



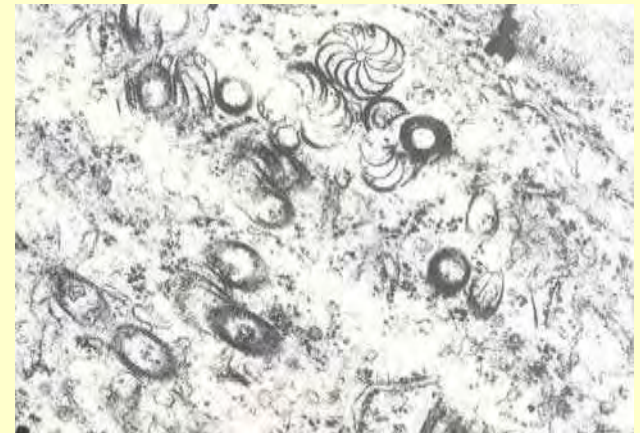
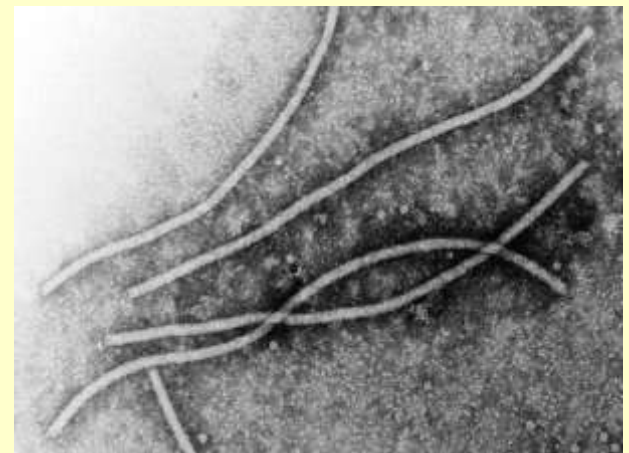
In vitro micrografting of different *Prunus* species with two cherry-adapted strains of *Plum pox virus*

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Plum pox virus, PPV

Family *Potyviridae*

- genus *Potyvirus*
- the biggest family of plant viruses (1/3 of known PV)
- virions :
 - flexible filaments
 - non-enveloped
 - helical symmetry
- cytoplasmatic inclusions
- genome :
 - linear (+) ssRNA
 - 5' terminus – VpG
 - 3' terminus – polyA
 - expression from a single ORF – polyprotein
 - co- and posttranslational autoproteolysis
(11 final polypeptides)



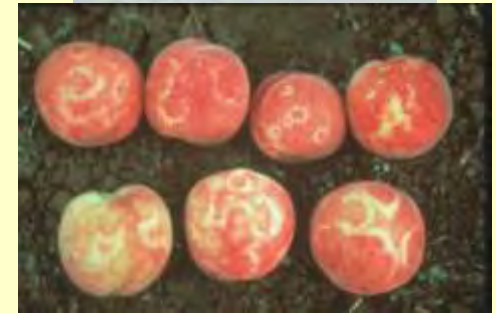
The natural host range

- all cultivated stone fruit crops (*Prunus spp.*: peach, plum, apricot, nectarine, almond, sweet and sour cherry), wild and ornamental *Prunus* plants

