Journal of Plant Diseases and Protection (2021) 128:353–368
https://doi.org/10.1007/s41348-020-00384-0

REVIEW

Mixed infection of plant viruses: diagnostics, interactions and impact on host

Pankhuri Singhal[1] · Sajad Un Nabi[2] · Manoj Kumar Yadav[3] · Abhishek Dubey[4]

Received: 29 May 2020 / Accepted: 17 September 2020 / Published online: 25 September 2020

Abstract
Globally, viral diseases cause huge economic losses in crops and their management is a big challenge to growers as well as researchers. Mixed infection is the existence of more than one virus in single plant, which results in varied symptoms at the same time. The presence of more than one virus always leads to difficulty in understanding the etiology of disease. Most of the viral diseases go unnoticed either due to the latent nature of infection of virus(es) or due to low severity of symptoms. But this might be true in case of single infection of the host by the concerned virus. When such viruses are seen causing infection in combination with other viruses at particular time, more severe disease symptoms can be observed. For any successful management of viral disease especially during mixed infection, detection and identification of plant viruses causative of the disease are of foremost importance. Several approaches like cocktail ELISA, multiplex PCR for known viruses and next-generation sequencing for both known and unknown viruses have been developed for detection of mixed infection of viruses. During mixed infection, several kinds of interaction commonly referred to as synergistic or antagonistic interactions are going on between and among the viruses, which aggravate the disease with more severe symptoms than with single infections. Here, we review the mixed infection of viruses, methods of detection, factors influencing, interactions and impact on plant during mixed infection.

Keywords Virus · Host · Vector · Detection

Introduction

Viruses are molecular pathogens and are ubiquitous in nature, found to be associated with wild as well as cultivated plants. According to the tenth ICTV report (2018b. v1), among the total viruses recorded, the plant viruses are grouped into 26 families, 118 genera, with 1516 plant virus species. Plant viruses pose a grave threat to the world agriculture due to frequent recombination events leading to the generation of new species and difficulty in understanding the disease caused by them in host due to varied symptoms. There has been an increase in the viral disease problems in the world due to large-scale free movement of planting material, changes in environmental conditions majorly due to global warming as well as increase in demand of agricultural produce in international trade. The losses can be quantified in terms of yield and quality of plant produce as well as economic and social consequences. A recent example of effect of changing environmental conditions leading to enhanced threat is the leaf curl disease caused by begomovirus complex becoming a key restraining factor in the production of cotton (Sharma and Rishi 2007). The three large groups of viruses, i.e., potyviruses, tospoviruses and begomoviruses, have been stated to have most economically important emerging viruses as members. Several workers have reported losses of as much as $30 billion worldwide per year due to viral diseases (Anderson et al. 2004; Sastry and Zitter 2014) which included severe losses of more than 25 million tons of cassava per year in India, Sri Lanka and Africa (Legg and Thresh 2000; Calvert and Thresh 2002; Thresh and Cooter 2005) due to *cassava mosaic virus* and in

✉ Pankhuri Singhal
pankhuri.agri47@gmail.com

[1] Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India

[2] ICAR-Central Institute of Temperate Horticulture, Srinagar 191132, India

[3] ICAR-National Rice Research Institute, Cuttack 753006, India

[4] ICAR-Division of Crop Research, Research Complex for Eastern Region, Patna, Bihar 14, India

the USA, a yearly loss of $100 million on potato production due to *potato leafroll virus* (PLRV) (Sastry and Zitter 2014).

Most of the viral diseases go unnoticed either due to the latent nature viral infection or due to low severity of symptoms. But this might be true in case of single infection of the host by the concerned virus. When such viruses are causing infection in combination with other viruses, at a particular time, more severe disease symptoms can be observed. The classical examples of enhanced disease symptoms due to mixed infection are of potato rugose mosaic disease caused by *Potato virus X* (PVX) along with *Potato virus Y* (PVY), potato crinkle disease caused by PVX and *Potato virus A* (PVA), tomato streak disease due to co-infection of PVX and *Tobacco mosaic virus* (TMV) (Jones and Barbetti 2012). In some of the cases, the severity level of the disease may lead up to 90% losses in yield caused by *Sweet potato chlorotic stunt virus* (SPCSV) and *Sweet potato feathery mottle virus* (SPFMV) (Karyeija et al. 2000). Cassava mosaic disease (CMD) epidemic caused due to mixed infection of *African cassava mosaic virus* (ACMV) and Uganda strain of *East African cassava mosaic virus* (EACMV) (Legg and Fauquet 2004); maize lethal necrosis (MLN) caused due to the co-infection with machlomovirus *Maize chlorotic mottle virus* (MCMV) and any of the potyvirus infecting cereals such *as Wheat streak mosaic virus* (WSMV), *Sugarcane mosaic virus* (SCMV), *Maize dwarf mosaic virus* (MDMV) or *Johnsongrass mosaic virus* (JMV) (Redinbaugh and Stewart 2018). Some mixed viral infections have been documented to be resulting from the complex interactions of more than three viruses such as grapevine leafroll disease which is caused due to the complex association of as high as 11 grapevine leafroll-associated viruses (Naidu et al. 2014, 2015). Though previous attempts have been made on discussion of mixed infections of viruses, through this review their modes of detection and information on their types of interactions with examples have been discussed in a distinct format.

Mixed infection of plant viruses

The plants in nature as well as cultivated habitats are variedly populous in terms of the number of associated viruses differing in their biological, epidemiological as well as socioeconomic significance. Mixed infections of viruses can be extraordinarily recurrent, such that their occurrence can be considered as a rule rather than exception. Mixed infections arise as a collaboration of several cross-interacting players that lead to complex events such as:

1. *Viruses* they act as generalists enabling infection of different host plants including both wild as well as cultivated plants. Due to frequent recombination and mutations, they generate new variants with acquired ability to infect new hosts thus possessing a large host range among crops. Also, being obligate parasites they try to live in harmony with the host in turn providing more scope for the coexistence of multiple viruses and occurrence of mixed infections.
2. *Hosts* a wide variety of wild and cultivated plants are available to act as hosts in the same environment providing the opportunity to viruses to have large host range and ability to survive throughout the year.
3. *Vectors* organisms involved in transmission of viruses are polyphagous and thus associated with multiple hosts, due to which they acquire virus from some plant and transmit to another which may already be infected by another virus in turn creating a possibility of mixed infection.

The interactions between these three factors argue in favor of more persistent occurrence of multiple viral infections in plants. Figure 1 depicts the three factors and their interactions leading to viral disease in the form of a smart art. Another mechanism probably playing a large role in leading to mixed infections is the inadvertent long distance transport of viruses facilitated by enhanced global movement of infected plants as well as people. As viruses can easily move from reservoir hosts to new hosts and adapt to new conditions thus when a plant is introduced in an area, the viruses associated with it tend to move to neighboring hosts resulting in mixed infections either via physical contact and vectors. An example of emergence of an already established virus of original host in a new host is of *Pepino mosaic virus* (PepMV) infecting pepino originally found in South America, which was further identified in greenhouse tomatoes in Netherlands in 1999 which was spread mainly due to seed dissemination. Eventually, strains of PepMV

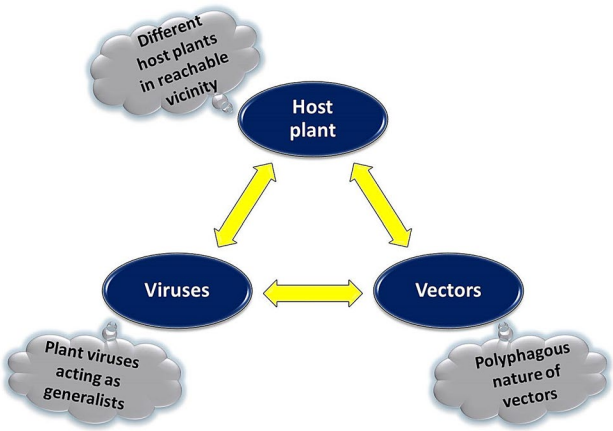

Fig. 1 Depiction of the three cross-interacting factors playing major role in the emergence of mixed infections of viruses along with a glimpse of their nature leading to mixed infections

evolved differencing in biological and genetic properties leading to further mixed infections in whole of Europe which became difficult to manage and require strict sanitation (Rojas and Gilbertson 2008).

Factors influencing outcomes of mixed infections

A number of factors are known to influence the possible outcomes of mixed infections of viruses, i.e., whether the effect will be positive, negative or neutral on each other as well as plant. Such factors may be in terms of either the concerned viruses themselves or the plant host and its cultivar they infect.

Co-infection and superinfection

The kind of interaction that will occur is extremely dependent on the time and order of arrival of viruses and infection of host in mixed infection. Co-infection refers to the simultaneous arrival of viruses and infection of host plant roughly at the same time by both viruses. At the early stages of infection, when the viral densities are low and a high number of healthy plants are available to act as host, the occurrence of co-infection is favored due to lack of competition for resources. Thus, co-infections are also known to increase the virus fitness. Superinfection refers to the arrival of concerned viruses in mixed infection at different time points (Saldana et al. 2003). Thus, the infection of one virus is preceded by the infection of the other virus involved in mixed infection. In later stages of infections as the disease spread, the viral densities increase with the reduction in the number of healthy host plants, and the superinfection becomes predominant and frequent due to increase in competition. The outcome of the superinfection is also influenced by the order of the infection of concerned viruses and also the time frame between the infections. As seen in case of papaya plants which when either co-infected by both viruses or first infected with *Papaya ringspot virus* (PRSV) followed by infection with *Papaya mosaic virus* (PapMV) resulted in synergistic interaction; but reversing the order of infection of viruses leads to antagonism, primarily due to the activation of defense responses against PRSV by early PapMV infection in turn establishing protective association with the host (Chavez-Calvillo et al. 2016). The superinfection laid the principle of cross-protection where the early infection by mild protecting strain prevents any consequent infection by severe strain of the virus.

