Review Paper:

Plant Virus Identification and Management

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Abstract

Plant diseases are a reason for relentless losses to mankind in numerous ways, which can be at a greater level or at a lesser amount in terms of severity. In fact many of the plant diseases are responsible for less vivid losses annually across the globe, though collectively they constitute considerable losses to farmers. Such infectious diseases affect the aesthetic values of plants either grown in fields, as landscape trees, in the home gardens etc. Among all the reported plant diseases, diseases caused by viruses are quite important to consider as there is still no precise cure to control them from spreading international boundaries. Occurrence of vector transmitted virus diseases and the harm they caused to crops (vegetable and fruits) are accounted to be growing in countries having a favourable tropical and subtropical environment.

In order to counter such problem/disease, an integrated approach to crop management should to be on priority basis ranging from growing virus-resistant crops to device strategies for appropriate control of plant-virus insect-vectors. However, in the developing nations, employing such management practices is more challenging and is rarely applied effectively. All the available management practices can be classified into two broad principles of action i.e. prevention (prophylaxis in some early writings) and therapy (treatment or cure).

Keywords: Plant viruses, Techniques, Management.

Introduction

Viruses are comprised of a miniature piece of nucleic acids (dsDNA, ssDNA, dsRNA or ssRNA) carrying genetic information enclosed in a quaternary structure i.e. coat-protein. Viruses are recognized to infect all kinds of living organism such as bacteria, animals, fungi, and plants. Nature of all viruses is obligate parasite, which means they are reliant on host cellular machinery to replicate.

Plant viruses regardless of their small size, are responsible for devastating diseases in crops, ornamentals and weeds plants. Symptoms caused by plant viruses in plants are leaf curling, mosaic patterns, crinkled leaves, stunted growth, yellowing of leaf veins, leaf distortion, leaf streaking, vein clearing and many more. Such symptoms caused by plant viruses range from mild to severe resulting in reduced plant growth, lower yield, inferior quality and economic loss over large area.

Accessibility of the diagnostic method is endowed with a superior elasticity, increased sensitivity, and specificity for quick judgment of virus diseases. Advancement of onsite recognition assays for diagnosis of coupled viruses at the aim of decision making is required to be taken up. For the duration of the previous eighty years, satisfactory accuracy and precision of visual disease estimation have often been accomplished by means of traditional disease measurement.[1,3,14,22]

Further it is predominantly imperative for viruses where disease liberated crops are the only efficient way to imprison the disease. With the beginning of molecular and serological based techniques, the diagnostic measures have made easier than the traditional methods. As per the inconsistency in pathogens genotypes, it is difficult to control. To battle the losses occurred, it is crucial to have quick and precise technique for recognition of the underlying pathogen, harshness of disease and mechanism of virulence. Progress in molecular biology and biotechnology in the past couple of decades enforced to build up speedy, definite and responsive techniques for the detection of plant pathogen.

A single diagnostic test or examination might render ample information on the individuality of a meticulous disease causing organism whereas an amalgamation of techniques is usually necessary for unambiguous virus judgment. The large adequacies of more user-friendly molecular based techniques are more responsive, precise, detailed, and much faster than conventional and serological based techniques.[2,4,17]

Diagnosis and Identification of Plant Viruses

Conventional and serological based techniques for identification of plant viruses includes:

(1) Electron microscopy [For study of external appearance / structural morphology or for detection of plant viruses electron microscopy is the best choice],

(2) Enzyme Linked Immuno Sorbent Assay (ELISA) [The enzyme linked immune-sorbent assay (ELISA) has been widely used among the various antibody based detection techniques. This is due to its ease to use, reliability and elevated sensitivity],

(3) Polymerase Chain Reaction (PCR) and Real Time Polymerase Chain Reaction (RT-PCR) [PCR (Polymerase Chain Reaction) and RT – PCR are the most popular thermostable amplification based techniques used for

detection and diagnosis of DNA and RNA plant viruses respectively],
(4) Loop Mediated Isothermal Amplification (LAMP) [The LAMP technique has been shown to be good approach for amplifying nucleic acid with high specificity, efficiency, and rapidity without the need for thermal cycler],
(5) Immuno Capture PCR (IC-PCR) and Immuno Precipitation PCR (IP-PCR) [Immunocapture is used to concentrate pathogenic cells prior to PCR, thus increasing detection sensitivity. It is a technique with increased detection sensitivity through multiple displacement amplification (MDA)],
(6) Nested PCR [The process is quite valuable when the virus concentration is very small, target gene is unstable and cannot be ensured by electrophoresis due to little amplification product],
(7) Multiplex PCR [In one time experiment, two more types of DNA or RNA viruses can be easily identified at the same time using gene specific primers. A number of plant viruses have been diagnosed using multiplex PCR in conjunction with ELISA],
(8) Co-operational PCR (Co-PCR) [A mount of four primers are needed to run nested-PCR and co-operational PCR. Nested-PCR makes use of two internal and two external primers to function, whereas co-operational PCR requires only one external primerand three internal primers to work functionally].[13,19,23,25]

