IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A REVIEW ON METHODS USED FOR PLANT VIRAL INFECTION

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ABSTRACT:

The biggest danger to sustainable agriculture is plant viral infections, which can cause multiple losses. Numerous viruses are affecting a variety of crops, thus new methods that can accurately identify the causative agents of viral infections are more and more needed for their efficient treatment and prevention. Numerous diagnostic techniques that are more sensitive and specific in identifying unidentified plant viruses are constantly being researched and developed. Controlling plant viral infections requires an early and precise diagnosis of these infections. For purposes of assembly, reproduction, intra- and intercellular mobility, and attracting vectors for dispersal, viruses depend on their hosts. Several viruses require chloroplasts to replicate. A crucial part of plant physiology, photosynthesis, is hampered when viruses multiply in chloroplasts. Chloroplasts are present in plants which is very essential for the replication of viruses. Some viruses replicate in chloroplast. When viruses replicate in chloroplast they inhibit the photosynthesis process, which is a very important process in plant physiology. This viral infection reduces the quality of crop yield, stunts crop growth, and results in low-quality products. To overcome this condition or this viral infection in plants use different techniques like PCR and ELISA. Many techniques are effective and can provide accurate identification of the viral causative agent. There are continuously developed new techniques for the detection method of viruses present in plants which have improved sensitivity and specificity. Assays utilizing enzyme-linked immunosorbents (ELISA), are created using the serological approach and are routinely utilized. However, ELISA's capacity to identify plant viruses is limited by factors including the target virus's antibody availability and the length of time needed to conduct the ELISA. Originally designed as a method for amplifying target DNA, the polymer chain reaction (PCR) has evolved into numerous variants and is now more sensitive than ELISA. Numerous plant virus detection systems, including those based on immunological detection, PCR technology, and hybridization-based

techniques like microarray, microscopic method, visual inspection method, dot stain immune binding assay nucleic acid-based method, etc. are described here.

KEYWORDS: Plant viral detection, Plant viruses, ELISA, PCR, Nucleic acid.

