

A Review Paper on Plant Virus Detection Methods

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ABSTRACT- Because fast changing climate facilitates the movement of viruses as well as their hosts, also vectors, from one nation or region to another, early and correct identification of plant viruses is critical to their management. The diagnosis of viral diseases is becoming increasingly crucial. So because indications of viral infections are not always distinct and might be mistaken with those of abiotic stressors, a suggestive diagnosis may not be the best approach. Agriculture is vital to every country's economy, and bacteria, fungus, and viruses represent a major danger to crops. Viruses, unlike any other ailment, are to blame for a significant share of the reduction in agricultural production. A variety of diagnostic procedures can be used to identify these viruses. There are direct and indirect solutions to this problem, each with its own set of benefits and drawbacks. The infection of the causative pathogen can be used to create diagnostic procedures. Observation of signals in indicator crops, electron microscopy, or serological techniques including such enzyme-linked Immunosorbent Assay have all been sometimes used identify virus in plants. They can be used to identify the best timing to start utilizing ELISA or PCR assays to detect plant viruses. Plant viruses can be recognized and treated if found early enough, result in a virus-free crop in the future. These techniques (ELISA or PCR) will become the future standards in diagnostics as the cost of these techniques (ELISA or PCR) decreases.

KEYWORDS- DNA, Detection, Disease, ELISA, Pathogen, RNA, Virus.

I. INTRODUCTION

Viruses are infectious germs that are too small to see with a light microscope, but they may still cause havoc. A tiny amount of nucleic acid is enveloped by a protein sheath in the simplest viruses. Viruses, like other creatures, include nucleic acid that contains genetic information that generally

specifies three or more proteins [1-5]. Viruses are all obligatory parasites that rely on their hosts' cellular machinery to replicate. Some have argued that viruses do not live outside of their hosts and they are not productive outside of the hosts. Deoxyribonucleic acid or ribonucleic acid are the two types of nucleic acids found in plant viruses (DNA and RNA). Light microscopy is unable to identify plant viruses because they are so little Depending on the genetic information carried by nucleic acids. Since the discovery of the tobacco mosaic virus, over a decade ago, more than 1100 plants viruses have been originate. Antiviral drugs haven't been developed for viruses like they've been for other plant infections, thus present treatments rely on indirect tactics to combat the In every plant management approach, the first step is to accurately identify the disease [6].

Viral infections may not always be effectively controlled by plant treatment following infection. It is therefore more effective to adopt management measures before an infection occurs in order to prevents the blowout of the disease It is one of the most effective techniques that farmers may use to reproduce healthy plant material In order for certification programs to be effective, they must include sensitive diagnostic procedures. Scientists have depended on advances in molecular biology or biotechnology over the last three decades to discover plant illnesses. Several methods for identifying or diagnosing plants infections have been discovered [7-10]. To determine the identity of a disease-causing organism, a single diagnostic test or assay may be sufficient. However, a combination of methods is typically necessary for an unequivocal diagnosis. Table 1 shows a list of the top seven plant viruses [11-13]. Viruses that have become well-known as a result of their scientific relevance include. Despite the fact that the most of these viruses continue to cause significant monetary losses in a variety of ways, their usage as sophisticated tools has lifted viruses to a high degree of scientific importance [14].

Table 1: The top seven plant viruses are depicted. The top-ranked plant viruses were identified by plant virologists connected with Genetic Plant Pathology, as shown in the table

| Name of plant Viruses | Writer of viruses description |
|---|-------------------------------|
| Tobacco mosaic viruses (TMV) | Karen-Beth G. Scholthof |
| Cucumber mosaic viruses (CMV) | Peter Palukaitis |
| Brome mosaic viruses | Paul Ahlukaitis |
| Tomato spotted wilt virus (TSWV) | Scott adkins |
| Plum pox virus (PPV) | Thierry Candresse |
| Tomato yellow leaf curl viruses (TYLCV) | Henryk Czosnek |
| Potato viruses X (PVX) | Cynthia Hemenway |

A. Tobacco Mosaic Virus (TMV)

Tobacco mosaic viruses are the most important plant viruses, according to a survey conducted by the plant virology industry. Mosaic plant diseases (including tobacco and tomato) are caused by single-stranded ribonucleic acid (RNA) viruses, notably those belonging to the nightshade family. Other crops, particularly tomatoes, are affected as well. Infected plants mechanically transmit viruses to normal plant leaves that have been scraped or damaged.