Transmission

- non-persistently by aphids
- vegetative transmission by humans

Symptoms of PPV infection

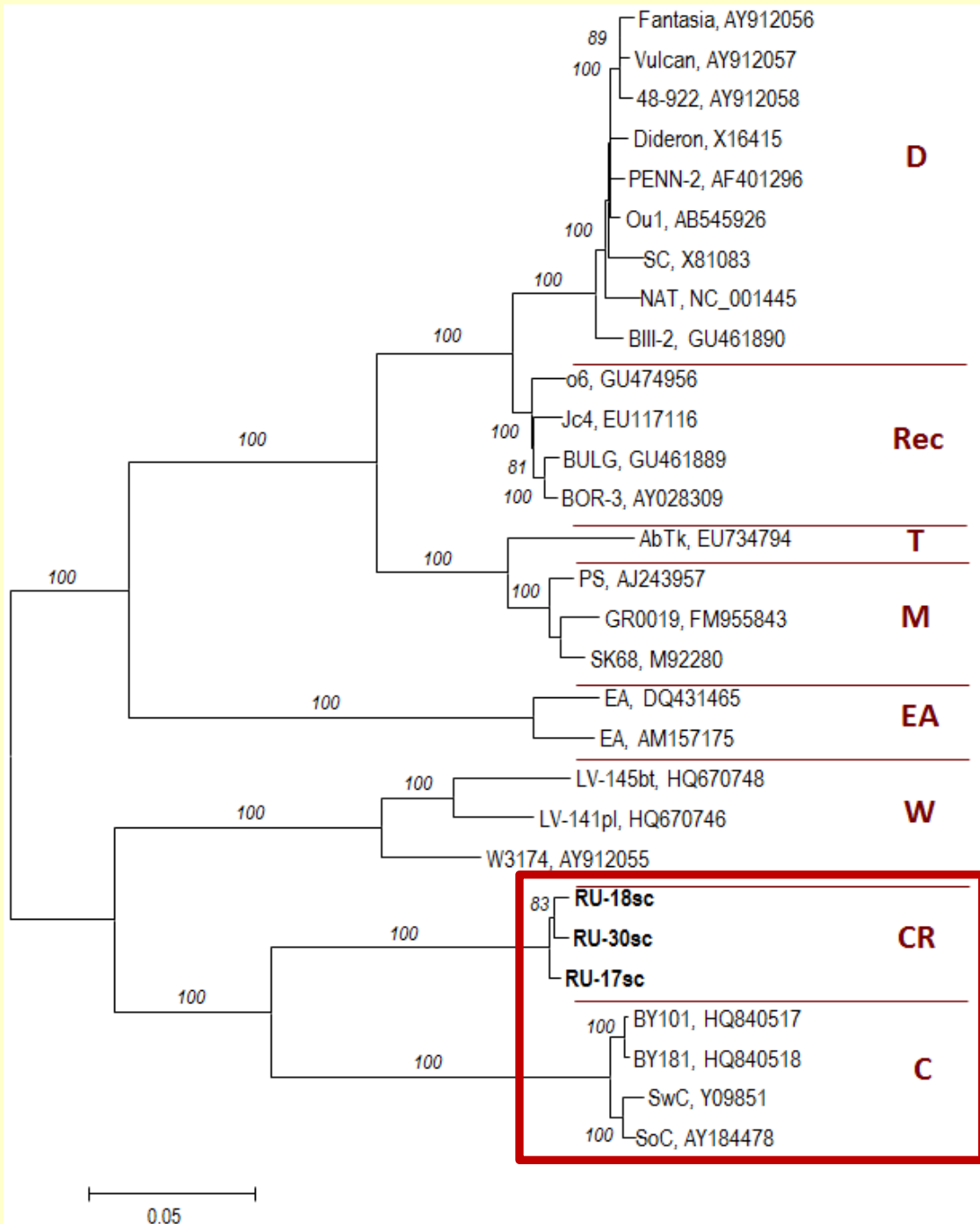


Strains of PPV

- PPV-M (Marcus)
- PPV-D (Dideron)
- PPV-Rec (recombinant) epidemiologically important

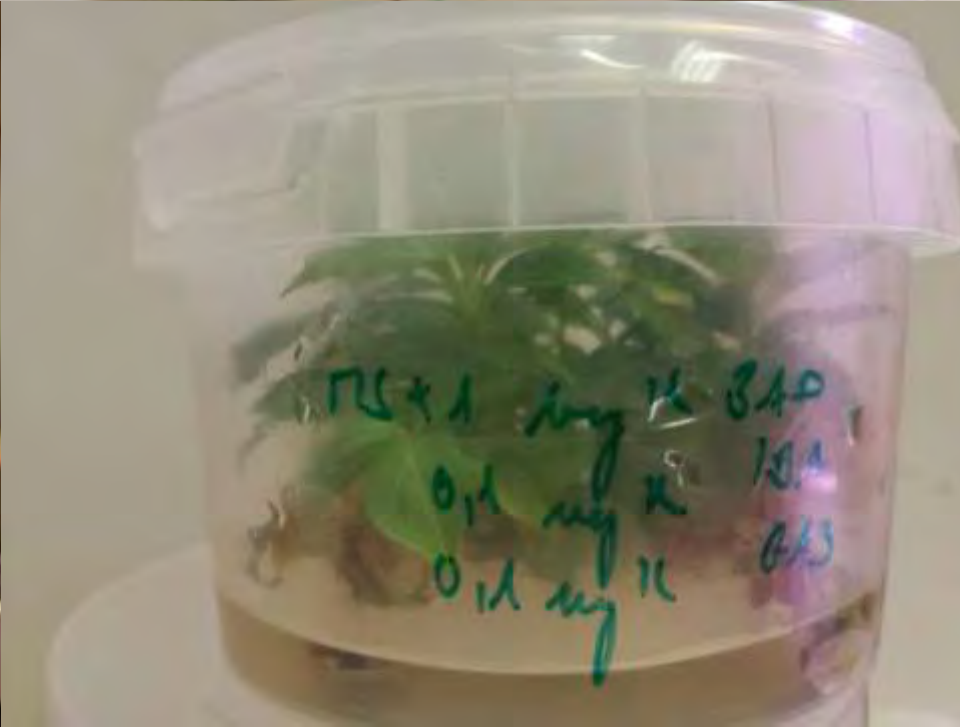
- PPV-EA (El Amar)
- PPV-W (Winona) geographically limited
- PPV-T (Turkey)
- PPV-An (ancestral, Albania)

