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REVIEW ARTICLE

An evaluation of nucleic acid-based molecular methods for the detection of plant viruses: a systematic review

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Abstract
Precise and timely diagnosis of plant viruses is a prerequisite for the implementation of efficient management strategies, considering factors like globalization of trade and climate change facilitating the spread of viruses that lead to agriculture yield losses of billions yearly worldwide. Symptomatic diagnosis alone may not be reliable due to the diverse symptoms and confusion with plant abiotic stresses. It is crucial to detect plant viruses accurately and reliably and do so with little time. A complete understanding of the various detection methods is necessary to achieve this. Enzyme-linked immunosorbent assay (ELISA), has become more popular as a method for detecting viruses but faces limitations such as antibody availability, cost, sample volume, and time. Advanced techniques like polymerase chain reaction (PCR) have surpassed ELISA with its various sensitive variants. Over the last decade, nucleic acid-based molecular methods have gained popularity and have quickly replaced other techniques, such as serological techniques for detecting plant viruses due to their specificity and accuracy. Hence, this review enables the reader to understand the strengths and weaknesses of each molecular technique starting with PCR and its variations, along with various isothermal amplification followed by DNA microarrays, and next-generation sequencing (NGS). As a result of the development of new technologies, NGS is becoming more and more accessible and cheaper, and it looks possible that this approach will replace others as a favoured approach for carrying out regular diagnosis. NGS is also becoming the method of choice for identifying novel viruses.

Keywords Plant virus detection · Molecular techniques · PCR · Isothermal amplification · Virus diagnosis

Introduction

Plant viruses are emerging as a significant threat to sustainable agriculture worldwide. According to the ICTV Master Species List (38.v3, 2022), 1850 plant-infecting viruses (assigned to 16 orders and 27 families) have been identified and taxonomically classified [75, 90, 165]. Each year, plant pathogens cause enormous economic losses on a global scale. These viruses pose a significant danger to the food and nutritional security of the world's population because they infect numerous crops, including corn, potato, rice, and wheat, which are staples in the diets of many nations. Changes in the ecosystem, specifically climate change, evolution, mutation, and global trade, have led to the transformation and development of novel viral strains and infections in the field of agriculture during the course of the last few decades [8, 137]. Moreover, each year a huge number of viruses infecting plants with varying host ranges wreak billions of dollars in damage. For instance, the barley yellow dwarf virus (genus: *Luteovirus*, family: *Luteoviridae*) affects several important cereal crops, primarily *Poaceae* species such as rice, oats, barley, maize, wheat, and rye [42, 148]. Recent critical analyses have highlighted and discussed pandemic and epidemic of plant viruses [83].

Plant viruses are transmitted mainly by vegetative propagation, mechanically or with the help of vectors through wounds in plants. It is hypothesized that wounding leads

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to a breach in the cuticle, the epidermal cell wall, and most likely the plasmalemma, allowing the cytoplasm to become accessible [159]. Vertical and horizontal transmission are the most common means of spreading plant viruses. Vertically, the virus is transmitted via vegetative propagation or through infected seeds, whereas horizontally, it's often transmitted through arthropod vectors (aphids, whiteflies, etc.), as well as through non-arthropod vectors (nematodes, fungi), or through contaminations due to humans or vertebrate animal activity through the crops (Fig. 1) [109, 141]. In contrast, the transmission of viruses through pollen happens both vertically and horizontally [141]. Vertical transmission occurs when infected pollen fertilizes healthy ovules, leading to the formation of infected seeds. These seeds will produce infected seedlings when they germinate. On the other hand, horizontal transmission happens when the viruses infect the mother plant that bears the ovules. Infected pollen is then released in large quantities to infect other healthy plants.

Unlike fungi and bacteria, where antifungal or antibacterial treatments are available, viruses are a significant concern due to the unavailability and high cost of antiviral therapies [137]. Also, the infection renders the plants unviable, and under field conditions, controlling the plant viral disease is challenging due to the absence of controlled regulations [97]. Remedial measures involve destroying the infected crop or controlling the virus-transmitting vectors. However, this requires timely and precise detection of the plant virus.

Viruses are much smaller than other disease-causing pathogens such as fungi and bacteria. Unlike fungi and bacteria which can be visualized using a light microscope, an electron microscope which is a sophisticated instrument is required for the visualization of virus particles [45, 81]. Such sophisticated instrumentation requires skill and training and cannot be routinely used for virus diagnosis. In a breakthrough diagnostic study in 1977, Clark and Adams used a serological technique to detect plant viruses, i.e., enzyme-linked immunosorbent assay (ELISA) [29]. Though in case of regular virus testing, ELISA has several advantages [155], there are still certain drawbacks, such as in-depth research necessitating specialized labs and virology experts to manufacture huge quantities of antisera and purify virions as well as other proteins as antigen. The production of antisera from the virus mixture is often time-consuming and highly expensive [21].

Researchers all over the world use a wide range of techniques to detect plant viruses (Fig. 2). These include: (1) microscopic techniques such as cryo-electron microscopy (cryo-EM), immune electron microscopy (IEM), immunosorbent electron microscopy (ISEM), transmission electron microscopy (TEM), (2) serological techniques like dot-blot immunoassay (DBIA), tissue-blot immunoassay (TBIA), direct tissue blot immunoassay (DTBIA), ELISA, (3) molecular techniques which includes various types of polymerase chain reaction (PCR) methods, isothermal amplification techniques (for instances, rolling circle amplification; RCA, loop-mediated isothermal amplification; LAMP, recombinase polymerase amplification; RPA, etc.), in addition to high-throughput sequencing methods, and (4) biosensor-based methods such as antibody-based biosensors, DNA/RNA-based affinity biosensors, etc. [50, 109].

In recent decades, various serological techniques such as tissue blot immune assays (TBIA), dot blot immunobinding assay (DBIA) have been extensively employed worldwide to identify pathogens infecting plants. But in recent times, the use of serological methods is decreased due to limitations like antibody availability, high cost, and increased time

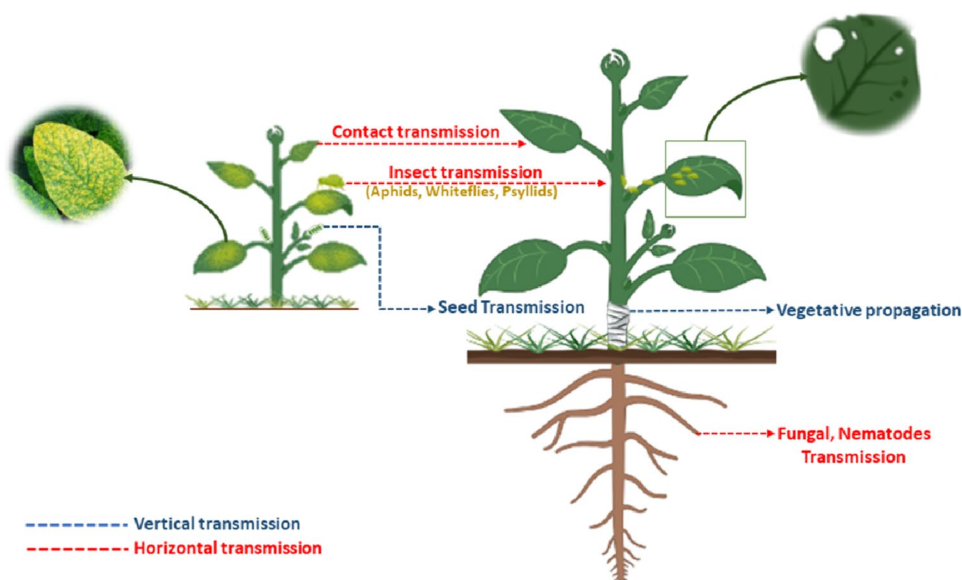

Fig. 1 Diagrammatic representation showing transmission of a plant virus from virus infected plant to an uninfected host plant; red dotted lines in the diagram represent the transmission of the virus horizontally, blue dotted lines represent vertical virus transmission

