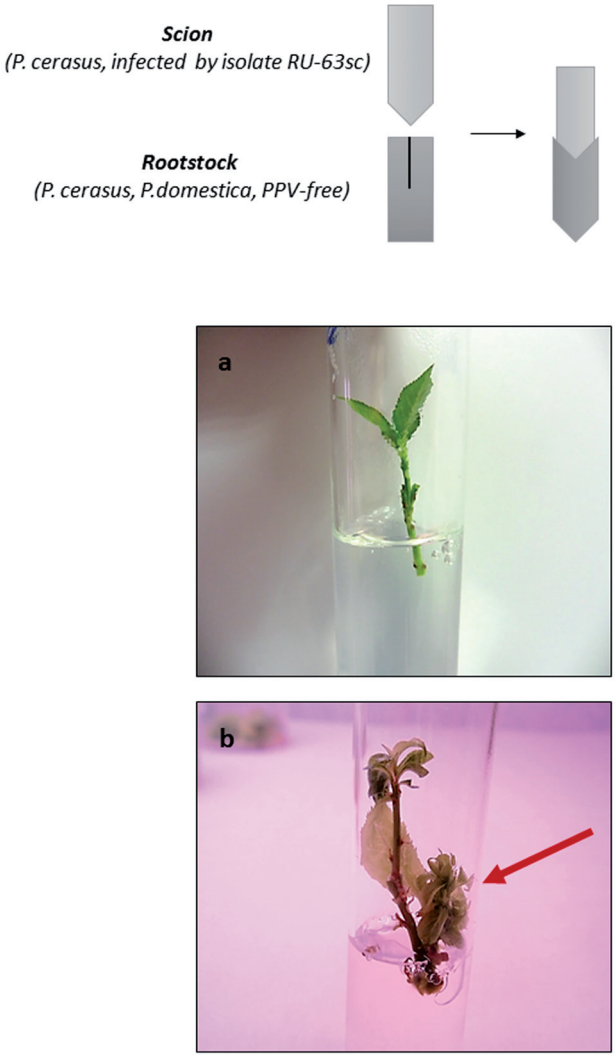

Fig. 1

Schematic representation of the *in vitro* micrografting technique used in this work (top) and micrograft development (bottom)
Micrograft after preparation **(a)** and after 21–28 dpm **(b)**. Red arrow indicates the newly emerged shoot used for DAS-ELISA testing.

rootstock by DAS-ELISA using commercially available kit (Bioreba AG, Switzerland) (Fig. 1). Alternatively, total RNA was isolated by NucleoSpin RNA Plant kit (Macherey-Nagel, Duren, Germany) and used as the template for RT-PCR. For the routine diagnostics of PPV presence, PPV-CR-specific primers reported before were used (*4*). Partial genomic sequence of 3,543 nucleotides spanning the region coding for partial P1, HC-Pro, P3, 6K1 and partial CI genes (nt positions 249–3802 based on the full-length RU-30sc sequence) was obtained by combining two overlapping fragments amplified with specific primers CR_249F (CGCCAAATCAGTAATGTGC) / CR_1723R (CGCTTGGCATGATACTCTC), and CR_1550F (AGAACTCGCACGCTACCAG) / CR_3802R (TGTGGTATGACATTTCCCG). Determined sequence was deposited to the GenBank database under accession number MG450667.

Success of *in vitro* micrografting is usually influenced by several factors, e.g. scion origin, its length and shape, rootstock age, used media, as well as the grafting method (*11*). Based on our experience, an optimal length of scion was 1–1.5 cm and length of rootstock 2–2.5 cm, enabling a safe and fixed connection of both parts. In case of successful micrografting, callus was formed at the graft union within 7–10 dpm and new shoots were grown from the rootstock and from scion leaf axils. In our hands, the micrografting procedure was successful in 96% (*P. cerasus*/*P. cerasus*) and 88% (*P. domestica*/*P. cerasus*), while PPV was proved only in 56% and 39 % for *P. cerasus* and *P. domestica* rootstock shoots, respectively. Irregular PPV distribution within woody plants (*12*) may be the reason for such low efficiency of virus transmission/detection.

