Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2019; 8(3): 3046-3051
Received: 04-03-2019
Accepted: 06-04-2019

**Rajamanickam S**
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

**Rajesh Kumar P**
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

**Karthikeyan G**
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

**Correspondence**
**Rajamanickam S**
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

# A potential tool for the detection of plant viruses through serological and molecular approaches

**Rajamanickam S, Rajesh Kumar P and Karthikeyan G**

**Abstract**
Plant viruses cause major losses to several agricultural and horticultural crops around the world. In contrast to other plant pathogens, there are no immediate strategies accessible yet to control infections and subsequently, the current measures rely on indirect strategy to deal with the plant viral diseases. Therefore, three strategies for detection and identification of viruses in plants. One of the strategies is physical perception by expertise and microscopic methods able to verify shape and size of viral particles. Second strategy is based on the viral coat proteins. These are agglutination tests, precipitation test, Enzyme-linked immunosorbent Assays (ELISA) and immunoblotting. In this strategy of detection of virus coat protein, precipitation methods are more precise and sensitive. The third method is nucleic acid based methods, so they are nucleic acid hybridization, PCR, RT-PCR, Real Time PCR, IC-PCR, Nano Cips, DNA microassay and Multiplex PCR. Nucleic acid based detection like DNA microarray and nano chip are more sensitive and accurate techniques to detection of the viral particles than other methods. Availability of these detection strategies provides greater flexibility, sensitivity and specificity for rapid detection of virus, epidemiological studies, plant quarantine, seed certification and breeding programs. Hence, these detection strategies to improve knowledge and strengthen skills for virus diagnosis of plant virology are essential for plant disease management.

**Keywords:** Plant viruses, serological, molecular approaches

## Introduction
Plant viruses cause severe constraints on the productivity of a large vary of economically essential crops worldwide. Symptomatic techniques for viruses fall into two general classifications: biological properties identified with the association of the infection with its host and vector (e.g., symptomatology and transmission tests) and fundamental properties of the virus itself (coat protein and nucleic acid). Detection strategies based on coat protein include precipitation/agglutination tests, enzyme-linked immunosorbent assays and immunoblotting. Viral nucleic acid-based techniques like dot-blot hybridization assays and polymerase chain reaction are more sensitive than alternative strategies. Current advances in biotechnology and molecular biological science have contend a major role within the development of quick, specific and sensitive diagnostic tests (Makkouk and Kumari, 2006) [25]. Even though wide used for virus detection, serological strategies have bound disadvantages. They are based on the antigenic properties of the virus coat protein that represents just about 10% of the total virus genome and therefore does not take into account the rest of the virus genome (Gould and Symons, 1983) [12]. There are situations where immunological procedures have limited application in particular for the detection of viroids, satellite RNAs, viruses which lack particles viruses which occur as extremely diverse serotypes and viruses that are poor immunogens or difficult to purify. Subsequently nucleic acid-based diagnostic assays may be the methods of choice. Nucleic acid-based virus detection systems make use of cloned DNA probes in a dot-blot assay or specifically designed primers in a polymerase chain reaction (PCR) test. The following methods are used to detect the plant viruses.

## Detection based on biological properties
**Microscope based detection -** Transmission electron microscope is better for virological studies than a scanning type which is used only occasionally. Contrast of the virus specimen increases easily by shadow casting with platinum + iridium, gold or uranium vapors deposited form a particular angle (Williams and Wyckoff, 1944) [35], but the resolution of the structure is limited to the size of particles of metal vapour and hence at present this is not a method much in vogue with the virologists (Gibbs and Harrison, 1976) [11]. Negative staining of virus particles involves use of electron dense stains like sodium phosphotungstate, ammonium molybdate, sodium silcotungstate, uranyl acetate or uranyl formate, etc.

This helps in resolving the particles outline as well as all the interstices up to which the stain equally well with all these stains yet sodium phosphotungstate is the commonly used one while uranyl formate results in best resolution of structure of the anisometric virions. A quick diagnostic method of whitefly transmitting *Tomato yellow leaf curl virus* is to search for virus-induced nuclear inclusions using a light microscope (Christie *et al*., 1986) [7].

