Development of ALSV-mediated VIGS in *Prunus* fruit trees

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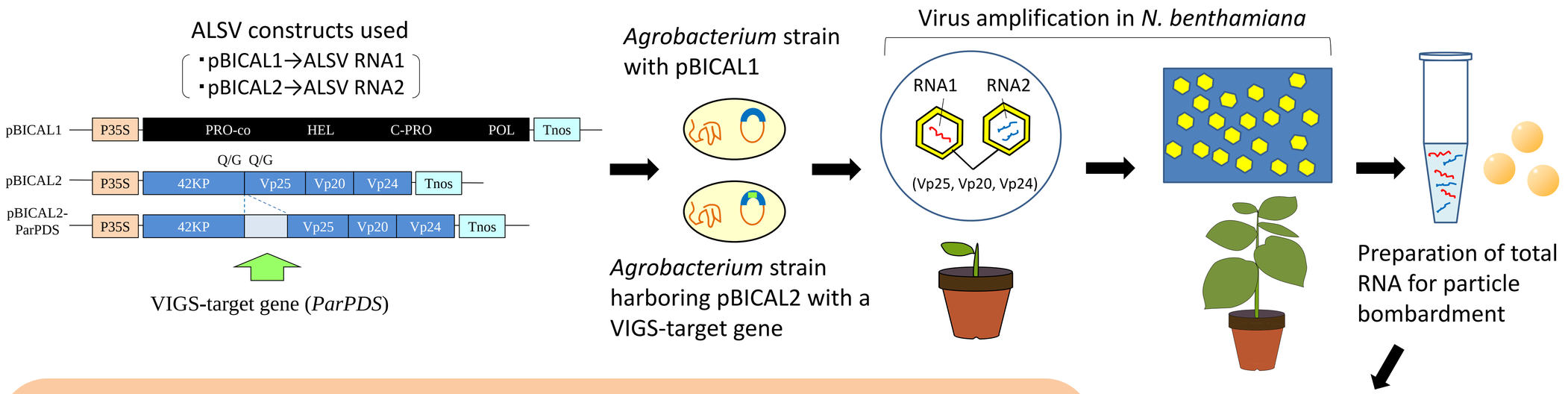
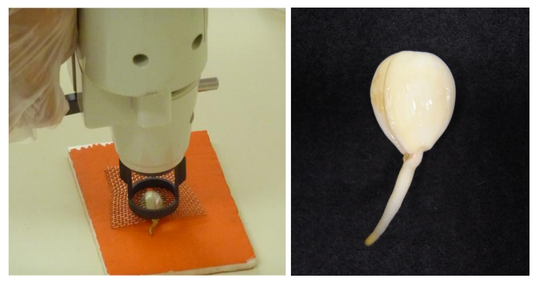
Introduction

Apple latent spherical virus (ALSV) vectors have been shown to effectively induce stable virus-induced gene silencing (VIGS) in a wide range of plant species including rosaceous fruit tree species, such as apple and pear. In this study, we attempted to develop a gene evaluation system for various *Prunus* fruit trees using the ALSV-mediated VIGS.

