An Overview on Plant Virus Detection Methods

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ABSTRACT: ***As fast climate change promotes the country-to-country movement of viruses, their hosts, and vectors, early and precise identification of plant viruses is critical to their management. Diagnosis of viral illnesses is becoming increasingly crucial. Because of symptoms of viral illnesses are not always distinct and might be mistaken with those of abiotic stressors, symptomatic diagnosis may not be the best option. Agriculture is an important part of every country's economy. Different diseases like as bacteria, fungus, and viruses pose a major danger to the crops. Viruses, more than any other disease are responsible for significant losses in the agriculture production rate. Many diagnostic technologies are available to protect the agricultural crops from these viruses. There are both direct as well as indirect tools which are quite useful to resolve this problem but these have certain drawbacks. The infection of the causative agent is used to develop diagnostic techniques. Perception of side effects in pointer plants, electron microscopy, or serological procedures, for example, compound connected immunosorbent test have all been utilized to distinguish infections in plants. "Enzyme Linked Immunosorbent Assay" (ELISA) or Polymerase "Chain Reaction" (PCR) these two techniques can be very useful in determining the best moment to start implementing control measures for the detection of plant virus. Future prospective if a plant virus is detected in early stage, it can be diagnosed and treated to ensure a virus-free plant. Because the cost of these techniques (ELISA or PCR) is decreasing and becoming more inexpensive, they will become the future standards in diagnostics.***

KEYWORDS: ***DNA, Detection, Disease, ELISA, Pathogen, RNA, Virus.***

1. INTRODUCTION

Plant viruses are made up of a coat protein and two kinds of Deoxyribonucleic acid, nucleic acid, as well as Ribonucleic acid (DNA and RNA). Plant viruses are too small to be detected with light microscopy. Depending on the nucleic acid that carries genetic information. More than 1000 plant viruses have been discovered since the Tobacco Mosaic Virus (TMV) was originally discovered over a century ago. Plant viruses have been linked to significant losses in agricultural production, plant quality, or plant products all around the world. They are particularly dangerous because of their difficulty in detecting and identifying them. Viruses, unlike other plant pathogens, have no direct control strategies available yet, thus existing approaches rely on indirect tactics to manage the illnesses. Accurate disease identification is a crucial first step in any plant management strategy.

Plant therapy after infection may not always result in efficient control of viral infections. As a result, the illnesses are best controlled if management measures are implemented prior to infection. Using healthy plant replication material is one of the most successful strategies that farmers may utilize [1]. The provision of sensitive diagnostic procedures is one of the factors required for effective certification programmed to generate such propagation materials [2]. Over the last three decades, advances in molecular biology and biotechnology have been used to build quick, precise, or sensitive approaches for detecting plant pathogens. Many techniques for detecting & identifying plant pathogens have been developed. A single diagnostic test or assay may be sufficient to determine the identification of a disease-causing organism, however, for an unambiguous diagnosis, a combination of techniques is usually required. List of top seven plant virus is shown in the Table 1.Viruses that have made a prominent presence due to their scientific significance include. Despite the fact that most of these viruses continue to inflict large economic losses in a variety of crops, their employment as advanced instruments has elevated viruses to a high level of relevance for scientists[3].]

Table 1: Illustrates top seven plant viruses. The table reflects the ranking list of plant viruses chosen upon by plant virologists linked with Molecular Plant Pathology [3].

Name of plant Virus	Author of virus description
Tobacco mosaic virus (TMV)	Karen-Beth G. Scholthof
Tomato spotted wilt virus (TSWV)	Scott Adkins
Tomato yellow leaf curl virus (TYLCV)	Henryk Czosnek
Cucumber mosaic virus (CMV)	Peter Palukaitis
Plum pox virus (PPV)	Thierry Candresse
Brome mosaic virus (BMV)	Paul Ahlquist
Potato virus X (PVX)	Cynthia Hemenway

1.1 Tobacco Mosaic Virus (TMV):

The greatest significant plant virus, according to this survey of the plant virology industry, is "tobacco mosaic virus". A "single-stranded" Ribonucleic acid (RNA) virus that causes mosaic plant disease (including tobacco and tomato), particularly those belonging to the nightshade family. Other crops, especially tomatoes, are also infected. The viruses are transfer mechanically from infected plants to normal plant leaves that have been scraped or damaged as shown in Figure 1[4].