I. INTRODUCTION:

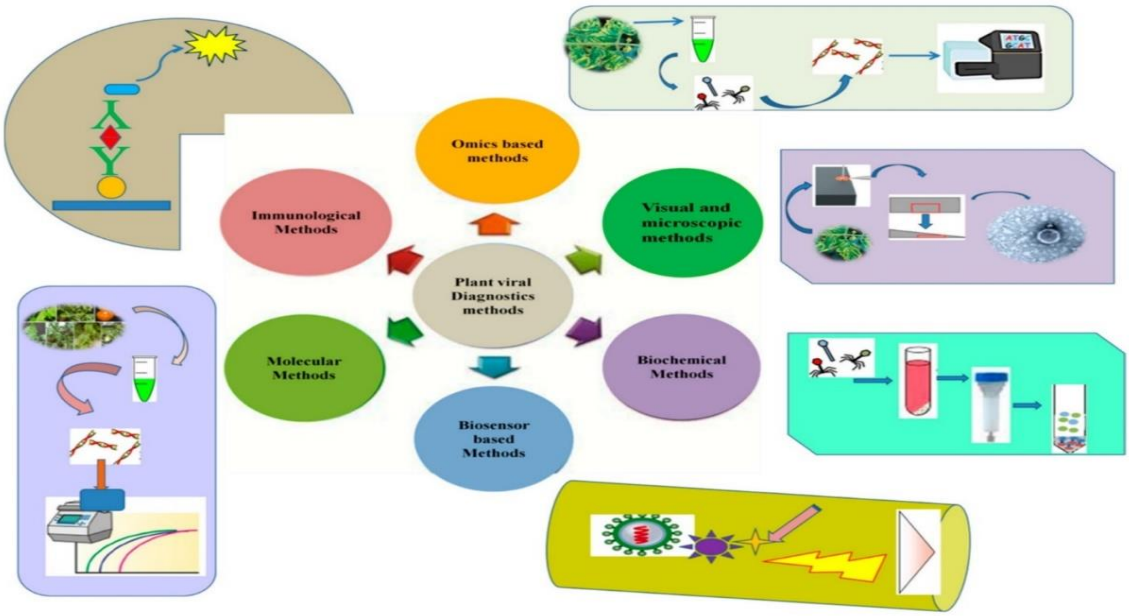
Generally, viruses are the acellular parasites and very small organisms as compared to other pathogens like fungi, bacteria, these pathogens are easily seen under the microscope but viruses are too small and they can see only under the transmission electron microscope. They are made up of either DNA or RNA which are coated by protective shell capsid.[1]The first recognized plant virus is tobacco mosaic virus (TMV) then now a day there are more than 1000 plant viruses identified. [2]Viruses are one of the main reasons in agriculture that will affect the quality as well as quantity of crop yield. [3] There are about more than 25 families of plant viruses identified which infect the various types of crops and causes economic loss. [4] Numerous pathogenic organisms, including viruses, oomycetes, nematodes, and fungus, constantly attack plants. The significant agricultural losses caused by these infections restrict global food production. An estimated 50% of newly discovered plant diseases are viral in nature, and new plant viruses are found on a daily basis [7]. Viruses are mandatory parasites that, when they enter a host cell, need a place to replicate. After entering the cell, the viral genome is used as a template to create multiple genomic copies that are then translated into viral proteins by transcription and translation, producing new viral particles in the process [8]. Numerous genetic, metabolic, and physiological alterations in plants were brought on by virus infection, including modifications to the chloroplasts' photosynthetic process [9]. The most genetically varied organisms that infect humans, animals, and plants are viruses. Since most viruses have a short genome and only encode a few proteins, it is challenging to regulate viruses using a number of techniques.[10] Viruses are the major source of emergent infectious illnesses in a range of crop plants, accounting for nearly half of all crop losses caused by viral infection. all the pathogens that cause disease in plants, viruses pose a significant risk to agricultural productivity. As a result, viruses pose a significant global agricultural production challenge by lowering the quantity and quality of food crops. It is known that more than 25 families of plant viruses can infect a wide range of crop plants worldwide [4]. Plant viral species are spread by two mode of transmission and the modes are vertical mode of transmission and horizontal mode of transmission. In vertical mode of transmission, the causative or infectious agents passed through parent plant by either vegetative propagation or sexual reproduction through the infected seeds. In horizontal mode of transmission spreading of viruses occurs due to vectors, tools which are used in agriculture and some external factors due to which causes the contamination [5]. Plants infected with a virus may exhibit a number of symptoms, including yellowing, chlorosis, necrosis, mosaic damage, and stunting. These signs might cause the plant to lose its ability to grow and reproduce.[11] Sometimes plant shows symptom like viral infection because of climate change, imbalance in nutrition requirement.[2] Due to viral infection in plants the physiology and biochemistry of that host plant cells will changed. Virus's damage the plants due to this sometimes plants get dead. They also change the chemical composition, metabolism process in plants also decrease the photosynthesis process due to reduce carbohydrates content in plants [6].

To accomplish quick and accurate detection, the chosen methods and techniques such as electron microscopy (EM), immuno-serological methods (ELISA), molecular approaches, and biosensor-based methods are currently widely used[4]. In order to improve the sensitivity and specificity of illness diagnosis, multiple technique combinations are typically used. The evaluation of plant viral dynamics is gaining attention; yet, compared to human and animal viruses, there are reportedly fewer viable methods for detecting viruses in infected plants [5]. The first was the introduction of antibody-based detection methods, specifically monoclonal antibodies and enzyme-linked immunosorbent assays [10, 12]. There are two types of immuno-diagnostic and molecular-diagnostic techniques currently available in the field of virology: protein-based techniques such as precipitation/agglutination tests, enzyme-linked immunosorbent assays (ELISA), dot immunoblotting assays (DBIA), and tissue blot immune binding assays (TBIA). Lawson stated that appropriate screening procedures were carried out in order to certify any plant free of specific pathogens using ELISA, DBIA, TBIA, PCR, and DNA probes [13].

