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Full Length Research Paper

Detection of potato leafroll virus isolated from potato fields in Tehran province in aphids by immunocapture reverse transcription polymerase chain reaction

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The surveys conducted during 2006 and 2007 revealed the infection of the virus in potato fields in Tehran province. Due to the important roll of aphids in transmission of the virus, immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) and reverse transcription polymerase chain reaction (RT-PCR) was developed using potato leafroll virus (PLRV) specific antibodies and specific primer pair (20 mer) located in the virus capsid gene to detect the virus in aphids. A 336-bp PCR product was detected from aphids (*Myzus persicae*) which had been fed on PLRV-infected plants. The PCR band was specific to PLRV as determined in PLRV-infected plants. This inquiry shows that this method is applicable to assess viroliferous nature of aphids in yellow -pan traps during season.

Key words: Luteovirus, immunocapture reverse transcription polymerase chain reaction, viroliferous aphids.

INTRODUCTION

Potato leafroll virus (PLRV), a species of the genus Polerovirus, family Luteoviridae, is an RNA virus that infects potato worldwide causing important damage (Robert and Lemaire, 1999). This virus is transmitted in persistent manner by a limited number of aphid species (Peters and Jones, 1981). *Myzus persiae* Sulz is the most efficient and important vector. Acquisition and transmission of PLRV by aphids is a prolonged process, requiring stylet penetratin to the phloem, followed by incubation period of a day or so and then another phloem penetration to transmit the virus (Bagnall, 1988). Because PLRV persists throughout the aphids life, it can be spread over longer distances by winged aphids and occurs in low titers in plant tissues and its vectors (Peters and Jones, 1981; Gildow, 1993). Using an electronic monitoring system, it has been reported that as little as 1.6 min of feeding time is enough to acquire PLRV from potato plant (Leonard and Holbrook, 1978). This short acquisition period and the persistence of PLRV during the entire lifetime of a viroliferous aphid make aphids one of the key steps in management of Luteoviruses, especially in vegetatively propagated crops like potato.

Today one of the most efficient strategies for the management of PLRV is the control of aphid vectors by systemic insecticides, but it is very difficult to control viroliferous aphids carried on wind currents moving into an area from other areas. The production of certified seed stocks in areas where migrant aphids arrive needs continuous monitoring of aphid flights and timely use of pesticides. The monitoring of aphids includes testing them for the presence of PLRV to determine if the aphids present a virus risk to the crop prior to the pesticides application. Knowledge of the percentage of viroliferous migrant aphids is essential from an epidemiological point of view. Detection of PLRV in aphids has been made using enzyme-linked immunosorbent assay (Clark et al., 1980), by dot-blot hybridization (Smith et al., 1993), reverse transcription polymerase chain reaction (RT-PCR) in groups of 5 - 10 aphids (Hadidi et al., 1993) and by RT-PCR in single aphids (Singh et al., 1995). The objective of this study is to show the possibility of detection of PLRV using immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) from aphids freshly collected from PLRV infected plants in order to use it as a forecasting system for PLRV.

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Figure 1. Upwardly rolled and erect leaves caused by PLRV in potato field.

MATERIALS AND METHODS

Virus isolate

Potato plants were collected from four main potato seed stock producing areas in Tehran province in 2006 - 2007. These plants which were naturally infected with PLRV in the field were selected on the basis of plant symptoms containing yellowing, upwardly rolled and erect leaves and plant stunting (Figure 1).

Screening of plant material for PLRV

The presence of the virus in collected samples was confirmed by monoclonal PLRV specific antibodies (IgG: AS-0741, Mab: AS-0060-11, RAM AP: AS-0060, DSMZ, Germany) in TAS-ELISA (Jordan, 1990).

Aphid vectors and virus propagation

The most efficient vector of PLRV, green peach aphid (*Myzus persicae* Sulzer.) was reared at 20 - 25°C under 16 h photoperiod on *Brassica napus*. Adult apterates were used in virus acquisition tests. In order to propagate the virus, it was transmitted by aphids on *Physalis floridana*. These plants served as the stock of PLRV for the greenhouse experiments. Aphids were placed in groups of 10 - 20 on PLRV- infected *P. floridana*. Due to the latent period of PLRV which is about 8 to 72 h (Eskandari et al., 1979), the aphids were collected at 72 h of exposure to PLRV–infected plants and used for total RNA extraction and IC-RT-PCR.

