



Detection and Characterization of Plant Viruses Infecting *Hibiscus rosa-sinensis* L.

8

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Abstract

An ornamental plant *Hibiscus rosa-sinensis* is cultivated as a topical perennial shrub. Viral pathogenesis upon this flowering plant shows variable symptoms like mosaic, vein banding chlorotic spot, ringspots, etc., and its host range, transmissibility, and morphology are the backbone for viral identification. Vector-virus relationship manipulates the vector behavior and activates proteins which on one half facilitate effective transmission adding in viral diseases and on other half promote genetic behavior, recombination, and evolution of viruses. Disease-causing microorganisms require diagnostic techniques which is necessary for efficient and specific for viral detection which may also contribute to early disease management. Further different approaches such as use of RT-PCR, various microscopic technique, chemical analysis, high-throughput sequencing, and serological and molecular methods are also described for virus identification. Failure in eco-agriculture practice led to considerable financial loss affecting directly dependent developing countries and indirectly developed nations. Therefore, their management is essential to avoid risk. The vast view and understanding of viral disease precisely on *Hibiscus rosa-sinensis* and also the peak of approaches engaged for management are summarized in this chapter.

Keywords

Ornamental plants · Hibiscus · Plant virus · Vector-virus relationship · Detection

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151

8.1 Introduction

Plant viruses are omnipresent to all the habitats that support primitive life along with most important emerging infectious viral diseases of plants. Amid the diseases that affect plants, viral diseases are significantly important as it can be rapidly transmitted by different mode without any effective approach to treat the virus-infected plants (Hull 2014). *Tobacco mosaic virus* (TMV) was the earliest virus that has been studied and was discovered by Martinus Beijerinck in 1898. These pathogenic viruses of plant are **nucleoproteins** parasitic with variations in size, physical structure, chemical composition, and number of genomic units. They are vectored by insects, mites, fungi, **nematode**, and parasitic plants and also transmitted by **vegetative propagation**, **pollen**, and seed and also by contact. Plant viruses migrate within a plant via **plasmodesmata** for between-cell movement and also via **phloem** for transport. Plant viruses could show divergent symptoms like mosaic patterns, streaking, and hypo- or hyperplasia and stunting vein clearing. Moreover, plants can also maintain viruses in them without any accessible symptoms. Furthermore, as obligate parasites of host plants, these viruses could cause utmost injury to plants. Ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) and a protein capsid are established as a nucleic acid composition in these viruses. Classification of plant viruses is based on their chemical compounds and morphologic, biologic, pathologic, and molecular characteristics. Simultaneously, these plant viruses with their host cell create a close biologic unit. Concurrently, some artificial and natural substances are supposed to inhibit virus replication and their cell-to-cell movement, and although these substances cannot act as viricides in vivo, still viral infection can cause biologic decline which contributes to the reduction in the competitive ability of weeds in agroecosystems. Therefore, viruses can indirectly contribute to weed control also.

Hibiscus rosa-sinensis L. (family Malvaceae) is a tropical perennial shrub often grown as an ornamental plant in tropical as well as subtropical region and is admired for its aesthetic value; in Indian landscape gardens from ancient times, this plant is popular. Among the medicinally important flowering plants, hibiscus is widely available and engaged by practitioner of natural health for contraception, menorrhagia, venereal diseases, controlling uterine bleeding, fever, and cough, refrigerant and vitalize in palpitation. It is reported to contain anthocyanins, flavonoids, cyclopeptide alkaloid, and vitamins. The pharmacological and clinical studies confirmed the therapeutic value of 1172 *Hibiscus rosa-sinensis* (Jadhav et al. 2009). However, due to viral diseases that results in yield losses in the form of reduced flowering along with immature, irregular, and deformed flowering. Leaves that are affected are severely curled, reducing the aesthetic value with stunted plants (Rajeshwari et al. 2005). In this reveiw, we are going to discuss the esteemed effect, geographical distribution, identification, and characterization of viruses and the novel emerging approaches for viral disease management of plant virus on *Hibiscus rosa-sinensis* L.