Plant host and cultivar

The outcomes are also influenced by the host as well as the cultivar infected by the concerned viruses in mixed infections. The infection of different hosts by concerned viruses of mixed infections may show alterations in the patterns and quantity of virus accumulations as well as the type and severity of symptom expression influencing the type of interaction between viruses, responses to host and also vector transmission. Such host-dependent alteration has been seen in case of co-infections with *Pepper golden mosaic virus* (PepGMV) and *Pepper huasteco virus* (PHV) of pepper resulting in antagonism as contrary to that of tobacco and *N. benthamiana* resulting in synergism (Mendez-Lozano et al. 2003). Infection of different host species resulting in different quantities of virus load has been reported in case of co-infections with *Tomato infectious chlorosis virus* (TICV) and *Tomato chlorosis virus* (ToCV) of *Physalis wrightii* plants, wherein titers of both viruses were found to be decreased, on contrary to co-infections of *N. benthamiana* plants wherein viral load of TICV was increased, while that of ToCV was found to be decreased (Wintermantel et al. 2008). Another classical example is of mixed infections by PVY and PVX of tobacco plants (*Nicotiana tabacum*), wherein enhanced diseases symptoms along with tenfold increase in PVX titers are reported, on contrary to mixed infections of *N. benthamiana* resulted in severe disease with systemic necrosis of leaves and stem leading to death of plant, but no change in the titer of PVX was observed (Gonalez-Jara et al. 2005). The outcome of mixed infection may also be cultivar specific, as reported in co-infections of three wheat cultivars with *Triticum mosaic virus* (TriMV) and *Wheat streak mosaic virus* (WSMV), wherein synergism with extensive leaf deformation, leaf bleaching and stunting was observed in Arapahoe and Tomahawk cultivars, while negligibility to mid-synergism was observed in case of Mace cultivar (Tatineni et al. 2010). They noticed that this might be due to the asymmetrical accumulation of co-infecting viruses (34-fold more efficient replication of WSMV as compared to TriMV) and hypothesized that the concentration of individual viruses in mixed infections is a vital aspect and should reach a threshold level for eliciting synergism. Many more examples of cultivar-dependent mixed infection outcomes include infection of PVX with TMV in tomato cvs. Fukuju No. 2, GCR 236 and GCR 237 (Balogun et al. 2005).

Interactions among plant viruses

In the course of mixed viral infections, the concerned viruses might interact among themselves in a variety that ranges from neutralism to synergism to antagonism, directly impacting the host plant as well as their

relationships with vector organism. In neutral, no changes in viral accumulation in terms of titer or pathogenicity are observed during mixed infection compared to single infections of individual viruses. Phenotype of the mixed infected plant remains same as that of the single infected plant. It is the most commonly occurring interaction as a number of plant viruses infect a single host at the same time. On the other hand if differences in single and mixed infections are observed, the interactions might be synergistic or antagonistic. In synergism, one virus or both viruses benefit from the presence of the each other in terms of viral titer, pathogenicity and increased dissemination by vector organisms. However in antagonistic interaction, the association of one virus is detrimental to the presence of the other, or both are detrimental to each other in mixed infection of plants. In many cases, synergisms and antagonism might occur together at the same time in a host, wherein one partner is alleviated and the other is suppressed. The interactions between concerned viruses may also lead to recombination, reassortment and complementation events leading to the generation of new variants of viruses. The devastating CMD appeared due to the emergence of highly virulent forms via reassortment and recombination between EACMV and ACMV. This was due to the exchange of capsid protein (CP) gene sequences of ACMV with homologous sequences of EACMV, giving rise to a highly virulent recombinant, *East African cassava mosaic virus Uganda-2* (EACMV-UG2) that has been implicated in this disease outbreak. This is an example of synergism wherein both viruses interact with each other in positive manner to aggravate the disease. The various types of interactions and their causes along with examples have been discussed below. Figure 2 depicts the types of interactions occurring in mixed infections and their possible outcomes on the concerned viruses.

Synergism

In most cases of mixed infection, aggravated disease with more severe symptoms than with single infections is observed commonly referred to as a synergistic interaction or synergism. The synergism between concerned viruses might be in terms of increased replication of either one or both the viruses, acquired ability to invade new plant tissues (movement) and helper dependence of one virus on other for transmission or other essential functions. Virus–virus interaction within mixed infections may result in the suppression of host defense mechanisms by one or both viruses. During mixed infections, the viral suppressor proteins (VSPs) have also been reported to play role in altering tissue movement patterns of other viruses (García-Marcos et al. 2009; Gonalez-Jara et al. 2005). However, in case of mixed infections involving a potyvirus, often the accumulation level of the potyvirus is unaltered, while that of the non-potyvirus increases significantly (Gonalez-Jara et al. 2005; Pruss et al. 1997). Pruss et al. (1997) identified that the potyviral P1 protein and HC-Pro (helper component protease) act as a pathogenicity enhancer of other viruses, thus by acting as a helper virus, by suppressing PTGS paving the way for further infection. The need for the effective suppression of RNA silencing mechanism of plant for successful infection has also led to the enhanced mixed infection of viruses. As reported in case of synergistic interaction among tripartite begomovirus combination of *Pepper golden mosaic virus* (PepGMV) DNA-A and DNA-A and DNA-B of *Pepper huasteco yellow vein virus* (PHYVV) inducing striking disease severity in *N. benthamiana*, pepper and tomato plants compared to the symptoms induced by PHYVV unaccompanied (Sharp et al. 1999). The open reading frame (ORF) 4 of DNA-A component (AC4 ORF) functioning as suppressor of gene silencing provides evidence for creation of selection

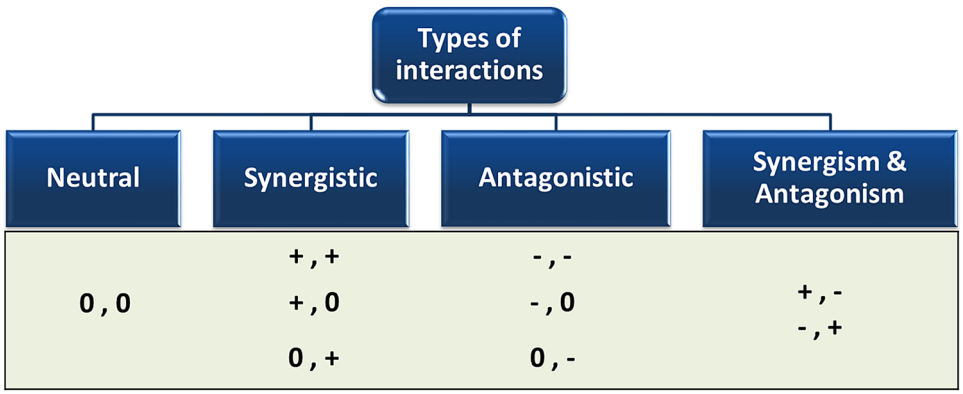

Fig. 2 Depiction of the kind of interactions that take place between the various multiple viruses infecting plants simultaneously. Beneath, each interaction is displayed the possible outcomes of such interactions on each concerned virus in mixed infection. 0 indicates no effect, while + indicates positive effect on the virus, and − indicates negative effect on the virus. Here, the possible outcomes are described in terms of two interacting viruses

pressure for maintaining more than one DNA-A component for efficient suppression of antiviral defense response (Vanitharani et al. 2004).

In most of the reported cases of synergism, increase in titer of only one virus has been observed such as for *Sugarcane mosaic virus*-MD-B and MCMV (Goldberg et al. 1987), PVX and PVY (Vance et al. 1991) and *Soybean mosaic virus* (SMV) and *Cowpea mottle virus* (CPMV) (Anjos et al. 1992). However, in case of synergism between WSMV and MCMV (Scheets et al. 1998), WSMV and TriMV (Tatineni et al. 2010), increase in concentration of both interacting viruses and with concomitant increase in disease severity has been observed. The synergisms between unrelated viruses are common, but instances of synergisms between viruses belonging to the same family have also been reported such as two Crinivirus spp. (Wintermantel et al. 2008), potyviruses (Tatineni et al. 2010) and begomoviruses such as mixed infection of ToLCNDV with monopartite begomoviruses, such as *Cotton leaf curl Kokhran virus*-Burewala strain (CLCuKoV-Bur) (Zaidi et al. 2016), *Bhendi yellow vein mosaic virus* (BYVMV) (Venkataravanappa et al. 2015), *Croton yellow vein mosaic virus* (CYVMV) (Singh et al. 2012), *Squash leaf curl China virus* (SLCCNV) and *Tomato leaf curl Ranchi virus* (ToLCRnV) (Kumari et al. 2011).

Types and cause of synergistic interactions

Helper dependence Helper dependence occurs when any of the viruses involved in mixed infection is faulty in one or more indispensable functions (dependent virus) which in turn can be supplemented by the other virus acting as the helper. Thus in this kind of interaction, there is a solitary assistance of helper virus to the dependent virus. In this case, usually the subviral agents or viral associated replicons are found in mixed infections in host along with helper virus in turn either reducing or exacerbating the symptoms caused by the helper virus. A general kind of dependence might be in terms of transmission of non-transmissible isolate of virus after encapsidation of nucleic acid within the capsid of either only transmissible isolate (complete dependence) or within capsid consisting of capsomeres of both isolates (phenotypic mixing) (Bourdin and Lecoq 1991). The example of famous groundnut rosette disease, caused by interaction of an umbravirus *Groundnut rosette virus* (GRV), its satellite RNA and luteovirus *Groundnut rosette assistor virus* (GRAV), wherein GRV and its sat-RNA are entirely dependent on the coat protein of GRAV for encapsidation as well as transmission by aphid vector (Murant 1990). This complete dependence on a helper virus coat protein for the encapsidation of the genome of dependent virus has been termed as transencapsidation or genome masking. It is a natures mode of maintaining mixed infections and has been most commonly reported in case of umbraviruses, which lack the genetic information to code for the coat protein and thus depend on the luteoviruses for encapsidation and in turn aphid transmission. The other classical example of small RNA replicons causing mixed infection by depending on the helper virus is of ST9 of *Beet western yellows virus* that acts as dependent virus on helper polerovirus for its aphid transmission and movement in turn enhancing replication of polerovirus leading to severity of symptoms (Passmore et al. 1993; Sanger et al. 1994). The Potato spindle tuber viroid (PSTVd) is a mechanically transmitted viroid, but after its transcapsidation in Potato leafroll virus (PLRV) capsid, it becomes specifically and efficiently transmitted by few aphid species, mainly the green peach aphid (*Myzus persicae*) (Querci et al. 1997). Similarly, in case of *Barley yellow dwarf virus*-MAV strain (BYDV-MAV) and *Cereal yellow dwarf virus*-RPV strain (CYDV-RPV), transmitted by aphids *Sitobion avenae* and *Rhopalosiphum padi*, respectively, in single infections, BYDV-MAV RNA becomes encapsidated in CYDV-RPV capsids and becomes transmissible by *R. padi* in mixed infection of barley, oat and wheat (Creamer and Falk 1990; Baltenberger et al. 1987).