II. Methods for plant viral detection: -

Numerous methods for plant viral diagnostics have been developed and commercialized. However, the practical application of each prevalent method is dependent on a variety of factors, including cost, sensitivity, rapidity, instrument availability, and disease stage. [14]Plants can exhibit a variety of physiological symptoms when infected with a virus. Historically, such visual examinations have been used to detect viral disease. The use of indicator plants in diagnostics. Moreover, several other quantitative high-throughput imaging-based methods have been developed.

FIG. 1 COMMON DIAGNOSTIC METHODS USED FOR PLANT VIRAL IDENTIFICATION DISEASE



DIAGNOSIS OF VARIOUS CROP PLANT. [11]

Serological and nucleic acid-based methods are becoming essential in plant disease diagnosis as a molecular biology techniques advance. NGS technologies developed in recent decades have also provide a platform for viral diagnosis. These techniques also have been used to detect and identify various viral species [15].

A. A method of visual inspection:

Visual inspections of infected plants and seeds were the most commonly used method for detecting plant pathogens. Symptoms of viral diseases affecting plants were proposed as a basis for virus taxonomy at the turn of the twentieth century. [11]The symptoms expressed in the host plants are usually used to characterize a plant viral disease with a known etiology. This is due to the ease with which symptoms can be identified, especially when they appear to be disease-specific [16]. External manifestations of viral infection in plants are closely associated with specific plant physiology disorders, and the symptoms are classified as mosaics and chloroses. Changes in the colors and shapes of the leaves occur in mosaics. The entire plant is affected by systemic lesions. Necrotic lesions are observed in some cases, which are also used for virus taxonomic identification—for example, viruses with necrotic mosaics and potatoes with streaks [17]. Crinkling, browning of leaf tissues, mosaics, and necrosis are all symptoms of viral diseases. Visual symptoms of viral diseases can take many different forms. The primary diagnosis of the disease and the extent of plant damage can be determined based on the species (variety) of the host and its resistance to the virus [18]. Visual inspections of viral disease diagnosis using symptoms are extremely difficult because multiple viruses may be present in the host, altering symptoms [19].

Young tobacco plants (Nicotiana glutinosa) can be used to detect tomato a sperms virus, and the leaves of Gomphrena globosa can be used to detect potato X-virus. These methods have numerous drawbacks, and their outcomes are dependent on the ages of the leaves and the technique used to rub the virus into the leaf. A method based on leaf halves was proposed for comparing these experiments. Control infected plants' juice was applied to one half of the leaf, and another variant's juice was applied to the other half. Following staining of the infected leaves with iodine testing for starch, which shows externally invisible infectious zones (infected leaf cells produce a low concentration of starch), an improved modification is provided [11].

A visual inspection can be performed using a variety of techniques, including visible light imaging, chlorophyll fluorescence imaging, hyperspectral imaging, and thermal imaging. However, these methods are primarily influenced by environmental and biological factors. Visual inspections are also ineffective in asymptomatic cases [20]. As a result, fluorescent proteins have remained effective investigative tools for deciphering biological processes and have been widely used as marker systems for the infectious process and viral particle quantification in host plants. One of the most effective methods for most viral diagnosis was the visualization of fluorescence tagged proteins in host plants using microscopic techniques [11].

