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Advances in diagnostics and management of viral and phytoplasmal diseases in floriculture

Accurate and timely diagnosis of plant viral and phytoplasmal infections plays a key role in effective disease control and maintaining floricultural productivity. This article discusses the advancements in the diagnostics and management of viral and phytoplasmal diseases in floriculture. Phytoplasmas are obligate parasites which solely depend on the propagative planting material or insect for the spread from one location to another. To achieve clean propagative material, certification of mother stocks that are phytoplasma-free besides maintenance of the mother plant under insect-proof net throughout the year is a must.

ORNAMENTAL plants encompass a wide variety of plant species, including flowering plants, shrubs, trees, vines, certain grasses and ferns, succulents and cacti chosen for their ornamental characteristics. Ornamental plants play a crucial role in enhancing the aesthetics, functionality, and environmental quality of our surroundings. Their aesthetic charm, cultural significance, environmental benefits, economic contributions, and positive impacts on health and biodiversity underscore their vital role in horticulture and landscaping. While they are valuable for their significance in enhancing biodiversity, they are equally notorious for acting as reservoirs for viruses for their spread to other food and cash crops. Among the pathogens, more than 40% of emerging ones are viruses and ornamental plants are the primary hosts of many threatening virus diseases. This is evident from historical Tulip colour breaking in Tulips to Iris yellow spot virus causing havoc in onion as well as impatiens necrotic spot virus spreading in greenhouses. All the segments of floriculture encounter significant manifestation of phytoplasma diseases as well. Due to inadequate awareness among the stakeholders, many a times, the affected plants are further propagated with a mistaken premise that the plants are novel variants. Thus, accurate and timely diagnosis of plant viral and phytoplasmal infections plays a key role in effective disease control and maintaining floricultural productivity. This article discusses the advancements in the diagnostics and management of viral and phytoplasmal diseases in floriculture.

Virus diseases

'Tulip colour breaking' marks the beginning of ornamental virology in history. Production and marketing of highly priced variegated tulips (*Tulipa* spp.; Liliaceae) by Dutch growers in the seventeenth century. The flower variegation, as we know now, is caused by the potyvirus *Tulip breaking virus* and other potyviruses. During the past several decades, the frequency of plant diseases caused by viruses has increased worldwide including India as well. The growing demands for high quality and disease-free planting material, increased volume of international seed and plant trade, high phytosanitary standards imposed by many countries for plant material imports, as well as the fact that a great number of viruses have a wide range of hosts, emphasize the significance of virus diseases of ornamentals.

Rose is an economically important crop of India and the world. There are reports of Strawberry latent ringspot virus infecting roses and Prunus necrotic ringspot virus (PNRSV-rose), as the most prevalent virus infecting rose in India. Roses are also reported to be attacked by Apple mosaic virus (ApMV), Arabis mosaic virus (ArMV) and Rose leaf curl virus, a begomovirus. Chrysanthemum is the third most important cut flower in India. A large number of viruses and viroids, viz. Chrysanthemum virus B, Tomato aspermy virus, Cucumber mosaic virus, Tomato spotted wilt virus (TSWV), Chrysanthemum stem necrosis virus, Chrysanthemum spot potyvirus, Chrysanthemum chlorotic stunt viroid, and Chrysanthemum stunt viroid are known to infect chrysanthemum in nature. Among the top six flowers in the growing floral export industry in India, *Gladiolus psittacinus* (Iridaceae) is a very important cut flower widely used in bouquets and floral baskets because of its very attractive inflorescences in a wide range of colours. Numerous viruses infect gladioli in nature viz. CMV, Bean yellow mosaic virus (BYMV), Broad bean wilt virus (BBWV), TSWV, Tobacco mosaic virus (TMV), Tobacco necrosis virus (TobNV), Tobacco black ring virus (TobBRV), Tobacco ring spot virus (TobRSV), Tomato black ring spot virus, Arabis mosaic virus, Tobacco streak virus, Soybean mosaic virus (SMV) and Strawberry latent ring spot virus (StrLRSV). Of these, CMV and BYMV are the most important viral

Mixed infection of CMV and BYMV in gladiolus

Tuberose mild mottle virus infection

pathogens of gladiolus. Tuberose occupies an important place in Indian floriculture industry being suitable for both cut and loose flower as well as it is source for essential oil. Tuberose is reported to be infected by Tuberose mild mottle virus and Tuberose mild mosaic Potyvirus. Jasmine (*Jasminum sambac*) is a popular flower and oil-bearing plant in the family Oleaceae. India is the second producer and exporter of jasmine in the world. Different viruses of genus *Orthotospovirus* and Cucmber mosaic virus (CMV) are reported to occur in Jasmine, Marigold, Gerbera etc.