On the other hand heteroencapsidation/heterologous encapsidation with phenotypic mixing, wherein the genome of a virus can acquire the properties of the particles of another virus partially, as seen in case of aphid non-transmissible potyvirus ZYMV isolate that during mixed infection with potyvirus (PRSV) produces heteroencapsidated particles in turn acquiring the ability of aphid transmission (Bourdin and Lecoq 1991). The non-transmissibility of ZYMV strain is due to modification in the triplet amino acid sequence (asp-ala-gly) located near the *N*-terminus of capsid protein. Recently, taxonomic studies indicated that the viral polymerases of viruses are significantly similar that in turn favor the capture and exchange of capsid genes in mixed infections of both viruses (Wolf et al. 2018). Heteroencapsidation to facilitate whitefly transmission has also been reported between pseudo-recombinants of two bipartite begomoviruses, tomato leaf curl New Delhi virus and Palampur virus in cucurbits and tomato (Kanakala et al. 2013).

Heterologous complementation/transcomplementation is another form of facilitation in which the non-structural proteins called the helper components act as bridges between virus particles in turn facilitating vector transmission (bridge hypothesis). As observed in case of *Potato aucuba mosaic virus* (PAMV), it is only contact transmitted in single infections, but when present in mixed infections in potato plants along with potyvirus, it acquires the ability of transmission by aphids. It has also been observed that even if in the plant PVY is not previously present, but the aphid vector has fed on PVY-infected plant, then PAMV becomes transmissible even from singly infected

plants. This facilitation is a result of transcomplementation through a conserved DAG motif in the *N*-terminus of PAMV which is a characteristic of potyviruses (Manoussopoulos 2001) along with the HC-transcomplementation in which HC-Pro of one virus can enable aphid transmission of another virus after acquiring of HC-Pro by the aphid which binds to the cuticular lining of aphid mouth parts (Froissart et al. 2002). Another example of such complementation is observed in case of previously mentioned mixed infections of TICV and ToCV, which enables TICV transmission by *Trialeurodes abutilonea* from mixed infected plants, which otherwise is a non-vector of TICV alone (Wintermantel et al. 2008).

Overcoming tissue tropism Tissue tropism refers to the specificity of a virus to be able to infect only some type of cells or tissues and exhibit restrictions to enter others. In mixed infections, the viruses acquire the ability to invade and infect new plant tissues, most commonly observed in phloem-restricted viruses (Mascia and Gallitelli 2016). As seen in case of *Tomato yellow spot virus* (TYSV) which localizes in mesophyll cells and *Tomato rugose mosaic virus* (ToRMV) which localizes in phloem in single infections in *N. benthamiana* plants. But in mixed infection of tomato by both viruses, ToRMV acquires the ability to invade mesophyll cells too along with phloem (Alvez-Junior et al. 2009). Similar alteration has also been seen in mixed infections of PVA and PLRV in *N. benthamiana* plants, wherein PLRV acquires the ability to infect different cell types in leaves along with phloem (Savenkov and Valkonen 2001). Similarly, in natural mixed infections of beans with *Bean golden mosaic virus* (BGMV) with any tobamovirus also enables the BGMV to invade mesophyll parenchyma along with phloem (Carr and Kim 1983). This could possibly due to the assistance of movement proteins of one virus in overcoming the movement deficiencies of the other. A similar phenomenon could also be seen in natural hosts such as zucchini squash co-infected with ZYMV that assisted CMV in systemic spread (Choi et al., 2002). The acquired ability to infect mesophyll cells or other leaf cells enhances the mechanical transmission as well as the transmission of virus by vectors by influencing the time duration of acquisition and inoculation. Not only the movement proteins but also the RNA silencing suppressor (RSS) proteins of viruses has been proven to play role in overcoming tissue tropism of unrelated viruses. Such phenomenon was shown for a DNA–RNA virus co-infection, wherein CMV 2b protein (RSS) and not the movement protein (CMV 3a protein) was found to mediate movement and increased replication of *Abutilon mosaic virus* (AbMV) in non-vascular cells along with vascular cells in *N. benthamiana*, tobacco and tomato (Wege and Siegmund 2007).

Altered host range It is a case of impact on both plant and viruses, wherein a plant infected by one virus becomes susceptible to the infection by other viruses also which are unable to infect that plant in single infections. This extension of host range may be due to heterologous encapsidation or genome masking as seen in case of *Southern cowpea mosaic virus* (SCPMV) which infects only cowpea and *Southern bean mosaic virus* (SBMV) which infects only common bean in single infections. But in mixed infections, SCPMV also acquires the ability to infect common bean systemically in encapsidated form (Hacker and Fowler 2000).

Antagonism

Antagonism is mainly due to activation of host defense responses by the infection of primary virus thus preventing the subsequent infection by secondary virus. Also, reduced rate of virus multiplications might be due to tough competition for host resources in mixed infections, leading to declined fitness in contrast to single infections. Generally, closely related viruses or viral strains while attacking the same host plant do not inhabit the same cells already conquered by their counterpart, subsequently occupying discrete niches, generally referred to as spatial separation/spatial exclusion. Such exclusions have been proved in mixed infections of *N. benthamiana* with *Tobacco vein mottling virus* (TVMV), *Clover yellow vein virus* (ClYVV) and *Plum pox virus* (PPV). The decreased titers of one or both viruses cannot be correlated with the symptom expression in many cases such as double infections of tomato by two begomoviruses—ToYSV and ToRMV, wherein though titers of both viruses were found to be reduced, the symptoms expressed were more severe in comparison with single infections. This might be due to the negative interference of tomato plants between two begomoviruses (Alvez-Junior et al. 2009).

Types of antagonistic interactions

Low synergism + high antagonism Many a times in mixed infections, low synergism and high antagonism are observed like in case of tomato plants infected with *Tomato torrado virus* (ToTV) and PepMV wherein slight increase in the titers of ToTV in early stages of infection is observed suggesting synergism, but the titers of PMV were strongly reduced at all the time points suggesting antagonism. Due to this slight increase in titers of one virus in some infections, the antagonisms are less studied because antagonism instead of exacerbation goes unnoticed (Gomez et al. 2010). The coexistence of synergism and antagonism has also been reported in case of mixed infections of ToYSV and ToRMV in *N. benthamiana* wherein titers of ToYSV were found to be slightly decreased, while that of ToRMV increased significantly (Alvez-Junior et al. 2009). The interactions between

these two viruses are also host dependent as indicated by the reduced concentrations of both viruses in doubly infected tomato plants.

Homologous interference/superinfection exclusion (SIE) Commonly referred to as cross-protection wherein the presence of one virus in a cell (Spatial separation) or entire host in few cases prevents or interferes with the subsequent infection by any another virus in turn protecting the host by excluding the superinfecting virus. The SIE is supposed to occur even if the principal virus is weaker than its counterpart, which is due to the maximized production of progeny particles of the first virus after infection of the cell (Beperet et al. 2014). This mechanism has been widely exploited to prevent viral infections in crops using avirulent or mild protecting strains such as PRSV, *Citrus tristeza virus* (CTV) and ZYMV (Ziebell and Carr 2010). The protection a mild strain offers to a normal (severe) strain has been demonstrated to remain stable for long time in Brazil in case of CTV (Rezende and Müller 1995). It has been observed that the cross-protection occurs exclusively between the isolates of the same strain and not that of different strains (Folimonova et al. 2010). The possible mechanism of cross-protection lies in the prevention of virus uncoating and disassembly along with interference in replication of the challenging virus by the protecting viral strain (Sarika et al. 2010). It also benefits the viruses in generating stable sequences by reduced recombination between homologous viruses (Syller et al. 2012). The phenomenon of superinfection exclusion has been demonstrated in mixed infections of *N. benthamiana* plants with TMV and *Hibiscus latent Singapore virus* (HLSV), wherein superinfections of HLSV prevented late infection by TMV of the host (Chen et al. 2012).

Impact on hosts

Plants

The mixed infections have varied impacts on host plants, apart from the enhanced symptoms and reduced yield in many cases such as the cassava brown streak disease outbreak in cassava due to mixed infection of *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV) leading to synergism (Jacobson et al. 2018). The other impacts might be in terms of increased gene expression as well as physiological modifications of the host plant in turn leading to changes in the metabolism of the plant more than that caused by single infections of viruses.

Host modification/altered gene expression

In mixed infection of plants by viruses, modification in terms of enhanced gene expression during simultaneous infection by concerning viruses might occur as host response to multiple infections which might be far greater in magnitude as compared to single infections. The synergistic interactions during PVX and PVY co-infection of *N. benthamiana* which lead to necrosis and finally death of plant have been studied for changes in gene expression on host. The gene responsible for chloroplast function was found to be downregulated, while an upregulation of genes controlled carbohydrate metabolism, protein synthesis and degradation, oxylipin biosynthesis and response to biotic stimulus and stress including induced expression of enzymes generating reactive oxygen species (ROS) as well as several mitogen-activated protein kinases (MAPKs). A severe oxidative stress was also found to be induced in *N. benthamiana* leaves, indicated by increased lipid peroxidation and generation of superoxide radicals in chloroplasts. During PVX–PVY co-infections gene silencing of alpha dioxygenase 1, delayed cell death was found to be induced. Thus, it was confirmed in co-infections of PVX–PVY the relative proportion of genes altered was far greater than the corresponding infections of either PVY or PVX singly (García-Marcos et al. 2009).

RNA silencing/PTGS pathways

RNA silencing or post-transcriptional gene silencing (PTGS) pathways are an endogenous cellular mechanism of eukaryotes that is exploited by plants for the prevention of infection by plant pathogens via processes such as chromatin modification, DNA methylation and transposon activity, by the activity of small RNAs (sRNAs). Thus, it is expected that when plants are simultaneously attacked by multiple viruses in synergy, they respond vigorously with an upsurge in the endogenous sRNAs to initiate the PTGS against invading viruses. In a study of mixed infection of wheat plant by the WSMV and the TriMV, a major change in the host endogenous sRNA profile was observed between single and mixed infections that lead to change in defense responses of the host (Tatineni et al. 2014). Examples of suppression of host RNA silencing pathways to enhance efficiency of mixed infection has already been cited earlier in this review.