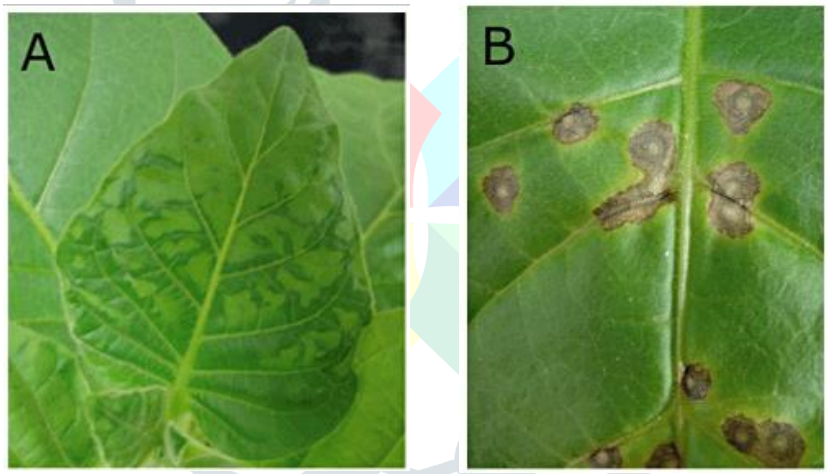

Figure 1: Illustrates the Tobacco mosaic virus. "(A) TMV-associated mosaic in Nicotiana tabacum CV. Turk plants following systemic infection. (B) Necrotic local lesions on N. tabacum cv. Glurk leaf, exhibiting Holmes' N-gene resistance after inoculation with TMV" [4]**.**

1.2 "Tomato Spotted Wilt Virus" (TSWV):

"Tomato Spotted Wilt Virus" (TSWV) is a serious disease that affects a variety of crops grown in subtropical and temperate climates. TSWV is a one-of-a-kind virus that belongs to its own viral family. TSWV has one of the most diverse host ranges of any plant virus. Each of the various hosts of the tomato spotted wilt virus has its own set of symptoms. Symptom expression will also differ between cultivars. Ring spots (brown rings or yellow rings) and various line patterns, black streaks on petioles as well as stems, necrotic leaf patches, or tip dieback are all frequent TSWV symptoms [5].

1.3 "Tomato Yellow Leaf Curl Virus":

"Tomato yellow leaf curl virus" (TYLCV) is a Deoxyribonucleic acid (DNA) belonging to the family Geminiviridae and the genus Begomoviral. TYLCV is the most harmful tomato disease, and it may be create in tropical as well as subtropical areas, inflicting significant economic losses. This virus spreads by the whitefly Bemis a tubacin, also known as the silverleaf whitefly and sweet potato whitefly, which belongs to the Aleyrodidae family as well as order Hemiptera. The tomato plant is the principal host for TYLCV, although it

has also been identified in eggplants, cucumbers, as well as other plants. Potatoes, beans, tobacco, and peppers are all common ingredients. Because of the fast expansion of TYLCV in recent decades, there has been a greater emphasis on research to better understand and control this dangerous virus. Serology has been used for the identification of plant viruses for further than half a century. The employment of improved immunodiagnostic techniques for the identification as well as detection of viruses, on the other hand, made viral detection simpler, more sensitive, and cost-effective. We shall outline the key aspects of the most widely utilized methods in recent years in this paper.

1.4 Plant Virus Detection Methods:

1.4.1 "Enzyme Linked Immunosorbent Assay" (ELISA):

For last three decades, "the enzyme linked immunosorbent assay" (ELISA) have been a often used approach for detecting viruses because it is extremely sensitive, simple, quick, and, most significantly, it can quantify viral concentration in plant tissue. An antibody tagged via an enzyme that may reacts with a substrate to create a colored, soluble in water products makes the interaction of the virus and particular antibody apparent. Serological approaches are usually preferred over other methods for detecting plant viruses because of their specificity, rapidity, and scope for standardization [6]. Traditional serological methods, on the other hand, cannot be utilized for many important viruses due to constraints such as low viral concentrations, incorrect particle shape, or the existence of virus in activators or inhibitors in plant extracts [7].

