Study of biological and molecular characterization of pepper-PVY isolated from Tehran pepper fields and it's comparison with other PVY isolates

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Abstract: Potato virus *Y* a type species of the genus potyvirus infects several crops in the family solanaceae. PVY isolated from field infected peppers was identified on the basis of host reaction, serological and molecular characterization. The result of ELISA, Immunoblot electrophoresis and Immuno Capture Reverse Transcription Polymerase Chain Reaction (IC-RT-PCR) indicated that pepper isolate of PVY shares the reported properties of the PVY but in host range studies some differences were observed. For this reason the sequence analysis was performed .This is the first report of PVY incidence in Iran pepper fields.

Keywords: Potato virus *Y* ❺IC-RT-PCR ❺Immuno blot electrophoresis

Introduction

Potato Virus Y (PVY)is the type member of the genus potyvirus in the family potyviridae (9) and it has a single positive sense genomic RNA about 10 Kb long, inside flexuous virions.

PVY is the cause of major diseases in solanaceous crops including potato, tobacco, pepper, tomato and also infects many non solanaceous weeds. the symptoms induced by PVY and its host range are highly variable. These traits are used for the classification of PVY strains. PVY isolates have been historically classified according to symptomatology and aphid transmiss-ibility in to PVY^O ,PVY^N and PVY^C groups (3), but recently ,a host indep-endent classification ,based on RFLP pattern of the coat protein(CP) gene after IC-RT-PCR has been developed (7), allowing the regrouping of PVY isolates in three main clusters: potato PVY^N, potato PVY^O and non potato PVY^{NP}. In this paper, we present a biological and molecular chararacter-ization of typical pepper isolate of PVY especially in compa-rison with other PVY isolates.

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Material and methods

359 Leaf samples with veinal necrosis, veinal clearing, mosaic and dwarfing symptoms were collected from 12 pepper farms in Tehran province during 2005 and 2006. Pepper leaf samples were tested for the presence of PVY. The double antibody sandwich ELISA was performed as previously described (2)using polyclonal antibody (DSMZ, AS-0137/403). ELISA plates (Nunc Maxisorb), coated with 1:1000 dilution of related IgG in carbonate coating buffer (15 Mm Na2co3, 35 Mm NaHCO3, and 5 Mm NaN3, PH 9.6) and incubated overnight at 4c̊. Samples were extracted with extraction buffer (1:5 wt/vol, 3 mM KCL, 3Mm nan3, 8 Mm Na2HPO4, 1 Mm NaH2PO4, and 0.13 M NaCL (PBS) containing 2% polyvinilpyrolidone (PVP)-24000 and 0.05% Tween 20, Ph 7.4). Wells were washed four times, at 5-min intervals, with washing buffer (0.05% Tween 20 in PBS), and 100 µl of plant extract was added to each well and incubated overnight at 4c̊. Wells were washed four times with washing buffer; then 100 µl of alkaline phosphatase conjugated IgG diluted in conjugate buffer (2% PVP-24000, 0.05% Tween 20, 0.2% bovin serum albumin (BSA) and 1 Mm MgCL2 in PBS, Ph 7.40) was added and incubated 3h at 37c̊. Wells were washed four times with washing buffer and incubated in 100 µl of

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[DOI: 10.21859/isv.1.4.31]

Fig. 2: Mosaic on N.rustica

Fig. 1: Mosaic on *N.tabacum CV.White Burley*

Fig. 4: Vein clearing and mosaic on *Datura* metal

Fig. 3: vein clearing and yellowing on C.- annuum

substrate buffer (1mg/ml *p*-nitrophenyl phosphate and 3 Mm NaN3 in diethanolamin buffer, Ph 9.8), absorbance was determined at 405 nm by an ELISA-reader after 30 min. Samples with absorbance values greater than or equal to three times the average of negative samples were considered infected (positive).

Due to PVY-pepper host rang (A. Fanigliulo et al., 2005), the PVY-pepper isolate was mechanically inoculated on Nicotiana tabacum cv.White Burley, Nicotiana glutinosa, Chenopodium amaranticolor, Cheno-podium quinoa, Datura stramonium, Datura metel, Physalis floridana, Capsicum annuum and Solanum tuber-osum with phosphate buffer (0.1M, PH:7).

