

SHORT COMMUNICATION

SEROLOGICAL DETECTION OF *CITRUS PSOROSIS VIRUS* IN SEEDS BUT NOT IN SEEDLINGS OF INFECTED MANDARIN AND SOUR ORANGE

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SUMMARY

Seed transmission of *Citrus psorosis virus* (CPsV) was investigated in mandarin cv. 'Grosso di Puglia' and in the Egyptian sour orange cv. 'Baladi'. Seeds collected from infected mandarin trees were subdivided into two lots, one of which was exposed to a hot water treatment (10 min at 52°C) followed by a fungicide treatment before testing by DASI-ELISA. In non-treated seeds, CPsV antigen was detected in seed coats (56.0% mean positive samples) and peeled seeds (7.5% mean positive samples). Detection rates were lower in treated seeds, *i.e.* an average of 16.0% positive seed coat samples and 3.0% positive peeled seed samples. None of 690 'Grosso di Puglia' mandarin and 202 'Baladi' sour orange seedlings obtained from treated and untreated seeds reacted positively in ELISA and no psorosis symptoms were observed in glasshouse-grown seedlings, nor were expressed by Dweet tangor grafted on 50 of these seedlings.

Key words: citrus, *Citrus psorosis virus*, seed transmission, ELISA, diagnosis.

A substantial number of plant viruses is seed-transmissible at least in one host (reviewed by Mink, 1993). Seed-transmission can be either direct because the embryo is infected or indirect, when the seed coat is contaminated (Stace-Smith and Hamilton, 1988; Mink, 1993).

Infectious diseases of citrus reported to be seed-transmitted are few (Mink, 1993) and some of the extant records are controversial. Thus, Davino *et al.* (1991) reported lack of seed transmission of *Citrus infectious variegation virus*, contrary to the findings by Wallace (1968), whilst transmission of psorosis through seeds (Bridges *et al.*, 1965; Childs and Johnson, 1966; Campiglia *et al.*, 1976) was questioned, as the disease

was apparently misidentified (Wallace, 1978; Roistacher, 1993).

Polyclonal antisera and monoclonal antibodies are now available for the sensitive detection of *Citrus psorosis virus* (CPsV) in infected citrus tissues (Garcia *et al.*, 1997; D'Onghia *et al.*, 1998; Alioto *et al.*, 1999; Potere *et al.*, 1999; Djelouah *et al.*, 2000). This gave us the opportunity to investigate serologically whether or not CPsV is present in citrus seeds and can be transmitted to seedlings.

CPsV-infected seed donors were three plants of mandarin cv. 'Grosso di Puglia' (GDP 1, 2, and 3) and a plant of Egyptian sour orange cv. 'Baladi', both of which showed psorosis symptoms and were ELISA-positive for CPsV (Potere *et al.*, 1999). One tree each of both cultivars free from CPsV was chosen as donor of presumably healthy seeds to be used as controls.

Seeds were extracted from fruits of infected mandarin and sour orange donors, selected, and thoroughly washed with running tap water. Mandarin seeds were divided in two lots, one of which was submitted to the treatment recommended for controlling *Phytophthora* and preserving seeds during storage, *i.e.* a 10-min hot water dip at 52°C followed by quick cooling and a 3 min dip in 1% solution of 8-hydroxyquinoline sulphate (Nauer and Roistacher, 1962). After thorough washing with tap water, treated seeds were dried at room temperature, placed in plastic bags, and stored at 4°C until used.

Seeds from psorosis-affected mandarin and sour orange were sown in plastic pots containing steam-sterilized UC soil mix (Nauer *et al.*, 1968) and kept in a climatized glasshouse at 24°C. A total of 320 seedlings were obtained from non-treated mandarin seeds, 370 seedlings from treated mandarin seeds, and 202 seedlings from sour orange. Seedlings from psorosis-free mandarin and sour orange were 55 and 23 respectively (Table 2).

Pistils from 270 flowers from infected GDP-1 mandarin and from 30 flowers from healthy GDP-4 mandarin were collected and tested for the presence of CPsV.

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Table 1. CPsV detection by ELISA in mandarin seeds.

Mandarin accessions	Non treated seeds				Treated seeds			
	Seed coats (infected/ tested)	%	Peeled seeds (infected/ tested)	%	Seed coats (infected/ tested)	%	Peeled seeds (infected/ tested)	%
GDP 2	16/82	20.0	16/82	20.0	6/140	4.3	2/140	1.4
GDP 3	170/204	83.0	6/204	3.0	70/200	35.0	12/200	6.0
Total	238/426	56.0	32/426	7.5	78/480	16.0	14/480	3.0
GDP 4 (-)	0/53	0.0	0/53	0.0	0/53	0.0	0/53	0.0

GDP: cv. 'Grosso di Puglia'; (-): CPsV-free.

Table 2. CPsV detection by ELISA in mandarin and sour orange seedlings.

Citrus accessions	Seedlings from non treated seeds (infected/ tested)	Seedlings from treated seeds (infected/ tested)
Pooled GDP-1-2-3	0/320	0/370
GDP 4 (-)	0/55	0/55
Baladi-1		0/202
Baladi-2 (-)		0/23

GDP: cv. 'Grosso di Puglia'; (-): CPsV-free.