8.2 Virus Disease of *Hibiscus*

Ornamental plants are economically important which are very popular throughout the world. Demand of these ornamentals plants is expanding constantly in international market. Moreover, it includes production of cut and potted flowers, cut foliage plants, as well as propagation material. However, both decorative value and quality of propagated material obtained from ornamental plants are diminished by viruses and viroids infection. Due to this reason wide range of ornamental plant species along with cultivars and their expanded geographical distribution, increases the diversity of virus infected them. However, viral phytopathogens can also spread from vegetative propagation. Natural reservoirs for plant viruses that promote circulation of viruses and their transmission to other economically important crops are generally perennial ornamental crops, and *Hibiscus rosa-sinensis* L. is one of them. Effective investigation, detection, and prevention of diversity among these viruses and viroids associated with ornamental plants are essential to conserve the further spread of infections (Mitrofanova et al. 2018).

Hibiscus rosa-sinensis Linn (*family*: Malvaceae), an attractive horticultural plant, is a tropical perennial shrub frequently cultivated as an ornamental plant. Five plant viruses that utmost infect *H. rosa-sinensis* that have been characterized previously are *Hibiscus chlorotic ringspot virus* (HCRSV, genus *Carmovirus*), *Hibiscus yellow mosaic virus* (genus *Tobamovirus*), *Eggplant mottled dwarf virus* (EMDV, genus *Nucleorhabdovirus*), *Hibiscus latent ringspot virus* (HLRSV, genus *Nepovirus*), and *Okra mosaic virus* (OkMV, genus *Tymovirus*) (Regenmortel et al. 2000) from China. In recent year, two novel tobamoviruses infecting *H. rosa-sinensis* were characterized in Singapore, Florida and two new begmoviruses were reported in Brazil (Adkins et al. 2006; Quadros et al. 2019). Virus isolated from *H. rosa-sinensis* in China was analyzed by biological and serological means along with virion properties and coat protein (CP) gene sequences, concluding that it was a *Tomato mosaic virus* (ToMV) that infects *H. rosa-sinensis*. Multiple studies have been done on RNA, DNA, and especially multiple alignments of amino acid sequences that demonstrated several substitutions in CPs of hibiscus-infecting ToMV isolates which might contribute to the variations in symptoms induced by them.

8.3 Geographical Distribution

The rapid spread of virus associated with *Hibiscus* draws a lot of attention from different countries. Virology experts are trying to understand the different mechanisms as the virus disease occurred in almost all part of the countries. Table 8.1 gives the valuable information field symptomatology and geographical spread of the virus associated with *Hibiscus*.

Table 8.1 Worldwide distributions of viruses infecting *Hibiscus rosa-sinensis*

<i>Genus</i>	Virus	Symptoms	Geographical origin	References
<i>Tobamovirus</i>	Hibiscus latent ringspot virus	Leaf mosaic and mottle	Western Nigeria	Brunt et al. (1980)
	Hibiscus latent Singapore virus	Chlorotic mottling	Singapore	Srinivasan et al. (2002)
	Hibiscus latent Fort Pierce virus	Diffuse chlorotic spots and mottle	Florida	Adkins et al. (2003)
	Tomato mosaic virus	Mosaic	China	Huang et al. (2004)
	Hibiscus latent Fort Pierce virus	Diffuse chlorotic spots and mottle	New Mexico	Allen et al. (2005)
	Hibiscus latent Fort Pierce virus—J	Diffuse chlorotic spots and mottle	Japan	Yoshida et al. (2014)
	Tomato mosaic virus—Ab10	Mosaic	Iran	Parizipour and Keshavarz-Tohid (2020)
	Tomato mosaic virus—Ah14	Mosaic	Iran	Parizipour and Keshavarz-Tohid (2020)
	Tomato mosaic virus—D09	Mosaic	Iran	Parizipour and Keshavarz-Tohid (2020)
	Tomato mosaic virus—M06	Mosaic	Iran	Parizipour and Keshavarz-Tohid (2020)
<i>Carmovirus</i>	Hibiscus chlorotic ringspot virus	Chlorotic spotting and ringspots	El Salvador and USA	Waterworth et al. (1976)
	Hibiscus chlorotic ringspot virus	Chlorotic spotting and ringspots	Australia	Jones and Behncken (1980)
	Hibiscus chlorotic ringspot virus	Chlorotic spotting and ringspots	Singapore	Wong and Chng (1992)
	Hibiscus chlorotic ringspot virus	Chlorotic spotting and ringspots	Taiwan	Li and Chang (2002)
	Hibiscus chlorotic ringspot virus	Chlorotic spotting and ringspots	New Zealand	Tang et al. (2008)
	Alfalfa mosaic virus	Mosaic	Spain	Parrella et al. (2012)
	Hibiscus chlorotic ringspot virus	Chlorotic spotting and ringspots	Iran	Pourrahim et al. (2013)