B. Microscopic technique:

Microscopic detection using modern light and high-resolution electron microscopes is one of the most useful methods for visualizing viruses in plant tissues [21]. Certain viruses cause clusters of viral particles to form in plant cells, similar to inclusions or Ivanovsky crystals, which can be seen under a light microscope. Each virus type has its own set of viral inclusions. Tobacco mosaic virus produces needle-shaped and hexagonal crystals, whereas Potato virus X and wheat mosaic virus produce spherical amorphous bodies [22]. Though microscopic methods have been found to be

useful for virus detection, they require a high level of skill and expertise for viral species identification. The use of transmission electron microscopy (TEM) to characterize viral particles in both crude and purified samples has improved their morphological characterization. TEM research has resulted in one of the first proposals to classify viruses based on their morphological and serological relationships, as well as some of their biological properties [23]. TEM allows for direct detection by first homogenizing the infected tissue and then staining it negatively [24]. Tomato yellow leaf curl disease (TYLCD), Potato virus S (PVS), Rice stripe mosaic virus (RSMV), Tomato brown rugose fruit virus (ToBRFV), and Pepino mosaic virus are examples of plant-infecting viruses. Potato virus M were observed and studied using electron microscopy techniques [25]. Other techniques, such as serology, are frequently combined with electron microscopy to increase detection sensitivity. In this context, immune electron microscopy (IEM), which essentially employs specific antiserum against viral antigen, has been developed. Several IEM variants, such as solid-phase immune electron microscopy (SPIEM) and immunosorbent electron microscopy (ISEM), are primarily used in viral diagnosis [11].

IEM can also be performed directly on raw serum without the need for immunoglobulin purification [24]. Microscopy techniques, in conjunction with serological methods, are thus directly used to detect localized viral infections within infected plants [26]. The ISEM assay was used to detect Bean Common Mosaic Potyvirus (BCMV), an important seed-borne pathogen, in young infected leaves of French beans [27]. IEM has also been used to treat yellow mosaic viruses (YMV) that infect legume plants. The presence of YMV was found in various parts of the seeds of a naturally infected black gram (Vigna mungo L. Hepper) plant [21]. In plant virology, electron microscopy has been useful in elucidating shape, size, and surface details for identification and classification. A confocal microscope (CM) was used in another related application of microscopy to detect indicator compounds or molecules. The CM was used in this study to identify co-localized heat shock proteins like HSP70 and HSP90 in tomato plants infected with Tomato yellow leaf curl virus (TYLCV). CM has also been used to detect viruses labeled with fluorescent proteins, allowing for the tracking of viral particles in plant intracellular tissues. Several researchers have used fluorescent viruses and/or plants that express marker proteins for green fluorescent protein (GFP) or red fluorescent protein (RFP).These techniques allow one to observe the infectious process and quantify the virus in plant tissues, as well as evaluate the host plants' natural or induced phyto immunity potential, such as virus-induced gene silencing. Furthermore, the presence of a protein GFP or RFP allows for real-time observation of changes in infected plant tissues using modern fluorescent and confocal laser microscopes. Cryo-electron microscopy (cryo-EM) is currently the most advanced microscopy technique used in the study of plant-virus interactions, as it can reveal nearly the atomic-level structures of viruses. Cryo-EM technology has helped us understand virus structural conformations, such as identifying specific nucleoproteins from negative-stranded RNA viruses, as well as providing a better understanding of viral infections by illuminating viral assembly and constituents. Thus, advances in microscopic techniques have aided us in interpreting the localization of viral infections via the detection of viral indicator molecules. Further advancements in these techniques may play a significant role in plant viral diagnosis and observations in the future [11].