The employment of the microplate technique of enzyme-linked immunosorbent test may greatly overcome these restrictions (ELISA). This study describes the use of this approach to the detection as well as quantitative testing of plant viruses. Although enzyme-labeled antibodies have been used to identify viral antigens on tissue slices for some time, their application in quantitative techniques is new. Enzyme immunoassays, in different forms, are rapidly being utilised in clinical pathology and immunology, where they are said to offer sensitivity levels equivalent to radio-immunoassay methods. The 'dual antibody sandwich' type of ELISA has already been proven to be appropriate for plant viruses.

Enzymes Linked Immunosorbent Assay (ELISA) is a widely used method for detecting plant viral particles. It provides additional benefits, including as fast, sensitive, and big sample detection in a short amount of time. To identify plant viruses, there are two types of ELISA methods are used, (direct and indirect ELISA). The ELISA test is influenced by a number of parameters, including the type of reagents being used how they are prepared, techniques of plant material extract, antibody kinds, and washing intervals, as well as incubation duration, must all be maintained. The plant virus was detected using the dot blotting method as shown in Figure 2[8].

Figure 2: Illustrates the tissue immune blotting test that is used to identify viral particles. These blotting methods are more sensitive than plate ELISA as well as more faster than other approaches[8].

1.4.2 "Polymerase Chain Reaction "(PCR):

Quick, straightforward, delicate, yet dependable methodology for viral nucleic corrosive detachment would empower the developing far reaching utilization of polymerase chain response (PCR) and turn around record polymerase chain response ("RT-PCR") for plant infection distinguishing proof. Test arrangement methodology incorporate entire plant nucleic corrosive extraction, infection disconnection, or viral cores detachment. Since they include the crushing of plant tissues, centrifugation, the utilization of natural synthetic substances, or ethanol precipitation, these cycles are in many cases tedious. Moreover, they make undeniably more popular nucleotide formats in more noteworthy amounts even with preferable immaculateness over those fundamental for PCR. A few straightforward methodologies for getting ready enormous quantities of plant tests for PCR applications like Random intensification of polymorphic DNA ("RAPD") have been created as of late. The most simple way is to add the plant tissue straightforwardly to the PCR response combination. This methodology, notwithstanding, will possibly work on the off chance that the PCR can oppose the presence of any inhibitory plant parts that might be added Grapevine, banana, or nut leaf extricates have all been demonstrated to restrain PCR.

Viral nucleic corrosive might be delivered and the presence of inhibitory mixtures fluctuated all through plant tissues. The thickness as well as satisfied of the cell divider, the presence of polysaccharides and responsive auxiliary metabolites, as well as the tissue type affected are for the most part components to consider. Almost certainly, the kind of viral contamination has an impact. The freedom of infections from leaves in a suitable cushion under high salt and soluble pH conditions, then again, may give adequate layout discharge for direct PCR usage. Most plants' dried leaves might be utilized as a wellspring of genomic DNA for PCR investigation, extraordinarily diminishing decreased the prerequisites contrasted with using new or frozen tissue. The possible job of Polymerase Chain Reaction (PCR) in the identification of viral particles at the sub-atomic level was illustrated by limitation section length polymorphism, portrayed in (Figure 3) [9].