(continued)

Table 8.1 (continued)

<i>Genus</i>	Virus	Symptoms	Geographical origin	References
	Hibiscus chlorotic ringspot virus IL	Chlorotic spotting and ringspots	Israel	Luria et al. (2013)
	Hibiscus chlorotic ringspot virus MGL1	Chlorotic spotting and ringspots	Turkey	Kamenova and Adkins (2004)
	Hibiscus chlorotic ringspot virus MGL2	Chlorotic spotting and ringspots	Turkey	Kamenova and Adkins (2004); Karanfil and Korkmaz (2017)
<i>Begomovirus</i>	Cotton leaf curl Multan virus	Leaf curl	China	Mao et al. (2008)
	Cotton leaf curl Burewala virus	Leaf curl	Pakistan	Akhtar et al. (2014)
	Cotton leaf curl Multan virus—01	Leaf curl	India	Srivastava et al. (2016)
	Cotton leaf curl Multan virus—02	Leaf curl	India	Srivastava et al. (2016)
	Cotton leaf curl Multan virus	Leaf curl	Philippines	She et al. (2017)
<i>Cilevirus</i>	Citrus leprosis virus C2	Green ringspots	Hawaii	Melzer et al. (2013)
	Hibiscus-infecting Cilevirus	Green ringspots	Florida	Avijit et al. (2018)
<i>Ilarvirus</i>	Tobacco streak virus	Necrotic streaks	Florida	Lewandowski et al. (2005)
	Hibiscus golden mosaic virus	Mosaic	Brazil	Allen et al. (2005)
<i>Rhabdovirus</i>	Eggplant mottled dwarf virus	Vein yellowing	Morocco	Lockhart (1987)
<i>Tospovirus</i>	Impatiens necrotic spot virus	Leaf chlorotic and necrotic	New Zealand	Elliot et al. (2009)
<i>Hostuviroid</i>	Hop stunt viroid	Upward curling leaf deformation	Italy	Luigi et al. (2013)

8.4 Detection of Viruses

8.4.1 Symptoms

Hibiscus plants shows viral symptoms annually on first young leaves during the February flush of growth when the plants are in full blooms (Wolfswinkel 1996) and basically exhibits systemic symptoms including dark and light green mosaic in

young leaves, leaf puckering with malformation on older leaves, and significant stunting. *H. rosa-sinensis* with rod-shaped virus particles were isolated which express systemic symptoms. Symptoms of *Hibiscus chlorotic ringspot virus* (HCRSV)-infected plant leaves range from generalized mosaic, mottle, to chlorotic ringspots as well as vein-banding patterns, severe stunting, and flower distortion (Gao et al. 2012). Likewise, we mentioned all the viral symptoms on *H. rosa-sinensis* in Table 8.1, respectively.

Identification of virus on Hibiscus plant, based on symptomatology alone is not reliable diagnostic indicators for viral infection of *H. rosa-sinensis* due to vegetatively propagated crop over a time hibiscus can accumulate multiple viruses. Based on various surveys, probability of some of the plants sampled for one study could be co-infected with one or more additional viruses which complicate symptomatology as well. Simultaneously, to overcome this problem of symptomatology, we use multiple methods like electron microscopy, IC-RT-PCR, chemical analysis, and sequence analysis.