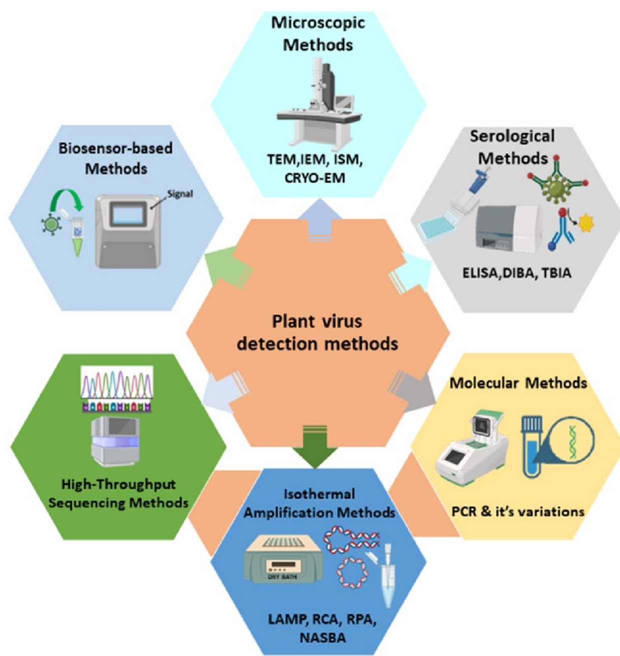

Fig. 2 Diagrammatic representation of various plant virus detection methods

consumption [81]. To overcome these problems, nucleic acid-based approaches are exploited worldwide as they are more rapid, accurate, and sensitive in detecting viruses [127]. The major advantage of practising techniques that are based on nucleic acid framework for the detection of plant viruses over several serological methods implies that molecular methods can detect virus at a very low titre or low virus concentration in comparison to serological ones i.e. proved in several instances for detection of plant viruses [17, 59, 151]. Many labs worldwide still use time-consuming and potentially clinically significant procedures like tissue culture for isolating viruses and serological techniques to establish the identification of the isolate [101].

According to the observation made from past decade, there has been a fair rise in the utilization of techniques based on molecules or nucleic acids. In 1990, molecular methods including polymerase chain reaction (PCR) were introduced to identify infection causing viruses in plants [163]. Since then, several variations of PCR and isothermal amplification techniques appear to be widely exploited for viral detection in plants. Real-time PCR provides better and more accurate results, hence remains superior and more responsive during diagnosis of particular plant viruses in comparison to serological procedures such as ELISA [37, 151]. To avoid substantial economic losses, it is imperative to evolve early and precise viral detection technologies for plants.

In this review article we have attempted to critically analyse and examine the present nucleic acid-based molecular approaches to detect various plant viruses, paying particular attention to instances where assays are required to be produced quickly, in situations like epidemics or pandemics. We have also discussed the advantages and disadvantages of the molecular techniques, which will help the readers to design and develop methods for the detection of several plant viruses. Supplementary Table 1 provides details of published reports that have used various nucleic acid-based molecular techniques to detect several plant viruses.

PCR and its variations

Conventional PCR

Polymerase chain reaction (PCR) is extensively utilized and most common molecular technique in virology owing to its high reliability, simplicity, and accuracy for the synthesis and target DNA amplification. Millions of copies of a certain DNA sequence may be generated using PCR in a single tiny reaction tube. [115]. A new cycle of DNA amplification begins when the denatured complementary strand of DNA has been annealed by pairs of oligonucleotides, often known as primers. DNA polymerase is subsequently directed to initiate DNA synthesis by the primers. Depending on the template, each response comes after the previous one. This procedure swiftly increases the size of specific DNA regions. Primers consist of two synthetic oligonucleotides, one that hybridizes to the complementary strand of double-stranded DNA (dsDNA) (3′→5′), and the another that binds to the other strand (5′→3′). DNA polymerase utilizes primers as substrates (a DNA-based enzyme) to bind and generate the complementary strand from the target DNA template. This is done by adding the deoxynucleotides (dNTP's) sequentially in a template-dependent manner [102, 139]. PCR involves three steps: firstly, denaturation, in which dsDNA strands get separated at 94 °C, and in the next step, primer annealing is there at 50–75 °C based on the primer melting temperature (T_m), followed by elongation or extension at 72 °C [72, 101, 102]. A programmable thermal cycler is used to set the temperature, the amount of time spent incubating at each temperature, and the number of cycles. DNA fragments that are quantified are then visualized by the help of agarose gel electrophoresis [102].

In the current scenario, PCR presents a common method for identifying plant viruses, especially DNA viruses such as genus *Babuvirus* (family—*Nanoviridae*) [152], genus *Begomovirus* (family—*Geminiviridae*) [66], and genus *Caulimovirus* (family—*Caulimoviridae)* [99]. There are sundry other variations of PCR technique like reverse transcription-PCR (RT-PCR), multiplex PCR, real-time PCR (qPCR), cooperational PCR (Co-PCR), and nested PCR i.e., utilized in phytopathology to detect several types of plant viruses of

different genera and families (Fig. 3). Table 1 summarizes the details of the different PCR techniques including the advantages and disadvantages of each method.

Reverse transcription-PCR (RT-PCR)

RT-PCR is an extremely sensitive and specific technique for the detection of RNA viruses (such as viruses from the *Bromoviridae*, *Betaflexiviridae* families etc.) [32, 92]. Reverse transcriptase, an RNA-dependent DNA polymerase enzyme, is added before the standard PCR step to produce complementary DNA (cDNA) from viral RNA, which enables targeted detection of RNA viruses by making use of specific primers [97, 169]. Viruses from virus-infected plant material and viral genetic material from a variety of other atmospheric mediums, including soil, fog, clouds, and streams can be detected with great precision using RT-PCR because of its extreme sensitivity [61]. In plant quarantine stations, RT-PCR was also utilized to detect RNA virus causing diseases for the purpose of enforcing quarantine regulations [89]. Since biological products cannot be stored at airports and harbours for lengthy periods of time and several samples should be analysed in parallel, it is crucial to achieve reliable and precise findings as rapidly as possible for quarantine purposes. This is why an RT-PCR based detection technique is preferable to others [88].

In recent times for identifying several RNA viruses, RT-PCR has been coupled with several other nucleic acid based techniques such as with real-Time PCR [37, 52], with nested PCR [39, 135], multiplex PCR [118, 125], as well as with LAMP [57] and RPA [56] for detection of plant RNA viruses in a timely, quick, precise, and sensitive manner.

