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Short communication

Using ELISA and PCR to test the potential for spread of plum pox virus by seeds of different stone fruit cultivars

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Abstract The enzyme-linked immunosorbent assay (ELISA) and the polymerase chain reaction (PCR) were used to test the potential for spread of plum pox virus (PPV) by seeds of the apricot (*Prunus armeniaca*) varieties 'Tyrinthos' and 'Bebecou', and the stone fruit rootstocks 'GF305' (*Prunus persica*) and 'Myrobalan' (*Prunus cerasifera*). Seeds originating from infected 'Tyrinthos', 'Bebecou', 'GF305', and 'Myrobalan' trees were stratified in metal boxes containing sand in a screenhouse. Leaves were collected from each of the seedlings and tested for PPV. No seedling was found infected with this pathogen. Our results suggest that at least in the cultivars tested, PPV is not seed transmitted.

Keywords seeds; spread; stone fruit; virus

INTRODUCTION

Plum pox virus (PPV), the causal agent of sharka disease of stone fruit trees (Dunez & Sutic 1988), is considered to be one of the most destructive fruit-tree viruses. This virus belongs in the potyvirus group and a number of members of the group are of economic importance. Potyviruses infect a wide range of plants, but most individual members have a relatively narrow host range. Several group members have been shown to have ''helper'' proteins for insect transmission. They are transmitted by aphids in a non-persistent manner and by sap inoculation. Some group members are seed transmitted such as pea seed-borne mosaic potyvirus (PSbMV) and lettuce mosaic virus (LMV) (Hollings & Brunt 1981).

PPV is present in most European and Mediterranean countries. The most widely used and convenient laboratory assay is some form of enzyme-linked immunosorbent assay (ELISA), which was first used for PPV detection over 20 years ago (Clark et al. 1976). However, the most sensitive procedures are those based on the polymerase chain reaction (PCR) (Wetzel et al. 1991). In this study, ELISA and PCR techniques were used to test the potential for spread of PPV by seeds of different stone fruit cultivars.

MATERIALS AND METHODS

One thousand seeds were harvested from trees of the Greek apricot (*Prunus armeniaca*) varieties 'Tyrinthos' and 'Bebecou' and the stone fruit rootstocks 'GF305' (*Prunus persica*) and 'Myrobalan' (*Prunus cerasifera*). All seed source trees tested positive for PPV by ELISA. Seeds were stratified in metal boxes containing sand in a screenhouse. The next spring (May), seedlings were transplanted in 1 litre pots containing sterile potting mix (3 peat : 1 perlite). At the same time, three leaves from the top, middle, and bottom of each seedling were collected from each of 400 plants of each cultivar. Leaves were pooled into 400 samples and then tested for PPV. It has been shown that ELISA and PCR are most sensitive when samples are taken in spring (Thomidis 2002). Similarly, Pasquini et al. (1999) used a total of 353 apricot seeds and 253 apricot seedlings to test transmission of PPV. Experiments were performed in two consecutive years on the same plants. Plants were kept in an insect-proof screenhouse.

Extracts were made by grinding samples (250 mg) in 5 ml PBS buffer (phosphate-buffered saline containing 0.5 ml Tween 20) (w/v), pH 7.2

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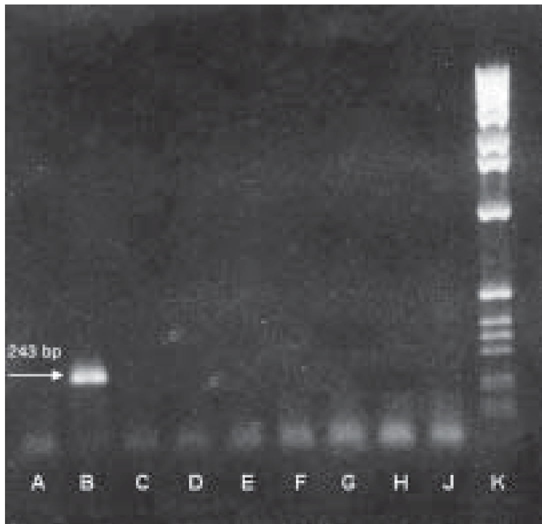

Fig. 1 Amplification products were analysed by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide. **A**, Healthy control (*Nicotiana clevelandii*); **B**, plum pox virus positive control; **C–J**, PCR products from crude plant extracts; and **K**, 123 bp DNA ladder (BRL).

supplemented with 2% (w/v) polyvinylpyrrolidone (PVP), and 0.2% (w/v) sodium diethyl-dithiocarbamate (DIECA). The ELISA tests were done in duplicate wells in polystyrene microtitre plates using a commercially available polyclonal anti-PPV serum and alkaline phosphatase congugate according to the protocol of the manufacturer (BIOREBA AG, Switzerland).

