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Current Status of Viral and Phytoplasma Diseases Affecting Gerbera Cultivation and Their Management

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Gerbera (*Gerbera jamesonii*) is a popular ornamental plant cultivated all over the world. It is grown in beds, pots and used as cut-flower in making bouquets and for decoration in ceremonial functions. Gerbera has been commercially cultivated by a large number of growers in India as a primary source of income, therefore, has high socioeconomic impact in floriculture industry. The gerbera cultivation areas in India are increasing day by day due to its uses and the market demands. Its cultivation has been hampered by a variety of diseases that affect its flower quality and quantity. Of them, the viral and phytoplasma diseases cause considerable losses in gerbera cultivation. In this review, we have described about the disease symptoms, detection methods and identification of causal virus and phytoplasma pathogens affecting gerbera production worldwide and their disease management strategies opted by the researchers for production of pathogen-free plants.

Keywords: *Gerbera jamesonii*, virus and phytoplasma diseases, disease management.

Gerbera (*Gerbera jamesonii*) is a popular ornamental plant of family *Asteraceae*. It is native to South Africa and commonly known as African daisy. The genus *Gerbera* consists of about 40 species and amongst them only one species namely *G. jamesonii* is under cultivation. Subtropical and Mediterranean climate is suitable for its growth and flower production. These climatic zones pass through the Israel, Italy, Spain, Portugal, Morocco, Colombia, Japan, South Africa, Australia and Southern India. The most economically gerbera producing countries are The Netherlands, Italy, Germany, France, and California. Gerbera is considered as the fifth most used cut-flower crop in the world after rose, carnation, chrysanthemum and tulip (http://en.wikipedia.org/wiki/Gerbera).

Gerbera has great ornamental value due to the typical capitulum inflorescence that displays a great variety of colors, and to the floral stem, which is highly valued by consumers as individual vase decorations and bouquet compositions (Mata et al., 2009). Gerbera cultivars of commercial importance throughout the world are Zingaro (red), Vista (red), Dustty (red), Fredorella (red), Silvester (white), Delphi (white), Salvadore (yellow), Rosaline (pink), Davaellen, Goliath, Cream Clementine (creamy white), Maroon Clementine (orange), Flamingo (Pale rose), Uranus (yellow), Fredenking (yellow), Terra

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queen (Pink), Valentine (pink), Fredaisy (pink), Labalga (lilac) etc. (https://vikaspedia.in/agriculture/cropproduction/package-of-practices/flowers/gerbera-cultivation). About 7 species have been recorded in India, distributed in the temperate Himalayas from Kashmir to Nepal at altitudes of 1,300 to 3,200 meters and stands fourth in important cut flower in India. The total cultivation area of gerbera in India is about 820 hectare with a cut flower production of 17, 840 MT and loose flower of 3,960 MT (Anonymous, 2015).

Gerbera is grown commercially in India for export and domestic market. Gerbera industry is supposed to be sunrise industry during the recent trading years for export point of view. Hence, the high quality cut flowers and millions of tissue cultured plants of gerbera are produced in the country. However, gerbera production is challenged by numerous bottlenecks and diseases caused by pathogens including insect, fungal, bacterial, nematodes, viral and phytoplasma that affect flower quality and quantity (Reddy, 2016). Among them, diseases caused by the viral and phytoplsama are considered as the most important due to their losses to floriculture trade caused by them and has been described further in this chapter.

Viral diseases affecting gerbera

Plant viruses are infectious particle composed of a protein coat and a nucleic acid core. Viruses are classified by the type of nucleic acid they contain, and the shape of their protein capsule. Till date only RNA viruses are reported on gerbera, which may be of two types: single or double stranded. The single stranded RNA viruses are further divided into two, positive sense and negative sense. In gerbera mostly single stranded positive sense RNA [ss(+)RNA] viruses such as Tobacco mosaic virus (TMV), Tomato black ring virus (TBRV), Cucumber mosaic virus (CMV), Tobacco rattle virus (TRV) are reported. Contrary to that, a very few reports of single stranded negative sense RNA [ss(-)RNA] viruses are also available in literature. The Tomato spotted wilt virus (TSWV) and Impatiens necrotic spot virus (INSV) are the tospovirus, and their genome consists of single stranded one negative and two ambisense single-stranded RNAs.

The viral diseases reported worldwide on gerbera are concentric rings and distortion of leaves caused by INSV and necrotic spot on leaf caused by TSWV and INSV reported from Serbia (Stanković et al., 2011) and from New Zealand (Elliott et al., 2009), respectively. Gerbera has been reported as the host for TMV and TBRV in China (Zhang, 2009). Color break on the petals, and deformed flowers on gerbera are reported due to CMV infection in India (Verma et al., 2004). TRV has been reported to infect gerbera from The Netherlands (Stouffer, 1965; Schmelzer, 1966). In 2002, Chrysanthemum stem necrosis virus (CSNV) has also been detected by ELISA in gerbera in Slovenia (Ravnikar et al., 2003). The summarized Table 1 represents the current status of viruses infecting gerbera until writing of this review.