8.4.2 Microscopy

Electron microscopy (EM) is an important tool for revelation and analysis of virus replication. Electron microscopy depends on its ability i.e. to image the whole spectrum of interactions with resistance and non-reactions in the case when new virus isolates or species. EM can determine functional features of viruses along with bottom line mechanisms of interactions relevant in nature and in synthetic virology analysis. Electron microscopy (EM) can be of three different types: transmission electron microscopy (TEM), immunoelectron microscopy (IEM) (Milne and Lesemann 1984), and cryogenic electron microscopy (cryo-EM). These are basically used for plant viral detection. The electron microscopy which is the most powerful technique available to virologists today has versatility especially as a universal means of virus detection and describes its development from descriptive tool.

8.4.3 Serological and Molecular Detection

In the last decades, for detection of plant viruses, various techniques have been developed which include rapid and specific serological (enzyme-linked immunosorbent assay, ELISA) and molecular techniques (molecular hybridization and DNA amplification) for the detection of plant viruses. Serological techniques are generally used for detection of viruses to the species level; sometimes they allow discrimination between virus strains (serotypes) using monoclonal antibodies (Myrta et al. 2000; Permar et al. 1990; Sheveleva et al. 2018). Binding of viral proteins with antibodies known as ELISA (Clark and Adams 1977) but in molecular hybridization are basically used to detect virus species, but its detection level can be modified to a certain level by using different probes and hybridization conditions we can say that

binding of viral nucleic acids with sequence-specific DNA or RNA probes, due to their sequence complementarities (Hull and Al-Hakim 1998).

The high sensitivity of these amplification techniques like in PCR can be a problem, contamination of reagents as well as instruments with amplicons from previous samples or sometimes cross-contamination between samples which we are using can produce false positives, reducing specificity. Simultaneously, universal detection in case of some plant viruses by ELISA with monoclonal antibodies failed for some plant virus isolates (Sheveleva et al. 2018).

8.4.4 High-Throughput Sequencing (HTS)

The most advanced and versatile technique since the nucleotide sequences which are frequently used in nowadays are HTS techniques that can be used for both to estimate the genetic variation along with structure of virus populations and to identify a virus sample at different taxonomic levels or we can say that discover new virus species, genera or families (Kreuze et al. 2009; Pecman et al. 2017; Verdin et al. 2017; Wu et al. 2015), according to their nucleotide or amino acid identity with known sequences in databases (Gen Bank) or the presence of sequence motifs. This is the most impressive technique for multiplex detection as it can identify and discover an unlimited number of viruses and virus variants within a plant (Jones et al. 2017).

8.4.5 RT-PCR

The amplification of multiple targets simultaneously in a single PCR reaction by using several primer pairs specific for each target is done in a multiplex PCR or RT-PCR technique. But the main problem with this RT-PCR is limitation in number of targets that can be amplified simultaneously since the more primers are used there will be the high probability of incompatibility between some of them. However, the number of products of different sizes that can be resolved by electrophoresis or the number of fluorescent dyes that can be used (Boonham et al. 2007) is also limited.

8.4.6 Features of Type of Molecular Technique for Plant Virus Detection and Diagnostics

The features of detection techniques that are costs, designability, versatility, multiplexing, sensitivity, specify, quantification, throughput screening i.e. number of samples analyzed simultaneously, rapidity, on-sited and easiness these features are not only during the application but also during the design or development as well. When we consider the sample for processing which is also a critical step and affects the rapidity, easiness and throughput of the detection process whereas if we take about versatility of a detection technique is a ability for different detection levels that

means at family, genus, species, strain or isolate respectively. Multiplexing is an ability to perform parallel analysis and on-site is to detect viruses on field with portable devices is known as on-site. Moreover, while comparing all molecular detection techniques, it was found that RT-PCR is the best technique in case of quantification of samples, whereas lateral flow is the finest among all in terms of rapidity, on-site and easiness. HTS is supreme among all, as it is best in the features like designability, versatility, multiplexing, specificity and sensitivity features. Moreover, it is good in quantification as well (Rubio et al. 2020). But this high-throughput sequencing is still too expensive for most routine analyses, that's why it is necessary to develop rapid as well as accurate detection techniques for each virus, being PCR the easiest to develop. In contrast, the setup of molecular detection techniques is a directed process that should be cheap, fast, and versatile, enabling to address different detection levels by considering the genetic variability of virus populations.

