PRELIMINARY REPORT ON THE APPARENT BREAKING OF RESISTANCE OF A TRANSGENIC PLUM BY CHIP BUD INOCULATION OF PLUM POX VIRUS PPV-S

T. MALINOWSKI[1], B. ZAWADZKA[1], M. RAVELONANDRO[2], R. SCORZA[3]

[1]Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland; [2]Station de Pathologie Vegetale, INRA Bordeaux, Villenave d'Ornon, France; [3]USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV, USA

Summary. – Five transgenic clones of *Prunus domestica* L. containing plum pox virus (PPV) coat protein (CP) gene and one non-transformed control clone were challenged with PPV-S in the field. Symptoms developed on C2, C3, C4, C6 and B70146 but not C5 trees inoculated by chip budding (CBI) (2/2, 2/2, 1/1, 2/2 and 2/2, positive/inoculated) in the first summer after inoculation. However, in the second year, symptoms appeared on CBI C5 trees. The presence of the virus in the plants was confirmed by enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) amplification of a fragment of viral polymerase gene. During two years, symptoms of infection developed on 3 to 4 of 8 non-inoculated trees of clones C2, C3, C4, C6 and B70146. Eight non-inoculated C5 trees remained symptomless and ELISA-negative as of spring 1998, in spite of the presence of aphid vectors and inoculum sources.

Key words: plum pox virus; transgenic plum; resistance

Introduction

Sharka disease caused by PPV, is a serious threat to plum production in Europe. Attempts to obtain resistant or immune varieties by means of conventional breeding have not yet been successful.

Recently, several clones of transgenic plum (*Prunus domestica* L.) (Scorza *et al.*, 1994) containing one or multiple copies of CP gene of PPV have been obtained. These plants were challenged, under controlled conditions, with PPV-M by CBI and with PPV-D by aphids. The C5 clone proved to be highly resistant to PPV (Ravelonandro *et al.*, 1997).

In this report, we describe preliminary results of field testing of transgenic plum clones including C5 for resistance against plum pox virus under high infection pressure with the isolate PPV-S.

Materials and Methods

Transgenic plums C2, C3, C4, C5, and C6 (Scorza *et al.*, 1994) and untransformed control plum B70146 were propagated by grafting on GF 8-1 rootstocks as described by Ravelonandro *et al.* (1997). Each clone was represented by ten trees. The field experiment was set up in an isolated plum orchard near Skierniewice, Poland in April 1996. The transgenic and control clones were planted together with sixty plum (*Prunus domestica* L.) trees. Trees infected with PPV were already present in this experimental plot and only moderate protection measures against insects were applied in order to favour aphid-borne infection. In August 1996, all plum trees and two trees of each test clone were inoculated by CBI. A tree infected with PPV-S (Wypijewski *et al.*, 1994) was used as an inoculum source.

The trees were observed for symptoms of infection on leaves each month throughout the growing season (from April to September). Standard double-antibody sandwich ELISA (DAS-ELISA) of PPV using reagents prepared in our laboratory and ELISA kits purchased from Loewe (FRG) were performed two or three times during the growing season. Selected trees were tested also by RT-PCR for the presence of PPV RNA. Primers developed by Korschineck *et al.* (1991), permitting the amplification of a fragment of PPV NIb cistron, were used in silicacapture-RT-PCR (SC-RT-PCR) tests according to a procedure described by Malinowski (1997).

Table 1. Occurrence of infection of transgenic clones of plum with PPV after two years of growing in field

Clone	Number of PPV-positive (symptoms and ELISA) trees after inoculation by	
	Chip budding	Aphids (naturally present in the orchard)
B70146	2/2	3/8
C2	2/2	4/8
C3	2/2	3/8
C4	1/1*	4/8
C5	**2/2**	**0/8**
C6	2/2	3/8
Total	11/11	17/48

*One C4 tree died probably due to replantation problems.

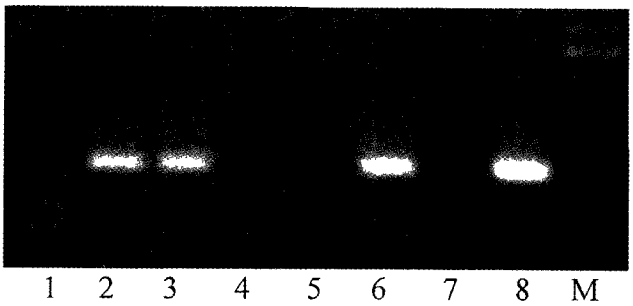

Fig. 1
Electrophoresis of SC-RT-PCR products of amplification of the 216 bp fragment of viral RNA polymerase gene
Nucleic acids were isolated from leaves of healthy plum (1), C5 symptom-positive tree No. 41 (2), C5 symptom-positive tree No 5 (3), C5 symptom-negative tree (4), C6 symptom-negative tree (5), C6 symptom-positive tree (6), B70146 symptom-negative tree (7), and B70146 symptom-positive tree (8). Size markers (M)

Results and Discussion

Natural aphid transmission

The first symptoms of PPV were observed in autumn 1996 on two non-CBI trees of clone C6. During the next two years, symptoms of infection developed on 3 – 4 out of 8 non-CBI trees representing clones C2, C3, C4, C6 and B70146 (control) (Table 1). The presence of PPV was confirmed by ELISA. The first symptoms appeared usually on single branches and during the next year spread to nearly entire trees.