Diseases caused by TSWV in gerbera

TSWV belongs to the genus *Tospovirus* of family *Bunyaviridae* is a spherical virus having diameter between 80-110 nm. Its genome consists of one negative and two ambisense single-stranded RNAs. The western flower thrips (*Frankliniella occidentalis*) is the vector that predominantly transmits TSWV globally and in greenhouses. TSWV infects

Table 1

Virus and phytoplasma diseases of gerbera

Viruses and Phytoplasma Diseases				
Pathogen	Natural Vector	Symptoms	Country	References
Tomato spotted wilt virus	Western flower thrips	Concentric rings and distortion of leaves	Serbia	Stankovic et al., 2011
			Southern Italy	Spano et al., 2011
			Venezuela	Marys et al., 2014
Chrysanthemum stem necrosis virus	Western flower thrips	Necrosis on leaf	Slovenia	Ravnikar et al., 2003
Impatiens necrotic spot virus	Thrips	Necrotic spot on leaf	New Zealand	Elliott et al., 2009
Cucumber mosaic virus	Aphids	Mottled on leaf and distorted petals with colour break symptoms on flower	Australia	Finlay, 1975
		Color break on the petals, and deformed flowers	India	Verma et al., 2004, Gautam et al., 2017
Tobacco rattle virus	Nematodes	Ring spotting and light green line patterns on leaf	Netherland	Hakkaart, 1968
			USA	Stouffer, 1965
			Germany	Schmelzer, 1966
Tomato black ring virus	Not known	Black ring on leaf	China	Zhang, 2009
Tobacco mosaic virus	Not known	Mosaic on leaf	China	Zhang, 2009
Candidatus Phytoplasma Asteris'	Leafhoppers	Virescence, phyllody and abnormal flower colour.	Southern Italy	Spanò et al., 2011a
Candidatus Phytoplasma Asteris' (16SrI group)	Leafhoppers	Severe leaf yellows, shortening of whole plant and flower deformation.	India	Gautam et al., 2015
		Virescence, phyllody and abnormal flower colour.	Italy	Bertaccini et al.,1996
Ca. Phytoplasma aurantifolia' (16SrII)	Leafhoppers	Phyllody symptoms (green flower) on Gerbera	Australia	Siddique, 2005

over 1000 plant species and causes significant economic damage to many agronomic and horticultural corps. Stunting is a common symptom of TSWV infection and generally more severe in young infected plants. TSWV also causes chlorotic or necrotic rings on the leaves of many infected hosts. However, wilt symptom of differ among hosts and can be variable even in a single host species. It may be speculated that the appearance of symptoms depends on virus strain, plant species and varieties, mode of infection, developmental stage of plant, time of symptom evaluation, environmental condition etc. The same symptoms can be caused by multiple viruses.

The infection of TSWV on gerbera was reported for the first time in Serbia based on disease incidence and transmission study (Stanković et al., 2011). TSWV was detected through double antibody sandwich-enzyme linked immuno-sorbent assay (DAS-ELISA) in 18 of 20 gerbera samples, subsequently validated through reverse transcription polymerase chain reaction (RT-PCR). The infecting viral strain was further identified as TSWV based on the high sequence identities found with a TSWV isolate of globe artichoke in Greece, and with other TSWV isolates of tomato, impatiens, and tobacco in Serbia. This was the first report infecting gerbera in Serbia, which had a devastating influence on its production (Stanković et al., 2011).

In 2011, Spanò and co-worker reported TSWV from *G. jamesonii* in Southern Italy. During survey, they noticed that greenhouse-grown *G. jamesonii* plants were showing severe malformations of flowers and necrotic spots on the leaves. Estimated disease incidence in the greenhouse was about 50% in cultivars Sporza and Dune, 20% in Lancaster and 10% in Poseidon. TSWV was detected in all samples tested by TSWV specific probe based dot blot hybridization assay. Two viral isolates obtained from Sporza were mechanically inoculated onto three plants each of tomato cvs. UC82, Faino, Diaz and Messapico, the latter two carrying the Sw5 resistance gene to TSWV isolates Sporza and Dune but not the local TSWV strain overcame the resistance and induced systemic necrosis. The two cultivars of tomato: UC82 and Faino were systemically infected by these TSWV isolates (Spanò et al., 2011b).

