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A BRIEF REVIEW FOR IDENTIFICATION AND DETECTION OF POTATO VIRUSES

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ABSTRACT

Potato is ranked fourth among the food crop and fifth for human consumption. It provides more yield and calories production as compare to cereals. Fungal, viral, viroid, bacteria, nematode, phytoplasmas and abiotic factors play a pivotal role for yield reduction of potato crop. Viruses known to infect potato in Pakistan include PVA, PVM, PVS, PVX, PVY, PLRV and PMTV. Increasing incidence of PVX and PVY in main potato growing areas of Pakistan is getting an alarming position and PLRV has caused significant yield losses. Present review article demonstrate different techniques for diagnostics of major potato viruses.

Key word: Potato, PMTV, PLRV, PVY, PVX, PVX, PVM, PVA.

INTRODUCTION

The cultivated potato (*Solanum tuberosum* L) is the world's leading staple food and vegetable crop and ranked fourth in production after rice, wheat and maize (Hopp *et al.*, 1988; Rauscher *et al.*, 2006). It provides 15 times more yield and calories production per unit area is higher than cereals (McGillivray, 1953). It has a potential to serve as a valuable food for the ever increasing world population as it contains higher dry matter, higher protein production and its adaptability to wide range of climates. It also provides protein, minerals, carbohydrates, vitamins (B & C) and high quality dietary fibers (Swaminathan, 1962). Potato crop was introduced in sub-continent in early 17th century by the British as a short duration crop. More than three dozen plant viruses, a viroid and phytoplasmas, fungal, bacterial and nematode can infect potato crop (Brunt *et al.*, 1989). A lower incidence (5-10%) of the viruses (singly or combined) infections in the current or previous crop hardly reduces the yields of crop. A higher virus incidence coupled with early or severe infection causes serious depressions in the tuber yield (Garg, 1987). Accurate estimates about the economic losses caused by viruses are lacking in potato crop. It was rough estimated that potato viruses may cause yield losses up to 50% in tuber yield. In Pakistan, climatic and soil conditions are highly conducive for autumn, spring and summer potato crops (Zanoni, 1991). Bacteria, nematode (Gondal *et al.*, 2012; Parveen *et al.*, 2013), fungus (Ashraf *et al.*, 2012; Mehboob *et al.*, 2013) and virus (Abbas and Hameed, 2012; Gul *et al.*, 2013) along with a biotic factors are the main problems in the country. Among viral diseases, PVX, PVY, PLRV, PVA, PVM, PVS and PMTV have been observed and caused up to 83% yield losses in potato crop (Mughal and Khalid, 1985). PVX, PVS, PVA, PVM and PVY are more common viruses while PLRV is prevalent in northern areas where insect vector pressure is high.

Enzyme linked immunosorbent assay (ELISA) has been extensively used in plant diagnostic laboratories of due to its automation, easy in use, sensitivity and a large number of samples can be tested (Abbas *et al.*, 2012). Significant nucleic acid research has revolutionized molecular diagnostics of plant viruses and molecular hybridization and PCR have been of particular significance in plant virology (Miller and Martin, 1988). Combination of serology and PCR (Immunocapture-PCR) became more sensitive and economical than conventional PCR because viral RNA purification was eliminated in this technique. Direct binding PCR (DB-PCR) and print-capture PCR (PC-PCR) are also important in detection of potato viruses (Rowhani *et al.*, 1995). Complementary DNA (cDNA) libraries of coat protein gene have been prepared by using the genomic RNAs of PLRV, PVX and PVY by coat protein (CP) gene specific sense and antisense primes. PVX has cloned and sequenced CP gene (613 bp) form Pakistani isolate (HE577130) and nucleotide evidence showed its maximum genetic similarity with USSR isolates of PVX (Jamal *et al.*, 2012) while this was first report of PVX form Pakistan. Abbas *et al.*, (2014) has reported recombinant strain of PVY on the basic of biological, serological and nucleotide evidence of CP gene from two Pakistani isolates (JQ425622 and JQ518266).