C. Serological method:

Serological methods for virus diagnosis, detection, and identification in plants are critical [15]. Traditional plant virus diagnosis requires a bioassay, an indicator plant, host range determination, symptomatology, virus particle morphology (size and shape), and vector relations [28]. A single diagnostic test or assay may provide sufficient information on the identity of a virus, but a combination of methods that are specific, sensitive, and inexpensive is usually required [29]. The first was the introduction of antibody-based detection methods, specifically monoclonal antibodies and enzyme-linked immunosorbent assays [30]. There are two types of immuno-diagnostic and molecular-diagnostic techniques currently available in the field of virology: protein-based techniques such as precipitation/agglutination tests, enzymes linked immunosorbent assay (ELISA), dot immunoblotting assay (DBIA), and tissue blot immunobinding assay (TBIA). According to Lawson, appropriate screening procedures have been carried out in order to certify any plant free of specific pathogens using ELISA, DBIA, TBIA, PCR, and DNA probes [31].

a) **Enzyme linked immunosorbent assay (ELISA):**

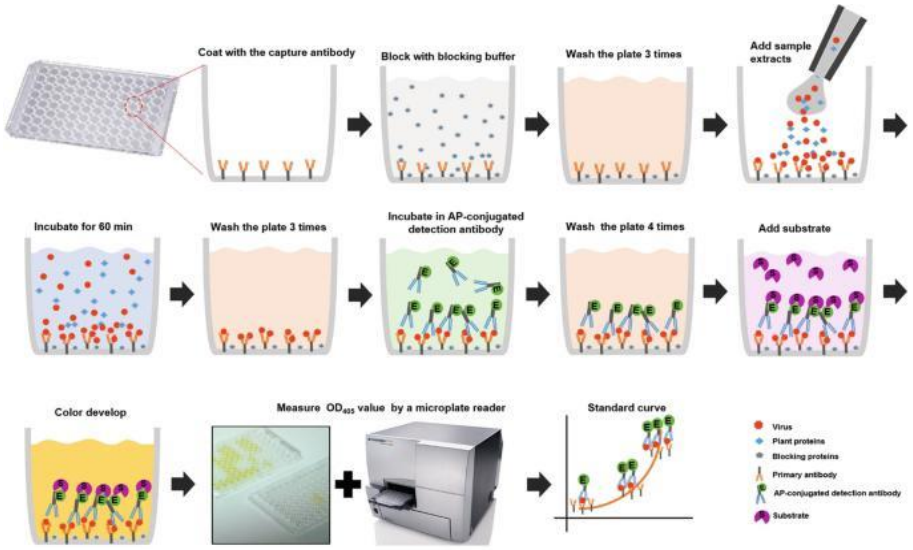
The use of enzyme linked immunosorbent assay (ELISA) for plant virus detection is well documented and has proven to be a very valuable detection tool for plant viruses. Furthermore, the test's specificity can preclude detection of even closely related strains of the same virus [32]. Clark and Adams discovered that, in contrast to nearly all other serological techniques in plant virology, which were based on the formation and detection of immune precipitates, the ELISA technique was based on the sensitive detection of non-precipitates reaction, which was made possible by the use of enzyme-labeled antibodies. For practical purposes, the ELISA technique's efficiency was independent of the antibody-to-antigen ratio. As a result, once the appropriate concentrations were determined, these were applicable for subsequent tests for detecting virus at all concentrations, and the reaction of enzyme-labeled antibody was a function of virus concentration, indicating that the technique has high quantitative potential [33]. Pesic and Hiruki discovered that the quantitative response over the concentration range of AMV tested indicated that ELISA was responsible for both variation in -globulin concentration and enzyme conjugated -globulin dilution. The minimum detectable amount of AMV, 1 ng/ml, was obtained using coating-globulin at a concentration of 2 g/ml and enzyme conjugated-globulin at 1/1000 and 1/2000 dilutions, or at 1 g/ml and 1/1000 dilution, respectively. An increased dilution of enzyme-conjugated -globulin 1/4000 reduced AMV antigen detection levels to 16 ng/ml with the coating -globulin at 2 g/ml and 250 ng/ml with -globulin at 1 g/ml. An enzyme-labeled antigen was used as a second antibody in indirect ELISA to detect the antigen antibody complex on the solid face. This eliminates the need to create specific enzyme conjugates for each antigen to be tested and eliminates extreme specificity, allowing for quantitative evaluation of strain relationships. Indirect ELISA was used to detect and confirm viral infection. The dissociation reactions of four plant viruses, citrus tristeza virus (CTV), carnation mottle virus (CarMV), carnation yellow fleck virus (CYFV), and tobacco mosaic virus (TMV), and their respective "y-globulin alkaline phosphatase conjugates, sandwiched to antibody microplates, were studied by Bar-Joseph. The double antibody sandwiches (DAS) of CTV and CarMV dissociated from the antibody-coated microplates after 60 minutes of treatment with 0.2 M glycine-HCI buffer pH