- PPV-C (cherry)
- PPV-CR (cherry Russia) specific to a host under field conditions



PPV-CR is most closely related to the PPV-C.

In vitro plant propagation



Multiplication and rooting of *in vitro* plants

Basal MS (Murashige and Skoog) culture medium for *Prunus sp.* :
sucrose (3%), **agar** (0.8%), **BAP** (1 mg/l), **IBA** (0,1 mg/l), **GA3** (0,1 mg/l)

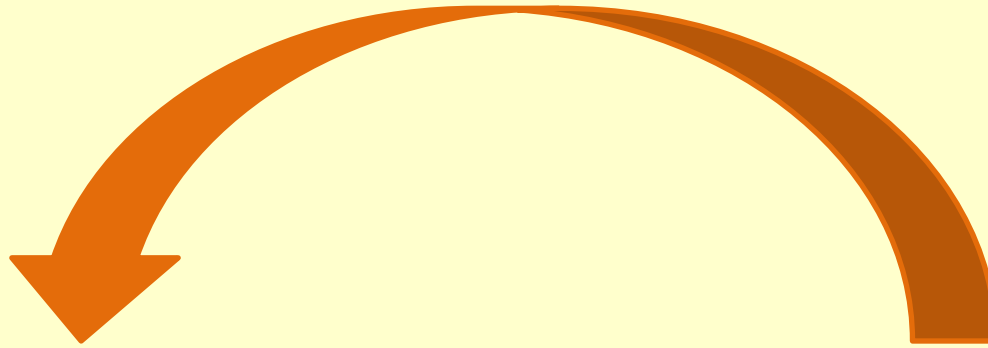
Culture medium (1/2 MS medium) for rooting of *P. avium*:
sucrose (3%), **agar** (0.8%), **IBA** (1 mg/l)

Culture medium (1/2 MS) for rooting of *P. cerasus* and *P. domestica*,
cv. Saint Julien:

Step 1.: elocation **sucrose** (3%), **agar** (0.8%), **GA3** (12,5 mg/l)

Step 2.: rooting **sucrose** (3%), **agar** (0.8%), **IBA** (4 mg/l)

In vitro micrografting

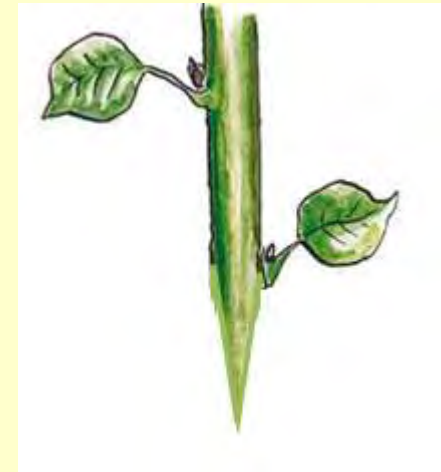


Rootstock

(without PPV infection)