PVY isolated was propagated and mainted in N.tabacum cv.White Burley. After various passages in this host symptoms were observed and the purification was done using the method described by Leiser and Richter (1978) with some modifications. Homogen-ized 100 gr leaf tissue in 200 ml 0.5 M sodium citrate buffer, PH7.4, containing 5mM EDTA and 15mM sodium DIECA. squeezed the homogenate through cheesecloth and centrifiuged for 15 min at 6000 rev/min. added triton x-100 to a final concentration of 3% (v/v) and stired for 30 min at 4C.centrifuged for 2h at 30,000g. Resuspended the pellets in 10mM sodium citrate buffer,PH7.4, containing 1M urea and 0.1%(v/v) 2-mercaptoethanol. Centri-fuged for 15 min at 15,ooo rev/min.layer the supernatant fluided over a cushion of 20% (w/v) sucrose and centrifuged for 2h at 50,000g. resuspend the pellets in 5mM borate buffer PH8, in 30mM Nacl, 3mM sodium citrate.

Two primers (NIa/F, NIa/R) previously described (4) were used as a control to amplify PVY strain groups. Another primers pairs were selected to specifically amplify PVY^{N}, PVY^{O}, PVY^{C} and PVY^{NTN} strains (1).

The nucleotide sequences of the coding region of PCR product of PVY was determined and was analyzed by multiple alignment with Blast Software to other isolates that were available in the Gen Bank.

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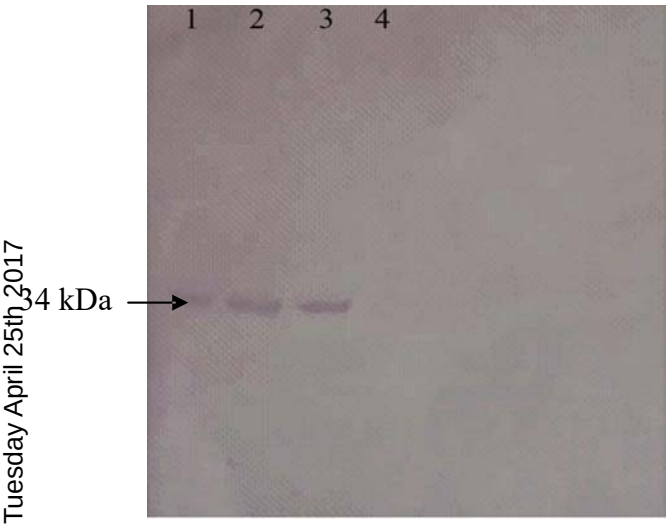

Fig. 6: western blotting with PVY antiserum(1/1000 dilution)
1, 2: Purified samples
3: Control +
4: Control -

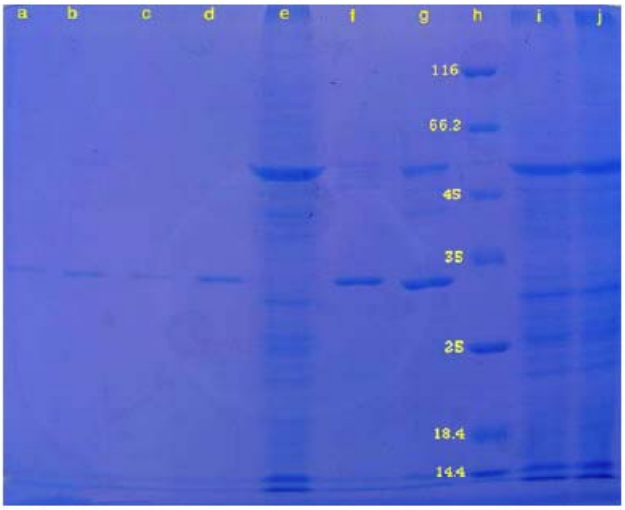

Fig. 5: a,b,c,d: complete purified samples
e: healthy samples
f,g: not complete purified samples
h:marker
i,j: plant samples

Results

The percent age of the collected samples from different fields which reacted positively in DAS-ELISA with PVY polyclonal antiserum was 59%. Host range studies showed that pepper isolate caused vein clearing and yellowing on C.annuum (Figure3), vein banding and mosaic on D.metel (Figure4), mosaic on N.tabacum cv.White Burley (Fig. 1) and N.rustica (Fig. 2) but didn't show any symptoms on P.floridana, C.amar-anticolor, C.quinoa and Solanum tuberosum.