Real-time PCR

Quantitative real-time PCR (qPCR) is another name for real-time PCR, which provides increased rapidity, specificity, sensitivity, reproducibility and quantitative measurement for early diagnosis of plant viruses along with a low risk of contamination due to minimal manual interaction with the PCR products [101, 135, 168]. This technique helps in the quantification and detection of target sequences in real-time reflected by fluorescent signals (proportionate to the amount of PCR product) using fluorescent probes (such as Molecular beacon, TaqMan) and dyes (such as SYBR Green, Eva Green) as well as primers (such as Scorpion primers, Sunrise primers) by labelling the amplicon during the amplification reaction [94, 101, 104, 168, 172]. Among the various dyes that have been extensively employed in real time PCR, SYBR Green is one of the simplest, cost effective, and most frequently utilized dsDNA-specific dye that fluoresces upon excitation after binding to dsDNA during the extension step and thus helps in quantifying the amplicon amount [145, 172]. The extent of fluorescence signal is directly related to the buildup of reaction products [127]. The dye binds to dsDNA's minor groove in a sequence-independent manner but not ssDNA's [172]. Though there are several advantages of using SYBR Green, such as there is no need to design a specialized probe and it is inexpensive and easy to use, still, there are some major drawbacks. These include low target specificity as well as the generation of false positive signals due to its ability to bind to any dsDNA present in the reaction, including primer-dimers [105, 127, 172]. Eva Green, a DNA-binding dye, is an alternative to SYBR Green which provides more sensitivity than SYBR green [104]. TaqMan Probes are the simplest and among the first to be put into practice and extensively exploited probes in qPCR [68]. These probes consist of oligonucleotides and contain a fluorescent reporter dye (such as Fluorescein amidite commonly known as FAM; 2′-chloro-7′-phenyl-1,4-dichloro-6-carboxy-fluorescein commonly known as carboxyfluorescein or VIC;) attached to the 5′ terminal. In contrast, the 3′ end is labelled with a covalently attached fluorescent quencher dye (e.g. TAMRA; 6-carboxy-tetramethyl-rhodamine) [68, 168]. TaqMan probes function by emitting fluorescence after the fluorescent reporter dye and quencher dye have been physically separated. This happens when the hybridized probe has been cleaved by the Taq DNA polymerase due to its 5′ exonuclease activity [73]. The main benefits of employing the TaqMan probe includes the easy design as well as multiplexing using distinct dye-labelled probes [105]. There are certain drawbacks, including reduced amplification efficiency due to a reduction in the temperature of strand extension, which is suboptimal for the *Taq* DNA polymerase activity [168]. In the qPCR technique, Molecular Beacon is another type of fluorescent hairpin oligonucleotide probe that is also labelled with a reporter fluorophore along with a non-fluorescent quencher dye (e.g., DABCYL; [4-(4′-dimethylamino) phenylazo] benzoic acid) on both ends [157]. The hydrolysing probes, i.e., TaqMan Probe and Molecular Beacon, depend upon fluorescence resonance energy transfer (FRET) for the quantitation [127]. Molecular Beacon forms a stem-loop structure when present in free solution, thereby bringing the reporter and quencher dye nearby, which emits no fluorescence [6, 157]. When hybridization of a molecular beacon with the target amplicon occurs during annealing phase, there is a change in the structural configuration. The reporter and quencher dye get separated, leading to the development of fluorescence [143, 157]. Sunrise primers (at the 5′ end consisting of a hairpin structure, labelled with a reporter fluorescent dye and a quencher dye) and Scorpion primers (like molecular beacon but act as a primer in amplification reaction) are also used in qPCR [172]. qPCR is more effective in higher concentrations of $MgCl_2$, primer, and dNTPs, whereas smaller amplicon size works better [97]. qPCR is rapid due to the reduced cycle time and absence of

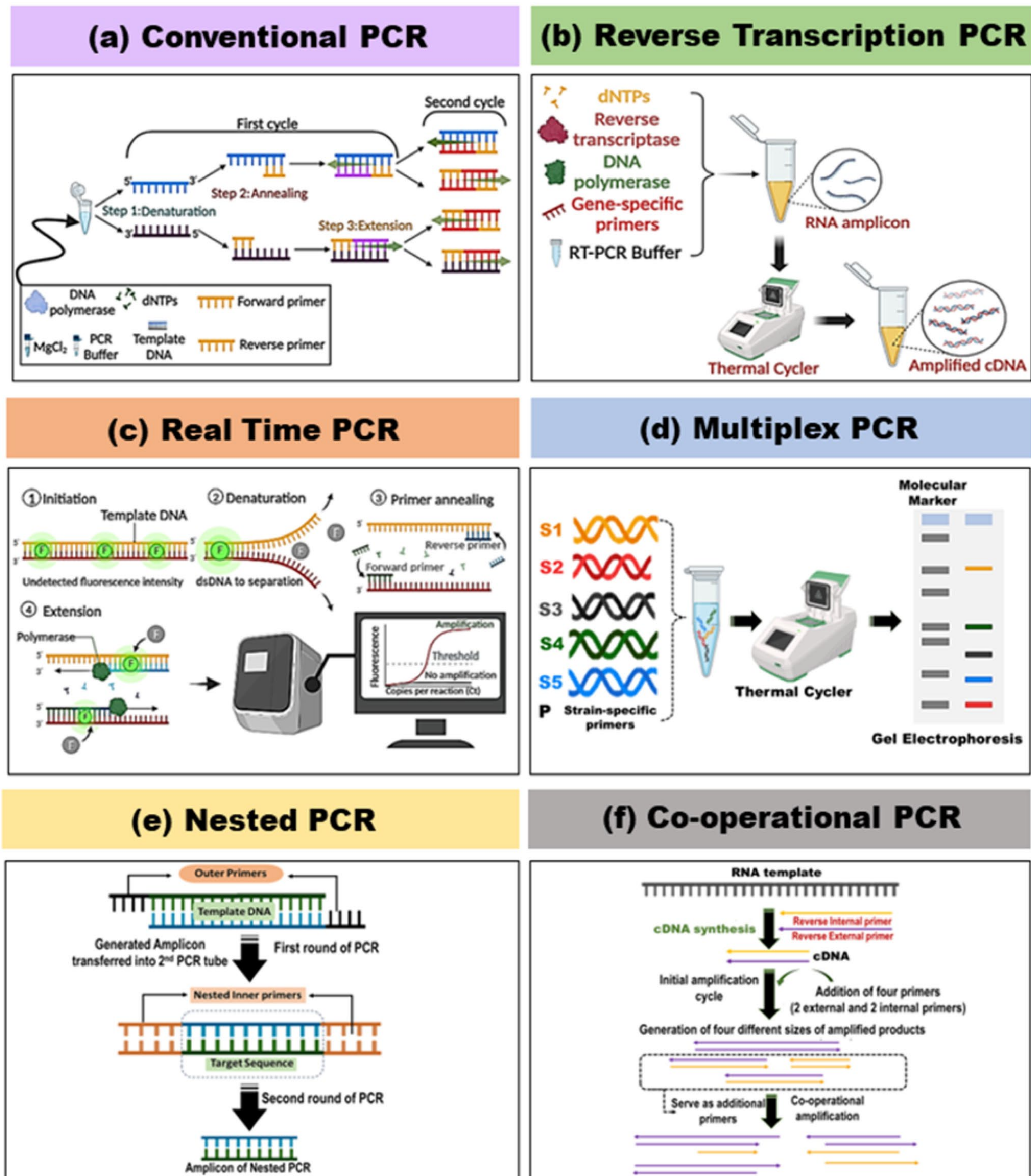

Fig. 3 Diagrammatic representation of various PCR amplification techniques; **a** conventional PCR amplification of dsDNA target, **b** amplification of RNA template using reverse transcription PCR (rt-PCR), **c** fluorescent dye-based real-time PCR (qPCR), **d** multiplex PCR using multiple DNA targets; S1–S5 signifies DNA of different strains, P signifies strain-specific primers, **e** nested PCR with target DNA template, **f** amplification of RNA target using co-operational PCR (Co-PCR)