P. cerasus RU74

P. domestica, cv. Saint Julien



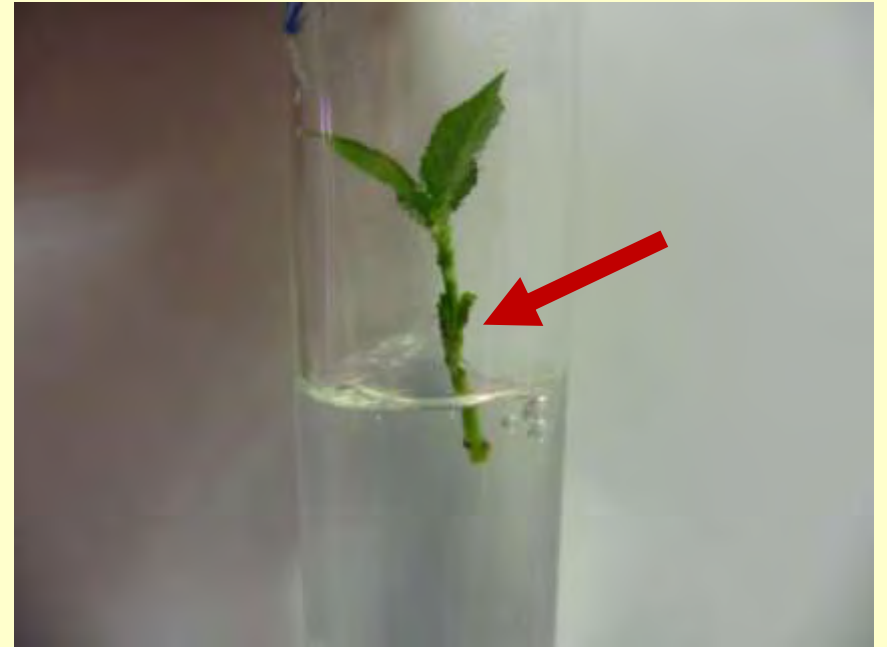
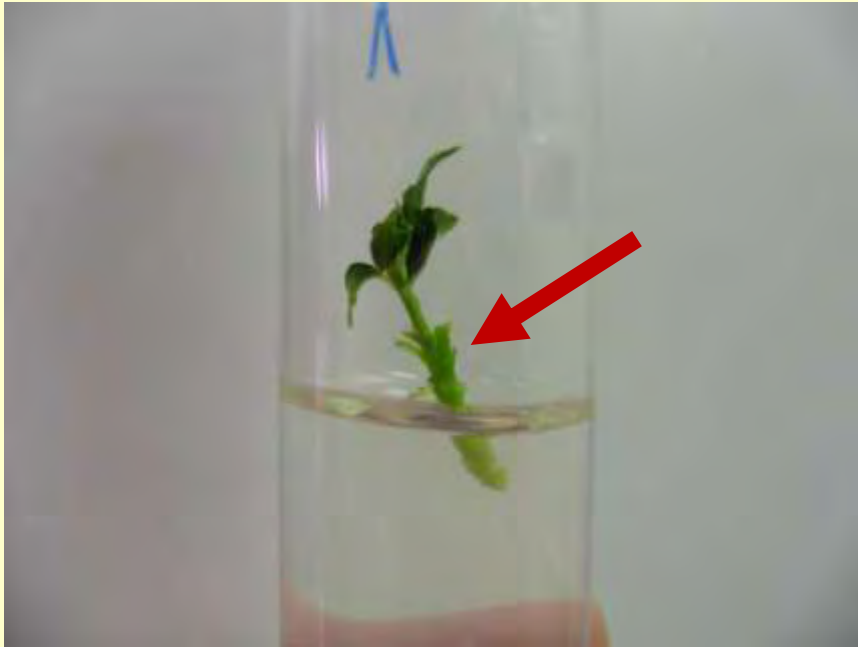
Scion

(infected plants)

P. cerasus RU63 → PPV-CR

P. avium → PPV-C

II. Methodology



Analysis of scion-to-rootstock virus transmission

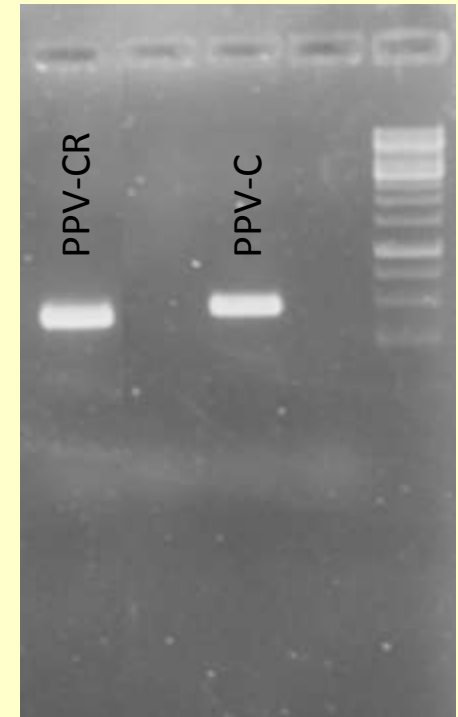
- 21 dp micrografting
- DAS-ELISA (PPV antibodies – Bioreba)
- specific RT-PCR detection of PPV-C and PPV-CR isolates