Diagnostics

Diagnostics plays an important role in virus disease management in ornamentals. Ornamental plants are a part of international trade. Thus right identification of viruses and their interception is a must to prevent the entry of exotic viruses into native biosphere. Rapid and robust diagnostics is imperative in production and ornamental plant nurseries to screen mother stocks for the production of disease-free planting materials. Diagnostic methods from symptomatology, serological methods like ELISA, IC-PCR, molecular techniques leading with PCR and sequencing is widely employed in detection of ornamental plant viruses. Advanced techniques like Next generation high throughput sequencing, metagenomics, high throughput real time PCR and on field detection methods like LAMP and RPA are useful in virus detection of ornamental plants. About 25 viruses from floricultural crops were detected by electron microscopy, immunosorbent electron microscopy, ELISA, PCR/RT-PCR/IC-RT-PCR and nucleic acid hybridization. Tomato chlorotic spot virus (TCSV) is an emerging orthotospovirus in ornamental crops. A reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay has been developed for easy detection of this virus in field. RT-LAMP has been successfully employed for detection of Tobacco mosaic virus from Chrysanthemum. Prunus necrotic ringspot virus (PNRSV) has been diagnosed with next generation sequencing from China rose. Machine learning, computer vision and generative AI can also be employed in the diagnostics of plant pathogens. There are reports of use of Hyperspectral Imaging and Outlier Removal Auxiliary Classifier Generative Adversarial Nets (OR-AC-GAN) for early detection of Tomato spotted wilt virus.

Management

Management of plant virus and viroid diseases is a matter of vital importance and concern to the farmer, horticulturist, forester and gardener. It is well established that the virus and viroid diseases in different crops cause enormous losses in terms of quantity and quality of products. Correct identification is the first step towards the management of the virus.

Cultural practices begin with clean cultivation and the use of clean planting material. Spread of Cucumber mosaic virus and Orthotospovirus in gerbera has been observed in tissue culture plants when mother plants are infected. The scopes of virus transmitted through seeds are also high. Thus, in both, seed propagated and vegetatively propogated plants, the mother stock needs to be disease-free. Use of virus-free certified planting material is imperative in management. In case of ornamental plants, awareness on this aspect is a must, as many a times virus infection is confused with novel plant types and propagated into further generations.

Exploiting the natural resistance to viruses is the most sustainable way of management of virus infection in plants. There are reports of PAMP triggered immunity (PTI) imparting natural resistance to virus infections as in tobacco and Arabidopsis where PTI-like responses were observed to the coat proteins of tobacco mosaic virus and potato virus X. Choosing natural source of resistance as a preventive strategy has been reported in management of Rose mosaic virus.

Genetic modification of ornamental plants has been used to introduce original traits of high commercial interest like improved floral morphology, new flower colour, early flowering, enhanced fragrance, stress tolerance or disease resistance. Transformations have been successful in atleast 50 ornamental plants including rose (*Rosa hybrida*), chrysanthemum (*Chrysanthemum morifolium*), petunia (*Petunia hybrida*), and carnation (*Dianthus caryophyllus*). Among them, only 7.9% were for virus resistance. Resistance to cucumber mosaic virus (CMV) was enhanced when a defective CMV replicase gene was transferred to the lilium. Chrysanthemum virus B coat protein gene has been expressed in transgenic chrysanthemum for inducing virus induced gene silencing. Transgenic poinsettia conferring resistance against Poinsettia mosaic virus (PnMV) has been produced through agrobacterium mediated transformation. CRISPR/Cas9 technology can be employed for molecular immunity against three geminiviruses, i.e. Tomato yellow leaf curl virus (TYLCV), Beet curly top virus (BCTV), and Merremia mosaic virus (MeMV) in *N. benthamiana* plants. Even though CRISPR/CAS is being employed in genome editing of ornamental plants, their exploitation for virus resistance is not explored widely.