Fifty plants of each cultivar were also tested for PPV by PCR using the protocol described by Wetzel et al. (1991). The sequences of the primers were 5′-CCCTCACATCACCAGAGCCA-3′ (forward primer) and 5′-CAGACTACAGCCTCGCCAGA-3′ (reverse primer) resulting in a 243 bp amplified fragment (Wetzel et al. 1991).

RESULTS AND DISCUSSION

The number of plants grown from seeds that originated from infected trees did not differ significantly from those that originated from healthy trees (Table 1). In both instances, plants showed good development.

Table 1 Number of plants grown from 1000 seeds of 'GF305' (*Prunus persica*), 'Myrobalan' (*Prunus cerasifera*), 'Bebecou', and 'Tyrinthos' (*Prunus armeniaca*) trees that were healthy and infected with plum pox virus (PPV). (Values within columns did not differ significantly according to Duncan's Multiple Range Test ($P = 0.05$).)

Origin of seeds	GF305	Myrobalan	Bebecou	Tyrinthos
Infected trees	452	680	723	655
Healthy trees	421	714	678	633

Table 2 Absorbance values (A_{405}) of the wells containing plum pox virus (PPV) control, healthy control, and the absorbance of the test samples in two consecutive years (a sample was considered positive if the absorbance values (A_{405}) was at least 3 times that of healthy controls). (Values are the mean of 400 samples.)

	GF305		Myrobalan		Bebecou		Tyrinthos	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
Healthy control	0.127	0.101	0.114	0.093	0.211	0.146	0.069	0.095
PPV control	0.927	1.176	0.987	0.759	1.512	1.025	0.649	0.852
Test samples	0.113	0.117	0.121	0.081	0.251	0.122	0.076	0.104

ELISA is widely used for the detection of PPV because of the speed and simplicity of the system. Adams (1978) first reported the use of ELISA for detection of PPV. Nevertheless, the reliability of this serological test is limited by frequent false results depending on the time of sampling. The most sensitive procedures are those based on PCR (Corvo & Santos 1995; Olmos et al. 1997).

Seed transmission is common for other viruses of the potyvirus group (Walkey 1991). For instance, Latham & Jones (2001) detected seed transmission of seed-borne mosaic potyvirus in *Lathyrus cinera*. McKirdy et al. (2000) reported seed transmission of bean yellow mosaic potyvirus in legumes. Jonansen et al. (1996), reported that the 5′ 2.5 kb of the 10 kb pea seed-borne mosaic potyvirus genome had a major influence on seed transmission, whereas the helper-component protease was a major determinant of seed transmission and the potyviral p1 protease exerted no measurable influence. Ligat & Randles (1993), showed that PSbMV infection can be transferred through the vegetative phase at a subliminal level, and reaches a relatively high concentration in floral parts and seeds. Thus PSbMV may be maintained at a high level of infection in seed in the absence of any apparent symptoms in the plant, and without requirement for horizontal transmission between plants by vectors.

No seedling was positive for PPV by ELISA and PCR techniques in this study (Table 2, Fig. 1). Similarly, some researchers reported evidence for non-transmission of PPV by seed in infected peach, apricot, and plum trees (Eynard et al. 1991; Triolo et al. 1993; Pasquini et al. 2000). Glasa et al. (1999) reported that PPV isolates are not seed transmitted in *P. cerasifera* ('Myrobalan'). In contrast, Myrta et al. (1998), detected PPV in apricot and plum seeds immediately after collection, in germinating seeds, and in 1-year-old seedlings. The uneven distribution of PPV in infected trees (Adams 1978) together with its frequent concentration at very low levels, requires further work on the potential for spread of this pathogen by seeds. Adams & Peterson (1980) reported that PPV moves to the roots of infected plum trees soon after infection. Also, it has been found that the virus may be less systemic in some cultivars (Kegler & Hartmann 1998). Our results suggest that at least in the cultivars tested, PPV is not seed transmitted.

The use of virus-free planting material may provide the most satisfactory means for the control of PPV diseases of stone trees.

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