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**

Name of researcher: Fouad Akad and Hanokh Czosnek, The Hebrew University  
Address (Email): akad@agri.huji.ac.il and Czosnek@agri.huji.ac.il  
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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 growing 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 hypersensitive 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\(^C\) strains 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:

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1     AAATTAAAC AACTCAATAC AACAAGCACA AAAACACTCA AAAAAAGCTTT 
TTTAATTTTG TTGAGTTTATG TTGATTCTCT TTAGTTGCAGT TTGTTTCGAAA
primer 2
61    CAACTCTAAC TCAAACCATTT CAATTCCGATT CTTCATCAA AAAACTCTTT
TTTATTTTGT TGAGTTATG TTGTATTCTT TTAGTTGCGT TTTTGTGAGT GTTTCGAAA
121   CAATTTGACTA GTATGCATTAC GTAATTGACT AAGTTATTTTC AATTGGCAGAG 
CTAATTGGAT ACCTGCTGAA TTCAATAGAT TTGGATGTAGA TTGGGAGGAA
primer 3
181   GACGCGCATTC GAGTTTGGGA GATACGACGTG ATGTGCTGAG TAGTTGACTA 
GCTGCTGCAA TTTCTTCTTC TTATATTAAA GGTGTGTTGA
241   CCATAGCTAAC AGTCCCTTT TTTGGCTGATT GCTGACTGAA GTGTGTTGACTA 
CTTGAGCTTAA TTTTGACGAA CAGTGGATCT TGAAAAACTT
301   CAACTCTAAC TCAAACCATTT CAATTCCGATT CTTCATCAA AAAACTCTTT
TTTATTTTGT TGAGTTATG TTGTATTCTT TTAGTTGCGT TTTTGTGAGT GTTTCGAAA
361   ACTATTCGAA CATCCAAGAA TGGTACTTGC ATGTATCGAT ACAAGACTGA TGCCCAGATT
2GTTCTGACT ACGGGTGTA
421   GCGCGCATTC AAAAGAAGCG CGAGGAGAGA GAAAGAGAGA AATATAATTT CCAAATGGCT
CGCGCGTAAG TTTTCTTCGC GCTCCTCTCT CTTTCTCTCC TTATATTAAA GGTGTGTTGA
481   GCGTCAAGTG TTTGCTGAAA GATCACTATT GCTGGTGGAG AGCCACCTTC AAAACTTGAA
AATAGCTTCA AAGACAAGCA AGTTGCTTCA AGACGCGCTG CAGTCTGAGA TTGGAGAGG
541   TCACAAGTG GCAAGGGGTT GTTCCCAACTA ACCTCAAGGA TGCAGCTTCA AAAAGATAT
AGTTGTTGAGG TTTGGGTTTTA AACAGTTTCG TCTTCTTCAA
601   CGCAGCCTCA AATTTGAGAG GGGACAAATG AACCACCTTA TCAAGCAGGT GAAGCAAATT
GCGTGCGGTT TTTACGTGCT CATAATTCGTT TCTTCTTCAA
661   ATGTCAACCA AAGGGAGGCT TGTTCAACGT ATTAGCAGAGA AAAATCCAGCA TGTTTCTATT
AATAGCTTCA AAGACAAGCA AGTTGCTTCA AGACGCGCTG CAGTCTGAGA TTGGAGAGG
721   AAAGAAGTTT GGGTACAGCA TGCGCAGCAG TGTTTACGAG GAGCTTCCAG
TTTCTTTCAA AACCCTTACAG TGGCAGCTGAA ACTACGCTT TTATGGGTTTTA AAGAGTCTT
781   AAGAGAGTTG ACTTTTGGATG TGTAATTTGATG CAGTGCTGAA TACGCTCCTG CAAACATTGAG
TTTCTTTCAA AACCCTTACAG TGGCAGCTGAA ACTACGCTT TTATGGGTTTTA AAGAGTCTT
841   ACGGACAAGT GGACTAAACCA ATGTTGTCGCT ATCAGATTTG CAGTGGATCT GATGCTGATT
TGCCCTTTCA AAGTTTTTTA TCAAGCAGGA TGACTAGATT GCTCCTCCTG CATAATTCGTT
prime 1
901   ATATTGAGTA ATACTAATCT CAAAGGAGTT GGTAGGTTTG GGTCAAAAGA GCTACGCTTGG
TTTCTTTCAA AACCCTTACAG TGGCAGCTGAA ACTACGCTT TTATGGGTTTTA AAGAGTCTT
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In order to detect PVY\textsuperscript{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 \textit{Hind}II 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 HindII 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’CTTCATCAAACAACTCTTTT3’. 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 PVYN strains and is different from all other PVY groups (PVYC strains, PVYO): 5’ATCTGGGCACTCAGTCTTG3’.

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

In the case of PVYN 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 PVYC infected material.

![Image of RFLP-PCR diagram]

**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 to 10 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 ddH₂O 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 ddH₂O 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 HindIII for 3 h.
   2. Subject the reaction products to agarose gel electrophoresis.

Results

The figure below shows the pattern obtained with PVY⁵ and PVY⁶

Lane 1 and 2: IC-RT-PCR using the two primers P1 and P2. The RT PCR products were incubated with HindII and subjected to agarose gel electrophoresis. In the case of PVY⁵, (lane 2) only the 400-bp fragment will appear (Cleaved with HindII). In the case of PVY⁶, only the 800-bp fragment will appear (lane 1) (not cleaved with HindII). 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 PVY⁵ (lane 4) two bands will appear: 400 and 800 bp. In the case of PVY⁶ (lane 3) only the 800-bp fragment will appear.
References

http://www.unece.org/trade/agr/meetings/ge.06/1999_09.r/pvyn.doc