In 2014, Marys and co-worker reported TSWV on gerbera in Venezuela based on symptomatic plants showed concentric rings, irregular chlorotic blotches, and deformation on leaves. Disease incidence was estimated at 30%. Mechanical inoculation with extracts of symptomatic leaves reproduced the typical concentric ring symptoms on indicator plants *Arachis hypogaea* L. cv. San Martín, *Capsicum chinense*, and *G. jamesonii* 6 to 15 days after inoculation. In initial tests, leaves from each 30 symptomatic gerbera and chrysanthemum species from several greenhouse facilities in Altos Mirandinos reacted positively when tested by DAS-ELISA with polyclonal antisera raised against TSWV (Marys et al., 2014).

Diseases caused by CMV in gerbera

CMV is the type member of genus *Cucumovirus* in the family *Bromoviridae*. It is reported to infect about 1287 plant species in 518 genera belonging to 100 families worldwide (Edwardson and Christie, 1986). CMV is transmitted by numerous species of aphids, through sap, seeds and dodder (Kaper and Waterworth, 1981; Dijkstra and Khan, 2006). CMV causes significant losses to most of the major crops, around the world, therefore is the bottlenecks to the crop production (Hull and Davies 1992; Raj et al. 2008) including gerbera (Gautam et al., 2014). CMV infection is characterized by severe chlorotic mosaic, greening of veins on leaves, color breaking in florets accomplished with flower deformations, and poor growth of the bloom (https://dfr.icar.gov.in/ForFarmers/InsectPests). It causes yellowing and mottling in gerbera leaves.

In 1975, J. R. Finlay first time reported infection of CMV on gerbera (*Gerbera jamesonii* Bolus) from two nurseries at Bundaberg and from a home garden at Brisbane, Australia based on mottled leaf and distorted petals with colour break symptoms on flower, virus transmission by mechanical inoculations and by aphid *Myzus persicae* transmission, electron microscopy and serological studies. During transmission study, gerbera seedlings mechanically inoculated with the virus developed similar symptoms in the systemically infected leaves, but later growth was often symptomless. The virus had-a host range similar to that described for CMV and was transmitted in a non-persistent manner by the aphid, *Myzus persicae*. During TEM, polyhedral particles of about 25 nm in diameter were observed in negatively stained preparations obtained from the leaf sap of infected *Nicotiana clevelandii*. The purified virus formed a single line of precipitation in gel diffusion tests against an antiserum to the Q strain of CMV, a strain originally isolated from capsicum in Queensland. A distinct strain of CMV was also isolated from gerbera that was not trans-

mitted by over 150 *M. persicae* in 6 tests, and it rarely produced systemic infection in cucumber plants. Single joining precipitation lines formed in gel diffusion tests of this virus against its homologous antiserum and antisera to the Q strain of CMV, Californian cucurbit strain of CMV, Queensland gladiolus strain of CMV, and the gerbera CMV strain described previously. CMV has been recorded as the cause of the disease in gerbera (Finlay, 1975).

In 2004, Verma and co-worker first time reported CMV on *G. jamesonii* in India based on virus transmission, ELISA, TEM of virus particle and its molecular characterization. The CMV was isolated from gerbera expressing color break on the petals, asymmetrical ray florets, and deformed flowers symptoms on gerbera growing in floriculture fields at the CSIR-Institute of Himalayan Bioresource Technology, Palampur, India and nearby nurseries. During host range or transmission study, the virus evoked chlorotic local lesions and systemic mosaic on many test species: *Cucumis sativus*, *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa*, and *N. tabacum* cv. Samsun. The virus was also transmitted in non-persist manner by *M. persicae* and *Aphis gossypii* and identified as CMV using methods of ELISA with CMV-specific antibodies. Polyhedral particles of 29 nm were observed with electron microscopy in leaf dips prepared from symptomatic gerbera leaves (Verma et al., 2004). For molecular detection, total RNA isolated from infected gerbera plant. CMV-specific primers were used to detect the virus through RT-PCR that produced an amplicon predicted size of 540 bp. Sequence alignment of the amplicons by BLAST resulted in 91 to 99% homology with the partial inter cistronic region and partial coat protein gene (1042-1574 bp) and identified as CMV subgroup-I. The natural occurrence of CMV on gerbera is reported earlier from Australia, India and China. This was the only report from India describing the CMV by ELISA and analysis of a small sequence amplified by RT-PCR from gerbera exhibiting colour break symptoms on petals, asymmetrical ray florets, and deformed flowers (Verma et al., 2004).

