European Journal of Plant Pathology **104:** 737–740, 1998.

Short communication

High level of resistance in potato to potato mop-top virus induced by transformation with the coat protein gene

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Accepted 6 July 1998

Key words: genetically engineered protection, pomovirus, *Spongospora subterranea*, resistance testing

Abstract

Transgenic plants of potato cvs Saturna and Pentland Marble expressing the potato mop-top virus (PMTV) coat protein (CP) gene were produced. Plants contained transgene RNA transcript and CP. In resistance tests made in a screenhouse, plants were grown in pots containing field soil infested with viruliferous *Spongospora subterranea* (the vector of PMTV). PMTV infection of the daughter tubers was determined by ELISA; 10% and 17%, respectively, of tubers from non-transformed control plants of Saturna and Pentland Marble became infected. However, tubers from six transgenic Saturna lines were immune to PMTV infection, and only one transgenic Pentland Marble tuber of 261 tested from four lines was infected. Spraing symptoms were not seen in any Saturna tubers, but less than 1% of transgenic Pentland Marble tubers developed symptoms compared to 12% of those from control Pentland Marble plants.

Potato mop-top virus (PMTV) is prevalent in potato crops grown in areas with cool climates and has been identified in Northern Europe, Canada, China, Japan and the Andean region of South America. PMTV is transmitted by the motile zoospores of the plasmodiophoromycete fungus *Spongospora subterranea* (Wallr.) Lagerh. (Jones and Harrison, 1969; Arif et al., 1995), the causative agent of powdery scab on tubers. Infection with PMTV can cause damage known as 'spraing' that occurs as brown arcs and circles in the tuber flesh of susceptible cultivars (Harrison and Jones, 1971). The cv. Saturna is widely used in the Scandinavian potato processing industry and is a particularly sensitive cultivar, outbreaks commonly occur in which up to 25% of tubers are affected by spraing (Sandgren, 1995). Such damage in the year of primary infection can have an immediate and important economic impact on production of potatoes for the processing market. High incidences of spraing can lead to whole batches of tubers being rejected for processing with consequential loss of income for farmer and processor.

Effective and environmentally acceptable chemical control of the fungal vector is not commercially available, and there are no sources of resistance or tolerance to PMTV that have been deliberately used in breeding programmes. The advantages of transgenic resistance to plant viruses using pathogen-derived sequences are well established and there are many examples where this approach has been successfully applied against potato viruses (reviewed by Acosta et al., 1995). The most common pathogen-derived transgene used for resistance is that encoding the coat protein (CP) from which is derived coat protein-mediated resistance (reviewed by Lomonossoff, 1995). Reavy et al. (1995, 1997) showed that transformation of the test species *Nicotiana benthamiana* with a translatable version of the CP gene from a Scottish isolate of PMTV conferred very strong resistance to infection with that isolate, a second Scottish isolate and four Scandinavian isolates of PMTV. Furthermore, this resistance was effective following manual, graft, or fungal inoculation (Reavy et al., 1995).

We report here on the transformation of potato cvs Pentland Marble and Saturna with the CP transgene

and expression of resistance to PMTV in screenhouse-grown plants following fungal inoculation. Fungal inoculation rather than mechanical inoculation was used to test resistance because mechanical inoculation of potato results in local lesion formation, but does not lead to systemic infection of the plant and progeny tubers (Harrison and Jones, 1970). Furthermore, fungal inoculation simulates the infection route in field conditions.

Petiole and stem tissue pieces of virus-free plants of cvs Saturna and Pentland Marble (kindly provided by the Scottish Agricultural Science Agency, East Craigs, Edinburgh) were transformed as described by Barker et al. (1992), using *Agrobacterium tumefaciens* containing plasmid PMTV-T CP/ROK2 (Reavy et al., 1995). The vector contained cDNA encoding the CP gene of PMTV (T isolate from Scotland) in a translatable context under the regulation of a cauliflower mosaic virus 35*S* promoter as described by Reavy et al. (1995). Plants of each independent transformed line were propagated *in vitro* before well-rooted plantlets were transferred to potting compost and grown in an aphid-proof glasshouse at approximately 20 °C. Transgenic plants among the primary transformants were identified by Northern blotting of total leaf RNA using a digoxigenin-labelled cDNA probe that hybridizes with transgene transcript RNA as described by Webster and Barker (1998). Bands of polyadenylated RNA transcript of the estimated size (650–700 nucleotides) were identified in the Northern blots. Of 37 primary transformants of cv. Pentland Marble, 30 produced transcript. All of 10 primary transformants of cv. Saturna produced transcript. Steady-state levels of RNA transcript varied among lines, but in general levels of expression were similar to those found in *N. benthamiana* containing the same transgene (Barker et al., 1998).