**Symptomatology**

Plant disease diagnosis is the identification of nature and causative of diseases or disorders based on the signs and symptoms. Identification of symptoms, signs and comparative symptomatologies of infectious and non infectious diseases are considered to be most essential for diagnosis of unknown plant disease. The viral diseases can be diagnosed quickly by visual examination of symptoms, quite simple when symptoms clearly are characteristic of a particular disease. Though, several factors like host plant, virus strain, time of infection, and the environment can influence the symptoms exhibited (Matthews, 1980) [25]. Virus could cause diseases in crop plants because of utilization of cellular substances during its multiplication inside the cells, taking up space in cells and finally due to disruption of cellular process. Single virus can affect or more different species of plants and even each plant species are affected by different kind of viruses. In case of *Pepper mottle virus* is member of the large and complex genus Potyvirus in pepper transmitted by *Aphis crocivora***,** *A. gossypii* and *Myzus persicae*. The *Pepper mottle virus* produced severe mosaic, malformation, crinkle, stunting, distortion with small fruits and mild mosaic on fruits and leaves (Khattab, 2006) [20]. The symptoms like yellow green mosaic, curling of leaf lamina and stunting of whole plant of cucumber (*C. sativus*), yellow mosaic in pumpkin (*C. maxima*), yellow spots on newly emerged leaves, mosaic, mild leaf curling and distortion on sponge gourd (*L. cylindrica*), yellow mosaic on pointed gourd (*Trichosanthes dioica*), upward curling, distortion symptoms on bitter gourd (*M. charantia*) and yellow mosaic on ridged gourd (*L. acutangula*) have been commonly observed in India (Raj *et al*., 2002) [30]. *Squash leaf curl virus* cause vein clearing symptoms in the tertiary veins of the younger leaves of pumpkin plants to prominent vein yellowing in secondary and primary veins as finally coalesced to a yellow (Saritha *et al*., 2011; Bandaranayake *et al*., 2014) [32, 4]. Sivaprasad *et al*. (2010) [34] reported that the symptoms of *Tobacco steak virus* (TSV) infected onion produced straw coloured, irregular necrotic lesions on younger leaves and flower stalks. Ladhalakshmi *et al.* (2006) [23] reported the occurrence of TSV for the first time in blackgram (*Vigna mungo*) in the majority of the blackgram growing areas of India. Symptoms of the disease consisted of brown necrotic lesions on young leaves, with brown streaks on petioles and stems, with death of plant in severe cases. Jain *et al*. (2005) [18] reported the occurrence of TSV for the first time in chillies (*Capsicum annuum*), which caused symptoms of necrosis of leaves and buds.

**Transmission tests**

Virus detection and identity technique strategies were grafting, mechanical and insect vector transmission of the plant viruses to susceptible indicator plants (Jones, 1993) [19]. Mechanical transmission by way of sap inoculation to herbaceous indicator plants can be performed with minimum facilities and characteristic symptoms produced via these plants allow each the detection and identity of many viruses. The *Capsicum chlorosis virus* isolates, from chilli and tomato were used to study biological host range. Twenty-five plant species belonging to six different families were mechanically inoculated. The virus isolates from tomato and chilli produced chlorotic lesions in *Vigna unguiculata* at 3–4 dpi and systemic mosaic symptoms in *Nicotiana benthamiana* at 6–7 dpi, which subsequently led to necrosis (Kunkalikar *et al*., 2010) [21]. The *Chilli veinal mottle virus* (ChiVMV), was successfully transmitted by *Aphis gossypii* (Glov.) to its natural host chilli pepper. The plants induced typical systemic vein mottling symptoms. The sap inoculums were prepared from *Chilli veinal mottle Potyvirus* (ChiVMV) infected plant showing mottling symptom and sap inoculated on indicator hosts. Among host plants tested, 5 different plant species *Nicotiana tabacum cv. Samsun, Nicotiana glutinosa, Nicotiana occidentalis, Chenopodium quinoa, Solanum nigrum, Datura metel and Physalis floridana* induced characteristic systemic mottling symptoms within 7 to 14 days of inoculation. The rest of the hosts remained asymptomatic and were DAS-ELISA (Hussain *et al*., 2008) [16]. Ladhalakshmi *et al.* (2006) [23] reported that the mechanical inoculation of plant sap from TSV infected blackgram onto various plant species *viz.*, *Vigna unguiculata* cv. 152, *Nicotiana tabacum*, *Chenopodium amaranticolor* and *Gomphrena globosa* resulted in necrotic lesions on the leaves and streaks on stems. Ali *et al*. (2009) [3] reported that, the extracts from TSV infected bean leaves were mechanically inoculated on to *Nicotiana benthamiana* and *Chenopodium quinoa* and found to be suitable propagation hosts for TSV.