8.4.7 Chemical Analysis

In this analysis, the effect of HCRSV infection on total titratable acidity, ascorbic acid, and total anthocyanin and flavonoid contents in sepals of Roselle plants has been evaluated (Shafie 2019). In this process, the acidity value, ascorbic acid, and flavonoids are detected high in Roselle sepals infected with HCRSV compared to sepals of healthy plants, whereas *Hibiscus chlorotic ringspot virus* infection significantly decreased the total anthocyanin content in sepals of infected plants compared with that of healthy ones, respectively. As a result, by applying the above analysis, we can detect the HCRSV infection.

8.5 Vectors of Hibiscus Virus

Plant virus affecting *H. rosa-sinensis* is transmitted by various means, which include transmission through vectors like insects, mites, fungi, nematode and parasitic plants, vegetative propagation, pollen, seed, and also by contact. Movement of viruses within a plant generally occurs through plasmodesmata for between-cell movement and via phloem for long-distance transport. Insect vectors specially whiteflies (*Bemisia tabaci*), a complex of morphologically indistinguishable species (Barro et al. 2011) are vectors of many plant viruses that includes several genera of these whitefly-transmitted plant viruses such as *Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus* and *Torradovirus* include several hundred species of emerging along with economically significant pathogens of important food and fiber crops [reviewed by Lapidot et al. 2014; Castillo et al. 2011; King et al. 2012]. Such plant viruses do not replicate within its vector nevertheless they are moved readily by adult whitefly from plant to plant. For most of these plant viruses, whitefly feeding is necessary for acquisition and inoculation, while for others only probing is required. Among these viruses, many of them are unable to transmit themselves by other mode. Thus virus cultures is maintained, biological as well as

molecular characterization (identification of host range and symptoms) (Cohen and Antignus 1994; Polston et al. 1993) ecology (Cohen 1990; Polston et al. 2006) all are require to show viruses can be transmitted to experimental hosts using the whitefly vector. New approaches to management, such as evaluation of cultural approaches or selection and development of resistant cultivars, new chemicals, or compounds, require the use of whiteflies for virus transmission.

8.6 Management

Considering efficient and durable control or management of viral diseases, it is necessary to consider the genetic diversity and evolution of virus populations and have specific, fast, and reliable diagnostic tools which should be necessary to implement.

Moreover, vectors are quite difficult to control in horticulture and agronomic production systems which were found to cause severe infection to green life. The severity rate of viral diseases is found higher in winter than in summer. Therefore, seed treatment is performed to control vectors in initial stage via systematic insecticides. The use of tolerant cultivars and suitable site selection are also important, to avoid mechanical transmission during pruning and propagation (Kamenova and Adkins 2004) implemented the everyday used of sanitation approach via pruning tool which was either applied for 1 min with 10% (wt/vol) NaOCl or 20% (wt/vol) NFDM to eradicate Hibiscus latent Fort Pierce virus or longer by using 2% (wt/vol) NaOH, 6% (vol/vol) NaOCl, 20% (wt/vol) TSP, may be effective procedures and has a practical significance for landscape maintenance workers, hibiscus growers, nurseries, and where frequent horticultural practices are means of virus spread and dissemination in the environment. Disease management should focus on virus reservoir, regulation of virus source, and control of load of vectors in the agroecosystem. Endemic and epidemics are risk factors for worldwide losses which include nature of weather, habitat, and agricultural practice which accelerate the development of population of vectors and reservoir in an area. Conventional approaches, such as cultural methods and biocide applications against plasmodiophorida vectors, nematode, and arthropod, have limited success at alleviating the impact of plant viruses. Initial planting of resistant cultivars is the most economical and effective way to control plant virus diseases. Natural sources of resistance have been utilized extensively to develop virus-resistant plants by conventional breeding. Non-conventional methods also give flourishing effects to confer virus resistance by transferring primarily virus-derived genes, including viral coat protein, movement protein, replicase, protease, defective interfering RNA, and non-coding RNA sequences, into susceptible plants. Non-viral genes (microRNAs, dsRNase, R genes, ribosome-inactivating proteins, RNA-modifying enzyme protease inhibitors, and scFvs) have also been used successfully to engineer resistance to viruses in plants. MicroRNAs and its molecular background provide a completely different new scope for plant protection against viruses. This would be very promising for those viruses having ambience or negative polarity as this would