After physical purification the A260/280 absorbance ratio was estimated. The A260/280 absorbance ratio of the isolate was 1.50 for purified virus preparation from *N.tabacum cv.White Burley*. SDS-PAGE of the coat protein extracted from purified virus prepara-tion gave bands at position of about 34 KDal (Fig. 5) and Western Blotting confirmed it as the PVY coat protein (Fig. 6).

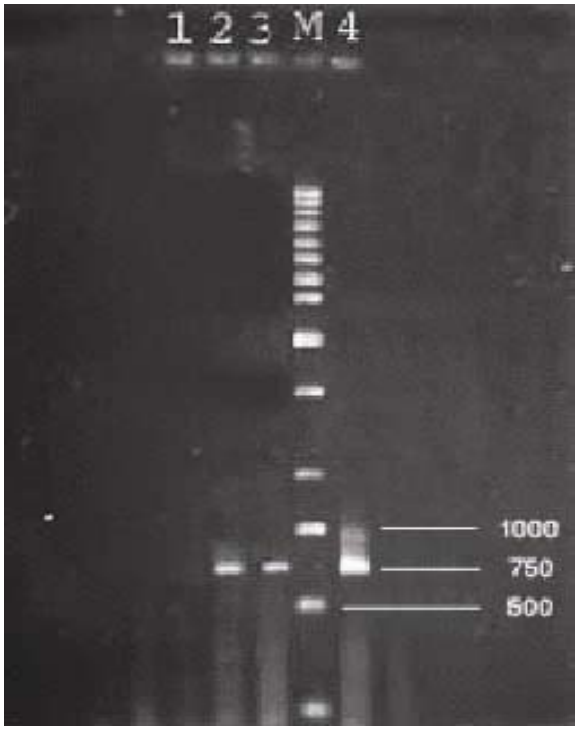

Fig. 8: IC-RT-PCR with PVYO primer 1: Healthy samples 2, 3, 4: infective samples, M: Marker

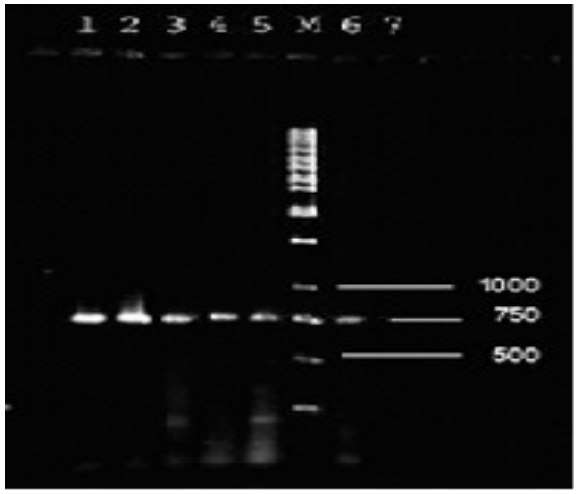

Fig. 7: IC-RT-PCR with PVY primer1-6: infective samples 7: healthy sample M: Marker

[DOI: 10.21859/isv.1.4.31]

The fragment of about 750 bp obtained after IC-RT-PCR by using specific primer pair of coat protein region of PVY(Fig. 7). Also for PVY strain detection primer pair o-8687F and o-9995R were used and the length of the amplified fragment was about 680 bp which determined this isolate as PVY^{O} but phylogenetic analysis of PVY isolates shows that pepper-PVY isolates from Tehran pepper fields has highest percentage of similarity with the non potato isolates :LYE84.2 (95%) and SON41 (90%).

Discussion

This is the first report of PVY incidence in Iran pepper fields. We present a biological, serological and molecular characterization of pepper isolate of PVY.

The symptomology results described above showed that typical pepper-PVY isolates were distinguished from potato isolates by host range (8). After phylogenetic analysis we noticed that pepper isolate has high similarity with LYE84.2 strain that was isolated from tomato (6) and with SON41 strain witch was isolated from *C. annuum* (5).

For this reason pepper infecting isolates could be classified within the PVY^{NP} group that expanding earlier suggestions made by Romero et al. (10).

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