Latest methods of virus detection includes:
(1) Nucleic Acids Sequence Based Amplification (NASBA) [With the help of RNase H, T7 RNA polymerase andreverse transcriptase, NASBA is employed for the direct amplification of RNA by PCR],
(2) Next Generation Sequencing (NGS) [This technique assists in the sequencing of either full or partial genomic sequences of viruses. Novel uncharacterized ssDNA, dsDNA, reverse transcribing, RNA viruses and viroids can characterize even the lack of previous knowledge or former sequence information],
(3) DNA Microarrays [Various viruses has been identified using this technique like *Potato leafroll virus* (PLRV), *Potato virus S* (PVS), *Potato virus A* (PVA), *Potato virus* Y (PVY), *Potato virus M* (PVM) and *Potato virus*X (PVX)],
(4) Lateral Flow Microarrays [Lateral flow microarrays (LFM) permit for speedy hybridization based nucleic acid recognition by means of a simple depicted colorimetric indication],
(5) Volatile Compounds for virus detection [Several volatile organic compounds are linked with host response to a disease. Volatile profiling is the measurement of volatile organic compounds and *Cucumber mosaic virus* (CMV) has also been investigated using VOC profiling],
(6) Tissue BlotImmunoassay (TBIA) [Mechanism of action and reliability of Tissue blot immunoassay in identifying plant viruses is parallel to that of ELISA to which antibody is applied],
(7) Quartz Crystal Microbalance Immunosensors (QCMI) [QCM has established to be victorious in recognition of plant viruses such as *Tobacco mosaic virus* (TMV), *Turnip yellow mosaic vir*us (TYMV) and *Cymbidium mosaic virus* (CyMV). The principal of QCM is the alternation in frequency and vibration due to mass changes in real time and QCM is applicable in vacuum, gas, and liquid condition],
(8) Affinity Biosensor [The largely accepted DNA probe is the single stranded DNA (ssDNA) bound on the electrodes having electro active indicators to determine hybridization between probe DNA and the complementary DNA analyte],
(9) Remote Sensing of Virus Diseases [Plant stress can be recorded by means of remote sensing through analyzing the variation in radiation emission due to virus infection. During preliminary stage, when diseased warning signs on the leaf surface are not observable, plants do response with respect to viruses such as the reduction of the photosynthesis rate which induces an increase of fluorescence and heat emission],
(10) Quantum dots (QD) [With the use of fluorescence resonance energy transfer (FRET) mechanism, in which energy is transferred among two light-reactive molecules, Quantum dots have been used for disease[10,16,21,24] detection].

Management Practices to tackle Plant Viruses

For management of plant viruses, many practices have been adopted by the farmers either to control the viral transmission agents (vectors) or to destroy the secondary sources of viruses other than natural (hosts like weeds). All the available management practices can be classified into two broad principles of action i.e. prevention (prophylaxis in some early writings) and therapy (treatment or cure). Earlier practices of prevention management include:
(1) Use of Insecticides [The methods to apply chemical insecticides are familiar to the farmers. In order to prevent economic losses, chemical insecticides are used as an easy approach by farmers. To control viruses seed lots can be treated with trisodium phosphate and dry heat (54–58 ^{O}C for one to two days) to reduce the virus loads of *Pepper mild mottle virus* (PMMoV) and *Maize dwarf mosaic virus* respectively],
(2) Soil Fumigation [Methyl bromide is primarily used for soil fumigation. Soil fumigants, when applied to soil produce gases controlling pests],
(3) Seed Selection [It is the best management for plant viral diseases as healthy seeds will produce disease (virus) free seeds. Use of certified vegetative seeds greatly helps to reduce the incidence of most viral diseases],
(4) Resistant Varieties [Virus resistant varieties are the best step towards production and management of disease as they are the cheapest and effective approaches to reduce the economic yield losses caused due to plant viruses]. With the augment of plant tissue culture, three famous methods have been employed to tackle viruses as a therapy (treatment or cure), these include (a) Heat therapy and Meristem tip culture [Heat treatment and Meristem tip culture are significant in various ways like small explants will be free of pathogens present in donor plants, lower risks of genetic instability, higher survival growth rate and cases are there in which crop/ varieties were saved from viruses], (b) In vitro

shoot grafting and callus culture [It deals with grafting of apical meristem on young root stock seedling in woody plants for elimination of viral infection] and (c) Protoplast fusion and somaclonal variations [Protoplast fusion is an important technique for the generation of hybrid plants among species which are incompatible. Somaclonal variations have been observed in many crops and used for developing virus resistant crops].[5,8,9,18,20]

Latest method for prevention of diseases included biotechnological approach, this includes genetic engineering which has revolutionarised the management practices against plant viruses, major ones are the genome editing tools like
(1) RNA Interference [RNA mediated silencing technology has now become the tool of choice for incorporating virus resistance in plants. A significant feature of this technology is the presence of dsRNA, which is not only the product of RNA silencing but also the potent trigger of RNAi. Upon RNAi induction, these dsRNAs are diced into short RNA fragments termed as siRNAs which are hallmarks of RNAi. Considerable virus resistance in transgenic plants can be created by exploiting the RNAi phenomenon of silencing specific genes by RNAi which is a desirable natural solution to this problem as disease resistant transgenic plants can be produced within a regulatory framework] and
(2) CRISPR/Cas System [Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR and associated protein-9 (Cas9) system are precisely suitable for gene knockout studies and now it has arose as the most dynamic genome editing technique. This wonderful editing technique was successfully employed in many crop species and proved virtuous results in confirming resistance against harmful plant viruses].[6,7,11,15]

Conclusion

Plant viruses account for foremost losses to a number of horticultural and agricultural plants across worldwide. Contrasting with rest of the plant infecting pathogens, there is not any straight away technique existing up till now to manage viruses.

Therefore, techniques for recognition and detection of viruses, equally in crops and carrier vectors, participate for a decisive role in virus disease management. Progress in molecular biology and biotechnology in the past couple of decades helped to enforce to build up speedy, definite and responsive techniques for the detection of plant pathogens.

In contrast to the rest of plant pathogens, there is no straight away technique existing till now to manage the viruses[12]. Therefore, techniques for recognition and detection of viruses, equally in crops and carrier vectors, have a decisive role in virus disease management. However, all these discussed methods necessitate research workforce with sufficient talent and knowledge to reform and carry out the analytical examination of viral diseases in very diverse environments.

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