accelerate reverse genetics. Very few genetically engineered and virus-resistant crops have been released for cultivation, and none is on hand in developing countries. However, a number of economically valued GEVR (genetically engineered virus-resistant) crops, transformed with viral genes, are of great interest in developing countries. The major issues encountering the yield and deregulation of GEVR crops in developing countries are chiefly socioeconomic matter related to bio-safety regulatory frameworks, intellectual property rights, expense to generate GE crops, and defiance by nongovernmental activists. Indication for satisfactory resolution of these of above mentioned factors, apparently leading to field tests and deregulation of GEVR crops in developing countries (Reddy et al. 2009).

Viruses exhibit resistance to external factors, and due to flexibility in their genome organization and expression mechanisms and also because of co-evolution and adaptability with host plant, the complexity of infection may lead to increase in number in future and become highly stable. These demonstrations can also contribute to expand host range. A greater understanding of co-evolution and recombination in viruses help in developing strategies for managing plant viral disease which could be done by in silico analysis (Morya et al. 2014). Thus, a good agronomical practice are required that minimize the activity of vectors that transmit virus and destroy infected host plants. Despite of this, there are still vital gaps and many unknown mechanisms regarding host interaction and host range and also the gene expression that needs to be understood. The advanced technology, multidisciplinary approaches generating integration data from transcriptomic, proteomics, metabolomics and genomic information invoke for various host plant may contribute further insights for sustainable diseases prevention in plants and suppressing virus reproduction.

8.7 Conclusion

Flowering plants have been widely used as curative agents for variety of ailments since the time immemorial. There, concentrated flowers as well as leaves extract can be found in various herbal preparations that are also available in the market today. *Hibiscus rosa-sinensis* L. is an important source of different types of compounds with diverse chemical structures along with pharmacological activities. Therefore, presence of such ample amount of chemical compounds indicates that *Hibiscus rosa-sinensis* L. could serve as a “prominent lead” for the development of unique agents having very good efficacy in various disorders in future. Further, more or less viruses are reported worldwide, out of which 32 viruses are housing in 8 genera summarized in this reveiw chapter which affects the economic aspects of *Hibiscus rosa-sinensis* L. generating huge yield loss. Moreover, *Hibiscus leaf curl*, a *begomovirus* disease, also causes yield losses of hibiscus flowers throughout India as well (Anon 1950; Mali 1980; Vasudeva et al. 1953). Although eight genera are emphasized here, a number of viruses associated with *Tobamovirus* and *Carmovirus* genera are profoundly studied for *Hibiscus* plant. Prominently these are transmitted by whiteflies especially in case of *begomovirus*. Although, damages caused by various pest have a considerable negative economic impact in agriculture and also

ongoing pharmacological research have been affected, being emergent viral diseases particularly most important among them (Anderson et al. 2004; Mumford et al. 2016). However, the tremendous ability of viruses to evolve and generate molecular as well as biological variation is a considerable difficulty for virus detection and disease management. Presently, when a new virus-like disease appears, number of approaches (like ELISA, PCR, RPA, HTS, electron microscopy, etc.) has been developed in the last few decades which could be used for its detection. In addition, an effective pruning and propagation sanitization tool was performed by Kamenova and Adkins (2004) to avoid mechanical transmission of viruses, thus minimizing productivity loss. Control of plant viral infection becomes a challenge because of the lack of precise management implications with *Hibiscus rosa-sinensis*. From this review, we found that very limited attempts are made to manage *Hibiscus* plant from viral infection. Therefore, by employing various serological and molecular techniques may provide a new insight to combat with challenges by identifying and characterizing the new viruses that can help researcher to manage them accordingly and make the world continuously benefited by pharmacological values of these plant.

For better productivity and yield, a combination of various management strategies should be used to control plant virus diseases. The use of new technologies coupled with cultural and biological practices provides best measures for combating plant viruses. There is also a need for government and other agencies to introduce greatly improved methods.

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