Table 1 Details of different PCR techniques used for the detection of plant viruses

PCR technique	Target	Components	Advantages	Disadvantages	Product detection	Relative cost*	References
Conventional PCR	DNA	PCR buffer, $MgCl_2$, dNTP mix, Forward oligonucleotide primer, Reverse oligonucleotide primer, Thermostable DNA polymerase	Enables the targeted amplification of intricate genomes with simplicity and accuracy Molecular diagnostic technique with extensive application	Qualitative Time consuming and post-PCR analysis increase risk of carry over contamination	Gel electrophoresis	++	63, 129
Reverse transcriptase PCR	RNA	PCR buffer, dNTPs, [α-^{32}P] dCTP (optional), RT buffer, random hexamer primers ([dN]6) or oligo-dT(oligo(dT)15/oligo(dT)18, Dithiothreitol, Reverse transcriptase enzyme, Taq DNA polymerase, RNasin (Ribonuclease Inhibitor)	quick, precise and can easily be coupled with other PCR techniques as well as isothermal techniques for detection of RNA viruses	Qualitative Time consuming and post-PCR analysis increase risk of carry over contamination	Gel electrophoresis	+++	10, 37, 39, 56, 57, 136
Real-time PCR	DNA/RNA	Real-time PCR master mix (SYBR Green/TaqMan assays), Forward oligonucleotide primer & reverse oligonucleotide primer or Probe	Quantitative, Real-time monitoring of the viral load rapid due to the reduced cycle time and absence of post-PCR analysis Lower risk of carry over contamination due to minimal manual interaction	The cost of detection equipment and consumables is high No amplification occurs after a particular threshold Though amplicon amount can be monitored, amplicon size cannot	Real-time thermal cycler with an attached system	+++	6, 58, 62, 101, 129
Multiplex PCR	DNA/RNA	PCR buffer, $MgCl_2$, dNTP mix, Primer combinations (depending upon the number of targets), Thermostable DNA polymerase	cost-effective as it helps to reduce the cost of reagents by eliminating the concept of detecting the viral pathogens individually	a limitation in resolving the products of various sizes on the agarose gel sensitivity and effectiveness of the technique is strongly influenced by the number of primers used	Gel electrophoresis	+++	43, 46, 97, 124, 137, 169

Table 1 (continued)

PCR technique	Target	Components	Advantages	Disadvantages	Product detection	Relative cost*	References
Nested PCR	DNA/RNA	PCR buffer, $MgCl_2$, dNTP mix, Outer oligonucleotide primer 1, Outer oligonucleotide primer 2, Inner oligonucleotide primer 1, Inner oligonucleotide primer 2, Thermostable DNA polymerase	Able to carry out in low viral load or the extract contains DNA polymerase inhibitors When carrying out in single tube, using two sets of primers will not re-amplify any non-specific product in the second cycle because the latter primers only amplify the internal priming sites of the amplicon of interest, thus reducing the chance of false positive result	risk of contamination due to two successive rounds of amplification in two distinct tubes	Gel electrophoresis (agarose or polyacrylamide gel)	+ + +	64, 97, 105, 123, 171
Co-operational PCR	DNA/RNA	PCR buffer, dNTPs, [α-^{32}P] dCTP (optional), RT buffer, External Oligonucleotides primer, internal oligonucleotide primer, Dithiothreitol, Reverse transcriptase enzyme, Taq DNA polymerase, RNasin (Ribonuclease Inhibitor)	It can be done in a single tube in one reaction in less time using capillary air thermal cyclers or metal block, thus minimizing the risk of contamination	It can be used with dot blot hybridization to avoid the usage of mutagenic ethidium bromide (EtBr)	Gel electrophoresis, Colorimetric detection	+ + +	15, 16, 97, 106, 107

*Indicated by the number of '+' symbols with more number representing higher cost

post-PCR analysis [101]. Unlike conventional PCR, qPCR does not require agarose gel electrophoresis, colorimetric analysis, or hybridization to confirm the amplified DNA, thereby taking lesser time. The technique has certain drawbacks, viz., (1) no amplification occurs after a particular threshold [58], (2) the equipment and reagents used in qPCR are too expensive for routine detection of viruses [6], and (3) though it is possible to monitor the amount of amplicon but it is not possible to detect the size of the amplicon [101].

Multiplex PCR

In recent times, multiplex PCR and multiplex RT-PCR both have become more popular for recognition of DNA and RNA targets, respectively due to their rapidity and reliability. Using primers appropriate for each target, this method allows multiple identification of a variety of diseases in a single reaction [43, 46, 97, 124, 137, 169]. In the case of multiplex PCR, different lengths of amplicons are preferred to avoid cross-reactivity [169]. The most complex part of carrying out multiplex PCR is primer designing because all the primers need to be of similar melting temperature (T_m) to anneal under the same PCR condition, and there should be no formation of primer-dimers as well as hairpins [43, 97, 124, 146]. By designing the correct primers, all the members of a particular genus can be detected by multiplex PCR by targeting the conserved genomic regions [109]. Using universal primers to amplify various DNA or RNA targets, such as the ones based on 16S rRNA gene sequence, remains unacceptable as it will make it harder to detect the less abundant targets [97]. After the reaction, the amplicons are identified and analysed based on their size using either agarose gel electrophoresis or by using polyacrylamide gels [70, 124]. Recognition of three to nine viruses simultaneously in a single assay can be performed by multiplex PCR [15, 54, 86, 111, 130, 156, 178].

In several occasions it's proven that multiplex PCR was more rapid and effective than ELISA in identifying several plant viruses [27, 98]. However, multiplex PCR has not yet supplanted conventional PCR, most likely due to the technical difficulties associated with preparing a reaction mixture containing multiple suitable primers [97]. In addition, differentiating between DNA amplifications of different gene sizes is complicated by the need to build distinct primers for each target DNA [169].

Due to multiple targets identification at a time in a single reaction, the major advantage of multiplex PCR is it is quick and cost-effective as it helps to reduce the cost of reagents by eliminating the concept of detecting the viral pathogens individually [43, 124].

The two significant drawbacks of multiplex PCR are that the sensitivity and effectiveness of the technique is strongly influenced by the number of primers used because there is a ceiling on the number of targets that may be quantified at once, so the more primers are employed, the more likely it is that some of them will be able to work together and secondly, there is a limitation in resolving the products of various sizes on the agarose gel [124, 137].

Multiplex PCR coupled with other PCR techniques like real-time PCR, nested PCR or RT-PCR as well as with isothermal amplification methods like multiplex LAMP, RPA is most commonly utilized for the recognition of multiple viruses infecting plants.

Nested PCR

Nested PCR is performed in plant virus detection to enhance the precision and effectiveness of the amplification reaction, especially when there is a low viral load or the extract contains DNA polymerase inhibitors [169, 171]. During nested PCR, two sets of primers are used to perform two separate rounds of PCR amplification (two external and two internal) targeting the same locus [97, 105, 114]. In the first round, the first set of primers help in the generation and amplification of the target fragment that will function as a template strand for the next set of primers, which will amplify the inner sequence only after annealing to the specific internal priming sites of the initially generated amplicon [105, 171]. The advantage of using these two pairs of primers is that if the former primers amplified any non-specific product in the first round, they are not re-amplified in the second round, as the latter primers only amplify the internal priming sites of the amplicon of interest [105]. The primary flaw with this technique is the risk of contamination due to two successive rounds of amplification in two distinct tubes [97, 105, 171].