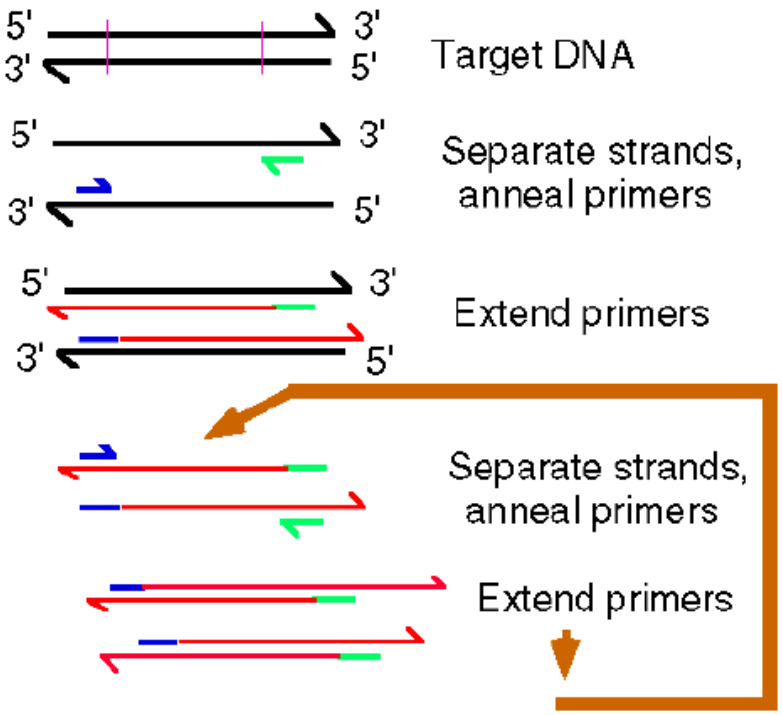

Figure 3: This Diagram showing viral identification at sub-atomic level by RFLP technique, Restriction section length polymorphism [9][10]**.**

2. LITERATURE REVIEW

Jeong, Joo-jin studied many sophisticated approaches to overcome the drawbacks of ELISAs. Since its inception as a method for amplifying target DNA, the polymerase chain reaction (PCR) has evolved into a wide range of variations with higher sensitivity than ELISAs. Plant viruses must be detected early and accurately if they are to be controlled. Since free trade agreements (FTAs) and fast climate change encourage

the spread of viruses, its hosts, and vectors from nation to country, viral illness detection is becoming increasingly essential. Because the symptoms of viral illnesses are difficult to distinguish from those of abiotic stressors, they are frequently mistaken. It's possible that a symptomatic diagnosis isn't the best option. Enzyme-linked immunosorbent assays, which were created on the basis of serological principles, have been widely utilised for the past three decades. But, due to several constraints, such as the availability of antibody for the target virus, the expense of producing antibody, the need for a high volume of material, and the time it takes to perform an ELISA, and ELISAs to detect plant viruses are becoming less common. This paper examines a variety of plant virus detection technologies, including immunological-based detection systems, PCR techniques, including hybridization-based methods like microarray. Some of the approaches have been tested in the field, while others are currently being refined to reach the degree of confidence required for real usage[11].

Luis, Rubio highlighted that the influence of plant virus genetic diversity as well as evolution on the creation and efficiency of different disease detection and control approaches is also investigated. High-throughput or the next sequencing allows multiplex detection, quantification, as well as the discovery of new viruses by enabling broad-spectrum and accurate viral identification. Plant viruses produce enormous economic losses and represent a hazard to agriculture's long-term existence. International commerce, climate change, and viruses' capacity to evolve fast all contribute to the frequent emergence of new viral diseases. Disease control is based on two strategies immunisation and cross-protection prophylaxis to prevent viral spread. The quick and accurate identification of the causative agent is critical for sickness treatment. For known viruses, diagnosis includes categorising a viral infecting a plant sample into a group of viruses with similar features, known as species [12].

S. A. Ghabrial studied about the approach for detecting plant viruses. The direct demonstration of the unfavourable affects of enzyme conjugation on antibody binding capabilities is offered. The authors develop an efficient and moderately sensitive radio immunosorbent assay (RISA) for identifying plant virus. The RISA strategy is a microplate procedure established with the understanding of a "twofold counter acting agent sandwich", and it is like the chemical connected immunosorbent examine (ELISA) then again, actually recurrence of a wave-globulin is utilized rather than the globulin catalyst form, and the bound 125I, globulin is separated from the twofold neutralizer sandwich by fermentation. “Cauliflower mosaic infection” (CAMV) or lettuce mosaic infection (LMV) might be distinguished at fixations as low as 5 and 2 ng/ml, individually, because of the radioactivity being corresponding to viral focus. With infections for which the ELISA values are too low to be in any way dependable, the RISA procedure may be beneficial [13].