Scheme of VIGS system using ALSV vectors in *Prunus*

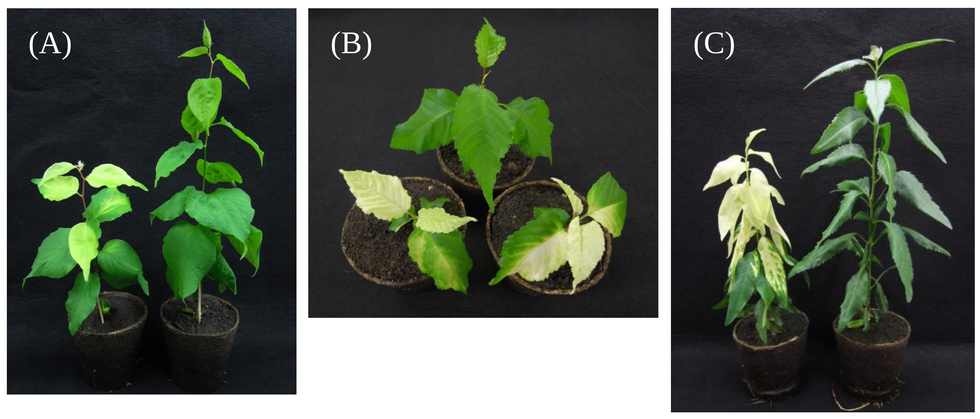


Disarmed *Agraobacterium* strain EHA105 harboring pBICAL1 and that harboring pBICAL2 with partial coding sequence of *ParPDS* (*Prunus armeniaca PDS*) were used to infect *Nicotiana benthamiana* to amplify virus particles. T-DNA regions of binary vector pBICAL1 and pBICAL2 were designed based on the sequences of ALSV RNA1 and RNA2, respectively. To produce and amplify recombinant ALSV particles, leaves of *N. benthamiana* were infected with pBICAL1/EHA105 and pBICAL2-ParPDS/EHA105 at the same time. The resultant ParPDS-ALSV was isolated from the infected *N. benthamiana* and used for inoculation to *Prunus* seedlings just after germination by a particle bombardment.



Viral inoculation to *Prunus* seedlings by a particle bombardment (Right, apricot seedling)

VIGS of *PDS* gene in *Prunus*



VIGS of *PDS* gene in apricot 'Shinshuomi' (A), sweet cherry 'Satonishiki' (B), and almond 'Nonpareil' seedlings infected with ParPDS-ALSV. Discoloration of the upper leaves was observed in infected seedlings.

Infectious rates of ALSV vectors into *Prunus*

Plant species	Cultivar	Number of infected plants/total inoculated plants	Infectious rate (%)
Apricot (*P. armeniaca*)	Heiwa	5/45	11.1
	Shinyo	2/18	11.1
	Shingetsu	3/24	12.5
	Shinshuomi	2/21	9.5
	Niigataomi	0/15	0
	Nanbuhachisuke	0/22	0
Sweet cherry (*P. avium*)	Satonishiki	6/30	20
Almond (*P. dulsis*)	Nonpareil	6/17	35.3
	Carmel	5/17	29.4
	Morcona	0/10	0
Peach (*P. persica*)	Ohatsumomo	12/14	85.7
Japanese apricot (*P. mume*)	Nanko	0/25	0
Japanese plum (*P. salicina*)	Sordum	0/20	0
European plum (*P. domestica*)	Sanctus Hubertus	0/27	0

Seven *Prunus* species [apricot (*P. armeniaca*), sweet cherry (*P. avium*), almond (*P. dulsis*), peach (*P. persica*), Japanese apricot (*P. mume*), Japanese plum (*P. salicina*), and European plum (*P. domestica*)], including several cultivars for apricot and almond, were used in this study. Total RNA extracted from *N. benthamiana* infected with ParPDS-ALSV was inoculated into cotyledons of *Prunus* seedlings just after germination using a helium gun. A typical *PDS*-silenced phenotype, characterized by uniform discoloration of the upper leaves, was observed with sweet cherry and some cultivars of apricot and almond several weeks after inoculation. However, our attempts to infect ALSV to Japanese apricot, Japanese plum, European plum, and the other cultivars of apricot and almond were unsuccessful. Although infectious rate of ALSV to peach was high, severe pale spot symptom was observed in the infected leaves.

Conclusions

This study showed that ALSV vectors could induce efficient VIGS in a certain range of *Prunus* species, including apricot, sweet cherry, and almond. The results strongly indicated that ALSV-mediated VIGS would be a powerful tool for evaluating gene functions in *Prunus*. However, the infectivity of ALSV vectors varied depending on the species or cultivars in *Prunus*. Furthermore, severe viral symptom was observed in infected peach seedlings. These results collectively suggest that the efficiency of the use of ALSV vectors for gene evaluation in *Prunus* varies depending on the species or cultivars.