All tests were by DASI-ELISA as described by Cambra *et al.* (1995) using the polyclonal antiserum A-322 (García *et al.*, 1997) and monoclonal antibodies (Potere *et al.*, 1999; Djelouah *et al.*, 2000) for antigen trapping and detection, respectively. Pistils, treated and non-treated seeds, and seedlings were individually tested. Mandarin seeds were peeled so that seed coats were tested separately from endosperm and embryos. CPsV-infected leaves of isolate IAMB-191X (D'Onghia *et al.*, 1998) were used as positive control, whereas pistils, seeds and seedlings from CPsV-free mandarin GDP-4 served as negative controls. Seedlings, grown in a glasshouse at 20-24°C, were observed for symptom expression and tested serologically five times over a 3 year period. Buds from Dweet tangor, one of the woody indicators for psorosis (Roistacher, 1993), were grafted onto 50 randomly chosen seedlings from CPsV-infected plants when they were one year old. Grafted plants were kept in a glasshouse at 20-24°C and observed for symptom expression for 4 months.

As shown in Table 1, CPsV was detected in seeds from diseased but not healthy mandarin accessions.

Virus was more frequent in non-treated than treated seeds (overall percentage of positive detection 32.0% versus 9.5%), suggesting that the hot water dip had influenced the results of serological tests, perhaps by denaturing target antigen. In non-treated seeds, the virus was more common in seed coats (20.0 to 83.0% incidence) than endosperm/embryos (3.0 to 20.0% incidence). The trend was the same in the treated seed lot, but positive virus detection was much lower (from 1.4 to 35.0% in seed coats against 0 to 6.0% in endosperm/embryos).

Although CPsV distribution within seed tissues (endosperm and embryo) was not investigated, it appears plausible that embryos were unlikely to be infected for none of the nearly 900 seedlings tested contained the virus as ascertained by ELISA (Table 2), symptom observation and, for a smaller number of them (50 seedlings), by indexing on Dweet tangor.

Based on the above, it seems plausible to conclude that no experimental evidence was obtained of seed-transmission of CPsV in mandarin and sour orange, thus confirming Wallace (1978) and Roistacher (1993) views.

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REFERENCES

- Alioto D., Gangemi M., Deaglio S., Sposato P., Noris E., Luisoni E., Milne R.G., 1999. Improved detection of citrus psorosis virus using polyclonal and monoclonal antibodies. *Plant Pathology* **48**: 735-741.

- Bridges G.D., Youtsey C.O., Nixon R.R., 1965. Observations indicating psorosis transmission by seeds of Carrizo citrange. *Proceedings of the Florida State Horticultural Society* **78**: 48-50.
- Cambra M., Asensio M., Gorris M.T., Perez E., Camarasa E., Garcia J.A., Moya J.A., Lopez Abella D., Vela C., Sanz A., 1995. Detection of plum pox potyvirus using monoclonal antibodies to structural and non structural proteins. *Bulletin OEPP/EPPPO Bulletin* **24**: 569-577.
- Campiglia H.G., Silveira M., Salibe A.A., 1976. Psorosis transmission through seeds of trifoliolate orange. *Proceedings 7th Conference of International Organization of Citrus Virologists, Athens* 1975, 132-134.
- Childs J.F.L., Johnson R.E., 1966. Preliminary report of seed transmission of psorosis virus. *Plant Disease Reporter* **50**: 81-83.
- Davino M., Areddia R., Pelicani L., Grimaldi V., 1991. Indexing of seeds of different citrus species for tristeza and variegation viruses. *Proceedings 11th Conference of International Organization of Citrus Virologists, Orlando* 1989, 368-372.
- Djelouah K., Potere O., Boscia D., D'Onghia A.M., Savino V., 2000. Production of monoclonal antibodies to citrus psorosis-associated virus. *Proceedings 14th Conference of International Organization of Citrus Virologists, Campinas* 1998 (in press).
- D'Onghia A.M., Djelouah K., Alioto D., Castellano M.A., Savino V., 1998. ELISA correlates with biological indexing for the detection citrus psorosis associated virus. *Journal of Plant Pathology* **80**: 157-163.
- Garcia M.L., De La Torre M.E., Dal Bo E., Djelouah K., Rouag N., Luisoni E., Milne R.G., Grau O., 1997. Detection of Citrus Psorosis-ringspot virus using RT-PCR and DAS-ELISA. *Plant Pathology* **80**: 157-163.
- Mink G.I., 1993. Pollen- and seed-transmitted viruses and viroids. *Annual Review of Plant Pathology* **31**: 375-402.
- Nauer E.M., Roistacher C.N., 1962. Long-term storage of heat treated seed. *California Citrograph* **47**: 140.
- Nauer E.M., Roistacher C.N., Labanauskas C.K., 1968. Growing citrus in modified UC potting mixtures. *California Citrograph* **53**: 456-458.
- Potere O., Boscia D., Djelouah K., Elicio V., Savino V., 1999. Use of monoclonal antibodies to citrus psorosis-associated virus for diagnosis. *Journal of Plant Pathology* **81**: 209-212.
- Roistacher C.N., 1993. Psorosis – a review. *Proceedings 12th Conference of International Organization of Citrus Virologists, New Dehli* 1992, 139-154.
- Stace-Smith R., Hamilton R., 1988. Inoculum thresholds of seed borne pathogens. Viruses. *Phytopathology* **78**: 875-880.
- Wallace J.M., 1968. Recent developments in the citrus psorosis diseases. *Proceedings 4th Conference of International Organization of Citrus Virologists, Gainesville* 1967, 1-9
- Wallace J.M., 1978. Virus and virus like diseases. *The Citrus Industry* **4**: 67-184.

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