RNA extraction

Total RNA was extracted from the PLRV- infected aphids, virus-free aphids and PLRV- infected plants with the RNeasy Plant Mini Kit (Qiagen) according to the manufacture's protocol. The RNAs were further used in RT-PCR assay.

Imuunocapture RT-PCR

The test was done without total RNA extraction. First like ELISA method (Clark and Adams, 1977), PCR tubes were coated with PLRV specific IgG diluted in coating buffer and incubated for 4 h in 37°C. After incubation, time tubes were washed with PBST. For the preparation of aphid's extract, viroliferous and non-viroliferous aphids were placed in 1.5 ml ependorf tube, frozen in liquid nitrogen and ground mechanically. After initial grinding, 200 - 400 µl of extraction buffer was added and samples were further mixed 10 - 15 s. PLRV-infected plants were extracted (0.1 g of infected leaf tissues in 1 ml extraction buffer) and used in the assay as a positive control. The extractions were added to IgG- coated tubes and tubes were kept overnight at 4°C. After this period, tubes were washed with PBST, dried and used in RT-PCR reaction.

Primer design

Specific primers which were designed according to the coat protein coding region of the virus genome, amplifying a fragment of 336 bp were used in RT-PCR and IC-RT-PCR (Singh et al., 1995).

cDNA synthesis and RT-PCR

The assay mixture contained 7 µl distilled water, RT-PCR buffer 5x, 0.5 mM dNTPs, 0.5 mM DTT, 5 pmol reverse primer, 20 units RNAse inhibitor, 5 units M-Mulve and 5 µl total RNA. For IC-RT-PCR method, 12 µl distilled water, RT-PCR buffer 5x, 0.5 mM dNTPs, 0.5 mM DTT, 5 pmol reverse primer, 20 units RNAse inhibitor, 5 units M-Mulv were added to the tubes which were mentioned before. Samples were incubated in 72°C for 3 min and transferred to 42°C for an hour. Aliquots (5 µl) of the reverse transcription reaction transferred to tubes containing 20 µl of PCR Mixture. The final conditions for PCR assay were as follows: 14.6 µl distilled water, 10x PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 2 pmol reverse primer, 2.4 pmol forward primer and 0.6 units Taq polymerase (Fermentase, Germany). Samples were amplified in 35 cycles using thermo cycler (Palm cycler, Gpool, Research, Corbett). Each cycle consisted of denaturizing at 94°C, primer annealing at 54°C and primer extension at 72°C, each step of 1 min duration and final extension at 72°C for 10 min.

Analysis of PCR product

Seven microlitres of PCR product was combined with 3 µl of loading dye and analyzed in 1% agarose gel and finally the gel stained with 0.5 mg/ml ethidiom bromide and photographed with gel documentation (White/ Ultraviolet transiluminotor- UVP- UK). A DNA ladder (Gene Ruller, TM 1 kb DNA ladder) was used on gel to determine the length of the amplified product.

RESULTS AND DISCUSSION

Evaluation of PCR product

The PCR amplification of PLRV RNA both in RT-PCR and IC-RT-PCR yielded an expected product of 336 bp (Figures 2 and 3). This product was detected in viroliferous and non-viroliferous aphids. The specificity of the PCR products from viroliferous aphids was confirmed with the fragment of 336 bp amplified in RT-PCR and IC-RT-PCR from PLRV- infected plants. There was no PCR band from virus-free aphids in both methods (Figures 2 and 3). However, in RT-PCR of viroliferous aphids, the

