CHARACTERIZATION OF PHENOTYPE RESISTANCE TO PLUM POX OF TRANSGENIC PLUMS EXPRESSING PLUM POX VIRUS CAPSID GENE

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Summary. – Resistance to plum pox virus (PPV) infection can be obtained in transgenic plants that express the virus capsid gene. An *Agrobacterium*-mediated transformation was used to introduce the PPV capsid gene into *Prunus domestica* plants. Over 11 regenerated plants (clones) were observed for the development of the disease symptoms and analysed for the presence of PPV by enzyme-linked immunosorbent assay (ELISA), Western blot analysis, and reverse transcription-polymerase chain reaction (RT-PCR) through 4 dormancy cycles. The level of protection against PPV was determined in the transformed plants, non-transformed plants, and a control transgenic plant "transformed" with the plasmid vector alone. One clone, C-5, appeared fully protected, while PT-6 and C-4 clones accumulated a low concentration of virus and the rest of the clones was entirely susceptible. Little is known about the mechanisms of resistance to virus infection in transgenic woody plants. To investigate this aspect, comparative studies based on the characteristics of resistant and susceptible clones have been started. A question, whether the phenotype resistance of clone C-5 is similar to that observed in transgenic herbaceous plants or not, has been addressed. Recent progress in this investigation is presented.

Key words: plum pox virus; capsid gene; expression; phenotype resistance

Introduction

Plum pox disease is one of the threats for stone fruit production in Europe. For the first time, it has been described by Atanassov (1932) in Bulgaria. At present, it is reported to be spread all over the Europe. Efforts to characterize the virus have been made and they showed through partial analysis of the genome that four major PPV strain types can be discriminated by their biological or serological properties: D, M, C (cherry) and El Amar. A lot of information about the genetic control of the disease can be collected but only a little can be considered (Kegler and Hartmann, 1998; Karayannis and Mainou, 1994; Dosba *et al.*, 1994). Today, many research laboratories are engaged in the control of PPV, but none reached this goal to date. Several measures have been developed to stop PPV dissemination , e.g. the roguing of diseased trees or a treatment of suckers with systemic herbicides.

In 1986 it has been shown via genetic transformation that transformed plants expressing the virus coat protein (CP) gene can resist the virus infection (Powell *et al.*, 1986). Exploiting the existing knowledge on the molecular biology of PPV, particularly the gene expression, the genetic engineering has been used to introduce the virus CP-mediated resistance into woody host plants of PPV (Laimer da Camara Machado *et al.*, 1992; Scorza *et al.*, 1994). The gene encoding CP has been successfully engineered into herbaceous *Nicotiana* (Ravelonandro *et al.*, 1993; Jacquet *et al.*, 1998) and woody *Prunus domestica* plants (Scorza *et al.*, 1994). The biotechnology-based control of PPV has been subjected to investigation.

Several clones of transgenic plum with one or multiple copies of PPV CP gene introduced into the plant genome were obtained (Scorza *et al.*, 1994). These plants were thoroughly tested under glasshouse conditions for the PPV resistance. The virus was introduced by grafting and aphids (Ravelonandro *et al.*, 1997). One plum clone, C5, has been

proved to be highly resistant. The analysis of the clones in greenhouse conditions also permitted to find some susceptible to the virus infection and others that can be considered tolerant showing a low amount of the virus.

Materials and Methods

A successful transfer of PPV CP gene into *Prunus domestica* plants was achieved by Scorza *et al.* (1994). Fourteen clones harbouring the CP gene were produced. As three of them (PT-A, PT-B and PT-C) failed in vegetative propagation, only 11 could be multiplied by grafting on GF 8-1 rootstocks (Ravelonandro *et al.*, 1997). Scions (10 – 30 cm long) were inoculated by chip budding and aphid feeding (Ravelonandro *et al.*, 1997). The scions were observed for the presence of the disease symptoms or assayed for PPV by double-antibody sandwich ELISA test. A reliable control of the incoming virus has been achieved by Western blot analysis and RT-PCR test (Ravelonandro *et al.*, 1997).

Results and Discussion

Susceptible clones

The clones inoculated by chip-budding or aphids displayed uniformly their susceptibility to PPV infection. Table 1 shows that the clones categorized as expressors or non-expressors of PPV CP gene were susceptible to PPV infection. Strong expressors like clones C2, C-3 and PT-4, as well as non-expressors like clones C-6 and PT-5 exhibited both the PPV symptoms and the PPV genome replication.

Recovering clones

The recovery reaction is known as a resistance phenotype shown by transgenic plants against a potyvirus infection (Baulcombe *et al.*, 1996). Woody host plants develop PPV infection during the vegetative growth while all susceptible plants show symptoms at this stage. At the end of the third dormancy cycle, no difference can be observed between susceptible and possibly recovering clones. Extended observation performed until the fourth dormancy cycle showed that C-4 clone displayed a delayed and shortened PPV infection in young leaves developing on the top of scions. Parallel PPV detection tests in control plants (non-transformed scions) revealed a possible form of recovery in transgenic woody plants. The analysis of PT-6 clone gave similar results.

Table 1. Properties of various clones of transgenic plums tested in greenhouse conditions

Clone	PPV coat protein level	Observed phenotype
C-2	H	Susceptible
C-3	H	Susceptible
C-4	H	Recovered?
C-5	0	Highly resistant
C-6	0	Susceptible
PT-2	L	Susceptible
PT-3	L	Susceptible
PT-4	H	Susceptible
PT-5	0	Susceptible
PT-6	H	Recovered?
PT-19	M	Susceptible

H = high, M = medium, L = low, 0 = zero

Resistant clone

In examining the transformed plants, one clone, C-5, did not show any form of infection. This result was checked after four dormancy cycles. The scions inoculated by chip-budding or aphids did not show PPV infection. Apart from the integration of multiple copies of PPV CP gene into the C-5 plum genome, this clone did not show any other differences in comparison with the rest of the transformed clones. The study of the resistance mechanisms displayed by C-5 clone is underway. Preliminary data indicate that this clone is developing a sequence homology-dependent resistance that leads to a CP gene silencing as well as to a block of PPV genome replication (Baulcombe , 1996).

The research on the possibility of exploiting the biotechnology-based control of PPV attack is continued. The results of plantation of C-5 plum in field under natural conditions will influence the choice of this clone as potential candidate in the breeding program.

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