Biological and Molecular Characterization of Various Isolates of *Potato Virus Y-N* (PVY-N) Strain Group

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Abstract

Fourteen *Potato virus Y* (PVY) isolates were characterized. They represented PVY^N strain only. However, application of serological and molecular genetic methods led to a more complicated characterization. For example, five isolates induced necrotic symptoms on tobacco plants typical of PVY^N, despite reacting as PVY^O serologically. Moreover, the PVY isolates were not identical according to molecular genetic properties. Typical PVY^{NTN} PCR products were observed for 11 isolates, but four of them (Hr220-5, Hr387-7, Nord 242 and Syn1Scot) did not produce potato tuber necrotic symptoms in infected cultivars. An immunocapture RT-PCR probing was developed using a set of 24 primer pairs derived from eight regions of the PVY genome. Using this method, five out of seven PVY^{NTN} isolates including the Czech standard PVY^{NTN} from the potato cv. Nicola were found to be identical. However, two PVY^{NTN} isolates and all the other probed PVY samples showed unique patterns, suggesting specific differences at the nucleotide level. This method enabled specific identification of individual isolates variability even within different PVY strains.

Keywords: *Potato virus Y*; *Solanum tuberosum* L; immunocapture RT-PCR; virus genome

INTRODUCTION

At the beginning of the eighties the potato tuber necrotic ringspot disease (PTNRD) appeared in potato crops (BECZNER *et al*. 1984) and now it is wide-spread in the world. These isolates called PVY-NTN constitute a new sub-group of PVY-N (LE ROMANCER *et al*. 1994) and further different sub-group of PVY-N isolates (Wilga-type) was reported in Poland (CHRZANOWSKA 1991) and then in France (GLAIS *et al*. 1998) and Czech Republic (DĚDIČ & PTÁČEK 2000).

Identification of PVY^{NTN} is based primarily on the development of necrotic symptoms on tubers of inoculated sensitive potato cultivars, but this is time-consuming. It has not been possible to select indicator plants or to produce MAbs that reliably distinguish PVY^{NTN} isolates from other members of PVY^N strain group. The methods based on the variability of genomic sequence have been more successful (BLANCO-URGOITI *et al*. 1996; WEIDEMANN & MAISS 1996; GLAIS *et al*. 1996; WEILGUNY & SINGH 1998). However, the RFLP analysis of the whole genome of a group of sequenced PVY isolates (GLAIS *et al*. 1998) has indicated a more complex nature of PVY genome possibly because of recombination events.

In this study a number of PVY isolates was investigated by biological, serological and molecular genetic methods. In order to characterize individual isolates, a system of immunocapture RT-PCR probing of the PVY genome has been used.

MATERIALS AND METHODS

Virus isolates originated from Canada, Czech Republic, France, Poland and Slovenia were maintained and evaluated on four varieties of potato and on tobacco plants (*N. tabacum* cv. Samsun). The presence of PVY was determined 10–21 days later by the development of characteristic symptoms and by a positive reaction in an enzyme-linked immunosorbent assay (ELISA). We

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used either the RNeasy Plant Total RNA Kit (Qiagen) for total RNA isolation or an immunocapture on ELISA plates for template preparation. First-strand cDNAs were synthesized by the Enhanced Avian RT-PCR Kit (Sigma) using reverse primers or random nonamers in 0.2 ml PCR tubes or in ELISA microplates. For the PCR, 5 μl of the reverse transcriptase reaction mixture (cDNA) was amplified in a total volume of 50 μl using the Enhanced Avian RT-PCR Kit (Sigma) and the primers and methods:

a) WEIDEMANN & MAISS (1996)
b) GLAIS *et al.* (1996)
c) WEILGUNY & SINGH (1998)
d) PTÁČEK *et al.* (2002).

For the RT-PCR probing system (PTÁČEK *et al.* 2002), the PCR and RT-PCR primers were derived from the sequence of a Hungarian isolate of PVY^{NTN} (EMBL database, Acc. No. M95491). Eight regions, numbered 1–8, located throughout the PVY genome were selected from alignment of PVY^{NTN} with PVY^{O} (Acc. No. D00441) at positions where the maximum difference in restriction sites was observed. Each of these regions was subdivided into parts I–III to obtain three fragments ranging in length from 440 to 220 bp. Twenty four primer pairs in total were derived from the PVY genome.

RESULTS AND DISCUSSION

Because of the economic importance of PVY^{N} to potato production, isolates mostly identified biologically as PVY^{N} were selected for this study. From serological analyses and tests on potato cultivars the 14 PVY^{N} isolates could be split into several subgroups. The first subgroup included nine isolates typical of PVY^{NTN} from which characteristic PCR products were obtained, using three published techniques for differentiating PVY^{NTN} (WEIDEMANN & MAISS 1996; GLAIS *et al.* 1996; WEILGUNY & SINGH 1998). From this subgroup the isolate from the cv. Nicola was selected as the standard PVY^{NTN}. The second subgroup of five isolates designated $PVY^{N}W$ (CHRZANOWSKA 1991) showed a positive reaction with PVY^{O}-specific antibody, and did not produced PTNRD symptoms. According to RT-PCR analysis, $PVY^{N}W$ isolates could be subdivided into two sets. The first produced PVY^{NTN}-specific RT-PCR products, while the second did not. The properties of two remaining isolates, Tu 660 Cal, Syn 1 Scot, were unusual. Tu 660 Cal, in contrast to the published data (MCDONALD & SINGH 1996), did not produce PTNRD on the sensitive potato cvs. used in this study. In addition, no PVY^{NTN}-specific RT-PCR products were observed, thus supporting the results of WEIDEMANN and MAISS (1996). For Syn 1 Scot, PTNRD was not observed, although it was positive for PVY^{NTN} in RT-PCR. It can be concluded that the RT-PCR methods evaluated do not reliably differentiate PVY isolates and that the PVY^{N} subgroup is very variable, thus confirming the work of others (GLAIS *et al.* 1998; DĚDIČ & PTÁČEK 1998; SINGH *et al.* 1998). The high diversity of PVY may mean that the virus undergoes fast evolutionary changes.

In order to identify PVY isolates quickly, without the need for sequencing, the method of immunocapture RT-PCR probing, using a set of 24 pairs of primers covering eight regions throughout the whole PVY genome was developed. Each region was subdivided into three parts in order to form three distinct cDNA products by RT-PCR, ranging from 220 to 440 bp. Initial experiments, using the standard PVY^{NTN} isolate of Czech origin from the cv. Nicola, showed that all selected primers led to amplification of specific products. Five out of seven PVY^{NTN} isolates were identical and were different from the two other PVY^{NTN} isolates, Tu 64–8, and Y-N Weq. The remaining probed PVY samples showed unique patterns, suggesting specific differences at the nucleotide level. Significant parts of these isolates belongs to $PVY^{N}W$ (CHRZANOWSKA 1991). According to our analyses, the remaining isolates have unique combinations of biological, serological and RT-PCR properties. The probing spectra of these isolates are also unique. Our analyses support the theory that some isolates might be recombinants as proposed by GLAIS *et al.* (1998).

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