B. Tomato spotted wilt viruses (TSWV)

Tomato spotted wilt viruses are a clever disease that affects a wide range of crops produced in both subtropical and temperate regions. TSWV is a one-of-a-kind virus with its own family of viruses. Tomato spotted wilt viruses have one of the broadest ranges of hosts of any plant virus. Each of the tomato spotted wilt virus's hosts has its unique set of symptoms. Cultivars will also have different symptom manifestation. TSWV symptoms include charcoal streaks on sepals and stems, necrotic leaf patches, as well as tip dieback and other ring spots (brown rings or yellow rings) as show in (Figure 1) [15].



Figure 1: TSWV is unique among tomato diseases in that it is caused by viruses, not a fungus as well as bacteria [16]

C. Yellow Leaf Curl Viruses in Tomato

Tomato Yellow Leaf Curl Virus is a Deoxyribonucleic Acid (DNA) virus that belongs to the Geminiviridae family and the genus Begomoviral. The Yellow Leaf Curl Virus in Tomatoes is the most devastating tomato disease, causing significant economic loss in both subtropical and tropical regions [17]. The whitefly Bemisia tabaci, also known as the silverleaf whitefly or sweet potato whitefly, belongs to the Aleyrodidae family of the order Hemiptera, and the viruses are transferred by it. TYLCV is most commonly found in tomato plants, although it has also been found in eggplants, cucumbers, and other plants. Ingredients such as potatoes, beans, tobacco, and peppers are all prevalent. Because of TYLCV's rapid spread in recent decades, more focus has been placed on studies to better understand and manage this deadly virus [18]. Serology has been used to detect plant pathogens for more than half a century. Improved immunodiagnostic technologies for virus monitoring and classification, on either hand, make viral detection simpler, better sensitive, or cost-effective.

D. Method for Detecting Plant Viruses

1) Enzymes Linked Immunosorbent Assay

Because it is very sensitive, simple, fast, or most significantly, it can measure viral quantity in plant tissue, the enzymes linked immunosorbent assay has been a commonly used method for identifying viruses over the past three decades. When an antibody is labeled with an enzyme which can combine with such a substrate to generate a colored, water-soluble byproduct, the interaction between the virus as well as the antibody is apparent. Because of their specificity, speed, and scope for standardization, serological techniques are typically favored over other methods for identifying plant viruses. Due to limitations such as low viral quantities, wrong particle shape, or the presence of virus inactivators or inhibitors in plant extracts, traditional serological techniques cannot be used for many essential viruses.

These constraints can be considerably alleviated by using the microplates approach of enzymes linked immunosorbent

assay (ELISA). This paper explains how to utilize this method to detect and test plant viruses quantitatively. Although enzyme-labeled antibodies have long been used to detect viral antigens on tissue slices, their use in quantitative methods is relatively recent. Enzyme immunoassays, in various forms, are quickly gaining popularity in clinical pathology and immunology, where they are said to have sensitivity levels comparable to radioimmunoassay techniques. The dual antibody sandwich type of ELISA has already been proven to be appropriate for plant viruses. The Enzymes Linked Immunosorbents Assay is commonly utilized technique for identifying virus particles in plants. It also has other advantages, such as rapid, sensitive, and large sample detection in a short period of time. Two distinct types of ELISA techniques are used to identify plant viruses: (direct as well as indirect ELISA). A variety of aspects impact the ELISA test, including the type of chemicals used and how they are made, plant material extract procedures, antibody types, and washing intervals, as well as incubation length. The dot blotting technique was used to detect the plant virus, as shown in Figure 2.