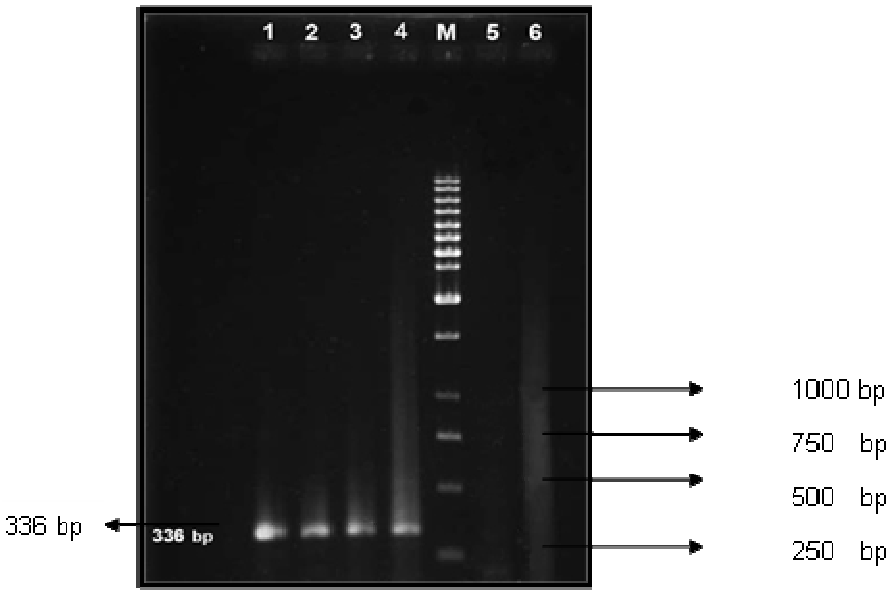

Figure 2. Agarose gel electrophoresis of IC-RT-PCR: Viroliferous aphids (Lanes 1, 2), PLRV-infected plants (Lanes 3, 4), non-viroliferous aphids (Lane 6). M: DNA ladder (Gene Ruler, 1 Kb).

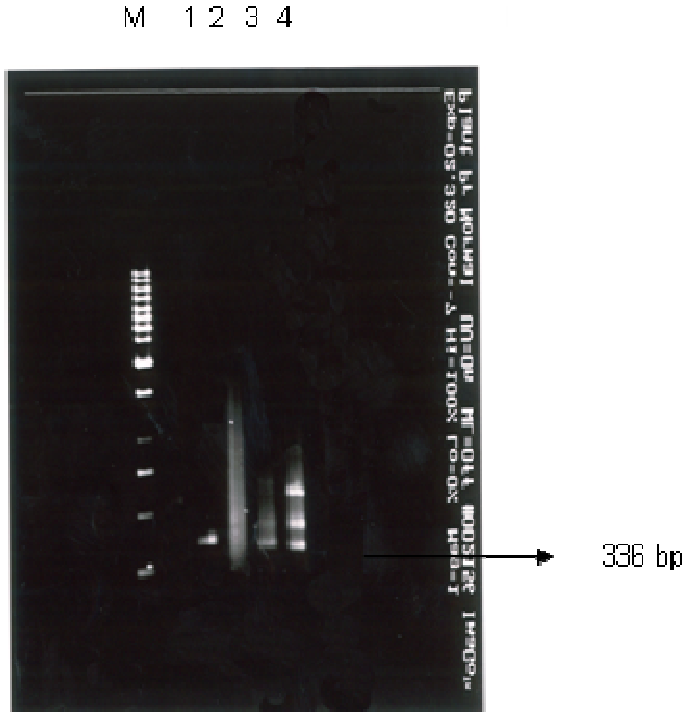

Figure 3. Agaros gel electrophoresis of RT- PCR: PLRV- infected plants (produced in IC-RT-PCR) (Lane 1), Virus-free aphids (Lane 2), PLRV- infected plants (Lane 3), Viroliferous aphids (Lane 4), M: DNA ladder (Gene Ruler, 1 Kb).

expected product of 336 bp was achieved with other unexpected bands (Figure 3, Lane 4).

This study demonstrated the feasibility of detecting PLRV from aphids exposed to PLRV -infected plants using IC-RT-PCR. As it can be seen, IC-RT-PCR gives more accurate result than RT-PCR. Therefore the IC-RT-PCR is applicable for detection of PLRV from aphids as RT-PCR has been shown to be effective for detecting PLRV in single aphids (Singh et al., 1995).