Identification and detection of viruses in potato: Bacteria, fungal and nematode disease can be effectively controlled by chemical methods but no chemicals exist that could be used as a direct field control of viral disease in potato crop. Sensitive and reliable confirmation of virus in seed stock and eradication of viral infected seed tubers is more effective and reliable method to prevent the viral infection in natural condition (Hull, 1993). The nucleic acid of plants virus is surrounded by protective protein coat and detection methods were evolved from these two components. Previously, plant viruses were detected on the bases of protein component through serological methods (ELISA) because these methods were found effective at early stage of detection. The protective protein coat contains only 2-6% of the genetic information of viral genome and these methods were not

effective for complete genome information (Hull, 1993). Viroids are not enveloped in protective protein coat and serological techniques cannot be applied for viroids confirmation. Viroids can be detected from bioassays and reverse gel electrophoresis technique but these methods are not suitable for large number of samples. Nucleic acid base research and application of recombinant DNA technology allow researcher to diagnose plant virus and viroids more effectively and molecular hybridization along with polymerase chain reaction (PCR) assay also using in scientific laboratories of plant virology.

Susceptible indicator host plants: Symptom expression is not a reliable criteria for confirmation of virus and its interpretation should be treated with caution because symptom formation is due to many factors such as growth stage, nutrition deficiency, insect sucking, environmental conditions, host genotype, time of infection, virus strain, etc but it is an initial step to disease diagnosis (Batool *et al.*, 2011). The symptoms development on a range of susceptible indicator host plants is a considerable diagnostic value but it is insufficient for identification of viruses. Host range study and its symptom development have significant value for studying new unknown virus or its strains because these host plants produced characteristic symptoms in the response of particular virus. A large number of herbaceous plants are reported as a susceptible indicator host plants to several viruses and produced systemic infection or local lesions when on mechanical transmission. *Chenopodium amaranticolor, C. annuum, Physalis floridana, C. album, Nicotiana glutinosa, N. rustica, N. tabacum cv. Samsun, N. benthamiana, Datura stramonium, D. metel, D. stramonium, Solanum demissum x,* S. *tuberosum Gomphrena globosa* and *Lycopersicon esculentum* were used as a test plants for potato viruses (Abbas *et al.*, 2012). Indicator plants rose in glass houses for experimental purposes but it is time consuming to test a large number of sample. The main drawback of this diagnostic method is that these host plants exhibit different symptoms in the response of strains of same viruses.

Serology: Antibodies produced by a hybrid cell (hybridoma) formed by fusion of a B lymphocyte cell with a mouse myeloma cell are known as monoclonal antibodies (Mabs) and they were produced first time against Tobacco mosaic virus (TMV). Mabs have been successfully prepared against more than 50 different plant viruses, including PLRV, PVA, PVM, PVS, PVX and PVY. Old immunological test procedures included chloroplast agglutination, microprecipitation tests and gel immunodiffusion (Hampton *et al.*, 1990). In order to increase the sensitivity of serological tests, the use of solid phase to adsorb an antibody or antigen with subsequent attachment of an antigen or antibody and the use of an antibody-enzymes conjugate to detect the antigen (ELISA) has become an important procedure in plant virus detection. In this chapter only methods which are commonly used for potato virus detection will be reviewed.