2.2. CYFV was eluted less efficiently in similar treatments. Acidification did not dissociate the TMV double-sandwich, but it could be partially dissociated under alkaline conditions (pH 12.1). The use of microplate recycling to reduce the cost of routine large-scale CTV screening is described. According to Lommel, indirect ELISA could be very useful as a routine plant virus detection tool for virus disease diagnosis and surveys where precise quantitative results are not required. The advantages of indirect ELISA are that the sample only needs to be macerated and added to the plate, crude antiserum can be used (though it should be cross-absorbed to prevent spurious host reaction), and a single, commercially available second antibody conjugate is used, which eliminates the problems of preparing and storing many different conjugated antisera, as Abd El-Aziz points out [32].

b) Dot blot immunobinding assay:

The blotting technique has become widely used for specific identification of nucleic acids and proteins. To detect protein, the antigen was spotted on a nitrocellulose membrane and incubated in test antibody, followed by incubation in peroxidase-conjugated second antibody to the first antibody and development in 4-chloro-1-naphthol. The procedure described above is known as a dot blot immunobinding assay (DBIA). It was used to screen hybridoma supernatants for monoclonal antibodies and pathological sera for multiple antibodies [34]. The benefits of DBIA include the ability to detect much lower amounts of virus due to the very small sample volume (2 l compared to 250 l for ELISA) or (4 l compared to 250 l for ELISA). The DBIA has a potential drawback in that a large volume 50 ml relatively concentrated (1 mg/ml) virus antiserum was required, but antivirus antibodies solution can be stored for at least 6 months and used on at least 600 samples without appreciable loss of sensitivity. It produced satisfactory results in modified DBIA using either polyclonal or monoclonal antibodies or both direct and indirect methods. The assay was superior to ELISA and immunosorbent electron microscopy methods in detecting 0.35 ng of purified cowpea mosaic virus (CpMV). A dot immunobinding assay detects polyvirus pictogram quantities [15].

FIG. 2 ELISA TEST [38].



The two faces of a nitrocellulose membrane were used in a tissue blot immunoassay to detect Bean yellow mosaic virus and the possibility of mechanical transmission from the printed membrane to the host plant. Different types of regular paper were evaluated as potential replacements for the commonly used nitrocellulose membrane (NCM) as the solid phase in the tissue-blot immunoassay (TBIA) used to detect Alfalfa mosaic virus, Bean yellow mosaic virus, and Broad bean stain virus (BBSV) in faba bean tissue, and Barley yellow striate mosaic virus in barley tissue. Among the many types of paper tested, Hewlett Packard (HP) non-glossy plotter paper proved adequate for detecting all of the viruses listed above. Satisfactory results were obtained after printing (blotting) the samples to be tested and blocking with 2% gelatin (for one hour at 37oC) or 0.1% Roche blocking reagent (for one hour at room temperature). This study could be used to detect BBSV in groups of 15 young lentil seedlings. When testing for phloem limited legume viruses such as Bean leaf roll virus (BLRV), Faba bean necrotic yellows virus (FBNYV), and Barley yellow dwarf virus (BYDV), non-glossy blotter paper from HP was less effective. When used for BLRV and FBNYV detection in faba bean tissues and BYDV detection in barley tissues, white paper (manufactured by Soporcel, Portugal) was slightly more sensitive. Because NCM accounts for 40%-50% of the cost of test reagents, using ordinary paper significantly reduced reagent costs [35].