Norman W. Schaad noted that conventional pathogen isolation procedures may take longer and be less trustworthy than quick real-time PCR diagnosis, which can lead to suitable control measures & eradication operations. Disease losses are maintained to a minimum, and control expenditures are kept to a minimum. Rapid-cycle real-time polymerase chain reaction (PCR) methods have the potential to revolutionise the way plant pathogens and diseases are detected and diagnosed. Highly accurate primers and fluorescence probe sequences might be produced to construct target amplicons to specific portions of a pathogen's genome as the genomics age progresses and more DNA sequence data becomes available. Diagnostic tests may now be completed quickly in the field or at distant locales other than the traditional diagnostic facility, due to portable real-time PCR equipment like the ones detailed here [14].

María M. López studied discusses the identification of bacteria and viruses that cause plant disease. A preliminary enrichment procedure in liquid or solid media is recommended to increase the sensitivity of bacterial detection methods. When enormous amounts of samples need to be analysed, serological & molecular techniques are now the best choices to assure safe and sustainable agriculture, it is necessary to detect dangerous viruses and bacteria in plant material, vectors, including natural reservoirs. In the last several years, the techniques for detecting infections have changed substantially, with the extraction of the target from the sample being vital for better detection. Imprinting and squashing plant material as well as insect vectors onto membranes has enhanced viral sample processing. Microarray technology is the most recent development in nucleic acid analysis, although it requires general DNA/RNA extraction and pre-amplification processes to enhance detection sensitivity [15].

3. DISCUSSION

Early and precise identification of plant viruses is critical for controlling them, therefore viral disease diagnosis is becoming increasingly crucial. Because the symptoms of viral illnesses are so diverse and might be mistaken with those of abiotic stressors, symptomatic diagnosis may not be suitable. Instead, enzyme-linked immunosorbent tests (ELISAs) can be used to diagnose viral disorders. They've been widely utilised since they were created on the basis of serological principles. However, due to several constraints, such as the availability of antibody for the target virus, the expense of producing antibody, the necessity of a high volume of material, and the time it takes to perform ELISAs, ELISAs to detect plant viruses are becoming less common. ELISAs have a number of drawbacks that can be overcome using a variety of sophisticated methods. The "polymerase chain reaction" (PCR) is a method that allows for the selective amplification therefore detection of target DNA sequences in nucleic acid mixtures. Through repeated rounds of denaturation, reannealing, or DNA synthesis at extreme temps, DNA polymerases are employed to amplify the target sequence, enabling an exponential rise in the amount of the DNA of interest. Recent advances in molecular detection technologies have resulted in the creation of more accessible, effective, and specific assays, allowing these tests to be used to identify plant diseases, including viruses. Growers, agricultural agronomists, and others will benefit from such tests and plant-health experts not to rely solely on symptoms or time-consuming diagnostic tests, and to allow for early virus identification[16].

4. CONCLUSION

Plant viruses are still among the leading causes of agricultural economic losses. It is critical to detect plant viruses in order to safeguard agricultural fields as well as the economy and there are two sorts of viral detection methods available (ELISA and PCR). Traditional procedures need a certain level of expertise. Advanced molecular techniques, on the other hand, are simple, quick, and sensitive. Control techniques for viral particle infection on plants are required. Crop management, viral particle transmission, and other techniques of control are examples. Major serological and molecular approaches are important for viral diagnostics to identify accurate disease and prevent misunderstanding with similar symptoms infecting plants with diverse strain. Due to many restrictions, such as the availability of an antibody for the target virus, the price of generating an antibody, the necessity for a significant amount of material, and the time it takes to run an Enzyme Linked Immunosorbent Assay. ELISAs to identify plant viruses are becoming less prevalent. ELISAs have a number of disadvantages that may be addressed utilising a variety of advanced ways. These tools may be highly beneficial for identifying when control measure should be applied. Furthermore, such diagnostic tests are key components of programme aiming at generating virus-free plant propagation materials. Because the cost of these procedures Enzyme Linked Immunosorbent Assay and Polymerase Chain Reaction (ELISA and PCR) is reducing and becoming more affordable, they will become the future standards in diagnostics.

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