(Clark and Adams, 1977) developed the Enzyme Linked Immunosorbent Assay (ELISA) technique for successful detection on plant viruses and this technique become more popular within short period of time due to its simplicity, adaptability, rapidity, sensitivity and accuracy. The double antibody sandwich (DAS-ELISA) test on a solid phase (usually plastic) is most commonly used. Specific antibody was used for trapping the plant viruses and a specific enzyme-labeled antibody (conjugate) is added. Enzyme substrate was added at the end and the reaction of virus is measured visually (development of color) or spectrophotometrically. A variation of the above method is the indirect ELISA, in which plates are coated with antigen, and the primary antiviral antibody of one animal species (e.g. rabbit) is added. Commercial kits for PVA, PVM, PVS, PVX, PVY, PLRV and others viruses are available and provide valuable results when potato leaves are tested (Abbas *et al.*, 2012). Positive, healthy and buffer or blank controls should be added in ELISA for more reliable results. In the certification laboratories of Swiss, ELISA is used for testing more than 20.000 seed potato tubers per day against PVY and PLRV (Salazar *et al.*, 1992). Thousands of potato plant samples are tested for potato virus (PVA, PVM, PVS, PVX, PVY, PLRV) with ELISA in Pakistan at Crop Disease Research Institute (CDRI) Islamabad under the National Agricultural Research Center. The minimum level of virus detection by ELISA is about 2ng/ml and these techniques are unable to detect the virus at initial stage of infection (low titer). This is one of the main reasons that serologically negative samples produced symptoms at the lateral stage of infection (Abbas *et al.*, 2013). Low titer (theoretically one viron) will multiply (billions of copies) within short period (few days) and healthy potato plants can be infected in the open field by mechanical inoculation along with insect vector through viral infected potato plants (Betancourt *et al.*, 2008).

In Dot-ELISA, antibodies or antigens are bound to nitrocellulose or nylon membranes have been used to detect PVX, PVS, and PVY and PLRV (Smith and Banttari, 1987) but this method is readily adaptable to field samples. Suspected potato sample's crude saps were spotted onto a membrane and allow it to dry at room temperature. Bovine serum albumin (BSA) is used for saturation the surface of membrane and primary specific viral antibody were introduced on the surface. Secondary antibody-enzyme conjugate and substrate were added and the reaction was observed from an insoluble colored product at the site of reaction. Tissue blotting is also a serological technique which has more resemblance with ELISA (Lin *et al.*, 1990) and this method is more reliable for the confirmation of PVX and PVY from tubers in the field (Bravo-Almonacid *et al.*, 1992). Blots are made by pressing the freshly cut tissue surface gently but firmly on a nitrocellulose membrane and antigens in tissue blots are detected by enzyme-labeled probes. This technique has much more importance because it provides specific, rapid and simple tool for large-scale diagnosis of plant viruses.

Immunosorbent electron microscopy (ISEM): Electron microscopy (EM) is used for the detection of size and shape of a particular virus and these basic steps play a pivotal role towards virus identification. EM also provides information on virus morphology to be obtained within minutes after sampling a diseased plant and it is used to examine viruses in crude extracts from infected plants. Immunosorbent Electron Microscopy (ISEM) is a highly sensitive technique which was developed by combining the electron microscopy and serology and it was introduced by (Debrick, 1973) for the detection of plant viruses. ISEM is more sensitive than ELISA for some viruses (Garg and Khurana, 1991) and thousand times more sensitive than conventional electron microscopy (Garg, 1987). Among potato viruses, PLRV is phloem-restricted with low titer and create hindrance in detection with conventional electron microscopic and ELISA.