This method can be combined with reverse transcriptase PCR, and hence several plant viruses can be detected with the help of nested RT-PCR which also exhibits high sensitivity as well as specificity [3, 14, 39].

Co-operational PCR (Co-PCR)

Co-PCR is a technique devised by Olmos et al. [121] (Spanish Patent 31 October 2000; P20002613) that helps in the sensitive and rapid identification of RNA viruses [26, 121]. Co-PCR utilizes only one reaction tube for its operation using a simple tetra primer to produce four distinct amplicons [121]. Initiation reaction occurs with the reverse transcription of two specific regions of the targeted RNA molecule. One of these regions is contained in the other, producing four different sizes of amplified products, i.e., a large, a small, and two medium amplicons, after the initial amplification cycle. This is mainly due to the action of different primer combinations [121]. Two major advantages of using Co-PCR are; first, it can be done in a single tube in one reaction in less time using capillary air thermal cyclers,

thus minimizing the risk of contamination in comparison to nested PCR [97, 106, 121]. Secondly, Co-PCR can be performed in combination with dot blot hybridization and thus the usage of mutagenic ethidium bromide (EtBr) is avoided [15, 16, 107]. In contrast to nested-PCR, only one set of external and three sets of internal primers are required in Co-PCR [121]. Primarily, the presence of PCR inhibitors is the most difficult aspect of traditional PCR. Co-PCR utilising down-diluted samples can circumvent this problem. Co-PCR indicated a low-signal product in undilute samples but a stronger signal in diluted samples [121]. Recognition of certain plant viruses in a single closed tube like cherry leafroll virus (CLRV) detection, demonstrates that, Co-PCR has a sensitivity at least 100-fold higher than nested RT-PCR as well as RT-PCR [121, 122]. When compared to other detection methods, especially in RNA virus detection, Co-PCR proved to be much more effective, quicker, simpler, and cheaper [121].

Isothermal amplification and its variations

Recently, isothermal amplification approaches have also been employed extensively due to their ability to identify plant viruses effectively. It provides more sensitivity, rapidity, and cost-effectiveness than conventional PCR [176, 177]. Many benefits of employing isothermal amplification methods over conventional PCRs include that it does not require any skilled individual or any thermal cycler, which requires high temperatures to function correctly. Hence, it's easy to use an isothermal amplification reaction as it can be done under simple laboratory conditions in a single reaction temperature without help from a skilled individual [174, 176]. Several isothermal amplification techniques are employed in various applications. In this review, we discuss some of these techniques used to identify plant viruses: loop-mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), rolling circle amplification (RCA) and recombinase polymerase amplification (RPA). Table 2 provides a comparative analysis of the different isothermal amplification techniques.

Loop-mediated isothermal amplification (LAMP)

In 2000, Notomi and his colleagues developed a technique called loop-mediated isothermal amplification (LAMP); it employs tailored primer sets to initiate polymerase-driven elongation of a gene's sequence [120]. The primers help in producing stem-loop structures, which facilitate the amplification process. Quantification of sequence is carried out by using a strand-displacing polymerase. This technique can develop a billion copies of DNA from fewer copies of target DNA under isothermal conditions within an hour or less by using three specific sets of primers and a DNA polymerase, i.e., *Bacillus stearothermophilus* DNA polymerase or Bst-DNA polymerase [120, 126, 127]. LAMP utilizes a set of three specifically designed primers to recognize and bind to eight distinct genomic regions [127]. During the non-cyclic step, two outer primers (F3 and B3) help in displacing the strand in addition to two inner primers (FIP and BIP), which aids in forming loop as they both have sense and antisense sequences corresponding to target DNA, whereas the loop primers (FLP and BLP) help in the acceleration rate of the amplification reaction [120, 127, 154]. LAMP technique and target gene detection can be performed in a quick time and at a steady temperature of 63 °C simply by incubating three sets of primers, DNA polymerase, the reaction mixture containing betaine, Tris–HCl, $MgSO_4$, KCl, dNTPs, Tween-20 and $(NH_4)_2SO_4$ [127]. LAMP has recently been a widely used detection technique as the assay can be carried out with little to no expense or need for specialized people; hence, it is cost-effective. The significant advantage of using LAMP is that its result can be interpreted through the unaided eye as there is an increase in the turbidity of the solution indicating the quantification of the targeted genomic region [129]. When run on an agarose gel, the amplified result shows up clearly or by real-time monitoring through spectrophotometric analysis [127]. Various fluorescent dyes, for example, EtBr [179], Calcein [149, 154], SYBR green I [128], Pico green [40], propidium iodide [71], SYTO 9 [119] are widely used for on-field detection. Though it has multiple advantages over conventional PCR, LAMP frequently results in false positives as it relies on detection using turbidity and non-specific dyes [53]. RT-LAMP is used for detecting RNA viruses, where a reverse transcriptase besides Bst-DNA polymerase [127] is added to the reaction mixture. The LAMP technique is highly specific and can be used for SNP typing. In contrast to wild-type alleles, the mutant allele carrying an SNP doesn't undergo DNA synthesis and LAMP amplification cycling. If DNA synthesis occurs due to miscopy, the amplification is stopped, which results in minimal amplification or a delay in completing every step. This minimal amplification is attributed to an SNP in the target gene. Over the years, researchers have significantly improved and modified the LAMP technique. Two such variations of the LAMP technique are multiplex LAMP (mLAMP): which allows the detection of two pathogens simultaneously by using a combination of two sets of LAMP primers [77] and micro LAMP (μLAMP): integration of LAMP assay in a microfluidic chip [49]. Other modified LAMP techniques are also available which include LAMP in combination with the lateral flow assay, electric LAMP (eLAMP), and lyophilized LAMP [36].

Table 2 Details of different isothermal amplification techniques used for the detection of plant viruses

Iso-thermal technique	Target	Components	Temperature	Time	Advantages	Disadvantages	Product detection	Relative cost*	References
LAMP	DNA/RNA	Bst-DNA polymerase, Six specific primers, Tris–HCl, $MgSO_4$, KCl, dNTPs, Tween-20, $(NH_4)_2SO_4$, fluorescent dye (Optional)	60–65 °C	< 1 h	The result can be interpreted through the naked eye, helpful for on-field detection, capable of distinguishing between a single nucleotide variation and amplifying a certain gene	less sensitive in the detection of complex samples due to the presence of inhibitors, complex primer designing	Gel electrophoresis, Real-time, Visual observation (Turbidity/colour change)	+	18, 112, 113, 120, 138
NASBA	RNA	DNA-dependent RNA polymerase (DdRp) from the T7 virus, AMV reverse transcriptase, RNase H, two specific primers, NTPs, dNTPs	41 °C	1.5–2 h	Amplification reaction performed in very quick time i.e., within 90 min, No risk of contaminating DNA amplification	Although amplification is performed isothermally, a single melting step is required to anneal the primers to the target	Gel electrophoresis, molecular bacon in real-time, colorimetric detection using specific labelled probe	+ +	30, 34, 97, 158
RCA	DNA/RNA (Circular)	Φ29 DNA polymerase/ T7 RNA polymerase, set of primers, dNTPs	30–65 °C	1 h	Identifying unknown viruses without obtaining the virus's sequence in advance	Only capable of detecting viruses containing circular DNA	Gel electrophoresis, fluorescence spectroscopy, or microscopy, flow cytometry	+ +	7, 18, 19, 33, 51
RPA	DNA/RNA	Recombinase Protein (uvsX), single-stranded DNA binding proteins (SSB), strand displacing polymerase, crowding agent (e.g., polyethylene glycol), set of primers, dNTPs	37–42 °C	10–20 min	Quick and sensitive, low incubation time, short reaction time, does not require annealing temperature for primers	Costly, needed extra special care for visualizing products using agarose gel electrophoresis	Gel electrophoresis, probes, endpoint detection (lateral flow assay), real-time assay	+ + +	9, 18, 96, 132