Daughter tubers were harvested from primary transformants to establish four transgenic lines of cv. Pentland Marble (designated AL lines) and 6 lines of Saturna (designated AM lines) which were selected for resistance testing. The lines chosen represented the range of steady-state RNA transcript accumulation from low to high level expression (relative levels of transcript expression are shown in Table 1). Lines were propagated once more in the glasshouse to produce seed-sized tubers for the resistance tests.

Plants of the transgenic lines appeared to be morphologically normal when grown in glasshouse and screenhouse environments. Tests were made to assess accumulation of endogenous PMTV CP in leaves of transgenic plants grown in the screenhouse and glasshouse by ELISA and immunoblotting. Both methods used monoclonal antibody SCR69 (Torrance et al., 1993). No extracts of transgenic lines gave an A_{405} value greater than that given by the non-transgenic controls in triple antibody sandwich ELISA (TAS-ELISA) as described by Barker et al. (1998). However, CP was detected in leaf extracts of all lines by immunoblotting, done as described by Barker et al. (1992). This contrasts with results obtained from lines of *N. benthamiana* expressing the same transgene, in which we found that CP could be readily detected by ELISA (Reavy et al., 1995). The reason for lower levels of CP accumulation in transgenic potato compared with *N. benthamiana* is not known. Translation of the CP transcript may not be as efficient in potato, or possibly potato provides a less stable environment for CP resulting in lower steady-state levels. As with transgenic *N. benthamiana* (Barker et al., 1998) a correlation was noted between the steady-state level of CP accumulation (detected by immunoblotting) and transcript accumulation (detected by Northern blotting). For example, AL5 and AL32 contained substantially higher levels of CP and transgene RNA transcript than AL1 and AL19.

For resistance tests, potato plants were grown and inoculated in an unheated screenhouse (gauzehouse). PMTV was transmitted by fungal inoculation using soil from a site (a farm at Braco, near Auchterarder, Perthshire, Scotland) known to be infested with PMTV-containing resting spores of *Spongospora subterranea*. Bait tests in which *N. benthamiana*, *N. debneyii* and potato were grown in soil from this site, provided no evidence of recovery of a soil-borne virus, other than PMTV, following inoculation of root extracts of bait plants to indicator test plants. Six or seven sprouted tubers of each transgenic line and 14 and 15 tubers of non-transformed control Pentland Marble and Saturna, respectively, were planted (in early May 1997) in 10 litre pots of sterilised peat-based compost in which 750 g of field soil had been mixed. Prior to mixing, soil was air-dried to eliminate nematode pests. For the non-inoculated control treatment, two tubers from each transgenic line and five tubers each of non-transformed control cvs Saturna and Pentland Marble were planted in compost without the addition of soil. The pots were embedded in sand to ameliorate the effects of rapid temperature fluctuations. Pots were connected to an irrigation system to provide a watering regime comprising alternating periods (one week) of being heavily-watered followed by a period of free

Table 1. Infection and spraing symptoms in progeny tubers of potato plants grown in PMTV-infested soil

Line	Relative concentration of RNA transcript[1]	No. infected/ no. tested[2]	No. with symptoms/ no. tested[3]
Pentland Marble transgenic line AL1	Low	0/80	1[4]/162
Pentland Marble transgenic line AL5	High	1/61	2/134
Pentland Marble transgenic line AL19	Low	0/60	0/134
Pentland Marble transgenic lineAL32	High	0/60	1/157
Pentland Marble WT control	-	27/155	44/375
Saturna transgenic line AM4	Medium	0/70	0/169
Saturna transgenic line AM5	High	0/70	0/133
Saturna transgenic line AM6	Medium	0/70	0/140
Saturna transgenic line AM9	Medium	0/70	0/154
Saturna transgenic line AM10	Medium	0/70	0/164
Saturna transgenic line AM12	High	0/70	0/126
Saturna WT control	-	16/160	0/255

[1] Relative steady-state levels of transgene RNA transcript assessed from intensity of bands in Northern blotting.
[2] Infection in tubers from plants grown in PMTV-infested soil determined by ELISA. Tubers from plants grown in PMTV-free compost (two plants of each transgenic line and five of each non-transformed wild-type (WT) cv.) were used as ELISA controls; no positive reactions were obtained from testing approximately 225 of these tubers.
[3] Symptoms of spraing in tubers from plants grown in PMTV-infested soil determined by slicing tubers and scoring spraing symptoms. Tubers from plants grown in PMTV-free compost were tested as controls; a total of 230 tubers from AL lines, 259 from AM lines, 53 from cv. Pentland Marble and 125 from cv. Saturna. No symptoms were identified in these tubers.
[4] Tuber with very slight symptoms.

draining. This regime is known to favour germination of resting spores of *S. subterranea*. Daughter tubers were collected in September and stored at 4 °C for 5 months until they were tested.