Polymerase chain reaction technology: A detection procedure of virus in infected samples must be sensitive, specific, rapid, easy to use, reliable and cost-effective and PCR offers further sensitivity and specificity to detect PVY and its strains. PCR has already revolutionized research in molecular biology and it increasingly applied in most molecular biology laboratories. New applications of PCR are being published at an increasing rate and it is evident that it will be used in may field of basic and applied research. RT-PCR methods are popularized and the main advantage of these methods is that viruses can be detected from total RNA using sap from dormant potato tubers (Singh *et al.*, 2008). Billions of nucleic acid copy was achieved through multiple cycles of three steps at different temperature which includes denature the DNA, anneal two oligonucleotide primers to the denatured (opened) DNA strands and primer extension by thermostable DNA polymerase to synthesize the target sequence whose ends are defined by the primers. The PCR products can be visualized thorough gel electrophoresis analysis. PCR required DNA fragment for amplification but important plant virus exhibiting RNA genome. RNA was converted to complementary DNA (cDNA) through reverse transcription (RT) and RT-PCR technology is 1000 times more sensitive than ELISA in term of detection sensitivity for potato viruses (Jeon *et al.*, 1996). The success of PCR depends upon specific sense and antisense primes for the amplification of viral genome. The nucleotide sequence of viral genome is available in GenBank (NCBI). Oligonucleotide primers must be 18-25 nucleotide residues in length, with no annealing 3' end, 50 % G+C content, no secondary structures and high G+C content at the 3' ends. Primer can also be designed for conserved or variable regions. Specificity of PCR will be affect by annealing temperature and successful amplification depends upon G+C content and primer length. Primers having 20 nucleotides require increase of 2°C for every addition of A or T and 4°C for G or C. RT-PCR was applied in detecting PVY, PLRV, PVX, PVA, PVS and PVM (Nie *et al.*, 2008; Abbas *et al.*, 2012; Nosheen *et al.*, 2013). RT-PCR has been applied for cloning, molecular detection and sequence analysis of CP gene of PVY and PVX (Jamal *et al.*, 2012).

Very expensive costly molecular biology grade consumable and costly equipment (thermocycler) are necessary of PCR and these techniques are prone to render false positives due to its extremely sensitivity coupled with the ease of contamination by contaminated reagents, gloves, hair, skin, aerosols, commercial preparations of *TaqDNA polymerase*, or even autoclaved material containing target sequences (Dwyer and Saksena, 1992). Only a limited number of samples can be tested in one run and it is the main reason that this technique is limited to scientific labs rather than plant diagnostic labs. Rapid response and high sensitivity enable PCR a more reliable method for testing the limited number of 'mother' seed stocks/plants. RT-PCR was optimized for Alfalfa mosaic virus (AMV), Impatiens necrotic spot virus (INSV), Tobacco rattle virus (TRV), Tomato spotted wilt virus (TSWV), PLRV, PMTV, PVA, PVM, PVS, PVX, PVY and Potato spindle tuber viroid (PSTVd). The ordinary (PVY^{O}), Common (PVY^{C}), necrotic (PVY^{N}) and recombinant (PVY^{NTN}, $PVY^{N:O}$ and $PVY^{N\text{-}wi}$) strains of PVY could be differentiated using multiplex primers (Crosslin and Hamlin, 2011).

Molecular hybridization: This technique is used for confirmation of viroids (Owens and Diener, 1981) and viruses (Salazar *et al.*, 1992). These methods are based on interaction between purines (A=T) and pyrimidine (G≡C) and results in the formation of a stable hybrid between the target sequences and those of the probe. These hybrids depend on number of hydrogen bonds, electrostatic and hydrophobic forces. Electrostatic forces rely on the phosphate molecules of the nucleic acid backbone whereas hydrophobic interactions are maintained between the staggered bases. The dot-blot hybridization is most common technique which involves the target nucleic acid and appropriate specific probes (Pallás *et al.*, 1998). Nonradioactive riboprobes were used for successful confirmation for PVS, PVX (Eweida *et al.*, 1990), PVY and PLRV (Hopp *et al.*, 1988). No universal protocols are available for molecular hybridization analysis and the choice of protocol depends on virus, host and probe (Pallás *et al.*, 1998).

Immunocapture-PCR: Potato viruses consist of two components (protective protein coat and nucleic acid) and molecular and serological tools differ in the target viral component, sensitivity, specificity and facility of automation. The serological and molecular tools were combined in a single technique termed as Immunocapture-PCR (IC-PCR) in which virus particle was captured (protective protein coat) and then was amplified (nucleic acid) with PCR (Nolasco *et al.*, 1993). This method removed the virus purification step which is necessary to eliminate the interfering plant cell components that affect the PCR-based methods and it is 250 times more sensitive than normal PCR. This technique was successfully applied to PLRV detection (Leone *et al.*, 1997).

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