Eight non-CBI C5 trees remained symptomless and ELISA-negative as of June 1998.

Trees inoculated by CBI

Symptoms developed on all CBI trees of clones C2, C3, C4, C6 and B70146 (2/2, 2/2, 1/1, 2/2 and 2/2, positive/inoculated) in the first summer after inoculation and in the second year (June 1998) on both inoculated C5 trees. Chlorotic pattern observed on C5 leaves was less pronounced and less diffused than that appearing on the other clones. In June 1998, a single leaf on each of several branches on both C5 trees displayed the symptoms. The results of symptom observation and ELISA are summarized in Table 1.

The presence of the virus in the symptomatic leaves collected from C5 trees was further confirmed by SC-RT-PCR amplification of the fragment of the viral polymerase gene (Fig. 1).

C5 proved to be highly resistant against inoculation with strains PPV-D and PPV-M in our earlier experiments carried out under glasshouse conditions. It is not clear, what factors contributed to the apparent breakdown of resistance in the field when the virus was introduced by CBI. It could be attributed to the different isolate of PPV used in this study, although C5 trees exposed to natural infection through aphid feeding did not show symptoms in this study. Furthermore, C5 has thus far been resistant to a number of PPV isolates in glasshouse studies (Ravelonandro, unpublished data).

It appears that the mechanism of resistance in clone C5 is one of co-suppression based on multiple transgene copies, low levels of transgene RNA, and the absence of detectable transgene protein (Scorza *et al.*, 1994). The co-suppression has been shown to be affected by the environment and plant development (Pang *et al.*, 1996). These factors may have influenced the expression of resistance in this field test. Thus far, only CBI C5 trees have become infected. This suggests that the presence of the chip bud inoculum may be of significance. This inoculum source may act as a virus reservoir. Following the dormancy, the co-suppression mechanism may require a resetting as has been shown in seedlings of co-suppressed herbaceous species (Tanzer *et al.*, 1997). The presence of virus in the chip bud tissue at this time may allow for a short period of infection before the co-suppression mechanism is "reset". The C5 transgenic plum clone may represent a useful model for studying the effects of the dormancy and other environmental and physiological factors on co-suppression. Observation of C5 and the other clones under test is continued.

Acknowledgements. We would like to thank Mmes Kowalska and Zielinska for technical assistance, in particular for performing the chip budding.

References

Korschineck I, Himmler G, Sagl R, Steinkellner H, Katinger HWD (1991): A PCR membrane spot assay for the detection of plum pox virus RNA in bark of infected trees. *J. Virol Methods* **31**, 139-146.

Malinowski T (1997): Silicacapture-reverse transcription-polymerase chain reaction (SC-RT-PCR): application for the detection of several plant viruses. *Diagnosis and Identification of Plant Pathogens. Proc. 4th Int. EFPP Symp.*, Bonn, 1996. Vol. 11. *Developments in Plant Pathology*, Kluver Academic Publishers, pp. 445-448.

Pang SZ, Jan FJ, Carney K, Stout J, Tricoli DM, Quemada HD, Gonsalves D (1996): Post-transcriptional transgene silencing and consequent tospovirus resistance in transgenic lettuce are affected by transgene dosage and plant development. *Plant J.* **9**, 899-909.

Ravelonandro M, Scorza R, Bachelier JC, Labonne G, Levy L, Damgsteegt V, Callahan AM, Dunez J (1997): Resistance of transgenic *Prunus domestica* to plum pox virus infection. *Plant Dis.* **81**, 1231-1235.

Scorza R, Ravelonandro M, Callahan AM, Cordts JM, Fuchs M, Dunez J (1994): Transgenic plums (*Prunus domestica* L.) express the plum pox virus coat protein gene. *Plant Cell Rep.* **14**, 18-22.

Tanzer MW, Thompson WF, Law MD, Wernsman EA, Uknes, S (1997): Characterization of post-transcriptionally suppressed transgene expression that confers resistance to tobacco etch virus infection in tobacco. *Plant Cell* **9**, 1411-1423.

Wypijewski K, Malinowski T, Musial W, Augustyniak J (1994): The nucleotide sequence of coat protein gene of Polish isolate of plum pox virus (PPV-S). *Acta Biochim. Pol.* **41**, 87-95.