Phytopathol. Mediterr. (2002) 41, 76–78

Eradication of *Potato virus Y* and *Potato leafroll virus* by chemotherapy of infected potato stem cuttings

GIANNI FACCIOLI and M. CHIARA COLALONGO

Dipartimento di Scienze e Tecnologie Agroambientali, Università
Via Filippo Re 8, 40126 Bologna, Italy

Summary. Eradication of *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV) by *in vitro* chemotherapy (50 mg l^{-1} of ribavirin+100 mg l^{-1} of DHT) of small potato stem cuttings (0.15×0.5 cm) of some potato cultivars is reported., Successful eradication seemed to depend on the duration of treatment and type of cultivar rather than on the virus. The best results were obtained with a 8-week treatment of cv. Monnalisa, Kennebec, and Désirée, i.e. 43 to 50% of PVY-free plants and 39 to 42% of PLRV-free plants. With the same type of treatment cv. Spunta, Primura and Liseta yielded only 14 to 21% of PVY-free plants and 15 to 27% of PLRV-free plants. Possible advantages over chemotherapy of the meristem tips are discussed.

Key words: potato, PVY, PLRV, sanitation, chemotherapy, ribavirin, DTH.

Introduction

Elimination of the main potato viruses by meristem tip culture, alone or combined with chemotherapy and/or thermotherapy, has been successfully employed for producing virus-free potatoes (reviewed by Faccioli, 2001). Stem-cutting culture coupled with chemotherapy and heat therapy has also been used with some success as an alternative to meristem tip culture (Griffith *et. al*, 1989; Sanchez *et. al,* 1991). Using this technique, Faccioli and Zoffoli (1998) eradicated *Potato virus X* (PVX) and *Potato virus S* (PVS), both of which invade meristem tips (Faccioli and Rubies-Autonell, 1982; Faccioli and Colombarini, 1996) from some popular potato cultivars. As described in the present paper, the experiments were extended successfully to *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV), neither of which infects meristematic tissues (Faccioli and Rubies-Autonell, 1982; Faccioli *et al*., 1988).

Materials and methods

Potato plants of cv. Désirée, Kennebec, Liseta, Monnalisa, Primura, and Spunta, were obtained by sowing class E certified seed-tubers in an air-conditioned greenhouse at 22±3°C under natural light conditions, supplemented in winter with an artificial 16-h photoperiod (Fluora 77R fluorescent tubes, 3 klux). Thirty days after sowing, the plants were checked for viral infections by DAS-ELISA as described previously (Faccioli and Colombarini, 1966) proving to be virus-free. Ten plants of each cultivar were mechanically inoculated with PVY

Corresponding author: G. Faccioli
Fax: +39 051 2091439
E-mail: faccioli@agrsci.unibo.it

strain N using infected sap from *Nicotiana tabacum* cv. White Burley, and 10 with PLRV by transferring groups of 10 *Myzus persicae* (Sulz) aphids, which had fed on PLRV-infected *Physalis floridana* plants for three days. Five days later the aphids were killed with a nicotine spray, and after a month all plants were ELISA tested for viruses.

Single-node stem cuttings (0.15 cm in diameter, 0.5 cm long) without subtending leaf were excised under a laminar-flow cabinet (Gelaire 200, class A) from plants infected with either virus, sterilized with a 1% water solution of Na-hypochloride for 20 min, soaked three times in sterile distilled water, and blotted on filter paper. Explants were placed in Magenta boxes containing 40 ml of solid Murashige and Skoog modified medium (MSm) (Faccioli *et al.,* 1988) to which 100 mg ml^{-1} of DHT (2,4-dioxo-hexahydro-1-3-5 triazine) and 50 mg l^{-1} of ribavirin (Sigma-Aldrich, Milano, Italy) were added. The boxes were placed in a growth cabinet at 22±11°C under a 16-h artificial light photoperiod (Fluora 77 R Osram, 3 klux). Comparable stem cuttings were grown on simple MSm medium as a control. Four weeks later, shoots developed from stem cuttings were tested by ELISA and those that appeared to be virus free based on the readings (A405<0.05 OD) were transferred to simple MSm medium for 10 days. Cuttings that were still infected were tranferred to fresh MSm containing DHT and ribavirin for a further 4 weeks, and then again placed on simple MSm medium like the others. Rooted plantlets were all transplanted to a mixture of sand, pit and soil (1:1:1) in a greenhouse and were ELISA-tested three weeks later for the presence of viruses. Data were worked out statistically with the χ^2 test.

The results (Table 1) show that, in general, it was more difficult to obtain healthy material from PLRV-infected than from PVY-infected stem cuttings and that the treatment was less successful against PVY and PLRV than against PVX and PVS (Faccioli and Zoffoli, 1998).

The factors that more strongly influenced virus eradication were the duration of treatment and the type of cultivar. Poor results were always obtained with a 4-week treatment i.e., from 7.5% to a maximum of 27.7% of PVY-free plantlets (cv. Spunta and Kennebec, respectively). The efficacy of the treatment compared with the control (100% infection) was statistically significant only with cv. Kennebec. Eradication was less effective with PLRV i.e. 2.5% and 11.1% of virus-free plantlets of cv. Liseta and Kennebec respectively, and was statistically not significant. However, with the 8-week

Table 1. Eradication of *potato virus Y*(PVY) and *potato leafroll virus* (PLRV) from potato stem cuttings (0.15×0.5 cm) of various cultivars by chemoterapy *in vitro* with ribavirin (50 mg l^{-1}) and DHT (100 mg l^{-1}).

