TECHNICAL SHEET No. 21

Virus Detection: *Potato virus Y* (PVY)

Method: Immunocapture RT-PCR, RFLP Immunocapture RT-PCR

General

Virus detected: PVY from potato
General method: IC-RT-PCR, RFLP-IC-RT-PCR

Developed by

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Goals

To develop a sensitive and specific method for PVY detection based in serology and PCR, easily and reliably method to distinguish between necrotic PVY^N and common PVY^C strains.

Introduction

PVY strains are pest quarantine in Israel, and therefore development of sensitive methods for their detection is pivotal for establishing virus free potato seeds. Israel imports all potato seeds for planting from Europe, where the PVY^N strain is present. The current method routinely used for PVY detection is ELISA.

PVY is a member of the Potyviridae, the largest plant virus family. For general information on PVY see the Web Site (life.anu.edu.au/viruses/ICTVdB/57010001.htm). Currently, three main strains of PVY are known: (i) PVY^O (ordinary or common strain) is widely distributed throughout the potato growning areas of the world. This strain induces mild to severe mosaic and can cause stem necrosis in some cultivars. (ii) The PVY^C is the stipple streak strain and produces systemic mosaic in some cultivars and a hypersenstive reaction in other cultivars. In tobacco, it produces the same symptoms as the PVY^O. (iii) PVY^N was recognized in the 1950's as causing serve veinal necrosis in tobacco. It was originally imported from Europe and South America. A new sub-group of PVY^N that causes superficial necrotic ring spots on tubers was first described in 1980. It is called potato tuber necrotic ringspot strain. The tuber symptoms develop during storage (Carnegie and van de Haar, 1999).

A monoclonal antibody assay was reported by Ellis (1996) for distinguishing the PVY^O and PVY^Cstrains from PVY^N. An ELISA assay is commonly used in the USA for detection of PVY^N. Recently, RT-PCR assays have been developed for detection of PVY^N (Nie and Singh, 2002a and 200b). The existence of recombinants between strains has been suggested (Glais et al., 2002), which makes for difficulties in detection and characterization. Currently, there are considerable efforts being made to better understand the relationship between the different strains of PVY, specifically their genomes and biological properties.

Materials and Methods

Design of primers

The figure below shows the partial sequence of the p1 protease gene of PVY-Hungarian N-strain (M95491) and the location of the primers used to discriminate between PVY strains:

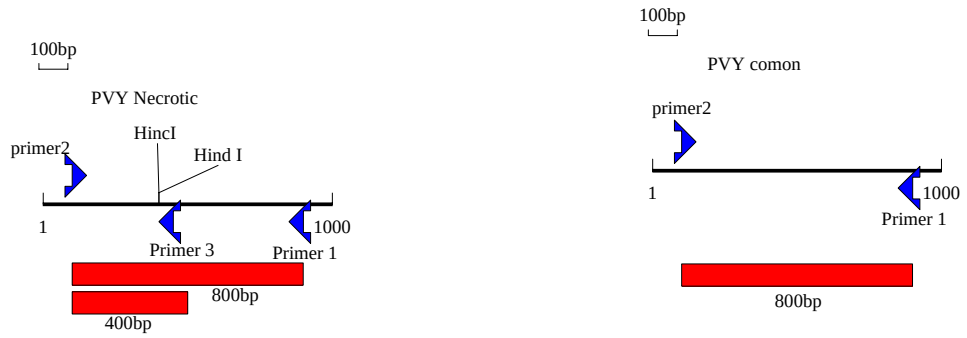
In order to detect PVY^N, we carried out a sequence comparison between PVY strains. The sequences were retrieved from GenBank and compared. Multiple alignments did not show many differences between the strains. Minor differences were found in restriction enzyme site (such as an *Hin*dII site) that allowed us to use the polymorphism PCR method (or RFLP-PCR).

RFLP-PCR is based on the different patterns obtained after the PCR products are incubated with restriction enzymes and separated by agarose gel electrophoresis. These polymorphisms were characteristics of the different PVY strains sequences.