**Purification of virus**

Purification is the process of separating the virus particles from host constituents and other chemicals present in sap. The purified virus must be free from contaminating cellular host components and must be obtained in a biologically active state. Purified virus is essential for basic characterization, identification, studying many physical and biochemical properties of viruses and also for production of antiserum which is used for serological studies. For virus purification the plant tissue is crushed to extract the virus containing sap. Most of the larger constituent of sap such as chloroplast, mitochondria, starch grains, fragments of cell walls sediments quickly and can be removed by brief low speed centrifugation. Sap constituent such as plant protein, ribosomes and microsomes resemble virus particles in size, composition and stability are the most troublesome host materials removed when purifying virus. Several steps are involved in virus purification and to follow the progress of purification, it is useful to assay the fraction for infectivity. General principles for purification of viruses are propagation of virus in choice of host plant, preparation of crude sap extract of virus, use of stabilizing agent to prevent the inactivation of virus during extraction, clarification, concentration of final preparation and testing for purity.

**Serological assay**

Serological or immunological assays have been developed and utilized effectively for some of years for the detection of plant viruses. These tests are extensively subdivided into liquid and solid phase tests. In the previous, both antigen and antibody react in solution to form a visible precipitate (precipitin or microprecipitin tests, gel diffusion assays) and agglutination of cells. In the later, assays are conducted on a strong surface together with on a microtitre plate or nitrocellulose membrane and the antigen–antibody reaction is

visualized with the aid of a appropriate detection device including an enzyme-labelled antibody.

**Enzyme-linked immunosorbent assay**

The enzyme-linked immunosorbent assay (ELISA) has been prominent for identification of viruses in plant material and vegetative propagules since it had been introduced to plant virology by Clark and Adams (1977) [8]. *Potato virus* Y from Potato was detected in greenhouse and field grown seven week old potato plants by local lesion host *Solanum demissum* and by way of ELISA tests. The virus was detected in 3-4 weeks by mechanical inoculation of local lesion hosts in greenhouse grown plants and in 4-5 weeks in field-grown plants. However, PVY was detected in these same plants by ELISA one week later. Virus concentration varied in plants inoculated at different leaf positions, reached a peak, then slowly declined. Smaller tubers (less than 30 g, and 30-60 g**)** from primarily infected field-grown plants gave rise to 'symptomless' plants, which had been now not identified by visual observation, at the same time as they have been diagnosed to be PVY infected by ELSA (Singh and Rojas, 1983) [33]. Extracts of the *Squash leaf curl Yunnan virus* infected Y23 squash leaves were tested by TAS-ELISA with MAbs raised to ACMV, ICMV and OLCV particles. The pattern of antigenic reaction of Y23 was readily differentiated with 14 MAbs in TAS-ELISA differed from the patterns reported for four already reported Chinese begomoviruses *viz*., TLCYNV, TCSV, TYLCCNV and *Tobacco leaf curl China virus* (TLCCNV) (Xie and Zhou, 2003) [36].

**Immunoblotting**

To detection of viruses in each plants and insect vectors were used by Dot immunoblotting assay (DIBA) (Makkouk *et al*., 1993) [26]. Dot immunobinding assay (DIBA) is a simple dot blot assay where antigen suspensions (1-5 µl each) are dotted on the nitrocellulose or nylon membranes and dried. After saturation of free protein binding sites with a blocking agent, the membrane is incubated successively with primary antibodies, labeled secondary antibodies and a substrate. The advantages of this technique are that it requires small amount of antigens over standard ELISA. It is cheaper, less time consuming and equally sensitive. The technique is similar to ELISA except that the plant extracts are spotted on to a membrane rather than using a microtitre plate as the solid support matrix. Unlike in ELISA, where a soluble substrate is used for colour development, a precipitating (chromogenic) substrate is used for virus detection in the DIBA. Hydrolysis of chromogenic substrates results in a visible coloured precipitate at the reaction site on the membrane.