Breakage of resistance

The breakage of resistance of the plant to the infection by a given virus leads to the transformation of the plant from nonhost to a host; thereby, extending the host range of the virus has been commonly observed in mixed infections of viruses. When a virus infects a particular cultivar of a plant which is otherwise resistant to some other virus/viruses, the

resistance might be broken facilitating invasion and infection by the other viruses. The breakage of resistance has been observed in tomato plants to TSWV co-infected with *Tomato chlorosis virus* (ToCV) (Garcia-Cano et al. 2006), in cucumber plants cv. Delila to CMV co-infected with ZYMV (Wang et al. 2004) and in sweet potato also to varied number of viruses when co-infected with SPCSV (Karyeija et al. 2000; Mukasa et al. 2006; Untiveros et al. 2007). In some mixed infections, the breakage of resistance remains uninfluenced by the order of infection of viruses such as in case of cucumber infection with CMV and ZYMV (Wang et al. 2004), while pre-infection of tomato plants with ToCV induced susceptibility to TSWV, but co-infections of both viruses did not. This might be due to the downregulation of defense responses in resistant tomato plants by pre-infection of ToCV (Garcia-Cano et al. 2006).

Vectors

Vectors play a key role in numerous viral infection cycles, since majority of plant viruses depend on the vectors for dispersion from plant to plant as well as field to field. Vectors are a mobile partner in plant–virus–plant interaction leading to spreading of infection. Thus, preference of vectors for multiple hosts at a time can favor occurrence of mixed infections. The changes caused by viruses while infecting plants influence the vector behavior and physiology in turn influencing the spread of the viruses (Bak et al. 2017). The presence as well as increased multiplication of viruses in mixed infections also influences the rate of their transmission by vectors. The transmission rate of a virus is generally estimated as the percentage of healthy plants in which the virus is inoculated by the viruliferous vector after feeding on the infected plants. The increased efficiency of transmission might be directly correlated with the increase in viral load of the concerned viruses as seen in case of aphid transmitted viruses (Barker and Woodford 1992; Gray et al. 1991; Pereira et al. 1989; De Bokx et al. 1978), whitefly transmitted criniviruses (Wintermantel et al. 2008) and mite transmitted potyviruses (WSMV and TriMV) (Tatineni et al. 2010). As stated earlier, mixed infections broaden virus distribution in the host and lead to invasion of new tissues by the viruses which in turn enhances the chances of virus availability for feeding vectors.

Direct vector manipulation

In mixed infections, the vectors are manipulated directly in a manner to be attracted to infected plants to acquire the virus and to the healthy plants to transmit the virus. As seen in case of rice co-infected with *Southern rice black-streaked dwarf virus* (SRBSDV) and *Rice ragged stunt virus* (RRSV) which are transmitted by white-backed plant hopper (WBPH) and brown plant hopper (BPH), respectively, in single infections. But in mixed infections, virus-free WBPH is more attracted to the infected plants, while the viruliferous WBPH is more attracted to healthy plants increasing the efficiency of transmission. On the other hand, BPH, a non-vector of SRBSDV, showed only preference for rice plants infected with SRBSDV over healthy plants, when they are viruliferous for RRSV thus favoring the occurrence of mixed infections in rice (Wang et al. 2014). The co-infections of *Cucurbit yellow stunting disorder virus* (CYSDV) and *Watermelon mosaic virus* (WMV) lead to prolonged feeding of melon plants by the aphids during a particular subphase of virus acquisition compared to single infected plants resulting in efficient transmission of potyvirus even during reduced WMV load in plants (Domingo-Calap et al. 2019).

Indirect vector manipulation via host

In some cases to favor the occurrence of mixed infections, the host may be modified in such manner in terms of visual, olfactory clues or nutritional alterations in order to increase the attraction of the vector to the host. As seen in case of potato plants mixed infected with PVY and PLRV transmitted by *Myzus persicae* and *Macrosiphum euphorbiae*, respectively, wherein for both vectors fecundity and preference increased in mixed infected potato plants either due to increase in sugars and amino acids in the phloem or some other sensory host modifications (Srinivasan and Alvarez 2007).

Detection and diagnosis

There have been numerous reports on single viruses infecting a host, but there has been a limitation in the detection of the number of pathogens infecting a host in mixed infections due to compromising detection factors such as specificity and sensitivity. In the past few years, there has been advancement in the multiplex or polyvalent detection methods for simultaneous detection of viruses based on either molecular hybridization or polymerase chain reaction (PCR). The viral metagenomics approach via high-throughput next-generation sequencing is now playing a vital role in revealing the extent of mixed infections in hosts by known as well as unknown viruses and virus-like organisms. Multiplex testing refers to the testing of a single sample for identification of more than one target pathogen, which can undeniably offer considerable cost and time benefits. It reduces the total handling time and the quantity of consumables and other reagents required, exclusively during testing of larger numbers of samples. It is a well-established fact that both cultivated and uncultivated plants, even being symptomless, are associated with number of viruses and virus-like agents

at a time; as most of the viruses are latent in nature (Nabi et al. 2018). This necessitates the development of multiplex detection methods for number of viruses to reduce the costs and enhance robustness and usefulness in routine diagnosis. Various methods are already in use for detection of multiple viruses or variants of same virus from same as well as different families. Some of the methods used for multiplex detection have been discussed below.

Biological assays

Biological indexing also referred to as biological assays or bioassays is a pathogen infection detection approach based on the symptom induction ability of pathogen on inoculation (either mechanical inoculation or grafting) on certain plants known as indicator plants (Gentit 2006). It can be efficiently used to detect the single infection of plant viruses. However, mixed infections of viruses are relatively common which can modify the expression of symptoms. A number of indicator plants highly susceptible to infections of many viruses' and virus-like diseases have been identified which can be used to detect an extensive range of pathogens. The efficacy of bioassay and probability of symptom expression in indicator plants by viruses in mixed infections has been studied by Vidalakis et al. (2004) for multiple citrus viruses. He observed that probability is significantly suppressed in co-infection:

1 Symptom expression by virus V1 can be suppressed when the virus V1 particles have a small title;
2 Symptom expression of V1 can be blocked in the presence of V2 virus, though these aspects are dependent on the V2 virus identity, strain and also on isolate (same for virus V1) as well as on indicator plant used;
3 Co-infection with certain other viruses will substantially reduce the number of symptomatic indicator plants in V1 and the efficacy of this indicator as a V1 indicator.

The involvement of latent viruses will minimize or suppress expression of the other virus resulting false-negative result. The mixed infections can also result in varied symptoms and delay in their expression.

Differences in the influence of temperature on pathogen replication can also impact the biological indexing results in plants with multiple viral infections (Biosecurity New Zealand 2009; Syller 2012; Constable et al. 2013). Such variability in symptom expression in mixed infection has been reported for mechanical inoculations with PVX and PVY of different indicator plants. Therein, external lesions appeared both in case of PVX and PVY inoculations of *Gomphrena globosa* L. and *Chenopodium amaranticolor*, respectively, while severe mosaic was observed for both on *Nicotiana tabacum* cv. White burley. However, variable symptoms such as vein banding and mild mosaic were observed on *Datura stramonium* for PVX and veins clearing, severe mosaic, malformation and leaf cup shape on *D. metel* for PVY (EL-Araby et al. 2009). Comparing the results of bioassays with that of serological or molecular tests, however, has shown the limitations for detecting and identifying many pathogens (Legrand et al. 2015).

Immunoassays

Immunoassays also referred to as the serology-based assays depend on the specific antigen–antibody interactions of the viruses wherein antibodies are designed against the specific proteins of the virus whose interaction indicates positive association of the virus in the assay.

Cocktail enzyme-linked immunosorbent assay (ELISA)

ELISA is a commonly used technique for the detection of viruses due to its reliability, sensitivity and specificity. But it is mainly used for detection of single pathogen due to the specific antibody–antigen reaction against the single target pathogen. Yet, research has been conducted on the fabrication of cocktail antibodies for simultaneous detection of multiple known viral infections. The specificity of the test lays in fact the production of color, if viruses are present, but the actual presence of specific virus is difficult to detect unless there is a different color or test line for each specific virus. So, the intensity of color indication gives proof of the presence of viruses in sample such as the production of polyclonal antibodies against the fused coat proteins (CPs) of PVX and PVY having the capability to detect the natural mixed infection of PVX and PVY causing rugose mosaic disease in potato via direct antibody-coated ELISA (DAC-ELISA) (Kapoor et al. 2013). Similarly, cocktail of polyclonal antibodies has also been designed for simultaneous detection of Cucumber mosaic virus (CMV), Groundnut bud necrosis virus (GBNV) and Papaya ringspot virus (PRSV) using either only CP sequences of first two or CP protein sequences of CMV and PRSV along with nucleocapsid protein (N) of GBNV via DAC-ELISA in Asteraceous, Cucurbitaceous, Iridaceous, Caricaceous and Solanaceous hosts at 1:500 dilution (Kapoor et al. 2014). The use of cocktail PAbs is very useful for indexing of virus-free plants especially in the vegetatively propagated crops. An alternative to the conventional ELISA is the use of magnetic microspheres/beads (6.5 µM) (Luminex xMAP technology) for linking of antibodies, instead of microtiter plate which is further used for the capturing of target pathogens. The use of paramagnetic beads has been employed for simultaneous detection of PVX, PVY and Potato leafroll virus (PLRV) in potato with equivalent sensitivity and specificity to DAS-ELISA and reduced time (Bergervoet et al. 2008). It has

been standardized for the simultaneous detection of three viruses (Watermelon silver mottle virus (WSMoV), Melon yellow spot virus (MYSV), Chilli vein-banding mottle virus (CVbMV)) along with fruit blotch bacteria (Acidovorax avenae subsp. citrulli) associated with watermelon (Charlermroj et al. 2013).

Antibody array

Array-based approaches for the immobilization of either antibodies or oligonucleotides for capturing and in turn detection of multiple target pathogens are a widespread diagnostic approach. Antibody array is an attractive technology in which captured antibodies are immobilized on the binding material followed by exposure to target samples for antigen–antibody reaction, which can then be detected by fluorescence, chemiluminescence or chromogenic substrates. A reliable and sensitive *antibody microarray was designed* for simultaneous identification of several plant viruses (*Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Strawberry latent ringspot virus* (SLRSV), *Raspberry ringspot virus* (RpRSV)) infecting grapevine and fruit crops, which was comparable to ELISA in sensitivity and specificity (Abdullahi and Rott 2009). Another visual antibody array was designed on nitrocellulose membrane for simultaneous and rapid detection of ten Solanaceae infecting pathogens, i.e., *Clavibacter michiganensis* subsp. *michiganensis*, CMV, PepMV, *Tomato aspermy virus* (TAV), *Tomato mosaic virus* (ToMV), *Tomato black ring virus* (TBRV), *Tobacco ringspot virus* (TRSV), *Tomato ringspot virus* (ToRSV), *Tobacco rattle virus* (TRV) and *Tomato spotted wilt virus* (TSWV) in tomato seed samples up to 3 months stability (Xiong et al. 2013).