*Indicated by the number of '+' symbols with more number representing higher cost

Nucleic acid sequence-based amplification (NASBA)

NASBA is a quick and efficient technique used to identify RNA targets under isothermal conditions using two specific primers along with three enzymes, i.e., T7 RNA polymerase (helps in the production of non-coding ssRNA, which is complementary to the target RNA), AMV (Avian myeloblastosis virus) reverse transcriptase (facilitates generation of cDNA through reverse transcription) and RNase H (helps in hydrolysing the RNA from the DNA-RNA hybrid complex) [30]. The major advantages of using NASBA are that it is possible to perform amplification reaction in isothermal conditions (41 °C) using a water bath [97, 106] which does not require much time for amplification, only 1.5–2 h is enough to carry out the amplification reaction [30]. Compared to PCR, NASBA requires fewer amplification cycles to generate a million copies of RNA [30]. The assessment of amplified NASBA products' can be done through the utilisation of gel electrophoresis, electrochemiluminescence, colorimetric assays, or employing internal specific probes such as a molecular beacon in real-time assays to identify amplified RNA [91, 97, 176].

Rolling circle amplification (RCA)

RCA technique is a form of enzymatic amplification used since the early 2000s, mainly for detecting plant viruses containing single-stranded circular DNA or RNA. It can also be used for the detection of the episomal form (circular) of dsDNA viruses such as Badnaviruses. The method makes use of a particular DNA polymerase, i.e., Φ29 DNA polymerase for ss-circular DNA viruses and T7 RNA polymerase for ss-circular RNA viruses [7, 76, 87, 95]. DNA polymerase obtained from the bacteriophage Phi29 is used due to several characteristics such as it shows 5′→3′ polymerization activity, 3′→5′ ssDNA proofreading and exonucleolytic activity [55]. It has high strand displacement activity, which helps generate the new template during amplification reaction through the displacement of complementary strand during DNA replication [19, 33, 82]. Some DNA polymerases such as Vent exo-DNA polymerase as well as Bst-DNA polymerase, can also be used in RCA to detect DNA viruses [7]. The four major components required to carry out the RCA reaction include a DNA polymerase (Φ29 DNA polymerase) along with a suitable buffer and short DNA or RNA primers followed by deoxynucleotide triphosphates (dNTPs) [65].

The basic concept of RCA demonstrates the formation of long ssDNA and RNA molecules from small circular ssDNA and RNA, respectively, using the corresponding polymerase under isothermal conditions (37 °C) using single or multiple primers [161]. Multiple repetitive sequences which are complementary in nature to the circular DNA template happen to exist in RCA products [7]. The product generated in the RCA process can be detected and visualized using several molecular approaches such as by using agarose gel electrophoresis or by using fluorescence spectroscopy, flow cytometry or microscopy after the incorporation of fluorescent dyes into the products via fluorophore-conjugated dNTP or hybridization of fluorophore-tethered complementary strands [7]. The primary benefit of utilizing RCA is that it uses random hexamer primers, which help to detect new viruses despite having no prior knowledge of the virus's sequence, by amplifying the circular template [18]. Other advantages of using RCA are that it doesn't require any thermal cycler like conventional PCR and doesn't require any thermostable DNA polymerase; thus, isothermal conditions are suitable for carrying out the reaction. It also works in biologically complex environments such as inside or on the cell surface [7]. Also, RCA is used to detect and differentiate episomal and endogenous Badnaviruses [79, 167].

Recombinase polymerase amplification (RPA)

The use of RPA is an additional tool for identifying plant virus, developed in the year 2006 (ASM Scientific Ltd.) and commercialized by Twist Dx Ltd. (Cambridge, UK) [132]. Compared to other isothermal amplification techniques mentioned earlier, RPA is a rapid detection method that can perform amplification reaction at a relatively low constant temperature and cost in a single tube without compromising specificity and sensitivity [80]. DNA and RNA viruses can both be detected with this approach. To amplify the target DNA/RNA, RPA utilizes single-stranded DNA-binding protein (T4 gp32), a recombinase (RecA from *Escherichia coli* or uvsX from T4-like bacteriophages) and strand displacing polymerase (DNA polymerase I from *Bacillus subtilis* or polymerase from *Staphylococcus aureus*) in the presence of explicit forward and reverse primers, dNTP's, ATP, cofactors (potassium acetate/magnesium acetate), recombinase loading factors (T4 uvsY protein) and crowding agents (Carbowax20M, a high molecular weight PEG) [93, 96, 132]. The RPA process begins with the hybridization of the recombinase-primer complex in the presence of a crowding agent (such as carbowax) and ATP, which further leads to the denaturation of the template DNA and formation of a D-loop structure by scanning the duplex DNA for homologous sequences [93, 96]. Separated strands are stabilized by utilizing single-stranded DNA-binding protein which aids in primer annealing to the template DNA [9, 93]. In the final step, the recombinase dissociates, allowing the strand displacing DNA polymerase to carry forward the amplification reaction at 37–42 °C. Due to the cyclic nature of the process, target sequence quantification is achieved [176]. For RNA viruses, the reaction mixture needs reverse transcriptase to generate the cDNA [9]. The primary benefit of RPA is that it doesn't require any thermocycler because the template

DNA denaturation, primer annealing, and elongation are enzyme-mediated [9, 18]. However, the method requires a PCR clean-up of RPA-generated amplicons before electrophoresis due to the presence of DNA-binding proteins, which increases the cost as well as assay time [9]. Through endpoint analysis RPA amplicon detection can be done: using agarose lateral flow assay, gel electrophoresis, bridge flocculation assay or colorimetric detection, and real-time: using fluorescent probes, fluorimeter, or ring-resonator technology [96]. In recent times, RPA coupled with lateral flow assays (LFA) has been employed to recognize certain RNA viruses [56, 78] and also few DNA viruses [25].

Microarray

Since the early 1990s, DNA microarrays have been used for speedy diagnosis of both common and rare plant viruses within a single species using the idea of nucleic acid hybridization [18, 137, 171]. The technique was developed initially for gene expression analysis, i.e., mRNA expression analysis [144]. However, this method has lately been implemented in a number of contexts, most notably in the detection of plant viruses. DNA microarrays may hold up to 30,000 individual probes for DNA samples. These probes hybridize to a target cDNA containing a fluorophore, silver or chemiluminescence label [161]. The microscopic spots are spotted on a rigid plane which can be glass slides, nylon membranes, and silicon wafers [171]. The basic steps to detect virus samples using the microarray technique are extracting and reverse transcription of viral nucleic acid and then labelling the probe during reverse transcription reaction with a fluorophore (fluorescein), silver, or dyes (Cy3, Cy5). After that, denaturation of the labelled target molecule occurs and then hybridization with the probes. Further, washing of slides after hybridization has been done to avoid cross-hybridization/ nonspecific hybridization or removing unhybridized cDNA on the array and then analysis, detection of target sequence due to the emission of fluorescence or chemiluminescence by bounded fluorescent-labelled target molecule [169].