**Detection based on virus nucleic acid**

Nucleic acid-based virus detection systems make use of cloned DNA probes in a dot-blot assay or specifically designed primers in a polymerase chain reaction (PCR) test. Both approaches have the potential to detect single nucleotide differences (Lopez, *et al*., 2003) [24]. These approaches will be summarized in the following paragraphs. There are situations where immunological procedures have limited application in particular for the detection of viroids, satellite RNAs, viruses which lack particles viruses which occur as extremely diverse serotypes and viruses that are poor immunogens or difficult to purify. Therefore nucleic acid –based diagonistic assays can be the strategies of choice. Nucleic acid-based virus detection systems make use of cloned DNA probes in a dot-blot assay or specifically designed primers in a polymerase chain reaction (PCR) test (Makkouk and Kumari, 2006) [25].

**Dot-blot assay**

This development in nucleic acid hybridization technology offers a good potential for virus detection. The target viral nucleic acid from a plant sample is spotted onto a solid matrix, commonly nylon or nitrocellulose membranes, and bound by baking. Free binding sites on the membrane are blocked with a nonhomologous DNA and a protein source. Thereafter, hybridization with a labeled probe is carried out. The label is then detected by autoradiography (for radioactive probes), or by a colorimetric reaction if an enzyme label is used. The Complementary DNA (cDNA) clones and particular to any region of the viral genome, are usually used as a probe to locate virus in plant extracts. To provide cDNA clones, the viral RNA is normally transformed to dsDNA (double stranded DNA) and cloned into suitable vectors (Sambrook *et al*. 1989) [31]. The sensitivity of dot-blot hybridization is about the same as ELISA. A modification of the dot-blot assay, squash blotting, has been used to detect some viruses. Recently Hsu *et al*. (2005) [14] developed modified hybridization technique namely, reverse dot blot hybridization, for rapid detection and identification of six Potyviruses. In this techniques the cDNA probe synthesised by RT-PCR with specific primers were immobilised on to nylon membrane and then hybridized with DIG labeled RT-PCR product amplified by Potyvirus specific degenerate primers.

**Lateral flow immuno assay**

Immunochromatographic measures (ICAs) fulfil these prerequisites to the greatest extent. ICA is performed utilizing test strips, multimembrane composites onto which explicit reagents have been pre-connected. Contact of the test strip with an example starts development along the layers of its fluid parts and immunoreagents, including a coloured marker conjugated with antibodies. After this development (in 10– 15 min), explicit resistant complexes are shaped on various parts of the test strip, which can be basically distinguished by the colour of the linked label. The above-portrayed highlights of ICA consider the testing of non-research center conditions in a brief timeframe with insignificant example readiness, without the utilization of extra reagents and gadgets, and with a straightforward identification and understanding of the obtained results. (Huang *et al*., 2016) [17].

**Polymerase Chain Reaction (PCR)**

The Polymerase Chain Reaction (PCR) has been used as the new standard for detecting a wide variety of templates across a range of scientific disciplines, including virology. The technique employs a pair of synthetic oligonucleotides or primers, every hybridizing to at least one strand of dsDNA target, with the pair spanning a region with the intention to exponentially reproduce. The hybridized primer acts as a substrate for a DNA polymerase, which creates a complementary strand via sequential addition of deoxyncleotides. The procedure can be briefed in three steps: (i) dsDNA separation at temperatures above 90ºC, (ii) primers annealing at 50-75C, and (iii) optimal extension at 72-78ºC. The rate of temperature change, the duration of the incubation at each temperature and the number of times each cycle is controlled by way of a programmable thermal cycle. The amplified DNA fragments will then be separated by agarose gel electrophoresis and the bands are visualized via staining

the resulting bands with ethidium bromide and irradiation with ultraviolet light. The specificity of PCR trying out is dependent on the primer sets used. There are virus species specific primers and genus specific primers.