Potato is one of the most important crops in Iran and one of the greatest seed stock producing areas-Damavand is located in the Tehran province. Except for a few companies that produces virus-free seed potatoes experimentally; there is not a bona fide company that produces disease-free seed potato in Iran. To meet this demand of farmers, seed potatoes have been imported from other countries (usually European countries), multiplied in main potato seed stock producing areas in Iran like: Damavand, Ardebil, Hamedan among others and distributed to the producers. Consequently seed potato production is slow and time consuming and as such virus diseases tend to increase with each multiplication year resulting in decrease in yield and quality of propagation material. On the other hand, different species of aphids especially green peach aphids which colonizing potato crops are an important problem for potato production in Iran. According to these facts, this finding has potential value for epidemiological study of PLRV and other Luteoviruses like Barley yellow dwarf virus, where annual epidemics are often caused by migrant viroliferous aphids (Martin, 1995). Also aphids can be collected from yellow-pan traps or suction traps and stored in 70% ethanol for identification and preservation (Foottit and Richards, 1993), and it has been shown that this type of storage

even after a year, does not have an adverse effect on the PLRV detection(Singh et al., 1995). The detection of PLRV from aphids by RT-PCR is 10^3- 10^4 fold more sensitive than other methods (Hadidi et al., 1993). In this study IC-RT-PCR has been more sensitive than RT-PCR (Figures 2 and 3) and due to the fact that there is no need for total RNA extraction in this method, it is more economic. As a result, IC-RT-PCR procedure described now could be a useful tool for epidemiological studies of PLRV and might be helpful in clarifying virus-vector interaction.

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REFERENCES

Bagnal RH (1988). Epidemics of potato leafroll in North American and Europe Linked to drought and sun spot cycles. Can. J. Plant Pathol. 10: 193-202.

Clark MF, Adams AN (1977). Characteristics of microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.

Clark RG, Converse RH, Kojima M (1980). Enzyme-linked immunosorbant assay to detect potato leafroll virus in potato tubers and viroliferous aphids. Plant Dis. 64: 43-45.

Eskandar F, Sylvester ES, Richardaon J (1979). Evidence for lack of propagation of potato leafroll virus in its aphid vector, *Myzus persicae*. Phytopathol. 69: 45-47.

Foottit RG, Richards WR (1993).The genera of the aphids of Canada, Homoptera: Aphidiodea and phylloxeroidea. In: The Insects and Arachnids of Canada. Part 22, Research Branch, Agriculture Canada, publication 1885.

Gildow FE (1993). Evidence for receptor-mediated endocytosis regulating luteovirus acquisition by aphids. Phytopathology, 83: 270-277.

Hadidi A, Montasser MS, Levy L, Goth RW, Converse RH, Madkour MA, Skrzeckowski LJ (1993). Detection of potato leafroll and strawberry mild yellow-edge luteovirus by reverse transcription-polymerase chain reaction amplification. Plant Dis. 77, 595-601.

Jordan RL (1990). Strategy and techniques for the production of monoclonal antibodies; monoclonal application for viruses. In: Hampton R, Balls E, De Boer S (eds) The Serological methods for detection of viral and bacterial plant pathogens, pp. 55-76. APS PRESS.

Leonard SH, Holbrook FR (1978). Minimum acquisition and transmission times for potato leafroll virus by green peach aphid. Ann. Entomol. Soc. Am. 710: 493-495.

Martin RR (1995). Luteoviruses. In: Singh RP, Singh US., Kohmoto K (Eds) Pathogenesis and host specificity in plant diseases, Histopathological, Biochemical, Geneticand Molecular bases, Vol. 3. Viruses and viroids. Pergamon Press (Elsevier Science), Oxford, pp. 99-114.

Peters D, Jones RAC (1981). Potato leafroll virus. In: Hooker WJ (Ed), Compendium of potato diseases, The American Phytopathological Society, St. Paul, MN, pp. 68-70.

Robert Y, Lemaire O (1999). Epidemiology and control strategies. In: Smith HG and Baker H (eds) The Luteoviridae, pp. 211-279. Wallingford: CABI.

Singh RP, Kurz J, Boiteau G, Bernard G (1995). Detection of Potato leafroll virus in single aphids by the reverse transcription polymerase chain reaction and its potential epidemiological application. J. Virol. Meth. 55: 133-143.

Smith OP, Damasteegt VD, Keller CJ (1993). Detection of potato leafroll virus in leaf and aphids extracts by dot-blot hybridization. Plant Dis. 77: 109.