Three primers were designed to amplify PVY strains and to allow the detection of the *Hin*dII polymorphic site of PVYN. The location of the primers on the sequence of PVY is indicated above. Primer 1, which is a complementary sense primer (identical in all strains), was used for first strand cDNA synthesis (from viral RNA purified): 5'TTCCAAAGTGTCCTTTGAG3'. Primer 2, which is a sense primer (identical in all the strains), was used to amplify the first cDNA strand: 5'CTTCATCAAACAAACTCTTT3'. Primer 3, is a second complementary sense primer, which is located between primer 1 and primer 2. The sequence of primer 3 is specific to PVY^{N} strains and is different from all other PVY groups (PVY^{C} strains, PVY^{O}): 5'ATCTGGGCATCAGTCTTG3'.

Discrimination between PVY strains using RT followed by PCR with three primers.

In the case of PVY^{N} infection, PCR amplification results in the production of two DNA fragments (one migrates at ~ 800bp and the other at ~ 400bp). In contrast, only one DNA fragment amplified (~ 800bp) with PVY^{C} infected material.



Immunocapture

1. Add 200 µl of anti-PVY (diluted 1:1000 in coating buffer) to each ELISA plate well or to PCR tube and incubate at 37°C for 3 h. We used both a commercial anti-PVY antibody (Bioreba) and an antibody from Prof. Gad Loebenstein, The Volcani Center.
2. Empty the ELISA cells/ PCR tube and wash 3 times with TBST.
3. Homogenize 25 mg of plant leaf or tuber with 5 ml of suitable extraction buffer.
4. Add 200 µl of the homogenate to the ELISA well/ PCR tube and incubate for 18 h at 4 °C.
5. Empty the ELISA well/ PCR tube and wash 3 times with TBST.
6. Dry the ELISA well/ PCR tube, add 5 to10 µl ddH2O and heat at 70 °C for 15 min.

RT-PCR polymorphism

The IC-PCR procedure was tested for the detection of various PVY strains. This method was based on PCR amplification of viral cRNA using three primers.

a) cRNA synthesis

1. To 5 µl IC add 8 µl ddH_2O and 1 µl primer 1 (100 pmoles); incubate at 70 °C for 10 min, followed by 30 min in an ice bath.
2. Add 1 µl of each dNTPs (25 mM each), 4 µl reverse transcriptase 5X buffer, 1 µl AMV reverse transcriptase (Promega); incubate at 42°C for 1h.
3. Heat for 10 min at 90 °C; adjust the volume to 50 µl with ddH2O.

b) PCR

1. The PCR reaction contains 5 µl from the reverse transcriptase reaction, 0.25 µl 25 mM dNTPs, 1 µl each primer 1, 2 and 3 (100 pmoles), 2.5 µl Taq 10x buffer and 1 unit *Taq* DNA polymerase; add ddH_2O to a final volume of 25 µl.
2. Cycling: One cycle: 95°C for 3 min, 50°C for 2 min, 72°C for 2 min. Thirty cycles: 95°C for 1 min, 55°C for 1 min, 72°C for 1 min. One additional cycle: 72°C for 10 min.
3. Analysis of reaction products. Subject the reaction products to 1% agarose gel electrophoresis.

c) RFLP PCR

1. Incubate the PCR products with *Hin*dIII for 3 h.
2. Subject the reaction products to agarose gel electrophoresis.

Results

The figure below shows the pattern obtained with PVY^N and PVY^C

Lane 1 and 2: IC-RT-PCR using the two primers P1 and P2. The RT PCR products were incubated with *Hin*dII and subjected to agarose gel electrophoresis. In the case of PVY^N, (lane 2) only the 400-bp fragment will appear (Cleaved with *Hin*dII). In the case of PVY^C, only the 800-bp fragment will appear (lane1) (not cleaved with *Hin*dII). Lane 3 and 4: IC-RT-PCR using three primers P1, P2 and P3. The IC-RT-PCR products were subjected to agarose gel electrophoresis. In case of PVY^N (lane 4) two bands will appear: 400 and 800 bp. In the case of PVY^C (lane 3) only the 800-bp fragment will appear.

References

Background on PVY: http://life.anu.edu.au/viruses/ICTVdB/57010001.htm

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