Lateral flow immunoassay (LFIA)

The LFIA also known as immune chromatographic assay is a point-of-care, rapid and cheap detection procedure for detection of multiple pathogens at a time and offers a lucrative approach for on-field, rapid and low-cost identification of multiple infections. It overcomes the disadvantage of labor-intensive and multistage detection of other methods. It is a portable device in the form of a strip made of nitrocellulose membrane imbibed with antibody conjugate at the test line where the antigen-positive reaction takes place in a chromatographic (Sajid et al. 2014). The LFIA was standardized for simultaneous detection of several potato viruses (PVY, PVX, PVA, PVM, PVS, PLRV) along with a bacterium (*Clavibacter michiganensis* subsp. *sepedonicus*) called as multiarray on a test strip (MATS) within 15 min (Safenkova et al. 2016). Similarly, in another attempt an alarm LFIA was designed for the detection of five potato viruses simultaneously (PVX, PVM, PVS, PVY and PLRV) through specific antibodies immobilized on the strip with sensitivity of 10–30 ng/mL (Safenkova et al. 2018).

Nucleic acid-based methods

The use of nucleic acids such as total DNA or RNA directly for detection of the presence of viruses in a plant host is a common aspect of detection, quarantine and certification owing to the sensitivity, specificity and rapidity along with cost-effectiveness. The primers or probes are designed against a specific region or entire genome of a specific virus which is further used to make several copies of the same (amplification) to make it detectable via gel electrophoresis or naked eye through chromatographic approaches.

Molecular hybridization

Molecular hybridization methods utilize the complementarity of the nucleic acid base pairs between the target sequences to be detected to the short labeled sequence, i.e., the probe for complementary base pairing. Variants of probes that can be employed are radioactive probes, non-radioactive probes, including biotin and digoxigenin (DIG) probes, single-stranded cDNA probes and riboprobes (Mühlbach et al. 2003). The non-radioactive probes can detect the target RNA molecules at femtomole level. A single hybridization assay can be effortlessly standardized for simultaneous detection of multiple viruses by mixing different DNA or RNA probes in a sole solution (probe mix) or synthesizing a single unique probe (polyprobe) containing partial complementary sequences of DNA (DNA probes) or RNA (riboprobes) to the plant viruses to be detected. Probe mixtures have been successfully used for the simultaneous detection of viruses in many crops such as tomato, i.e., CMV, TSWV, PVY, *Tomato yellow leaf curl virus* (TYLCV), *Alfalfa mosaic virus* (AMV) and ToMV (Saldarelli et al. 1996); carnation, i.e., *Carnation mottle virus* (CarMV), *Carnation vein mottle virus* (CVMV), *Carnation Italian ringspot virus* (CIRSV), *Carnation ringspot virus* (CRSV) and *Carnation latent virus*(CLV) (Sánchez-Navarro et al. 1999); stone fruit trees, i.e., *Apple mosaic virus* (ApMV), *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) (Saade et al. 2000); geranium, i.e., *Pelargonium line pattern virus* (PLPV) and *Pelargonium flower break virus* (PFBV) (Ivars et al. 2004); and artichoke, i.e., *Artichoke latent virus* (ArLV), *Artichoke Italian latent virus* (AILV), *Artichoke mottled crinkle virus* (AMCV), CMV, *Bean yellow mosaic virus* (BYMV), *Pelargonium zonate spot virus* (PZSV), *Tomato infectious chlorosis virus* (TICV), TMV, TSWV and *Turnip mosaic virus* (TuMV) (Minutillo et al. 2012). Numerous reports have been published for simultaneous detection of several viruses in crops using polyprobes (Pallás et al. 2018) after its first development by Herranz

et al. 2005 and have recently been designed with the capacity to detect 18 pathogens, including 13 and 12 viruses, five and four viroids in grapevine and tomato, respectively (Sánchez-Navarro et al. 2018a; Sanchez-Navarro et al. 2019). Though nowadays PCR has been taken up as a routine diagnostic protocol, hybridization is considered valuable technique as it balances sensitivity with ease of use, time and cost. The hybridization approach can play a crucial role in studying the basis of interactions between different viral populations infecting a host and giving insights on the phenomenon of superinfection exclusion at the cellular level (Pallás et al. 2018).

Array-based methods

Microarray and macroarray are multipathogen diagnostic protocols that work on the principle of hybridization by base pairing between complementary sequences of the target and specific probes previously immobilized on an array which is generally a microscopic slide. The two types of arrays are distinguished on the basis of size of sample spots wherein microarray contains spot sizes of less than 200 µm, while macroarray contains spot sizes of 300 µm or more. Such arrays are designed using the nucleotide sequence data of viruses available in public databases for synthesizing oligonucleotide probes followed by their printing on the slides allowing the simultaneous detection via colorimetric, fluorescence or electric currents to detect the hybridization with the target. A cDNA microarray designed using microtube hybridization has been used for the detection of mixed infection of lily viruses (CMV, *Lily mottle virus* (LMoV), *Lily symptomless virus* (LSV) and *Plantago asiatica mosaic virus* (PAMV)) from leaves and roots of lily bulbs (Sugiyamaa et al. 2008) and for the detection of 11 potato viruses and PSTVd in potato (Agindotan and Perry 2008). A number of microarrays have been designed for multiplex detection of plant viruses (Pallás et al. 2018). Microarrays can also be designed for the simultaneous detection of five maize pathogens (MaizePath microarray) including bacteria and viruses such as MDMV and SCMV (Krawczyk et al. 2017).

Multiplex polymerase chain reaction (mPCR) and multiplex reverse transcriptase PCR (mRT-PCR)

The mPCR and mRT-PCR are the most commonly used assay for routine diagnosis of mixed infection in crops for simultaneous detection of viruses in a single tube due to their robustness, reliability and cheapness. However, the number of targets to be detected determines the sensitivity of this approach, as for each target a primer pair has to be used in the cocktail. More the number of primer pairs, more are the chances of primer dimer formation or non-specific amplification. Though use of variants such as nested PCR (Papayiannis et al. 2011), dual priming oligonucleotide (DPO) primers (Kwon et al. 2014) and magnetic nanobeads (Deng et al. 2014) leads to increased sensitivity and specificity, they also contribute to increased cost. To enhance diagnosis sensitivity in multiplex PCR, it can be coupled with a DNA chip method wherein biotinylated mPCR products of target viral sequences are captured via immobilized DNA oligonucleotide probes on a DNA biochip. Further, the signals of hybridization are detected using a streptavidin–alkaline phosphatase (Strep–AP) conjugate by chemiluminescence. This combination of PCR and hybridization method provides sensitive, specific, accurate and easy interpretation of the results as compared to conventional methods and can be efficiently standardized for the detection of multiple pathogens. Selvarajan et al. 2011 designed an mRT-PCR for detection of *Banana bunchy top virus* (BBTV) and *Banana streak MY virus* (BSMYV) simultaneously in banana samples. Multiplex RT-PCR for four viruses in garlic and for four viruses and a fastidious greening bacterium in citrus has been optimized in India (Majumder et al. 2014; Pramesh and Baranwal 2014; Meena et al. 2016). Simultaneous detection of four kiwifruit viruses (*Actinidia virus 1*(AcV-1), *Actinidia virus A* (AcVA), *Actinidia chlorotic ringspot-associated virus* (AcCRaV) and *Citrus leaf blotch virus* (CLBV)) using primers designed from CP genes for amplification with cDNA of dilution up to 10^{-4} (Peng et al. 2020).

Real-time PCR/quantitative PCR (qPCR)

An alternative to the conventional detection is via real-time detection through qPCR/qRT-PCR which enables ongoing monitoring and absolute quantification along with amplification of the target with the aid of either fluorescent dyes (SYBRGreen or EvaGreen) or probes (TaqMan). The real-time PCR is 1000-fold more sensitive as compared to hybridization protocol (Boonham et al. 2014). It can be easily adapted for the detection of 2–5 viruses and is particularly useful for studying mixed infections due to concurrent quantification of the viral load of each concerned virus. Since first detection, it has been regularly used for detection of multiple viruses with the latest reports including real-time quantification of two sugarcane-infecting badnaviruses (*Sugarcane bacilliform IM virus* (SCBIMV) and *Sugarcane bacilliform MO virus* (SCBMOV)) with 1000-fold greater sensitivity than conventional PCR (Sun et al. 2018).

The real-time PCR can also be carried out on microarrays (micromatrices) for multiplex detection and analysis in real time. It offers the advantage to time reduction due to lyophilization of reaction mixture components in the wells as well as thermal conductive nature of microchip which reduces the time by 20–30 min. Such real-time PCR-based micromatrix system has been designed for simultaneous detection of key potato pathogens, including

PLRV, PVX, PVA, PVS, PVM, PMTV, PVY (PVY and PVY^{NTN} forms), PSTVd, and other bacteria and fungi (Nikitin et al. 2018).

The multiplex detection via TaqMan is confined to four targets due to multiple partially overlapping fluorescent signals due to the usage of multiple reporter and quencher dyes in turn limiting the number of assays that can be precisely performed in a single tube. Perhaps, true multiplex testing necessitates a number of parallel TaqMan assays leading to added cost and reduced time benefits (Van der Vlugt et al. 2015). The Luminex approach, as discussed earlier, involves polystyrene, array of carboxylated paramagnetic beads internally dyed with generic fluorochrome precoupled with oligonucleotide sequences for simultaneous detection of up to 150 targets which are PCR/RT-PCR-amplified products using specific primers followed by target-specific primer extension (TSPE). It is comparatively more reliable method than conventional RT-PCR with less scope for optical error. The approach has been adapted for the multiplex detection of begomovirus isolates in tomato (van Brunschot et al. 2014), three lily viruses—*Lily mottle virus* (LMoV*), Lily symptomless virus* (LSV) and CMV in lily (Lim et al. 2016), and three cucurbit-infecting viruses—*Zucchini yellow mosaic virus* (ZYMV), *Cucumber green mottle mosaic virus* (CGMMV) and CMV in cucurbits (multiplex LiquiChip assay) (Kuan et al. 2018).

A unique technique was designed by combining the use of molecular beacons with nucleic acid sequence-based amplification (NASBA) system for simultaneous amplification and detection in a closed tube referred as AmpliDet. This system was standardized for simultaneously detecting PLRV and PVY in seed potatoes with sensitivity of up to 10 fg of purified RNA (Klerks et al. 2001).