In the DNA microarray technique, two basic categories of probes are mainly employed to design the arrays, i.e., cDNAs and oligonucleotides [169]. For a successful detection using microarray, careful design of the probe, which includes the length of the probe, melting temperature (T_m), GC content, and the secondary structure is crucial [137]. Three types of DNA microarray platforms are available commercially, i.e., glass DNA microarray—prefabrication of cDNA fragment on glass slide using micro spotting, nylon membranes where PCR fragments are arrayed and in-situ oligonucleotide synthesis by using "chip" or high-density oligonucleotide microarrays [22, 97]. The first potato viruses were discovered and identified in 2002 by using DNA microarray [23]. Multiple potyviruses (strain and species level) were detected using an oligonucleotide microarray [170]. Ten tomato viruses were detected using a fluorescent-based microarray system by developing a tomato virus chip (Combimatrix) [153]. In 2010, a new oligonucleotide-based microarray was designed using an automated probe to detect 169 plant viruses from 13 genera [175]. Fifteen grapevine viruses were detected by a single-colour microarray hybridization system using oligonucleotides ranging in length from 27 to 75 nucleotides [1]. In addition to plant virus diagnosis, microarray technique has been utilized for phylogenetic or taxonomic analysis, for instance, differentiation and detection of cucumber mosaic virus (CMV) serogroups and subgroups [35], genotyping of plum pox virus (PPV) strains along with several (44 different) grapevine viruses [47, 131]. The advantages of using the DNA microarray technique include rapidity, reproducibility, and specificity in terms of detection. The drawbacks include a lack of flexibility and the high cost of designing microarray chips and probes. DNA microarray has the advantage that it may be programmed to identify any virus whose genome sequences are stored in a database [161]. Table 3 summarizes the details of DNA microarray technique used for detection of plant viruses.

Next-generation sequencing (NGS)

The development of DNA sequencing evolved as a revolution in virus detection, discovery and diagnosis. In 1977, Frederick Sanger invented a rapid DNA sequencing technology using a method known as chain termination [140]. At the same time, Maxam and Gilbert developed the DNA sequencing method using the chemical degradation method [108]. These methods also known as first-generation sequencing methods were dominant till the mid-2000s until the emergence of NGS [41]. In 2009, NGS was used for the first time to identify viruses infecting plants using RNA and small RNA as an input for sequencing [2, 4, 85]. In order to spot potential viruses, it's important to analyse the sequence data, then create the contigs and then scan the genomes using BLAST search against databases of plant viruses [18]. In recent times, metagenomics is evolving for the purpose of virus detection where the total genetic material (i.e., DNA/RNA) of infected plants are isolated and sequenced on NGS systems followed by viral sequence analysis [109]. NGS is classified into two distinct categories, i.e., second-generation sequencing and third-generation sequencing [109]. In second-generation sequencing, DNA is first used to create random libraries of DNA fragments, or RNA undergoes reverse-transcription into cDNA by making use of random primers or oligo (dT). A vast number of

Table 3 Details of microarray and NGS methods used for the detection of plant viruses

Technique	Types	Advantages	Disadvantages	Relative cost*	References
DNA microarray	1. Spotted Glass DNA microarrays (glass microscope slides with Poly-lysine coated) 2. High-density oligonucleotide In-situ, Synthesized arrays (on chip, Affymetrix technology, inkjet printing technology etc.) Self-assembled arrays	Ability to detect multiple pathogens concurrently Can be programmed to identify any virus whose genome sequences are stored in a database	Lack of flexibility and the high cost of designing microarray chips and probes	+ + +	22, 24, 69, 97, 161
Sequencing	**1. First-generation sequencing:** *Sanger sequencing* (long reads, up to 1000 bp though have a limited throughput capacity) **2. Second-generation sequencing:** Sequencing by hybridization Sequencing by synthesis *454 pyrosequencing (discontinued), Illumina and Ion-Torrent* (short reads, 100–300 bp but have huge throughput) **3. Third-generation sequencing:** *PacBio or Singe Molecule Real Time (*SMRT*)* (long fragments, up to 30–50 kb) **4. Fourth-generation sequencing:** *Nanopore* (ultra-long reads at high throughput while sequencing single molecules)	Efficiently identify virus and viroid sequences cost-effectively from the given sample of interest without any prior knowledge of the sequence investigation of intra as well as inter-host-virus variability and virus-host interactions, with the emergence of new viral pathogens being discovered	Low sensitivity due to the relatively small size of the viral genome viral genome variability leading to failure of resequencing protocols and presence of host genome sequences in the viral genome making it necessary to sequence the whole genome	+ + +	5, 13, 147

*Indicated by the number of '+' symbols with more number representing higher cost

short sequences are generated by repeatedly amplifying these libraries by cloning, attaching them to synthetic DNA adapters, and sequencing them. Third-generation sequencing eliminates the need for clonal amplification and allows for the sequencing of individual molecules in real-time, which speeds up the DNA-preparation process and yields lengthy reads of several kilobases [60, 67, 137, 160, 162]. Multiple sequencing platforms have now been developed and are commercially available such as Illumina sequencing systems (Illumina), Roche 454 pyrosequencing (Roche Diagnostics), Ion Torrent (Life Technologies) and SOLiD system (Life Technologies), Polonator (Dr. George Church's laboratory, through Azco Biotech) [13, 44]. The sequencing data generated has played a crucial part in phytopathology especially for plant virologists in several aspects such as diagnosis of viral infections and drug resistance mechanisms including molecular epidemiology of plant pathogenic viruses [13]. The key advantages of NGS are its potential to efficiently identify virus and viroid sequences cost-effectively from the given sample of interest without any prior knowledge of the sequence, investigation of intra as well as inter host-virus variability and virus-host interactions, with the emergence of new viral pathogens being discovered [5, 13]. Though there are several advantages of NGS over conventional molecular techniques in plant pathology, it has certain pitfalls which include low sensitivity due to the relatively small size of the viral genome, failure of resequencing protocols due to genome variability and presence of host genome sequences in the viral genome making it necessary to sequence the whole genome [13]. In Kenya, it was initially reported that viruses responsible for causing papaya ringspot disease in papaya were identified as cowpea mild mottle virus and cucumber vein-clearing virus from *Betaflexiviridae* family (Genus *Carlavirus*), moroccan watermelon mosaic virus from *Potyviridae* family (Genus *Potyvirus*), along with papaya mottle-associated virus from *Betaflexiviridae* family (Genus *Carlavirus*). These viruses were sequenced using Illumina MiSeq sequencing [116]. Several viruses infecting tobacco mostly of genus *Potyvirus* from the family *Potyviridae*, and two viruses of genus *Tobamovirus* from the family *Virgaviridae*, were detected using Illumina HiSeq sequencing [3]. Various members of the genus *Ilarvirus* from family *Bromoviridae* infecting prunus trees were also detected using Illumina MiSeq [84]. Several other plant viruses of different families were detected using NGS mostly Illumina MiSeq and Illumina HiSeq platforms [11, 48, 84, 103, 117, 164, 166]. Several plant viruses may now be detected using whole-genome sequencing with the help of the Oxford Nanopore MinION. Table 3 summarizes the details of sequencing techniques used for detection of plant viruses.