**Reverse transcription-polymerase chain reaction (RT-PCR)**

For RNA viruses, an initial step to transcribe the RNA viral genome to its complementary DNA (cDNA) (Reverse Transcription) by using the enzyme reverse transcriptase (RT) is needed. In view of that, the PCR process followed for the detection of RNA viruses is known as RT-PCR. *Potato virus Y* (PVY) is one of the most significant and destructive viruses located in potato. It is the kind member of the genus *Potyvirus* in the family Potyviridae. The viral genome consists of a single-stranded, positive-sense RNA. *Potato virus Y* (PVY) is one of the most important viruses of potato world-wide, several strain groups are recognized. In the past two decades, novel PVY variants have appeared causing necrotic symptoms on potato tubers. Implicated are two groups of recombinant strains: PVYNW and PVYNTN, and NA-PVYNTN. At the same time, the first two are recombinants between PVY-N- and O-strains the latter is a recombinant between an N-strain and an unknown PVY strain or other *Potyvirus*. A few drawbacks were found with recently published primers used in RT-PCR based differentiation of PVY strains as some described isolates could not be identified efficiently. Consequently we developed new primers using both recently available sequences and newly generated complete sequences of PVY strains. The reliability of those newly developed primers and processes was successfully tested on nearly 100 biologically and serologically characterised PVY isolates. The evolution of new PVY-N strains has complicated the diagnosis, which requires a combination of bioassay, serological and molecular assays. To simplify the identification and differentiation of various PVY-N strain groups, a competitive (single antisense and multiple sense primers) reverse transcription-polymerase chain reaction (RT-PCR) was used (Nie and singh, 2002). Typical potyvirus inclusions are not always found in leaf strips and virus seems to be unevenly distributed in its hosts and it often appears to have a low titer in many of the plants tested. The diagnosis of *Squash vein yellowing virus* (SqVYV) causal agent of watermelon can be difficult using microscopic studies. There is no antiserum to this virus available at this time. Currently, the best test is a RT-PCR assay or a nested RT-PCR assay using primers based on the sequence of the capsid protein gene of SqVYV (Adkins *et al*. 2008) [1].

**Real time PCR**

The ability to visualize the progress of amplification in a quantitative way turned into welcomed by using research workers. This technique has furnished insight into the kinetics of the PCR reaction and it is establishment of "real time" PCR. The monitoring of accumulating amplicon in real time PCR has been feasible by way of the labelling of primers, probes and amplicon with fluorogenic molecules. The elevated pace of Real time PCR is largely because of decreased cycle times, elimination of post –PCR detection methods and the use of fluoregenic labels and sensitive strategies of detecting their emissions. Real time PCR has demonstrated increasingly valuable diagnostic device for plant viruses. But, it requires an initial high capital funding to collect the needed equipment, as compared to other techniques. Hasiow *et al*. (2008) [13] detected and diagnosed tomato pathogen *Pepino mosaic virus* (PepMV) member of *Potexvirus* genus in tomato by using fluorescence dye SYBR Green in real time reverse transcription polymerase chain reaction (RT- PCR).

**Multiplex PCR**

Multiplex settled polymerase chain response (PCR) is a variation of PCR in which at least two loci are at the same time intensified in a similar response and this strategy has been effectively applied in numerous territories of DNA testing, including analyses of deletions, mutation, virus gene detection and polymorphisms, quantitative assays and reverse transcription PCR. A multiplex nested RT-PCR had been developed for instantaneous and sensitive detection of the viruses like CMV, CLRV, SLRSV, and ArMV in a single compartmentalized tube. This newly advanced technique combines the advantages of multiplex RT-PCR with sensitivity and reliability of nested RT-PCR executed in a single closed tube. It enables the simultaneous detection of numerous viral RNA targets in a single analysis, performed with woody plants. It additionally saves time and reagent costs because it may be finished in a single reaction, despite the fact that accurate design of well matched primers is needed. The compartmentalization of a single eppendorf tube with a pipette tip allowed multiplex-PCR and nested PCR to be combined effectively. Therefore, sensitivity is expanded at least hundred-fold over that of multiplex RT-PCR for the detection of viruses. The multiplex RT-PCR becomes established to be hundred-fold more sensitive than conventional PCR (Lopez *et al*., 2003) [22].