PPV-C_8552F 5'-GCTCGACTCCAACATAGTGG-3'

PPV-C_9026R 5'-CCGCGTGTTTGATAAGTCAG-3'

PPV-CR_8597F 5'-ATGATGTGACGTTAGTGGAC-3'

PPV-CR_9023R 5'-TCGTGTGTTAGACAGGTCAAC-3'

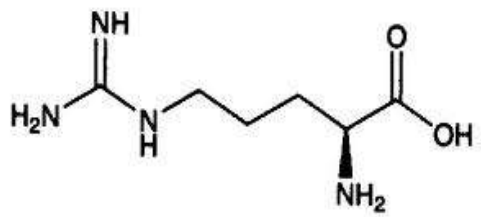


Sequence analysis of the P1-HC-P3 genomic part of cherry-to-plum transmitted PPV-CR

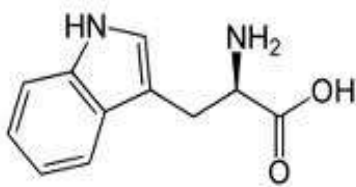
RU63sc_CHERRY	98	QIIEKKLKQQYKEEHERFQWLNGPENVVTHIETTNEETPTWVFPDVPKRPTSKTPSTKR
RU63sc_STJULIEN	98
RU63sc_CHERRY	158	HVIFDKVRMSEATLQLFMRKVANNAKANGQKVEIIGTKHVVGNYIRKSQCLTYFRTHVRHL
RU63sc_STJULIEN	158
RU63sc_CHERRY	218	NGLKPRYDITLDEATKKIVQVFANTSGFKHVHSGEITPGMSGFVLNPRNISDPMQVHDT
RU63sc_STJULIEN	218	D.....
RU63sc_CHERRY	278	DLFIVRGKHNSILVDARCPVSKEHSNELVHYSDPGKKFWVGFTNSFAQCKLRETDHQCTS
RU63sc_STJULIEN	278
RU63sc_CHERRY	338	DLDVQECGYVAALVCQAIIPCGKITCLQCAEKYAYMSQQEIRDRFSTVIEQHEKTVMMDTY
RU63sc_STJULIEN	338

● Nagyová, A., Kamencayová, M., Glasa, M., Šubr, Z.W. (2012). **The 3' - proximal part of the Plum pox virus P1 gene determinates the symptom expression in two herbaceous host plants.** In *Virus Genes*, 2012, vol. 44, no. 3, p. 505 - 514.

	120		140
pIC-PPV	GPDAIVNQIS	VDKCEASVRY	PFPHIIEKP
BOR-3	W
SK68	W
	220		240
pIC-PPV	SKPRYDLVLD	EATKKILQLF	ANTSGFHHV
BOR-3
SK68
	320		340
pIC-PPV	TNSFMQCKLR	ETDHQCTSDL	DVKECGYVA
BOR-3
SK68
	420		440
pIC-PPV	NYEAFKDITH	MIGERKEAPF	SHLNKINEL
BOR-3
SK68
	520		540
pIC-PPV	REYHAKRFFR	NYFDVIDVSE	GYRRHIVRE
BOR-3
SK68



Arg₁₃₈



Trp₁₃₈



● Calvo M, Malinowski T, Garcia J.A.: **Single amino acid changes in the 6K1-C1 region can promote the alternative adaptation of Prunus- and Nicotiana-propagated Plum pox virus strain C isolates to either host.** In *Molecular Plant-Microbe Interaction*, 2014, vol. 27, no.2, p. 136-149.

Conclusion

- *In vitro* plant propagation and micrografting help to overcome rooting problems and slow growth of some woody species
- Problem – irregular distribution of PPV in *in vitro* plants
- All tested genotypes could be infected by PPV-C and PPV-CR
- Change the aminoacid in the RU-63sc polyprotein after the passage from cherry to plum host → adaptation (future research)

Thank You for Your
Attention

