TECHNICAL SHEET No. 24

Virus Detection: PVY, PVX and PLRV

Method: MULTIPLEX – RT-PCR

General

Virus detected: PVY, PVX and PLRV in one reaction
General method: multiplex-RT-PCR

Developed By

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Goals

The m-RT-PCR assays developed is a reliable, rapid, and sensitive method for the detection of these three viruses in one reaction. The use of the m-RT-PCR assays are recommended for applications where improved sensitivity over standard RT-PCR is necessary for the early detection of infection and for quarantine and breeding programs.

Introduction

Potato *(Solanum tuberosum* L.) often becomes infected with two to three different viruses. The most common viruses affecting potato crops throughout the world are *Potato virus Y* (PVY, potyviruses), *Potato virus X* (PVX, potexvirus) and *Potato leaf roll virus* (PLRV, luteovirus). Egypt imports all potato seeds for planting from Europe, where theses viruses are present.

The current method routinely used for detection of these viruses is ELISA and there are no procedures to detect these three viruses in one step reaction. Multiplex-RT-PCR for PVY strains has been reported by Nei and Singh (2002).

Materials and Methods

Viral RNAs were extracted from potato samples infected with potato viruses (PLRV, PVX and PVY) using RNeasy Plant Mini kit (QIAGEN® cat # 74903) as follows:

1. Grind sample under liquid nitrogen to a fine powder using a mortar and pestle. Transfer to an appropriately sized tube.
2. Add 450 µl of either Buffer RLC to a maximum of 100 mg of tissue powder. Vortex vigorously. A 1-3 min incubation at 56 ºC may help to disrupt tissue.
3. Apply lysate to the QIAshredder spin column (lilac) sitting in a 2-ml collection tube, and centrifuge for 2 min at maximum speed. Transfer the flow-through fraction from QIAshredder to a new tube without disturbing the cell-debris pellet in the collection tube.
4. Add 0.5 volumes (usually 225 µ1) ethanol (96-100%) to the cleared lysate and mix well by pipeting.
5. Apply sample (usually 675 µ1), including any precipitate, which may have formed, onto an RNeasy mini spin column (pink) sitting in a 2-ml collection tube. Centrifuge for 15 sec at 10,000 rpm.

6. Pipet 700 µ1 Buffer RWI onto the RNeasy column, and centrifuge for 15 sec at 10,000 rpm to wash. Discard flow-through and collection tube.
7. Transfer RNeasy column into a new 2-ml collection tube. Pipet 500 µ1 Buffer RPE onto the RNeasy column, and centrifuge for 15 sec at 10,000 rpm. Discard flow-through and reuse the collection tube in step 8.
8. Add 500 µl Buffer RPE to the RNeasy column, and centrifuge for 2 min at maximum speed to dry the RNeasy membrane.
9. Transfer RNeasy column into a new 1.5-ml collection tube, and pipet 30-50 µl of RNase-free water directly onto the RNeasy membrane. Centrifuge for 1 min at 10,000 rpm to elute the RNA. Repeat if the expected RNA yield is >20 µg.

Design of Primers

In order to detect PVY, PVX and PLRV in one-step reaction, we along with M. K. Nakhla (University of Wisconsin-Madison), designed three specific primers for each virus to amplify the full-length of the coat protein gene. The viral sequences were retrieved from GenBank and compared.

Table (1) The oligonucleotide sense and complementary sense primer pairs used for m-RT-PCR and RT-PCR

a- The oligonucleotide sense and complementary sense primer pairs used for m-RT-PCR

Primer name	**Nucleotide sequence (5` to 3`)**	**Polarity**	**Expected amplicon size (bp)**
Potato virus Y PVYCPv*BamH1* PVYCPc*EcoR1*	5' TCAAGGATCCGCAAATGACACAATTGATGCAGG 3' 5' AGAGAGAATTCATCACATGTTCTTGACTCC 3'	sense comp.	801 bp
Potato virus X PVXCPv*EcoRI* PVXCPc*NcoI*	5`-GATAGAATTCAGATGACTACACCAGCCAACACC-`3 5`-TACGCGTCGGTTCCATGGACGTAGTTATGG TGG-`3	sense comp.	700 bp
Potato leaf roll virus PLRVCPvEcoR1 PLRVCPcNco1	5'AATAGAATTCTAATGAGTACGGTCGTGGTTARAGG 3 5'AAAA		

with final step for 10 min at 72°C. The reaction products were subjected to agarose gel electrophoresis.

Results

Fig. (1.): Detection of PVY, PVX and PLRV by RT-PCR and multiplex-RT-PCR from cDNAs primed by specific sense and complementary primers for each virus as shown in Table 1. Lane M, DNA leader molecular size markers; the sizes in bp are indicated on the left hand margin. Lane 1, amplified PVY by RT-PCR, lane 2, multiplex RT-PCR in which two pairs of viral specific primers for PVY and PVX were present in the reaction and two viral RNAs, and lane 3, multiplex RT-PCR in which three pairs of viral specific primers for PVY, PVX and PLRV were present in the reaction and three viral RNAs. The amplified products were: PVY (800 bp), PVX (700 bp) and PLRV (550 bp).

Discussion

Multiplex PCR is increasingly used because it improves the efficiency of diagnostic PCR (Johnson, 2000). In the near future multiplex PCR will probably be adapted for the simultaneous detection of viruses of one particular crop and for the simultaneous detection of other major plant pathogens such as viruses, viroids, bacteria, and fungi in the same reaction, as already demonstrated for viruses and viroids (Nie and Singh, 2001). An increase in sensitivity would probably be achieved if multiplex PCR in a single closed tube were developed based on this technology.

These techniques demonstrate the feasibility of multiplex RT-PCR based on specific primer design for the identification of several potato viruses i.e., PVY, PVX and PLRV, in a single step reaction. Such a method should increase both the sensitivity and specificity of the diagnosis and thus reduce the possibility of false negatives.

References

Nie, X., and Singh, R.P. 2001. A novel usage of random primers for multiplex RT-PCR detection of virus and viroid in aphids, leaves, and tubers. J. Virol. Methods 91:37-49.

Nie, X., and Singh, R.P. 2002. A new approach for the simultaneous differentiation of biological and geographical strains of *Potato virus Y* by uniplex and multiplex RT-PCR. J. Virol. Methods 104:41-54.