Molecular Studies of *Potato Mop-Top Virus* (PMTV) in Transgenic *N. benthamiana* and *S. tuberosum*

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Abstract

Resistance to *Potato mop-top virus* (PMTV) was studied in *Nicotiana benthamiana* and potato (*Solanum tuberosum* cv. Saturna) transformed with the coat protein (CP) gene of PMTV. In *N. benthamiana* plants mechanically inoculated with PMTV, RNA2 was detected in leaves and roots in several plants in the absence of RNA3. When *N. benthamiana* was grown in infested soil, viral RNA was detected in roots, but no systemic movement of PMTV to above-ground parts was observed. The incidence of PMTV infections was reduced in the CP-transgenic potato plants grown in an infested field in Sweden. However, in infected tubers, all three virus RNAs were detected.

Keywords: *Potato mop-top virus*; *Spongospora subterranea*; *Solanum tuberosum*; *Nicotiana benthamiana*; transgenic resistance

INTRODUCTION

Potato mop-top virus (PMTV, genus *Pomovirus*) is one of the most economically important plant viruses in the Nordic countries. It causes symptoms known as spraing: brown arcs and circles develop in the esh of tubers and make them unmarketable. The genome of PMTV consists of three single stranded positive sense RNA molecules, RNA1, RNA2 and RNA3 (SCOTT *et al.* 1994). RNA1 encodes the viral replicase (SAVENKOV *et al.* 1999). RNA2 contains four ORFs of which three encode putative proteins similar to the triple gene-block (TGB) proteins involved in the cell-to-cell movement of other viruses (LAUBER *et al.* 1998). RNA3 encodes the coat protein (CP) and a read-through protein (RT protein), the latter might be involved in vector transmission (REAVY *et al.* 1998). PMTV is transmitted in zoospores of *Spongospora subterranea*, which is a pathogen itself causing powdery scab on potato. In the resting spores of *S. subterranea* PMTV can survive in the soil for many years. The CP gene of PMTV was introduced to *Nicotiana benthamiana* and potato *Solanum tuberosum* cv. Saturna under control of the CaMV 35S promoter. The aim of this study was to test the CP-transgenic lines of potato for resistance in field conditions and to compare expression of resistance to PMTV between roots and leaves.

MATERIALS AND METHODS

Plant material. The transgenic lines of *N. benthamiana* (REAVY *et al.* 1995) and potato cv. Saturna (BARKER *et al.* 1998) transformed with the CP gene of PMTV-T driven by CaMV 35S promoter have been described. The T_1 progeny of *N. benthamiana* line W5 was grown from seed.

Field experiments with potatoes. The field trial with the transgenic Saturna lines was carried out in a field known to contain viruliferous *S. subterranea* in Brunskog, Halland, Southern Sweden (permit 22-1387/99, National Board of Agriculture, Sweden) (GERMUNDSSON *et al.* 2002).

Experiments with N. benthamiana. *N. benthamiana* plants were inoculated with PMTV by two different

methods: by mechanical inoculation to leaves or by growing the plants in soil infested with viruliferous *S. subterranea*.

Virus detection by ELISA. The eight largest tubers from each line and block (32 tubers per line in total) and the uppermost fully-expanded leaves of *N. benthamiana* plants were selected for ELISA to detect PMTV (GERMUNDSSON *et al.* 2002). Samples with an A_{405} value greater than twice that of the negative control were deemed to be PMTV-infected.

Extraction of viral RNA. The sample for RNA-extraction was taken at the same time and from the same part of the tuber as the sample for ELISA. Extraction of RNA from potato tubers was done as described by GERMUNDSSON *et al.* (2002). Total RNA extracts were prepared from leaves and roots of *N. benthamiana* as described by VERWOERD *et al.* (1989).

Dot-blot hybridisation. PMTV RNA2 and 3 were detected with specific probes obtained by PCR amplification from cDNA of PMTV. The probes were non-radioactively labelled with digoxigenin-11-UTP by using DIG RNA labelling kit (Boehringer Mannheim). Total RNA extracted from potato tubers, or roots or leaves of *N. benthamiana*, was dotted onto a nitrocellulose membrane (Bio-Rad) and cross-linked with UV-light. Hybridisation with probes specific to PMTV RNAs and detection of signals using antidigoxigenin-AP, CSPD and Lumi-Film (Boehringer Mannheim) were carried out according to manufacturer's instructions.