The topical application of dsRNA, hairpin RNA, and artificial micro RNA and trans-active siRNA molecules on plants can be used in virus disease management. It has been reported that bacterially-expressed dsRNA imparts protection to orchid plants against Cymbidium mosaic virus.

In vitro cultivation of meristematic tissue coupled with therapies has been used to grow virus-free plants

Marigold

China aster

Annual chrysanthemum

Gerbera

Jasmine

Chrysanthemum

in gladiolus. *In vitro* chemotherapy of gladiolus cormels with 30 mg/L ribavirin amended in the MSg3 medium was found optimum for elimination of Bean yellow mosaic virus (BYMV) from gladiolus. A combination of chemotherapy and electrotherapy resulted in the highest rate of regeneration and percentage of BYMV-free plants.

Understanding the biology and behaviour of these insect vectors is of paramount importance in developing the effective plant disease management strategies. However, the complexity of vector-pathogen-host plant interactions and the continual evolution of these vectors and pathogens makes the disease management strategies a challenging endeavour. By growing crops under protected cultivation, we can keep the vectors away from the crop. Enhanced management of flower bud production of iris and tulip in Tasmania is achieved through the use of reflective mulches in the virus control. Behavioural manipulations involve exploiting the vector's behaviour to manage their populations. The techniques include use of coloured sticky traps, trap crops, pheromone traps, and push-pull strategies. Parasitoids and predators can be employed for biological control of vector population. Under field conditions, potyvirus dispersion can be controlled using mineral oil to minimize aphid vector transmission efficiency, as it interferes with the binding of potyvirus particles to the stylets of aphids.

Phytoplasma diagnostics and management

In the history of phytoplasma diseases, the link of flower crops is persistent whether it is green peonies or yellows in aster. The most common and widely distributed yellow group of phytoplasma has been discovered on China aster and thus it is known as aster yellows. As the symptoms of phytoplasma infections is clearly evident in flowers through the manifestation of phyllody which make their identification easier. And this 'phyllody' is the major threat in flower production as flowers are not produced and instead leafy structures are formed. The phytoplasma infection in flower crops act as source for infection of other food and cash crops as well. Sandal spike was the first phytoplasma disease reported in India. Thereafter, a large number of phytoplasma diseases were described, which included brinjal little leaf disease, grassy shoot disease of sugarcane, rice yellow dwarf disease, sesamum phyllody, white leaf disease of *Cynodon dactylon*, little leaf disease of *Acanthospermum hispidium* and yellowing disease of *Urtochloa panicoides*. Following the development of molecular tools, phytoplasmas have been characterized from as many as 60 plant species including ornamentals in India. Aster yellows phytoplasma group (16SrI) are the most common group of phytoplasmas associated with more than 31 diseases of ornamentals, tree species, vegetables, sugarcane, fruit crops and pulses in India.

Diagnostics

Japanese group of plant pathologists and entomologists discovered phytoplasmas as cause of plant diseases with the report of association of 'MLOs' (mycoplasma like organisms) in mulberry dwarf diseases 1967 through transmission electron microscopy. Further reports on aster yellows and corn stunt appeared from USA in the same decade. Phytoplasmas formally called mycoplasma-like organisms (MLOs), are plant pathogenic bacteria in the class Mollicutes, that lack rigid cell walls, surrounded by single membrane and are pleomorphic with a size of 80-800 nm. Until the early 1980s, phytoplasma diseases were diagnosed by transmission electron microscopy (TEM) because of their small size and the difficulty of artificial culturing. In the 1980s, fluorescent microscopy techniques like direct fluorescence detection (DFD) and DAPI staining were developed where DFD detects auto fluorescence of necrotic phloem cells and DAPI detect phytoplasmal DNA. ELISA, the popular diagnostic technique for viruses could not become popular for phytoplasma diagnostics as antibody preparation was difficult. Around 1990, advances in molecular biology enabled direct detection of phytoplasmal DNA by nucleic acid hybridization and the polymerase chain reaction (PCR). The *16S rRNA* gene-based PCR has become the gold standard in phytoplasma diagnostics. Recently, loop-mediated isothermal amplification (LAMP) technique is gaining popularity in direct on-field diagnostics of phytoplasma diseases. Invention of Next generation sequencing enabled drawing the picture of complete genome of phytoplasma and recently two draft full genome of Phytoplasma Strains associated with Sugarcane Grassy Shoot (SCGS) and Bermuda Grass White Leaf (BGWL) diseases have been reported from India.