Discussion

In order to identify plant viruses, Morris and Dodds (1979) have employed nucleic acid-based approaches targeting viral DNA or RNA fragments [38]. The nucleic acid-based PCR technique developed by Mullis et al. [115] revolutionized plant virus diagnostics. PCR may increase the concentration of a single DNA strand by as much as 10^9-folds in just two hours [123]. As a result, the sensitivity and efficiency of viral identification are dramatically improved. There have been a number of variants and extensions to PCR, such as RT-PCR, quantitative PCR, multiplex PCR, etc., which have become popular in identifying viruses infecting plants. Attributed to rapid degradation of RNA in ambient conditions, and the fact that viruses infecting plants are mostly RNA viruses [134], reverse transcription is usually used to transform fragile RNA into complementary DNA which is comparatively more stable and then subsequently amplified using PCR. Over time, RT-PCR has recently advanced as the most preferred approach for detecting viral infections in plants due to its capacity to identify viruses in low quantities [110, 142]. A quantitative PCR can be used to determine the titre level of a virus in a sample by measuring the quantity of DNA remaining in given sample after each round of PCR amplification [150]. A promising approach, loop-mediated isothermal amplification (LAMP) was established by Notomi et al. [120]. In contrast to conventional PCR methods, LAMP is capable of amplifying DNA without the need for a high-precision thermocycler. Due to its ease, speed, affordability, and availability, it has gained popularity in identifying plant viruses. To effectively identify plant viruses, nucleic acid based approaches have one key benefit over several serological methods i.e. the methods can detect virus at a very low titre or low virus concentration in comparison to serological ones and this has been proved in several instances for detection of plant viruses [17, 59, 151].

In the last decade, several new nucleic acid-based methods have been used for investigating and identifying plant viruses. With an aim to efficiently sequence the entire viral genome, high-throughput sequencing method such as next-generation sequencing (NGS) proves to be an effective technique [12, 21]. NGS has assisted in the discovery of novel viruses and new hosts for existing viruses which offers an exhaustive framework for identifying and researching viruses that infect plants [20, 173].

Cross-contamination can be avoided with these techniques, but carefully collecting plant tissues and processing the samples takes a lot of time and effort. Several detection methods also need high-end machinery and pricey supplies [31]. These costs make testing many plants for viruses impractical, making it impossible to get

statistically valid samples from large-scale industrial production farms. Instead, Luo et al. [100] suggested sampling a small number of plants at random, in a traditional field pattern such as an X or W pattern, or strategically based on a visual valuation of the field's disease state. The risk of hit-or-miss outcomes due to an insufficient test rate is unacceptable in mission-critical sectors like nurseries [100].

Diagnosing plant viral infections has come a long way, but new technology could overcome many challenges. Plant viruses have been better understood because of the advent of molecular diagnostic techniques, but identifying the plant phenotypes brought on by viral infections is still difficult because of the intricate connections between viruses, environmental variables, and host genomes. Symptoms are frequently not evenly distributed throughout the plant [74]. Some virus strains cause no visible symptoms in infected plants, whereas others cause rapid decline [173]. It's possible that viruses infecting a plant won't always make it sick. Although there haven't been many studies on the persistence of viruses in the host, it has been observed that certain infected plants can recover. Disease detection is difficult because of the complexity of plant virus infections. Moreover, interactions between the virus, the host plant and the environment can lead to a vast range of symptoms in viral diseases. Co-infection in plants makes it harder to identify viruses. Symptoms of viral diseases can be easily misdiagnosed as those of other pathogens [28], such as fungi, bacteria, nematodes, or viroids, or as the result of abiotic stresses, such as those caused by insufficient levels of essential nutrients (such as phosphorus or potassium) or an excess of water. Air and soil temperatures, soil types, and edge effects are all environmental factors that must be taken into account. Plants may exhibit similar stress responses to the virus if subjected to mechanical or chemical harm, such as herbicide injury. These intricate permutations may impede reliable viral identification. Therefore, proper viral disease identification requires in-depth knowledge of plant health and persistent monitoring across time. Access to reliable ground truth data over many time periods, disease intensities, and geographical locations is crucial for developing an accurate viral disease prediction model using optical sensing technologies. It is nevertheless challenging and laborious to gather massive amounts of data. Ground truth data for model training can be easily gathered using methods like visual evaluation. Some diseased plants may not show any outward signs of infection. Hence chances of potential false negatives may occur with this approach. Despite the fact that groundtruthing relies on accurate and consistent laboratory testing processes, their prohibitive price points mean that only a small amount of ground-truth data can be collected, thus weakening the model. However, no diagnostic technique can ensure a 100% success rate. In the work of Pietersen and Harris [133], for instance, RT-PCR results are unpromising in recognizing GLRaV-3 in Richter 99 (*V. berlandieri*, *V. rupestris*), a grapevine rootstock.

Conclusion and future perspectives

Serological as well as nucleic acid-based techniques have both been used for detection of plant viruses. Detection using nucleic acid-based techniques manifests better specificity over the serological ones. Its downside include the need for specially trained personnel, potentially significant costs, and problems with result analysis brought on by carryover contamination. Antibody-based detection methods as well as immunoassays, on the other hand, are typically more robust, inexpensive, and user-friendly but they lack specificity and sensitivity in detecting plant viruses, thus cannot usually match the sensitivity of techniques used to detect nucleic acids. Therefore, whether or not to use nucleic acid detection or immunoassay approaches will depend heavily on the requirements of the assay. The efficient and precise requirements of the assay, the assay's cost, the urgency of the diagnosis (for instance, in the event of emergent strains that are creating an epidemic or pandemic), and the setting in which the assay will be performed. Compared to conventional detection techniques, molecular techniques provide several advantages, including improved sensitivity, specificity, and speedy detection. There are benefits and drawbacks to each of these methods, however, still they have the potential to revolutionize plant virus diagnostics by providing solid instruments for quick and efficient detection of viral infections observed in plants. In addition, these techniques can be utilized to study viral populations, genetic diversity, and evolution, providing valuable information about the mechanisms behind viral pathogenicity and adaptation. The biotechnological techniques for detecting plant viruses are anticipated to advance and change in the years to come in terms of future scope. Developments in technology and bioinformatics are probably driving the development of increasingly more precise and sensitive detection techniques. Furthermore, the accuracy and speed of virus identification and characterization will likely be improved by incorporating machine learning and artificial intelligence in the data processing.

To increase the accuracy of disease prediction, cutting-edge data processing methods and high-performance computers could be used. There is a need for the development of detection technologies that differ in price and accuracy due to the fact that newly evolving viruses and their variants present additional risks. The availability of NGS will improve the utility of immunoassays and other techniques for detecting nucleic acids. The fast expansion of NGS's use is being fuelled by researchers' efforts to learn more about the wide

range of viral genomes and the roles they play, which in turn is increasing the number of assays to facilitate virus detection. Molecular techniques for plant virus detection continue to advance, offering rapid, sensitive, and targeted approaches for virus characterization. These techniques have the potential to dramatically advance virus-resistant plant breeding and the diagnosis of plant viruses, ultimately enhancing global food production. With a promising future, these techniques are expected to expand their applications beyond diagnostics, encompassing various aspects of plant biotechnology.

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Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Conflict of interests The authors declare that they have no conflict of interest.

Ethical approval This study being a systematic review does not require any ethical approval.

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