**Microarray**

The technique is based on the hybridization of labeled samples with thousands of unique immobilized probes. There are few publications of plant virus detection using microarrays or macroarrays. Detection of different isolates of *Cucumber mosaic virus* (CMV) or *Plum pox virus* (PPV), four different cucurbit-infecting Tobamoviruses and up to 11 viruses infecting cucumber or potato have been reported (Agindotan and Perry, 2008; Bystricka *et al*., 2005) [2, 6]. Boonham, *et al*. (2005) [5] reported that, RNA was extracted from plants infected singly with PVX, PVY, PVS (ordinary and Andean strains) and from plants infected with mixed infections of PVY and PVX. Labelled cDNA was produced and used to probe the microarrays, in each case, detection occurred with the homologous capture probe. The exploitation of microarray methodology has been examined to investigate its potential in the field of viral diagnostics, using a range of potato viruses as a model system. Engel *et al*. (2010) [10] designed probe for grape infecting virus blongs to Bromoviridae, Bunyaviridae, Closteroviridae, Comoviridae, Flexiviridae, Tombusviridae and Tymoviridae. To determine the performance of the microarray on virus-infected plants analyzed by the microarray and by RT-PCR with viral primers, results obtained with grapevine samples with single or mixed infection showed consistency between the microarray and the PCR analyses. The microarray was especially useful for samples with viral co-infections.

**References**

1. Adkins ST, Webb S, Baker C, Kousik CS. *Squash vein yellowing virus* detection using nested polymerase reaction demonstrates the cucurbit weed *Momordica*