Reverse transcriptase loop-mediated isothermal amplification (RT-LAMP)

RT-LAMP is a rapid, specific, sensitive and robust detection technique also suitable for on-site diagnosis (Notomi et al. 2000). The high specificity is the subsequent result of usage of set of 4–6 primers. It works under isothermal conditions at temperatures ranging between 60 °C and 65 °C. Results can be interpreted via naked eye or UV light or by observing the amplification curves using a portable fluorometer (Fischbach et al. 2015; Lenarcic et al. 2013; Boubourakas et al. 2009). It also has the added advantage of multiplexing for simultaneous detection and recently has been adapted in banana testing for detection of *Banana bunchy top virus* (BBTV), *Banana streak OL virus* (BSV-OL) and CMV (Zhang et al. 2018).

Next-generation sequencing (NGS)

NGS is a highly sensitive approach having the potential to identify the complete spectrum of viruses and virus-like agents (including known and known ones) infecting a given host thus enabling multiplexing in an efficient manner (Jo et al. 2018). Metagenomics has also been proved fruitful in elucidating the existing state of viruses in plants as well as the roles different viruses play in virus–virus and virus–host interactions. To identify mixed infection, NGS was employed to simultaneously detect the causal agents of a sweet potato viral disease, i.e., Sweet potato chlorotic stunt virus and Sweet potato feathery mottle virus using small RNA profiling from co-infected plants. Along with these two viruses, they could also identify the infection of two new badnaviruses and a new mastrevirus (Kreuze et al. 2009). NGS can also be efficiently employed to determine the etiology of a disease as used in case of declining disease in Syrah wine grape which was considered as an emerging disease. The results confirmed the decline to be a manifestation of mixed infection that encompassed seven different RNA genomes including four viruses and three viroids (Al Rwahnih et al. 2009). It has also been used to identify the causative viruses of emerging and devastating maize lethal necrosis disease and results indicated it to be a manifestation of mixed infection of MCMV and SCMV (Adams et al. 2009). Apart from the ability to detect and identify viruses of different families and genera, the co-infections of multiple isolates or variants of a virus can also be identified via NGS. In apple, the NGS analysis revealed 14 definite isolates of *Apple stem pitting virus* (ASPV) and five variants of *Apple chlorotic leaf spot virus* (ACLSV) (James et al. 2017). The identification of isolates of a single virus may be of grave significance due to the far greater effect on disease symptoms and interactions than between viruses belonging to different families. The NGS proves to be the best available approach to confirm the mixed infections of crop and identification of viruses leading to disease symptoms in the host provided the lacuna of cost and time can be overcome.

Conclusion and future perspectives

Each and every cultivated and wild plant is infected by multiple viruses which may be known or unknown belonging to same or different families/genera. Understanding the ecology and epidemiology of mixed infections is an integral aspect of plant virology to decipher the impact it poses on the plant community. Moreover in mixed infections, due to frequent mutations, recombination and reassortments in the viral genome, variants and pseudo-recombinants of viruses evolve at a quick pace which might be deadlier, making the study of various concerned component viruses, of mixed

infections, of paramount importance. This is where a sensitive, specific and rapid diagnostic protocol, which can be multiplexed for simultaneous detection of large number of viruses, becomes necessary, as detection is a critical aspect for management of plant viruses due to lack of commercially available chemistries to destroy them. Thus, research and resources need to be directed toward the robust, quick and cost-effective diagnostic protocols and equipments. In mixed infections, responses and changes can be noted on all the interacting components, i.e., plants, viruses and vectors in terms of gene expression and protein synthesis. An upsurge in the gene expression and protein synthesis in plants lead to increased energy utilization due to vigorous retort of plants to multiple viral infections. Mixed infections alter the immune defense in host and cause phenotypic changes most commonly. But other changes in terms of breakdown of resistance of cultivars and uninfected hosts possess the biggest threat to agriculture. Thus, elucidating the key activities and point of interactions between plants and viruses must be taken up.

Acknowledgements Corresponding author is highly thankful to all co-authors for their contribution and valuable time

Compliance with ethical standards

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

References

Abdullahi I, Rott M (2009) Microarray immunoassay for the detection of grapevine and tree fruit viruses. J Virol Methods 160:90–100

Adams IP, Glover RH, Monger WA, Mumford R, Jackeviciene E, Navalinskiene M et al (2009) Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology. Mol Plant Pathol 10:537–545

Agindotan B, Perry KL (2008) Macroarray detection of eleven potato-infecting viruses and potato spindle tuber viroid. Plant Dis 92:730–740

Al Rwahnih M, Daubert S, Golino D, Rowhani A (2009) Deep sequencing analysis of RNAs from a grapevine showing syrah decline symptoms reveals a multiple virus infection that includes a novel virus. Virology 387:395–401

Alvez-Junior M, Alfenas-Zerbini P, Andrade EC, Esposito DA, Silva FN, da Cruz ACF, Ventrella MC, Otoni WC, Zerbini FM (2009) Synergism and negative interference during co-infection of tomato and *Nicotiana benthamiana* with two bipartite begomoviruses. Virology 387:257–266

Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agro technology drivers. Trends Ecol Evol 19:535–544

Anjos JR, Jarlfors U, Ghabrial SA (1992) Soybean mosaic potyvirus enhances the titer of two comoviruses in dually infected soybean plants. Phytopathology 82:1022–1027

Bak A, Cheung AL, Yang C, Whitham SA, Casteel CL (2017) A viral protease relocalizes in the presence of the vector to promote vector performance. Nat Commun 8:14493

Balogun OS, Teraoka T, Kunimi Y (2005) Influence of the host cultivar on disease and viral accumulation dynamics in tomato under mixed infection with potato virus X And tomato mosaic virus. Phytopathol Mediterr 44:29–37

Baltenberger DE, Ohm HW, Foster JE (1987) Reactions of oat, barley, and wheat to infection with barley yellow dwarf virus isolates. Crop Sci 27:195

Barker H, Woodford JAT (1992) Spread of potato leafroll virus is decreased from plants of potato clones in which virus accumulation is restricted. Ann Appl Biol 121:345–354

Bergervoet JHW, Peters J, Jose RCM, van Beckhoven G´eW, van den Bovenkampb, James W, Jacobson C, Jan M, van der Wolf (2008) Multiplex microsphere immuno-detection of potato virus Y, X and PLRV. J Virol Methods 149:63–68

Beperet I, Irons SL, Simón O, King LA, Williams T, Possee RD, López-Ferber M, Caballero P (2014) Superinfection exclusion in alphabaculovirus infections is concomitant with actin reorganization. J Virol 88:3548–3556

Biosecurity New Zealand (2009). Citrus (Citrus), Fortunella (Kumquat) and Poncirus (Trifoliate orange) post-entry quarantine testing manual. Ministry of agriculture and forestry, biosecurity, Auckland, pp. 58

Boonham N, Kreuze J, Winter S, van der Vlugt R, Bergervoet J, Tomlinson J et al (2014) Methods in virus diagnostics: from ELISA to next generation sequencing. Virus Res 186:20–31

Boubourakas I, Fukuta S, Kyriakopoulou P (2009) Sensitive and rapid detection of peach latent mosaic viroid by the reverse transcription loop-mediated isothermal amplification. J Virol Methods 160:63–68

Bourdin D, Lecoq H (1991) Evidence that heteroencapsidation between two potyviruses is involved in aphid transmission of a non-aphid transmissible isolate from mixed infections. Phytopathology 81:1459–1464

Calvert LA, Thresh JM (2002) The virus and virus diseases of cassava. In: Hillocks RJ, Thresh JM, Belloti AC (eds) Cassava: viology, production and utilization. CABI Publishing, Wallingford

Carr RJ, Kim KS (1983) Evidence that bean golden mosaic virus invades non-phloem tissue in double infections with tobacco mosaic virus. J Gen Virol 64:2489–2492

Charlermroj R, Himananto O, Seepiban C, Kumpoosiri M, Warin N et al (2013) Multiplex detection of plant pathogens using a microsphere immunoassay technology. PLoS ONE 8(4):e62344

Chavez-Calvillo G, Contreras-Paresdes CA, Mora-Macias J, Noa-Carrazana JC, Serrano-Rubio AA, Dinkova TD, Carrillo-Tripp M, Silva-Rosales L (2016) Antagonism or synergism between papaya ringspot virus and papaya mosaic virus in *Carica papaya* is determined by their order of infection. Virology 489:179–191

Chen Z, Tan JY, Wen Y, Niu S, Wong SM (2012) A game-theoretic model of interactions between hibiscus latent Singapore virus and tobacco mosaic virus. PLoS ONE 7:e37007

Chikh-Ali M, Gray SM, Karasev AV (2013) An improved multiplex IC-RT-PCR assay distinguishes nine strains of *Potato virus Y*. Plant Dis 97:1370–1374

Choi SK, Yoon JY, Ryu H, Choi JK, Palukaitis P, ParkWM, (2002) Systemic movement of a movement-deficient strain of *Cucumber mosaic virus* in zucchini squash is facilitated by a cucurbit-infecting potyvirus. J Gen Virol 83:3173–3178

Constable FE, Connellan J, Nicholas P, Rodoni BC (2013) The reliability of woody indexing for detection of grapevine virus associated diseases in three different climatic conditions in Australia. Aust J Grape Wine Res 19:74–80

Creamer R, Falk BW (1990) Direct detection of transcapsidated barley yellow dwarf luteoviruses in doubly infected plants. J Gen Virol 71:211–217

De Bokx JA, Van Hoof HA, Piron PGM (1978) Relation between concentration of potato virus YN and its availability to *Myzus persicae*. Eur J Plant Pathol 84:95–100

Deng XG, Zhu F, Chen YJ, Liu J, Zhu T, Li JY et al (2014) A more sensitive and rapid multiplex RT-PCR assay combining with magnetic nanobeads for simultaneous detection of viruses in sweet potato. Eur J Plant Pathol 140:111–117

Domingo-Calap ML, Moreno AB, Diaz-Pendon JA, Moreno A, Ferees A, Lopez-Moya JJ (2020) Assessing the impact on virus transmission and insect vector behavior of a viral mixed infection in melon. Phytopathology 110:174–186

El-Araby WS, Ibrahim IA, Hemeida AA, Mahmoud A, Soliman AM, El-Attar AK, Mazyad HM (2009) Biological, serological and molecular diagnosis of three major potato viruses in Egypt. Int J Virol 5(2):77–88

Fischbach J, Xander NC, Frohme M, Glökler JF (2015) Shining a light on LAMP assays-a comparison of LAMP visualization methods including the novel use of berberine. Biotechniques 58:189–194