Infecting virus	Cultivar	Virus free/total plants					
		4 week treatment			8 week treatment		
		No.	%	Control	No.	%	Control
PVY	Désirée	11/88	12.5 n.s.	0/18	31/72	43.1* [a]	0/15
PVY	Kennebec	13/47	27.7*	0/16	22/47	46.8* [a]	0/13
PVY	Liseta	9/67	13.4 n.s.	0/15	8/58	13.8 n.s. [a]	0/18
PVY	Monnalisa	12/50	24 n.s.	0/13	19/38	50 ** [a]	0/12
PVY	Primura	4/31	12.9 n.s.	0/15	7/26	26.9 n.s. [a]	0/11
PVY	Spunta	3/40	7.5 n.s.	0/13	10/37	27 n.s. [a]	0/12
PLRV	Désirée	3/52	5.8 n.s.	0/11	20/51	39.2* [a]	0/12
PLRV	Kennebec	6/54	11.1 n.s.	0/12	20/48	41.7*	0/14
PLRV	Liseta	1/40	2.5 n.s.	0/13	6/40	15 n.s.	0/13
PLRV	Monnalisa	5/46	10.9 n.s.	0/13	16/41	39 *	0/15
PLRV	Primura	2/42	4.8 n.s.	0/16	8/40	20 n.s.	0/15
PLRV	Spunta	3/37	8.1 n.s.	0/15	7/34	20.6 n.s.	0/14

*,**, Treatment different from control at P=0.05 (*) or at P=0.01 (**).
n.s., not significant.
[a] Differences among cultivars significant at P=0.05.

treatment the number of virus-free plantlets increased remarkably and reached 50% with the PVY-infected cv. Monnalisa, and 41.7% with the PLRV-infected cv. Kennebec.

Based on the overall sanitation effect of the 8-week treatment, the cultivars under study can be arranged in two distinct groups including: (i) Monnalisa, Kennebec, and Désirée, with 43–50% PVY and 39–41.7% PLRV elimination; (ii) Spunta, Primura, and Liseta, in which eradication was 13.8–27% for PVY and 15–20.6% for PLRV. Statistical differences among cultivars within the treatment were significant only for PVY.

Although the two viruses behave quite differently in the plant, since PLRV is phloem-limited and PVY is not, it seems that the cultivar rather than the type of relationship with the host accounts for the difference in the virus eradication rate. It is therefore important before venturing on mass sanitation to carry out preliminary tests to determine whether or not the method is suitable for any given cultivar. For instance, with the cv. Monnalisa, Kennebec, and Désirée, stem cutting culture combined with DHT-ribavirin treatment is suitable for the production of PVY- and PLRV-free material because of the simplicity and rapidity of this technique, even though better results can be obtained with meristem-tip culture.

If the present results are examined together with those of previous experiments involving PVX and PVS (Faccioli and Zoffoli, 1998) it is possible to conclude that cv. Monnalisa and Désirée are more efficiently sanitized from four viruses (PVX, PVY, PLRV, PVS) than the other cultivars tested, wheres cv. Primura is resistant to PVY and PLRV elimination, and cv. Spunta can be satisfactorily cleaned only from PVX.

The present method seems to be more effective than heat therapy combined with alternating temperatures and chemotherapy (ribavirin), applied *in vitro* to potato stem cuttings as reported by Griffith *et al*. (1989), who found a reduction in PLRV and PVY titre, but only a low percentage of PVY-free plants. Results were also better than those of Sanchez *et al*. (1991), who reported a low survival rate (20 to 75%) of 11 heat-susceptible *Solanum* genotypes, out of a total of 34 tested, and low percentages of PLRV- and PVY-free plants (9.1 and 25.9%, respectively) in the surviving material.

Acknowledgments

Thanks are expressed to Prof. G. Schuster, Botanisches Institut, Bereich Pflanzenphysiologie, Leipzig, Germany for the generous gift of DHT, and to Prof. G.P. Martelli, Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Italy, for critical revision of the manuscript.

Literature cited

Faccioli G., 2001. Control of potato viruses using meristem and stem-cutting cultures, thermotherapy and chemotherapy. In: *Virus and Virus-like Diseases of Potatoes and Seed-potatoes*. (G. Loebenstein, P.H. Berger, A.A. Brunt, R.G. Lawson, ed.), Kluwer Academic Publishers, Dodrecht, The Netherlands, 365–390.

Faccioli G. and C. Colombarini, 1996. Correlation of potato virus S and virus M contents of potato meristem tips with the percentage of virus-free plantlets produced *in vitro*. *Potato Research* 39, 129–140.

Faccioli G. and C. Rubies-Autonell, 1982. PVX and PVY distribution in potato meristem tips and their eradication by the use of thermotherapy and meristem-tip culture. *Phytopatologische Zeitschrift* 103, 66–76.

Faccioli G., C. Rubies-Autonell and R. Resca, 1988. Potato leafroll virus distribution in potato meristem tips and production of virus-free plants. *Potato Research* 31, 511–520.

Faccioli G. and R. Zoffoli, 1998. Fast eradication of potato virus X (PVX) and potato virus S (PVS) from virus-infected potato stem-cuttings by chemotherapy. *Phytopathologia Mediterranea* 37, 9–12.

Griffith H.M., S.A. Slack and J.D. Dodds, 1989. Effect of chemicals and heath therapy on virus concentration in *in vitro* potato plantlets. *Canadian Journal of Botany* 68, 1515–1521.

Sanchez G.E., S.A. Slack and J.H. Dodds, 1991. Response of selected *Solanum* species to virus eradication therapy. *American Potato Journal* 68, 299–315.

Accepted for publication: March 24, 2002