Management

Phytoplasmas, the obligate parasites, are solely dependent on the propagative planting material or insect for the spread from one location to another. Thus the right intervention at these two epidemiological factors can contain the disease spread to new locations but it is very difficult to achieve. Strategies to cure the already infected plant are very rare and with those available, success rate is very low. The possible solutions to manage phytoplasmal diseases are discussed below.

Introduction of infected planting material to a new area where the potential vectors are present can damage the entire plantation of the crop in the area as exemplified in the case of Limonium in 2004 by Weintraub. To achieve clean propagative material, certification of mother stocks that are phytoplasma-free besides maintaining the mother plant under insect-proof net through-out the year is a must. Production of phytoplasma-free planting materials through meristem tip culture and their certification is inevitable to maintain the propagative material disease-free. In case of grafting or budding, both buds/scions and rootstock has to be phytoplasma-free.

The removal of diseased plants is the most effective method to curtail the spread of phytoplasma in the infected field. In case of herbaceous plants, the entire plant has to be uprooted and removed, while in case of trees and shrubs, symptomatic shoots have to be removed.

Weeds are the notorious hosts acting as reservoirs for threatening plant pathogens and it is the same here as they host both phytoplasma and its insect vectors. In Germany, if the leaf hopper vector *Hishimonus* sp develops on *Convolvulus arvensis*, 80% of insects harbour phytoplasma compared to 5% in case of those develop on Ranunculus.

Insect-proof screening is the best available method for excellent vector control which in turn helps in curtailing the transmission. Mulching with plastic sheeting or reflective mulches will not only repel the insect vectors but also affect the growth of alternative hosts, thus reducing the incidence of phytoplasma and its spread. The compositions of vegetation in and around the field or landscape or orchard or vineyard reflect on the presence and dispersal of phytoplasma and its vectors. Thus overall management of the cropping system is a must to manage phytoplasma diseases.

Ranunculus phytoplasma-infected plants showing strong stunting and rosette shape symptoms were watered with 300 mL oxytetracycline per plant three times per week for two months. It did not produce phytotoxicity at either concentration of 1 or 100 mg/L. A reduction in symptomatology was observed with the production of yellow flowers, typical of the healthy variety. Two weeks after the end of the treatment, the symptoms reappeared, confirming the bacteriostatic effect of the antibiotic.

Production of phytoplasma free planting materials through tissue culture by using warm water (38°C) combined with a shoot tip (5–8 mm) tissue culture method. Other antimicrobial molecules used as alternative to antibiotics such as kinetins showed that they were ineffective in phytoplasma elimination. Micropropagated periwinkle shoots infected with a '*Ca*. P. asteris' strain (hydrangea virescence, HyV strain) immersed in sterile water containing decreasing concentrations of PAPII; only the 4% of infected shoots grown on a medium enriched with PAPII at dilutions of 1:10 and 1:100 for 15–150 days were found to be free from phytoplasmas.

A technology based on plasma-activated water (PAW), characterized by the presence of reactive oxygen and nitrogen species (RONS) in liquid, was tested on phytoplasma-infected micropropagated shoots and plants, in orchards and in greenhouse cultivation systems, found to enhance the plant defence mechanisms, health status of the treated plants and a delay in the phytoplasma symptom appearance.

Development of eradication of any disease requires the complete knowledge about the pathogen which is lacking in case of phytoplasma due to the difficulty in its culture '*in vitro*'. The most important challenge to the phytoplasmologists across the globe is the culturing of this pathogen in artificial media.

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