Figure 2: The tissue immune blotting test is shown, which is used to detect viral particles. According to the plate ELISA methodology, this blotting method is more sensitive and quicker than other techniques

2) Plant virus detection method (PCR)

The most often utilised techniques for screening propagation elements, seeds, or other crop samples for specific viral infection or latent presence include electron microscopy or immunological detection systems including such ELISA, PCR, but also microarray. Rapid, easy, sensitive, and reliable processes for viral nucleic acid separation would enhance the growing widespread use of polymerases reactions including reverse transcription polymerases chemical changes for plant virus identification. Sample preparation techniques include extracting nucleic acids from whole plants, isolating viruses, and separating viral nuclei. These procedures are often time-consuming since they need the grinding of plant cells, centrifugation, the application of organic chemicals, but also ethanol precipitation. This approach, on the other hand, will only work if the PCR can withstand any possibly interfering plant element. Grapevine, banana, even peanut leaf extracts have been found to inhibit PCR [19].

Because of the presence of inhibitory compounds, the nucleic acid released by viruses varies amongst plant tissues. The presence of polysaccharide (like starch) or a sensitive secondary metabolite (phenolic), as well as the types of tissues impacted, are all considerations to consider (e.g. phloem). It's also possible that the kind of viral infection (systemic or localized) influences the outcome. On one side, viruses liberated from plants in a suitable solvent under high salt or alkaline pH conditions may produce sufficient template releases for direct PCR [20,21]. On the one hand, viruses released from plants in a suitable solvent under high salt as well as alkaline pH conditions may offer sufficient templates for direct PCR [22]. The prospective relevance of Polymerases Chain Response inside the molecular markers of viral particles was described using restriction fragment length polymorphism (Figure 3).

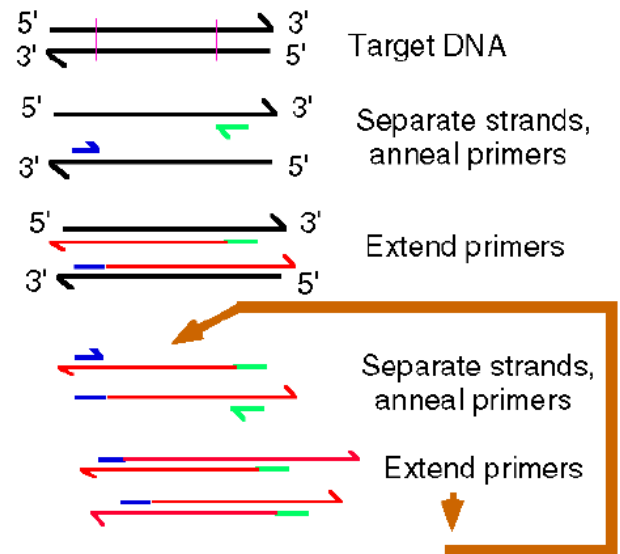


Figure 3: The Restrictions fragment length polymorphism (RFLP) approach for viral detection at the molecular level is depicted in this diagram [22]

II. LITERATURE REVIEW

Neil Boonham et al. studied about plant viruses are an intrinsically heterogeneous group with no nucleotides sequence kind (ribosomal RNA sequence) in common, unlike cellular diseases. As globalization of trade, particularly in ornamentals, or the potential implications of climate change enhance viral but also vector movement, the diagnostic environment is altering. Some of the methods used to test seed, other transmission materials, and field samples again for presence of specific viruses include biological indexing, electrons microscopy, antibody-based detection, which include enzyme-linked immunosorbent assay, polymerase chain reaction (PCR), but also microarray detection. Microarray detecting has the highest parallel and yet specific testing capability, but it might be used to identify single as well as combination of viruses with a sensitivity comparable to ELISA using current technologies. Even in "multiplexed" applications, PCR-based approaches offer the best sensitivities of the techniques outlined, but they also have

limited parallel detection capabilities. Several aspects of microarray technology are offered and studied for both existing but also developing technologies, such probe design, array building, assay target preparations, hybridization, washing, scanning, or interpreting [23].