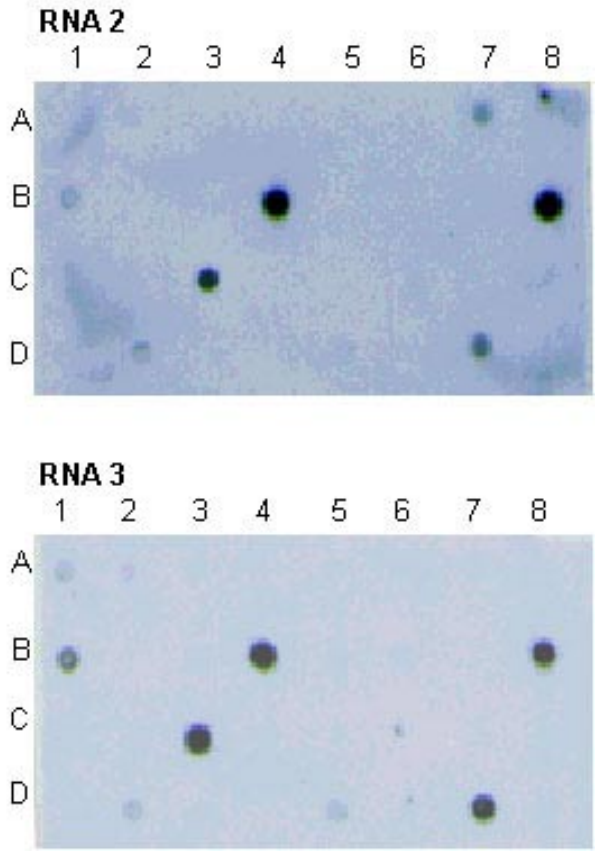

Figure 1. Detection of PMTV RNA2 and RNA3 in potato tubers using dot-blot hybridisation. The two panels show the same blot hybridised with different probes. The four tubers (A–D) in each column are from the same Saturna line. Columns: 1, line AM4; 2, line AM5; 3, AM6; 4, AM9; 5, AM10; 6, AM12; 7 and 8, represent two lines of non-transgenic cv. Saturna. Detection of the PMTV RNAs shown here was consistent with detection of PMTV in the same tubers by ELISA

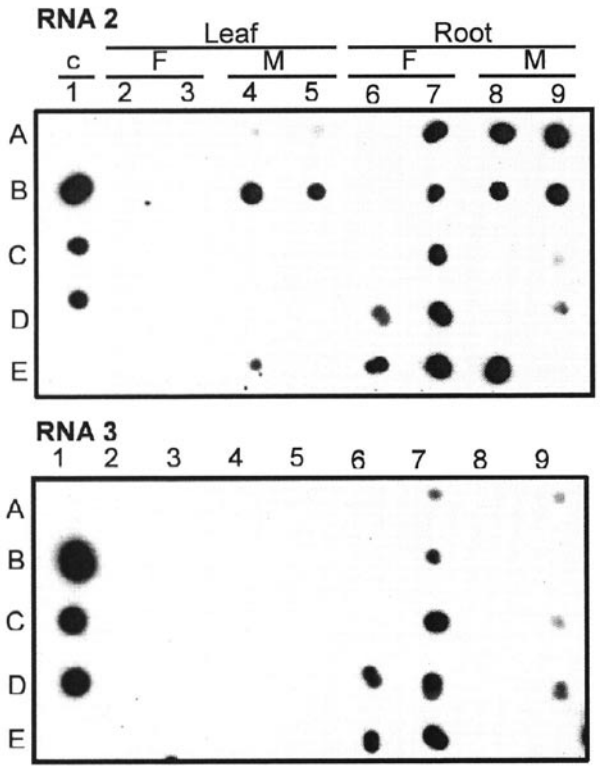

Figure 2. Detection of PMTV RNA2 and RNA3 in roots and leaves of *N. benthamiana* using dot-blot hybridisation. The leaves and roots of 20 transgenic plants and a few non-transgenic control plants were tested. **F**; transgenic plants grown in soil infested with viruliferous *S. subterranea*. **M**; transgenic plants grown in non-infested soil where one leaf was mechanically inoculated with PMTV. Controls: A1, leaf of a non-transgenic, healthy plant; B1, root of a non-transgenic, PMTV-infected plant; C1, leaf of a non-transgenic, PMTV-infected plant; D1, the roots of the plant of C1

RESULTS

Some PMTV-infected tubers were found in the transgenic potato lines but the incidence of infection was lower (7%) than in the non-transgenic lines (20%). PMTV RNA2 and RNA3 were detected in potato tubers using dot-blot hybridisation (Figure 1). In contrast, neither RNA2 nor RNA3 was detected in leaves of *N. benthamiana* plants growing in infested soil. However, in roots both RNA2 and RNA3 could be detected in 7 out of 10 plants, which indicates that there was no systemic movement of PMTV to the above-ground parts. In transgenic *N. benthamiana* plants mechanically inoculated with PMTV no RNA3 was detected in leaves but RNA2 was detected in the leaves of 5 out of 10 plants. In roots RNA2 was detected in 7 out of 10 plants but RNA3 was detected only in 3 out of 10 plants and in very small amounts.

DISCUSSION

Our data indicate that the CP gene-mediated resistance to PMTV restricts accumulation of RNA3, and expression of resistance is different depending on whether the host plant is inoculated mechanically or by the natural vector. Furthermore, the data indicate that expression of transgenic resistance can differ in roots and leaves. Finally, our data imply that in this particular case, the transgenic resistance may function through two mechanisms, one that is protein-based and another that is based on RNA silencing. Our work emphasises the importance of testing the resistance of transgenic plants in several locations because of the influences a particular environment may have on the expression of resistance.

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