In order to pinpoint possible changes in the 5´-proximal part of the viral genome after micrografting, we compared the sequences obtained from infected rootstocks with the original RU-63sc sequence. In the *P. domestica* rootstock we detected a single non-silent mutation in the P1 gene leading to asparagine to aspartate exchange in amino acid position 218 (numbered based on the complete RU-30sc polyprotein). On the other hand, no PPV sequence change was observed in the case of the sour cherry/sour cherry passage. This result further highlights the role of the P1 protein as a potential factor relevant for virus-host adaptation as reported previously (*13, 14*). Despite lower observed efficiency of virus transmission, the *in vitro* micrografting of woody hosts represents an essential alternative to use of herbaceous plants for research of PPV because it better mimics natural conditions. As we showed, this technique enabled also combination of different scion/rootstock species and relatively fast evaluation of genome changes due to the natural selection.

Acknowledgments. The research was supported by the grant APVV-0174-12 from the Slovak Research and Development Agency and by the grant VEGA 2/0124/15 from the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and Slovak Academy of Sciences. *P. cerasus* positively detected for PPV-CR (isolate RU-63sc) was kindly provided by Dr. Y. Prikhodko (Federal State Organization All-Russian Plant Quarantine Center, Russia).

References

1. Garcia JA, Glasa M, Cambra M, Candresse T, Mol. Plant. Pathol. 15, 226–241, 2014. https://doi.org/10.1111/mpp.12083

2. Sihelská N, Glasa M, Šubr Z, J. Integ. Agr. 16,510–515, 2017.

3. Crescenzi A, d'Aquino L, Comes S, Nuzzaci M, Piazzolla P, Boscia D, Hadidi A, Plant Dis. 81, 711–714,1997. https://doi.org/10.1094/PDIS.1997.81.7.711

4. Glasa M, Prikhodko Y, Predajňa L, Nagyová A, Shneyder Y, Zhivaeva T, Subr Z, Cambra M, Candresse T, Phytopathology 103, 972–979, 2013. https://doi.org/10.1094/PHYTO-11-12-0285-R

5. Calvo M, Malinowski T, García JA, Mol. Plant Microbe Interact. 27, 136–149, 2014. https://doi.org/10.1094/MPMI-08-13-0242-R

6. Pathirana R, McKenzie MJ, Plant Cell. Tiss. Org. 81, 11–18, 2005. https://doi.org/10.1007/s11240-004-2498-y

7. Wang Y-Q, Chinese Sci. Bull. 56, 3511–3517, 2011. https://doi.org/10.1007/s11434-011-4816-1

8. Murashige T, Skoog F, Physiol. Plant. 15, 473–497, 1962. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

9. Paunovic S, Ruzic D, Vujovic T, Milenkovic S, Jevremovic D, Biotechnol. Biotec. EQ. 21, 417–421, 2007. https://doi.org/10.1080/13102818.2007.10817486

10. Onay A, Tilkat E, Isikalan C, Namli S, In Jain SM, Häggman H (Eds): Protocols for Micropropagation of Woody Trees and Fruits. Springer, Dordrecht, 2007.

11. Rehmann HU, Gill MIS, J. Horticulture 2, 151, 2015.

12. Martinéz-Goméz P, Dicenta F, Phytopatol. Mediterr. 40, 157–164, 2001.

13. Salvador B, Delgadillo MO, Saénz P, García JA, Simón-Mateo C, Mol. Plant-Microbe Interact. 21, 20–29, 2008. https://doi.org/10.1094/MPMI-21-1-0020

14. Valli A, López-Moya JJ, García JA, J. Gen.Virol. 88, 1016–1028, 2007. https://doi.org/10.1099/vir.0.82402-0