Folimonova SY, Robertson CJ, Shilts T, Folimonov AS, Hilf ME, Garnsey SM, Dawson WO (2010) Infection with strains of Citrus tristezavirus does not exclude superinfection by other strains of the virus. J Virol 84:1314–1325

Froissart R, Michalakis Y, Blanc S (2002) Helper component trans complementation in the vector transmission of plant viruses. Phytopathology 92:576–579

Garcia-Cano E, Resende RO, Fernandez-Munoz R, Moriones E (2006) Synergistic interaction between *Tomato chlorosis virus* and *Tomato spotted wilt virus* results in breakdown of resistance in tomato. Phytopathology 96:1263–1269

García-Marcos A, Pacheco R, Martiáñez J, González-Jara P, Díaz-Ruíz JR, Tenllado F (2009) Transcriptional changes and oxidative stress associated with the synergistic interaction between potato virus X and potato virus Y and their relationship with symptom expression. Mol Plant Microbe Interact 22:1431–1444

Gentit P (2006) Detection of plum pox virus: biological methods. EPPO Bulletin 36:251–253

Goldberg KB, Brakke M (1987) Concentration of *Maize chlorotic mottle virus* increased in mixed infections with *Maize dwarf mosaic virus*, strain B. Phytopathology 77:162–167

Gomez P, Sempere RN, Amari K, Gomez-Aix C, Aranda MA (2010) Epidemics of *Tomato torrado virus*, *Pepino mosaic virus* and *Tomato chlorosis virus* in tomato crops: do mixed infections contribute to torrado disease epidemiology? Ann Appl Biol 156:401–410

Gonalez-Jara P, Atencio FA, Martinez-Garcia B, Barajas D, Tenllado F, Diaz-Ruiz JR (2005) A single amino acid mutation in the plum pox virus helper component-proteinase gene abolishes both synergistic and RNA silencing suppression activities. Phytopathology 95:894–901

Gray SM, Power AG, Smith DM, SeamanAltman AJNS (1991) Aphid transmission of barley yellow dwarf virus: acquisition access periods and virus concentration requirements. Phytopathology 81:539–545

Hacker DL, Fowler BC (2000) Complementation of the host range restriction of southern cowpea mosaic virus in bean by southern bean mosaic virus. Virology 266:140–149

Herranz MC, Sánchez-Navarro JA, Aparicio F, Pallás V (2005) Simultaneous detection of six stone fruit viruses by non-isotopic molecular hybridization using a unique riboprobe or 'polyprobe'. J Virol Methods 124:49–55

Ivars P, Alonso M, Borja M, Hernandez C (2004) Development of a non-radioactive dot-blot hybridisation assay for the detection of Pelargonium flower break virus and pelargonium line pattern virus. Eur J Plant Pathol 110:275–283

Jacobson AL, Duffy S, Sseruwagi P (2018) Whitefly-transmitted viruses threatening cassava production in Africa. Curr Opin Virol 33:167–176

Jo Y, Lian S, Chu H, Cho JK, Yoo A-H, Choi H et al (2018) Peach RNA viromes in six different peach cultivars. Sci Rep 8:1844

Jones RAC, Barbetti MJ (2012) Influence of climate change on plant disease infections and epidemics caused by viruses and bacteria. CAB Rev 7(22):1–32

Kanakala S, Jyothsna P, Shukla R, Tiwari N, Veer BS, Swarnalatha P, Krishnareddy M, Malathi VG (2013) Asymmetric synergism and heteroencapsidation between two bipartite begomoviruses, *Tomato leaf curl NewDelhi virus* and *Tomato leaf curl Palampur virus*. Virus Res 174:126–136

Kapoor R, Mandal B, Paul PK, Chigurupati P, Jain RK (2014) Production of cocktail of polyclonal antibodies using bacterial expressed recombinant protein for multiple virus detection. J Virol Methods 196:7–14

Kapoor R, Mandal B, Paul PK, Jain RK (2013) Simultaneous detection of potato viruses Yand X by DAC-ELISA using polyclonal antibodies raised against fused coat proteins expressed in Escherichia coli. J Plant Biochem Biotechnol. https://doi.org/10.1007/s13562-013-0251-5

Karyeija RF, Kreuze JF, Gibson RW, Valkonen JP (2000) Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. Virology 269:26–36

Klerks MM, Leone GOM, Verbeek M, van den Heuvel JFJM, Schoen CD (2001) Development of a multiplex AmpliDet RNA for the simultaneous detection of *Potato leafroll virus* and *Potato virus Y* in potato tubers. J Virol Methods 93:115–125

Krawczyk K, Uszczynska-Ratajczak B, Majewska A, Borodynko-Filas N (2017) DNA microarray-based detection and identification of bacterial and viral pathogens of maize. J Plant Dis Prot 124:577–583

Kreuze JF, Perez A, Untiveros M, Quispe D, Fuentes S, Barker I et al (2009) Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses. Virology 388:1–7

Kumari P, Singh AK, Sharma VK, Chattopadhyay B, Chakraborty S (2011) A novel recombinant tomato-infecting begomovirus capable of transcomplementing heterologous DNA-B components. Adv Virol 156:769–783

Kwon JY, Hong JS, Kim MJ, Choi SH, Min BE, Song EG et al (2014) Simultaneous multiplex PCR detection of seven cucurbit infecting viruses. J Virol Methods 206:133–139

Cheng-Ping K, Wen-Shi C, Tso-Chi Y (2018) Use of fluorescent microsphere-based assay for detection of three cucurbit-infecting viruses. Plant Dis 102:2324–2329

Legg JP, Thresh JM (2000) Cassava mosaic virus disease in East Africa: dynamic disease in a changing environment. Virus Res 71:135–149

Legrand P (2015) Biological assays for plant viruses and other graft transmissible pathogens diagnoses: a review. Bulletin OEPP/EPPO Bulletin 45(2):240–251

Legg JP, Fauquet CM (2004) Cassava mosaic geminiviruses in Africa. Plant Mol Biol 56:31–39

Lenarcic R, Morisset D, Mehle N, Ravnikar M (2013) Fast real-time detection of potato spindle tuber viroid by RT-LAMP. Plant Pathol 62:1147–1156

Lim MS, Kim SM, Choi SH (2016) Simultaneous detection of three lily infecting viruses using a multiplex Luminex bead array. J Virol Methods 231:34–37

Majumder S, Baranwal VK (2014) Simultaneous detection of four garlic viruses by multiplex reverse transcription PCR and

their distribution in Indian garlic accessions. J Virol Methods 202:34–38
Manoussopoulos IN (2001) Acquisition and retention of potato virus Y helper component in the transmission of potato aucuba mosaic virus by aphids. J Phytopathol 149:103–106
Mascia T, Gallitelli D (2016) Synergies and antagonisms in virus interactions. Plant Sci 252:176–192
Meena RP, Baranwal VK (2016) Development of multiplex polymerase chain reaction assay for simultaneous detection of clostero-, badna- and mandari-viruses along with huanglongbing bacterium in citrus trees. J Virol methods 235:58–64
Mendez-Lozano J, Torres-Pacheco I, Fauquet CM, Rivera-Bustamante RF (2003) Interactions between geminiviruses in a naturally o ccurring mixture: *Pepper huasteco virus* and *Pepper golden mosaic virus*. Phytopathology 93:270–277
Minutillo SA, Mascia T, Gallitelli D (2012) A DNA probe mix for the multiplex detection of ten artichoke viruses. Eur J Plant Pathol 134:459–465
Mühlbach HP, Weber U, Gomez G, Pállas V, Duran-Vila N, Hadidi A (2003) Molecular hybridization. In: Hadidi A, Flores R, Randles JW, Semancik J (eds) Viroids. CSIRO, Collingwood, pp 103–114
Mukasa SB, Rubaihayo PR, Valkonen JPT (2006) Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfected sweetpotato plants. Plant Pathol 55:458–467
Murant AF (1990) Dependence of groundnut rosette virus on its satellite RNA as well as on groundnut rosette assistor luteovirus for transmission by*Aphis craccivora*. J Gen Virol 71:2163–2166
Nabi SU, Mir JI, Sharma OC, Singh DB, Zaffer S, Sheikh MA et al (2018) Optimization of tissue and time for rapid serological and molecular detection of apple stem pitting virus and apple stem grooving virus in apple. Phytoparasitica 46(5):705–713
Naidu R, Rowhani A, Fuchs M, Golino D, Martelli GP (2014) Grapevine leafroll: a complex viral disease affecting a high value fruit crop. Plant Dis 98:1172–1185
Naidu RA, Maree HJ, Burger JT (2015) Grapevine leafroll disease and associated viruses: a unique pathosystem. Annu Rev Phytopathol 53:614–634
Nikitin MM, Statsyuk NV, Frantsuzov PA, Dzhavakhiya VG, Golikov AG (2018) Matrix approach to the simultaneous detection of multiple potato pathogens by real-time PCR. J Appl Microbiol 124(3):797–809
Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loop-mediated isothermal amplification of DNA. Nucl Acids Res 28:e63i–63vii
Pallás V, Sánchez-Navarro JA, James D (2018) Recent advances on the multiplex molecular detection of plant viruses and viroids. Front Microbiol 9:2087
Papayiannis LC, Harkou IS, Markou YM, Demetriou CN, Katis NI (2011) Rapid discrimination of tomato chlorosis virus, tomato infectious chlorosis virus and co-amplification of plant internal control using real-time RT-PCR. J Virol Methods 176:53–59
Passmore BK, Sanger M, Chin LS, Falk BW, Bruening G (1993) Beet western yellows virus-associated RNA: an independently replicating RNA that stimulates virus accumulation. PNAS USA 90:10168–10172
Pechinger K, Chooi KM, MacDiarmid RM, Harper SJ, Ziebell H (2019) A new era for mild strain cross-protection. Viruses 11(7):670
Peng Q, Qiu L, Yang T, Ning J, Xu Q, Dong J, Xi D (2020) A multiple reverse transcription PCR assay for simultaneous detection of four main viruses in kiwifruit. Eur J Plant Pathol. https://doi.org/10.1007/s10658-020-01950-w
Pereira AM, Lister RM, Barbara DJ, Shaner GE (1989) Relative transmissibility of barley yellow dwarf virus from sources with differing virus contents. Phytopathology 79:1353–1358
Pruss G, Ge X, Shi XM, Carrington JC, Vance VB (1997) Plant viral synergism: the potyviral genome encodes a broad-range pathogenicity enhancer that trans activates replication of heterologous viruses. Plant Cell 9:859–868
Querci M, Owens RA, Bartolini I, Lazarte V, Salazar L (1997) Evidence for heterologous encapsidation of potato spindle tuber viroid in particles of potato leafroll virus. J Gen Virol 78:1207–1211
Redinbaugh MG, Stewart LR (2018) Maize lethal necrosis: An emerging synergistic viral disease. Ann Rev Virol 5:301–322
Rezende JAM, Müller GW (1995) Mecanismos de proteção entre os vírus e controle de viroses de vegetais por premunização. Revisão Anual de Patologia de Plantas 3:185–226
Rojas MR, Gilbertson RL (2008) Emerging plant viruses: a diversity of mechanisms and opportunities. In: Roossinck MJ (ed) Plant virus evolution. Springer, Berlin Heidelberg, p 27
Saade M, Aparicio F, Sánchez-Navarro JA, Herranz MC, Myrta A, Di Terlizzi B et al (2000) Simultaneous detection of three ilarviruses affecting stone fruits by non-isotopic molecular hybridization and multiplex RT-PCR. Phytopathology 96:1330–1336
Safenkova IV, Pankratova GK, Zaitsev IA, Varitsev VA, Vengerov YY, Zherdev AV, Dzantiev BB (2016) Multiarray on a test strip (MATS): rapid multiplex immunodetection of priority potato pathogens. Anal Bioanal Chem. https://doi.org/10.1007/s00216-016-9463-6
Safenkova IV, Panferov VG, Panferova NA, Varitsev YA, Zherdev AV, Dzantiev BD (2018) Alarm lateral flow immunoassay for detection of the total infection caused by the five viruses. Talanta. https://doi.org/10.1016/j.talanta.2018.12.004
Sajid M, Kawde A-N, Daud M (2014) Designs, formats and applications of lateral flowassay: A literature review. J Saudi Chem Soc. https://doi.org/10.1016/j.jscs.2014.09.001
Saldana J, Elena SF, Sole RV (2003) Coninfection and superinfection in RNA virus populations: a selection-mutation model. Math Biosci 183:135–160
Saldarelli P, Barbarossa L, Grieco F, Gallitelli D (1996) Digoxigenin labelled riboprobes applied to phytosanitary certification of tomato in Italy. Plant Dis 80:1343–1346
Sánchez-Navarro JA, Cañizares MC, Cano EA, Pallás V (1999) Simultaneous detection of five carnation viruses by non-isotopic molecular hybridization. J Virol Methods 82:167–175
Sánchez-Navarro JA, Cooper CN, Pallás V (2018a) Polyvalent detection of members of the Potyvirus genus by molecular hybridization using a 'Genusprobe'. Phytopathology. https://doi.org/10.1094/PHYTO-04-18-0146-R
Sánchez-Navarro JA, Fiore N, Fajardo TVM, Pallás V (2018b) "Simultaneous detection of the 13 viruses and 5 viroids affecting grapevine by molecular hybridization using a unique probe or 'polyprobe'". In: Proceedings of the 19th conference of the international council for the study of virus and virus-like diseases of the grapevine, Santiago
Sanchez-Navarro JA, Corachán L, Font I et al (2019) Polyvalent detection of twelve viruses and four *viroids affecting tomato* by using a unique polyprobe. Eur J Plant Pathol 155:361
Sanger M, Passmore B, Falk BW, Bruening G, Ding B, Lucas WJ (1994) Symptom severity of beet western yellows virus strain ST9 is conferred by the ST9-associated RNA and is not associated with virus release from the phloem. Virology 200:48–55
Sarika Akram M, Iquebal MA, Maimuddin K (2010) Prediction of MHC binding peptides and epitopes from coat protein of Mungbean yellow mosaic India virus-Ub05. J Proteom Bioinform 3:173–178
Sastry KS, Zitter TA (2014) Management of virus and viroid diseases of crops in the tropics. Plant Virus Viroid Dis Trop 2:149–480
Savenkov EI, Valkonen JPT (2001) Potyviral helper-component proteinase expressed in transgenic plants enhances titers of potato

