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A Unique Form of Transgenic Resistance to Potato Mop-Top Virus Induced by Transformation with the Coat Protein Gene

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S u m m a r y

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Transgenic lines of *Nicotiana benthamiana* and potato were obtained that express a translatable transgene encoding the coat protein (CP) gene from potato mop-top virus (PMTV). Transgenic lines differed considerably in steady-state levels of transgene RNA transcript and CP which were positively correlated. The transgene conferred strong resistance in all lines of *N. benthamiana* to manual inoculation with the PMTV isolate from which the transgene sequence was derived and also to five other isolates. Greater than 88% of transgenic plants (mean from all lines) were immune to infection. Transgenic plants of potato cvs Saturna and Pentland Marble expressing the same transgene were obtained and resistance tests made on plants grown in pots containing field soil infested with viruliferous *Spongospora subterranea* (the vector of PMTV). PMTV infection was determined by ELISA of the progeny tubers after harvest. 10% and 17%, respectively, of tubers from non-transformed control plants of Saturna and Pentland Marble became infected. Tubers from six transgenic Saturna lines were immune to PMTV infection and only one transgenic Pentland Marble tuber of 261 from four lines was infected.

I n t r o d u c t i o n

Potato mop-top virus (PMTV) is transmitted by motile zoospores of the plasmodiophoromycete fungus *Spongospora subterranea* (JONES & HARRISON 1969) the causative agent of powdery scab on tubers. Infection can cause damage known as 'spraing' that occurs as brown arcs and circles in the tuber flesh of susceptible potato cultivars (HARRISON & JONES 1971). The cv. Saturna is a

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particularly sensitive cultivar; outbreaks commonly occur in which up to 50% of tubers are affected by spraing (SANDGREN 1995). The virus 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. 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. We describe a transgene encoding the CP of PMTV which confers very strong resistance in *Nicotiana benthamiana* and potato which could be used in commercial agriculture.

Materials and Methods

Plant transformation and assay of steady-state levels of transgene RNA transcript and CP

N. benthamiana and potato were transformed using *Agrobacterium tumefaciens* containing plasmid PMTV-T CP/ROK2 (REAVY & 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 35S promoter. 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. Steady-state levels of transgene transcript were estimated by densitometry of the Northern blots using the Bio Image Intelligent Quantifier PC program. Steady-state levels of transgene expressed CP were estimated by ELISA.

Resistance tests

Seed, collected from transgenic *N. benthamiana* plants, was sown to generate T_1 generation plants that were tested for resistance. Daughter tubers were harvested from primary transformants of potato to establish four transgenic lines of cv. Pentland Marble and six lines of cv. Saturna which were selected for resistance testing. The lines of *N. benthamiana* and potato chosen for the tests represented the range of steady-state RNA transcript accumulation from low to high level expression.

Transgenic seedlings of *N. benthamiana* were manually inoculated with PMTV, and 18 days after inoculation systemic tissue was tested using a sensitive infectivity assay as described by REAVY & al. 1995. For resistance tests on potato, plants were grown in an unheated screenhouse (gauzehouse), and PMTV was transmitted by fungal inoculation using soil from a site known to be infested with PMTV-containing resting spores of *S. subterranea.* 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 10 litre pots of sterilised peat-based compost in which 750 g of air-dried field soil had been mixed. Daughter tubers were collected and stored at 4°C for five months until they were tested by ELISA. Transgenic seedlings of *N. benthamiana* were also inoculated with PMTV in similar bait tests in the glasshouse.

Results and Discussion

Transgenic lines of *N. benthamiana* differed considerably in steady-state levels of transgene RNA transcript and CP which were positively correlated. A mean of 88% transgenic T_1 plants from seven lines were resistant to manual PMTV inoculation using inoculum containing virus particles (freshly macerated leaf tissue). Resistance was identified by failure to develop symptoms and the inability to recover infectious virus using a sensitive infectivity assay. Many of the 12% of

transgenic plants that gave a positive infectivity assay did not produce symptoms and virus was not detected in repeat assays on the same plants. The resistant plants did not express a 'recovery' phenotype and resistance was not overcome by using inocula containing viral RNA (total leaf RNA extracts). All lines were highly resistant to PMTV irrespective of the steady-state levels of transgene RNA transcript and CP. Transgenic T_1 seedlings of *N. benthamiana* were also challenged in a bait test in which they were grown in soil containing viruliferous *S. subterranea*. In these tests only two plants out of 99 became infected. T_1 plants were also highly resistant to graft inoculation (REAVY & al. 1995, BARKER & al. 1998)

A non-translatable version of the CP transgene was developed in which three codons at the 5' end of the gene were mutated to termination codons. When this transgene was transformed into *N. benthamiana*, it did not confer resistance to infection with PMTV, although the development of symptoms in some lines was slightly delayed (BARKER & al. 1998).

Resistance tests were made on transgenic seedlings of five transformed lines of *N. benthamiana* using six PMTV isolates. Plants were as resistant to two Swedish, two Danish and a second Scottish isolate as they were to the Scottish isolate from which the CP transgene had been cloned (REAVY & al. 1997).

PMTV CP was not detected in leaves of transgenic potato lines by ELISA, but could be detected in leaf extracts of all lines by immunoblotting. 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 & al. 1995). 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 & al. 1998) a correlation was noted between the steady-state levels of CP (detected by immunoblotting) and transcript RNA (detected by Northern blotting).

ELISA tests were made to detect virus in tubers harvested from the inoculated potato plants. 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 six transgenic lines of Saturna, none were infected whereas 10% of tubers from non-transgenic control plants were infected. Of 261 tubers tested from the four 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. No symptoms were found in tubers of either non-transgenic or transgenic lines of Saturna. Spraing symptoms were identified in 12% of control Pentland Marble tubers but only four tubers (<1%) from the transgenic lines produced symptoms.

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 & al. 1998). Thus, although it depends on coat protein translation to be effective, it mediates very strong resistance. Resistance conferred

by the PMTV CP transgene in *N. benthamiana* is effective against manual inoculation with Scandinavian isolates of PMTV (REAVY & al. 1997). There is remarkably little variation in the amino acid sequences of CP genes of PMTV isolates from Scotland, Scandinavia and South America (MAYO & al. 1996, REAVY & al. 1997) and we can expect the transgene used in these studies to be effective against a range of PMTV isolates. The same transgene also confers very strong 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. 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. 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.

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