D. Nucleic Acid Based method:

Nucleic acid-based techniques are extensively used in a variety of diagnostic domains, such as environmental investigation, food safety, and clinical diagnostics. The breadth of applications for molecular and genomics approaches in the detection of infectious diseases has increased with their fast growth. Nucleic acid (DNA or RNA)-based methods have gained widespread acceptance in the field of viral diagnostics because of their increased sensitivity. The isolation of the nucleic acid (either DNA or RNA), amplification, and product analysis are the three main processes in any nucleic acid-based investigation. Since it immediately reveals the outcome, the final step is the most crucial. Nucleic acid-based diagnostic methods have been created in a variety of ways; however, the majority of these methods require complex equipment and take a long time. Recent developments in NGS approaches may offer a way to conduct analysis both in resource-constrained settings and on-the-go [11].

a. PCR:

The Polymerase Chain Reaction (PCR) is a crucial technique employed for the molecular identification of many diseases. A great deal of work has gone into the PCR-based study of viral detection. Among the most popular techniques for virus detection [2]. PCR-based techniques identify the DNA or RNA signatures of the virus by utilizing molecular primers to amplify a specific area of the viral genetic material. PCR-based techniques that employ particular sets of degenerate primers are frequently employed to detect viruses in a variety of host plants. For instance, using primer sets created for various viruses, the tomato plant, which is grown all over the world and infected with multiple viruses, including the tomato mosaic virus, tomato leaf curl virus of New Delhi, tomato leaf curl virus of Gujarat, and tomato leaf curl virus of Palampur, was tested for viral infections. Although conventional PCR is still widely regarded as the most important method for diagnosing plant viruses, its usage in this field is still rather limited because of the difficulties in achieving ideal conditions and the length of time required for detection[36].

The genomes of the majority of plant viruses are RNA-based. In this regard, one of the most promising methods for plant virus diagnostics is the use of reverse transcriptase PCR (RT-PCR), which operates on the basis of cDNA synthesis from RNA. RT-PCR has been used on a wide range of RNA genome-based viruses in plants and is more sensitive, dependable, and economical. The Pepino mosaic virus (PepMV), which has emerged as a virus that affects tomato crops worldwide in recent years, can be effectively analyzed using RT-PCR. PepMV is a mechanically transmitted Potexvirus with an RNA-based genome. Numerous investigations have effectively identified and detected various PepMV genotypes in tomato crops. Reliable quantification of various Pepino mosaic virus genotypes has been reported using a newly devised one-step RT digital PCR, which has been determined to be more appropriate than RT-PCR alone. A wealth of information is accessible regarding pertinent quantitative molecular biology techniques, particularly RT-PCR assays, which are extensively employed in the diagnosis of a wide variety of plant viral diseases [11].

Nevertheless, using these methods to detect divergent forms of known viruses has its limitations. Therefore, for more meaningful and sensitive results, PCR and RT-PCR-based procedures need to be refined. These strategies have been developed concurrently by several groups during the past few decades. Among them, the techniques for detecting plant viral infections that were based on the idea of combining the sensitivity of ELISA and PCR eventually came to be known as immunocapture PCR or immunocapture RT-PCR (IC-PCR, IC-RT-CPR). These methods involve capturing the viral agents using virus-specific antibodies attached to PCR tubes or microwell plates, then PCR or RT-PCR of the viral particles. In addition to offering two-way specificity, IC-PCR techniques have been shown to be useful for the direct detection of viruses from unpurified or partially purified plant extracts [37]. Additionally, it may be able to prevent the requirement for nucleic acid (DNA or RNA) isolation. Therefore, robust diagnostic techniques are being successfully employed for plant virus detection, especially in plant species or tissues that contain inhibitory compounds. Such hybrid methods, which could provide double sensitivity, are more adaptable [11].