Three 16S rRNA primers were efficiently tested simultaneously in one experiment by Paola Caruso et al. for the accurate identification of *Ralstonia solanacearum* in watercourses. The method is based on the Co-operative polymerase chain reactions (Co-PCR), which allows the primers to function together and concurrently. The assay's specificity was less than 1 cfu/ml when tested on heat-treated samples collected spiked with *R. solanacearum* but also containing indigenous microbiota up to 105 cfu/ml. The novel Co-PCR approach was shown to be more sensitive than existing traditional PCR tests when tested on Spanish ambient water samples. 31.3 percent of something like the samples were positive with the newly devised test, compared to 13.7 percent or less with the old processes. The Co-PCR improves *R. solanacearum* detection sensitivity and will be a good approach for the routine *R. solanacearum* detection in ambient samples collected or epidemiological studies [24].

P. K. Maheshwar et al. developed a precise but also sensitive reverse transcriptions nested polymerase chain reaction test (RT-nPCR) for detecting Citrus tristeza virus (CTV) from healthy and infected citrus samples. Two sets of primer pairs were created by matching nucleotide sequences available in the GenBank database for distinct genotypes of CTV. The RT-nPCR reaction elements, as well as the different heat treatment parameters or reaction condition, were all fine-tuned. By sequencing the PCR data from direct as well as nested-PCR reactions, overall specificity of both primer pairs was confirmed. The presence of CTV specific amplicons in asymptomatic sample coming from affected orchards was used to assess the test's sensitivity. Because the RT-nPCR approach developed in this study is specific but also effective in detecting CTV, it might be utilised for both diagnosis or surveillance [25].

III. DISCUSSION

Plant viruses must be identified early and precisely in order to be controlled, therefore viral disease detection is becoming increasingly important. Symptomatic diagnosis may not be suitable since viral illnesses have such a wide variety of symptoms that they might be mistaken with those caused by abiotic stressors. Instead, enzyme-linked immunosorbent tests (ELISAs) might be used to diagnose viral infections. They've been frequently utilised because they were created using serological principles. ELISAs to detect types of viruses, on the other hand, are becoming less prevalent due to a number of factors, such as the lack of antibodies for both the target viruses, the high cost of manufacturing antibodies, the requirement for a large amount of material, or the length of time it takes to execute ELISAs. A number of faults in ELISAs may be rectified utilizing a variety of modern approaches. The polymerase chain reaction (PCR) is a method that uses selective amplification to identify target DNA sequences in nucleic acid mixtures. Repeated rounds of denaturation, reannealing, as well as DNA synthesis at high

temps are utilised by DNA polymerases to amplify the target sequence, allowing for an exponential rise in the amount of DNA of interest. As a result of recent advances in molecular detection technologies, more accessible, effective, but also specific screenings have been created, allowing these tests to be used to diagnosis plant diseases, particularly viruses. Growers, agricultural agronomists, but most will profit from such testing and plant-health specialists since they will enable for early viral detection rather than depending exclusively on symptoms or time-consuming diagnostic procedures.

IV. CONCLUSION

Plant viruses are still one of the most common causes of economic losses in agriculture. Plant viruses must be detected in order to protect agricultural areas and the economy, and there are two types of viral detection technologies available (ELISA or PCR). Traditional techniques need a certain amount of knowledge. On the other hand, advanced molecular methods are simple, fast, and sensitive. Plant infection by viral particles necessitates the use of control techniques. Crop management, viral particle transmission, or other regulatory techniques are all examples of this. To detect accurate disease as well as avoid misunderstandings with identical symptoms attacking plants with different strains, major molecular or genomic methods are required for viral diagnostics. Because of several of limitations, also with a lack of antibodies for the target pathogen, the cost of producing an antibody, the requirement for a large volume of material, as well as the time required to perform an Enzyme Linked Immunosorbent Assay. Plant virus detection ELISAs are becoming less common. ELISAs have a number of flaws that may be addressed using a range of advanced techniques. Furthermore, diagnostic tests like these are essential parts of a program aimed at developing virus-free improved planting materials. So because cost of these techniques, like as Enzymes Linked Immunosorbents Assay or Polymerase Chain Reaction (ELISA or PCR), is falling and becoming more affordable, they will become the future diagnostic gold standards.

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