Individual tissue extracts from five eyes and from the stolon attachment point of each tuber were taken using a tuber drill (Labec 'Sapex 01', Bioreba AG, Switzerland). Each extract was automatically diluted 1 in 5 with extraction buffer, and 100 μl expelled directly into the well of a microtitre plate and tested by TAS-ELISA as described by Barker et al. (1998). The tuber was deemed to be infected if one of the six extracts gave a positive reaction, i.e. an A_{405} value greater than twice the value given by a negative control. The majority of infected samples gave A_{405} values >5 times greater than those given by negative control samples, and the mean number of positive extracts per infected tuber was 3.3. Erratic distribution of PMTV in infected tubers is well known (Arli, 1996). After ELISA, tubers were thinly sliced with a knife and symptoms of spraing noted. In most cases spraing took the form of well-defined arcs of brown tissue in the tuber flesh but brown flecks were seen in a few cases. None of approximately 225 tubers from uninoculated control plants (a selection from all transgenic and non-transgenic lines) gave positive reactions in ELISA. Approximately half of the harvested tubers from inoculated plants were tested by ELISA and all tubers were tested for visible spraing symptoms. From ELISA, it was found that of 420 tubers tested from the 6 transgenic lines of Saturna, none were infected whereas 10% of tubers from non-transgenic control plants were infected (Table 1). Of 261 tubers tested from the 4 transgenic lines of Pentland Marble, only one tuber gave a positive reaction in ELISA whereas 17% of tubers from non-transgenic control plants were infected (Table 1). No symptoms were found in tubers of either non-transgenic or transgenic lines of Saturna, but while spraing symptoms were identified in 12% of control Pentland Marble tubers only 4 tubers ($<1\%$) from the transgenic lines produced symptoms. The single infected tuber from transgenic Pentland Marble line AL5 identified by ELISA also had readily distinguishable symptoms of spraing, but the other three tubers with symptoms did not give positive results in ELISA. Using soil with a greater load of viruliferous resting spores might have given a higher level of infection in control plants. Arli (1996) has shown that there is a poor correlation between development of spraing symptoms and virus detection in tubers by

ELISA, although in comparative tests ELISA detected more infected tubers than either symptom assessment or RT-PCR.

This is the first report of a transgene that confers resistance to PMTV in potato. The resistance observed in transgenic lines of cvs Saturna and Pentland Marble resembles that described in lines of *N. benthamiana* transformed with the same gene (Reavy et al., 1995; Barker et al., 1998). Thus, as with *N. benthamiana*, all potato lines are highly resistant, irrespective of their steady-state levels of CP and transcript accumulation and the resistance is effective against fungal inoculation. Studies of *N. benthamiana* transformed with the PMTV CP gene showed that the characteristics underlying the resistance conferred by this transgene seem to be unique (Barker et al., 1998). The transgene seems to be as effective against fungal inoculation of PMTV in potato as it is in *N. benthamiana*. Resistance conferred by the PMTV CP transgene in *N. benthamiana* is effective against manual inoculation with Scandinavian isolates of PMTV (Reavy et al., 1997). There is remarkably little variation in the amino acid sequences of CP genes of isolates of PMTV from Scotland, Scandinavia and South America (Mayo et al., 1996; Reavy et al., 1997) and we can expect the transgene used in these studies to be effective in potato against a range of PMTV isolates. Field trialling of these transgenic lines in different locations and in soils with differing inoculum loads will be necessary to verify this and to examine possible environmental effects on the expression of resistance.

Acknowledgements

This work was funded by the Scottish Office Agriculture, Environment and Fisheries Department (SOAEFD). We are grateful to Wendy Smith for help with the ELISA and to Sheila Dawson for help with tuber harvesting. We are also grateful to Sturdy Concrete Garages and to Mr. Kirk, Over Ardoch Farm, Braco for access to soil samples.

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