leafroll virus but does not alleviate its phloem limitation. Virology 283:285–293
Selvarajan R, Sheeba MM, Balasubramanian V (2011) Simultaneous detection of episomal *Banana streak Mysore virus* and *Banana bunchy top virus* using multiplex RT-PCR. Curr Sci 100:31–34
Scheets K (1998) Maize chlorotic mottle machlomovirus and wheat streak mosaic rymovirus concentrations increase in the synergistic disease corn lethal necrosis. Virology 242:28–38
Sharma P, Rishi N (2007) Cotton leaf curl disease, an emerging whitefly transmissible begomovirus complex. Plant Viruses 1:127–134
Sharp LP, Hou Y-M, Garrido-Ramirez ER, Guzman P, Gilbertson RL (1999) A synergistic interaction between geminivirus DNA components results in increased symptom severity and viral DNA levels in plants. Phytopathology 89:S71
Singh AK, Chattopadhyay B, Chakraborty S (2012) Biology and interactions of two distinct monopartite begomoviruses and betasatellites associated with radish leaf curl disease in India. Virol J 9(1):43
Srinivasan R, Alvarez JM (2007) Effect of mixed viral infections (Potato virus Y-Potato leafroll virus) on biology and preference of vectors *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae). J Econ Entomol 100:646–655
Sugiyamaa S, Masutab C, Sekiguchic H, Ueharad T, Shimurab H, Maruta Y (2008) A simple, sensitive, specific detection of mixed infection of multiple plant viruses using macroarray and microtube hybridization. J Virol Methods 153:241–244
Sun S, Ahmad K, Wu X, Chen J, Fu H, Huang M, Gao S (2018) Development of quantitative real-time PCR assays for rapid and sensitive detection of two badna virus species in sugarcane. BioMed Res Int. https://doi.org/10.1155/2018/8678242
Syller J (2012) Facilitative and antagonistic interactions between plant viruses in mixed infections. Mol Plant Pathol 13:204–216
Tatineni S, Graybosch RA, Hein GL, Wegulo SN, French R (2010) Wheat cultivar-specific disease synergism and alteration of virus accumulation during co-infection with Wheat streak mosaic virus and Triticum mosaic virus. Phytopathology 100:230–238
Tatineni S, Riethoven JM, Graybosch RA, French R, Mitra A (2014) Dynamics of small RNA profiles of virus and host origin in wheat cultivars synergistically infected by Wheat streak mosaic virus and Triticum mosaic virus: virus infection caused a drastic shift in the endogenous small RNA profile. PLoS ONE 9:e111577
Thresh JM, Cooter RJ (2005) Strategies for controlling cassava mosaic virus disease in Africa. Plant Pathol 54:587–614
Untiveros M, Olspert A, Artola K, Firth AE, Kreuze JF, Valkonen JPT (2016) A novel sweet potato potyvirus open reading frame (ORF) is expressed via polymerase slippage and suppresses RNA silencing. Mol Plant Pathol 17:1111–1123
van Brunschot SL, Bergervoet JHW, Pagendam DE, de Weerdt M, Geering ADW, Drenth A et al (2014) A bead-based suspension array for the multiplexed detection of begomoviruses and their whitefly vectors. J Virol Methods 198:86–94
van der Vlugt RAA, van Raaij H, de Weerdt M, Bergervoet JHW (2015) Multiplex detection of plant pathogens through the luminex magplex bead system. In: Lacomme C (ed) Plant pathology: techniques and protocols, methods in molecular biology. Humana press, New York
Vanitharani R, Chellappan P, Pita J, Fauquet C (2004) Differential roles of AC2 and AC4 of cassava Gemini viruses in mediating synergism and suppression of posttranscriptional gene silencing. J Virol 78:9487–9498
Vance VB (1991) Replication of potato virus X RNA is altered in coinfections with potato virus Y. Virology 182:486–494
Venkataravanappa V, Reddy CNL, Jalali S, Reddy MK (2015) Association of tomato leaf curl New Delhi virus DNA-B with bhendi yellow vein mosaic virus in okra showing yellow vein mosaic disease symptom. Acta Virol 59:125–139
Vidalakis G, Garnsey SM, Bash JA, Greer GD, Gumpf DJ (2004) Efficacy of bioindexing for graft-transmissible citrus pathogens in mixed infections. Plant Dis 88:1328–1334
Wang Y, Lee KC, Gaba V, Wong SM, Palukaitis P, Gal-On A (2004) Breakage of resistance to cucumber mosaic virus by co-infection with Zucchini yellow mosaic virus: enhancement of CMV accumulation independent of symptom expression. Adv Virol 149:379–396
Wang H, Xu D, Pu L, Zhou G (2014) Southern rice black-streaked dwarf virus alters insect vectors' host orientation preferences to enhance spread and increase Rice ragged stunt virus co-infection. Phytopathology 104:196–201
Wintermantel WM, Cortez AA, Anchieta AG, Gulati-Sakhuja A, Hladky LL (2008) Co-infection by two criniviruses alters accumulation of each virus in a host-specific manner and influences efficiency of virus transmission. Phytopathology 98:1340–1345
Wege C, Siegmund D (2007) Synergism of a DNA and an RNA virus: enhanced tissue infiltration of the begomovirus *Abutilon mosaic virus*(AbMV) mediated by *Cucumber mosaic virus* (CMV). Virology 357:10–28
Wolf YI, Kazlauskas D, Iranzo J, Lucia-Sanz A, Kuhn JH, Krupovic M, Dolja VV, Koonin EV (2018) Origins and evolution of the global RNA virome. MBio 9:e02329–e2418
Xiong L, Gao L, Liu Q, Xia J, Han X, Liu Y (2013) A simple, rapid and visual antibody array for the simultaneous detection of multiple plant pathogens. Anal Methods 5:2413
Yeh SD, Cheng YH (1989) Use of resistant *Cucumis metuliferus* for selection of nitrous-acid induced attenuated strains of papaya ringspot virus. Phytopathology 79:1257–1261
Zaidi SS, Shafiq M, Amin I, Scheffler BE, Scheffler JA, Briddon RW, Mansoor S (2016) Frequent occurrence of *Tomato leaf curl New Delhi virus* in cotton leaf curl disease affected cotton in Pakistan. PLoS ONE 11:e0155520
Zhang J, Borth W, Lin B, Melzer M, Shen H, Pu X, Sun D, Nelson S, Hu J (2018) Multiplex detection of three banana viruses by reverse transcription loop-mediated isothermal amplification (RT-LAMP). Trop Plant Pathol. https://doi.org/10.1007/s40858-018-0257-6
Ziebell H, Carr JP (2010) Cross-protection: a century of mystery. Adv Virus Res 76:211–264