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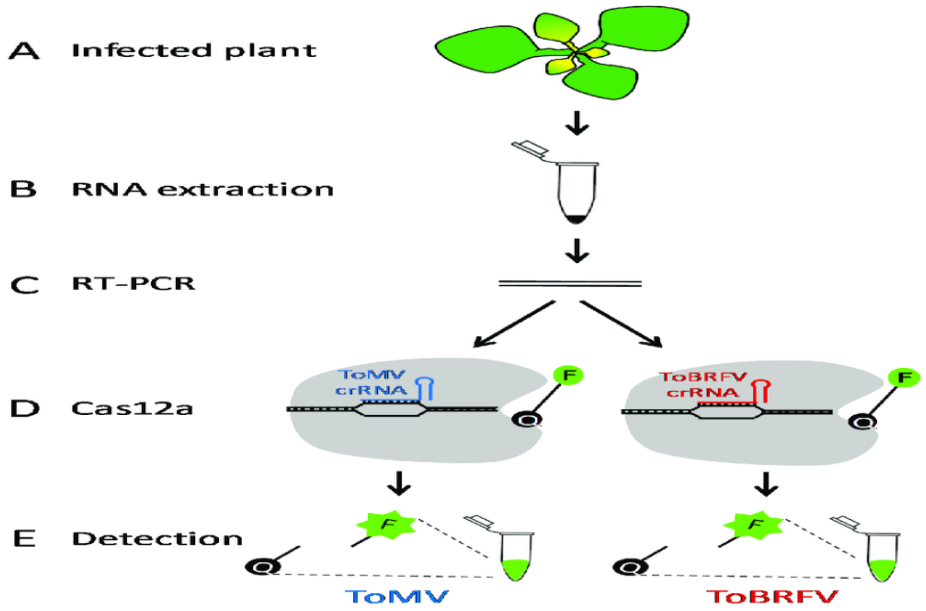

FIG.3 PCR METHOD. [38]

b. Next generation sequencing based method:

Biological research was dominated by first-generation sequencing, or Sanger sequencing, prior to the development of next-generation sequencing methods. These approaches' superior throughput and relative affordability kept them attractive for analysis even after high-throughput sequencing technology emerged. NGS systems are gaining popularity in many biological research domains because of their large data-generating capacity, rapid delivery, affordability, and ease of use. The term "omics" refers to a number of biological fields, including metagenomics, genomics, proteomics, metabolomics, and others, that use advances in next-generation sequencing (NGS) technologies to analyze different cellular components. Among these, the study of a collection of all the DNA or RNA from a diverse population of species is known as metagenomics. The field of metagenomics is being developed to detect viruses. These methods involve extracting total nucleic acid (DNA or RNA) from plant samples that have been infected, sequencing them on NGS platforms, and identifying viral sequences using bioinformatics tools [11].

III. CONCLUSION:

Plant viral diseases caused by several pathogens. Plant viral diseases have been shows to negative impact on crop health and decreases in crop yield. Several economic losses have been estimated per day due to plant viral diseases. There is no any chemical to manage this infection. Plant diseases caused by viruses can be effectively control at initial stage of disease development, or also control by planting virus free plant. The methods based on serological and molecular biology based have been used for viral diagnosis. PCR based assay has better sensitivity over ELISA and PCR method is faster than ELISA. Monitoring plant health and fast detection of pathogens are essential to reduce viral disease. commonly used diagnostic method for the detection of plant pathogen have some limitation like time required to complete test, low sensitivity, restricted ability to detect several pathogens simultaneously, and required sufficient knowledge regarding genome sequence. Metagenomic has identified many new viral entities. Further

developments in bioinformatics tools using NGS methods could facilitate potential technological advancements in disease diagnosis that will help to reduce crop losses due to viral diseases.

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