*charantia* is a reservoir host. Plant Disease. 2008; 92:1119-1123.
2. Agindotan B, Perry KL. Macroarray detection of eleven potato-infecting viruses and Potato spindle tuber viroid. Plant Dis. 2008; 92:730-740.
3. Ali MA, Winter S, Dafalla GA. Tobacco streak virus infecting faba bean (*Vicia faba*) reported for the first time. Plant Pathology. 2009; 58:406.
4. Bandaranayake WMEK, Wickramarachchi WART, Wickramasinghe HAM, Rajapakshe RGAS, Dissanayake DMKK. Molecular detection and characterization of begomoviruses associated with cucurbitaceae vegetables in Skingri Lanka. J Natl. Sci. Found Sri Lanka. 2014; 42:239-245.
5. Boonham N, Walsh K, Smith P, Madagan K, Graham I, Barker I. Detection of potato viruses using microarray technology: towards a generic method for plant viral disease diagnosis. J of Virol. Methods. 2003; 108:181-187.
6. Bystricka D, Lenz O, Mraz I, Piherova L, Kmoch S, Sip M. Oligonucleotide-based microarray: a new improvement in microarray detection of plant viruses. J Virol. Methods. 2005; 128:176-182.
7. Christie RG, Ko NJ, Falk BW, Hiebert E, Lastra R, Bird J *et al*. Light microscopy of geminivurs-induced nuclear inclusion bodies. Phytopathology. 1986; 76:124-126.
8. Clark MF, Adams AN. Characteristics of the microplate method of enzyme linked immuno-sorbent assay for the detection of plant viruses. J Gen. Virol. 1977; 34:475-483.
9. De Boer SH, Lopez MM. New grower-friendly methods for plant pathogen monitoring. Annu. Rev. Phytopathol. 2012; 50:197-218.
10. Engel EA, Escobar PF, Rojas LA, Rivera PA, Fiore N, Valenzuela P. A diagnostic oligonucleotide microarray for simultaneous detection of grapevine Viruses. J of Virol. Methods. 2010; 163:445-451.
11. Gibbs A, Harrison B. Plant Virology: The Principles. Edward Arnold, London, 1976, 292pp.
12. Gould RG, Symon RH. Molecular biological approaches for relationship among the viruses. Annual review of Phytopathology. 1983; 21:179-199.
13. Hasiow B, Borodynko N, Pospieszny H. Development of a real time RT-PCR assay for detecting genetically different *Pepino mosaic* virus isolates. J of Plant Protec. Res. 2008; 48(3):295-301.
14. Hsu YC, Yeh TJ, Chang YC. A new combination of RT-PCR and reverse dot blot hybridization for rapid detection and identification of potyviruses. Journal of Virological Methods. 2005; 128:54-60.
15. Hsu YC, Yeh TJ, Chang YC. A new combination of RT-PCR and reverse dot blot hybridization for rapid detection of Potyviruses. J Virol. Methods. 2005; 128:54-60.
16. Hussain S, Yasmin T, Fahim M, Hameed S, Haque MI. Transmission and host range studies of pakistani isolate of *Chilli veinal mottle* virus. Pak. J Bot. 2008; 40(6):2669-2681.
17. Huang XL, Aguilar ZP, Xu H, Lai W, Xiong Y. Membrane-based lateral flow immunochromato graphic strip with nanoparticles as reporters for detection: A review. Biosens. Bioelectron. 2016; 75:166-180.
18. Jain RK, Bag S, Awasthi LP. First report of natural infection of *Capsicum annuum* by Tobacco streak virus in India. Plant Pathol. 2005; 54:257.
19. Jones AT. Experimental transmission of viruses in diagnosis. In: Diagnosis of plant virus diseases, (Eds, Matthews, R.E.F). CRC Press, Boca Raton, Florida, USA, 1993, 49-72.
20. Khattab EAH. Biological, serological and molecular detection of *Pepper mottle* virus infecting pepper plants in egypt. Egypt. J Phytopathol. 2006; 34(2):121-138.
21. Kunkalikar SR, Sudarsana P, Rajagopalan P, Zehr UB, Ravi KS. Biological and molecular characterization of *Capsicum chlorosis* virus infecting chilli and tomato in India. Archives of Virology. 2010; 55(7):1047-1057.
22. Lopez MM, Bertolini E, Olmos A, Caruso P, Gorris MT, Llop P *et al*. Innovative tools for detection of plant pathogenic viruses and bacteria. International Microbiology. 2003; 6:233-243.
23. Ladhalakshmi D, Ramiah M, Ganapathy T, Reddy MK, Salaheddin K, Merin B *et al*. First report of the natural occurrence of *Tobacco streak virus* on blackgram (*Vigna mungo*). Plant Pathology. 2006; 55:569.
24. Lopez M, Bertolini E, Olmos A, Caruso P, Gorris MT, Llop RP *et al*. Innovative tools for detection of plant pathogenic viruses and bacteria. Int. Microbiol. 2003; 6:233-243.
25. Makkouk K, Kumari S. Molecular diagnosis of plant viruses. Arab J Plant Prot. 2006; 24:135-138.
26. Makkouk KM, Hsu HT, Kumari SG. Detection of three plant viruses by dot-blot and tissue-blot immunoassays using chemiluminescent and chromogenic substrates. Journal of Phytopathology. 1993; 139:97-102.
27. Matthews REF. Host plant responses to virus infection. In: Comprehensive virology, virus-host interaction, viral invasion, persistence and diagnosis, (Eds) Fraenkel-Conrat, H. and Wagner, R.R. Plenum Press, New York, USA. 1980; 16:297-359.
28. Nie X, Singh RP. A new approach for the simultaneous differentiation of biological and geographical strains of *Potato virus Y* by uniplex and multiplex RT-PCR. J Virol. Methods. 2002; 104:41-54.
29. Nie X, Singh RP. Probable geographical grouping of PVYN and PVYNTN based on sequence variation in P1 and 5′-UTR of PVY genome and methods for differentiating North American PVYNTN. Journal of Virological Methods. 2002; 103:145-156.
30. Raj SK, Srivastava A, Chandra G, Singh BP. Characterization of *Cucumber mosaic* virus isolate infecting gladiolus cultivars and comparative evaluation of serological and molecular methods for sensitive diagnosis. Current Science. 2002; 83(9):1132-1137.
31. Sambrook J, Fritschi EF, Maniatis T. Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, New York, 1989.
32. Saritha RK, Bag TK, Loganathan M, Rai AB, Rai M. First report of Squash leaf curl china virus causing mosaic symptoms on summer squash (*Cucurbita pepo*) grown in Varanasi district of India. Archives of Phytopathology and Plant Protection. 2011; 44(2):179-185.
33. Singh RP, Rojas SJ. Detection of *Potato virus Y* in primarily infected mature plants by ELISA, indicator host, and visual indexing. Canadian Plant Disease Survey. 1983; 63(2):39-44.
34. Sivaprasad Y, Bhaskara Reddy BV, Rekha Rani K, Raja Reddy K, Sai Gopal DVR. First report of Tobacco streak ilarvirus infecting onion (*Allium cepa*). New Disease Reports. 2010; 22:17.

35. Williams RC, Wycoff RGW. The thickness of electron microscopic objects. J Appl. Phys. 1944; 15:712.
36. Xie Y, Zhou XP. Molecular characterization of Squash leaf curl Yunnan virus, a new begomovirus and evidence for recombination. Arch Virol. 2003; 148(10):2047-54.