In 2014, Gautam and co-workers reported CMV on *G. jamesonii* based on complete RNA3 genome sequences associated with severe chlorotic mosaic and flower deformation disease in two cultivars (Zingaro and Silvester) growing in a polyhouse at CSIR-NBRI, Lucknow, India (Fig. 1). The disease incidence was found at 16.27% (70/430) in cv. Zingaro followed by 11.57% (50/432) in cv. Silvester, as calculated on the basis of the actual number of diseased plants found out of the total number of plants in the particular plot surveyed. The transmission of causal virus was attempted using the leaf sap of naturally infected gerbera plants of cvs. Zingaro and Silvester, separately on some recipient host seedlings. During sap transmission tests, the virus was successfully transmitted from naturally infected gerbera to healthy gerbera seedlings which developed similar chlorotic mosaic symptoms at 40-45 dpi. The inoculations of sap taken from cultivars Zingaro and Silvester also induced more or less similar local and necrotic lesions and systemic mosaic symptoms on *C. sativus*, *C. annuum*, *P. hybrida*, *N. glutinosa*, *N. tabaccum* cv. White Burley and *N. rustica* at 30-35 dpi. Further, the complete RNA3 genome of CMV was amplified by RT-PCR using CMV-RNA3 primers from three infected gerbera leaf samples. The amplicons obtained were cloned sequenced (Accession numbers: JN692495, JX913531, and JX888093). These sequences shared 90-95% identities with various strains of CMV reported worldwide and close phylogenetic relationships with several other strains of CMV of subgroup IB. Therefore, CMV isolates associated with severe chlorosis and flower deformation disease in two cultivars (Zingaro and Silvester) of *G. jamesonii* were identified as the members of subgroup IB (Gautam et al., 2014).

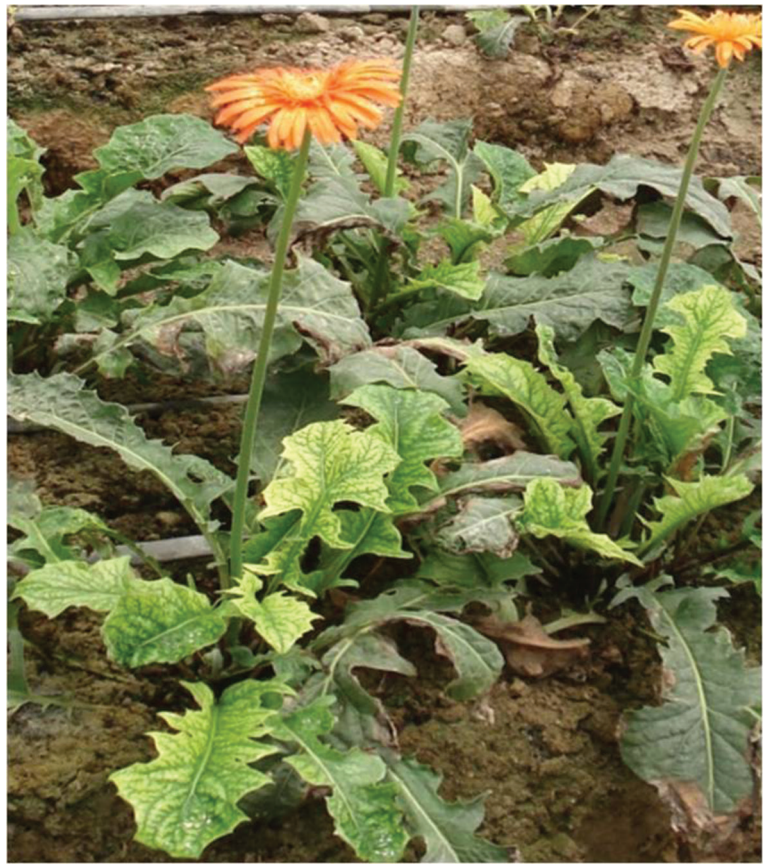

Fig. 1. Naturally infected gerbera plants exhibiting severe chlorotic mosaic and flower deformation symptoms under polyhouse condition

Diseases caused by TRV in gerbera

TRV is an important plant pathogenic virus of family *Virgaviridae* of genus *Tobravirus*. It is transmitted by nematodes of the genera *Trichodorus* and *Paratrichodorus* (Fauquet et al., 2005). It can also be mechanically and seed transmitted. It has a linear, single-stranded, positive-sense RNA divided into two segments, RNA 1 (6791 nt) and RNA 2 (1905 nt). The virus particles of TRV are rod-shaped and of 190 × 22 nm to 80-110 × 22 nm in size. Over 400 species of plants from 50 families of vegetables, ornamentals, and weed are susceptible to infection. The common symptoms of TRV infection on gerbera include mottling, cholortic or necrotic local lesion, ringspots or line patterns, and systemic necrosis (https://dfr.icar.gov.in/ForFarmers/InsectPests).

The occurrence of TRV in gerbera has been reported by Stouffer (1965), who found it in field grown plants in the USA, and by Schmelzer (1966) in the German Democratic Republic. In 1967, Hakkaart received some gerbera plants with virus like symptoms from a commercial grower in The Netherlands. The leaves showed ring spots and light green line patterns, which in older leaves often became necrotic. Sap from affected leaves was

inoculated on several plant species. Three of these: *N. tabacum* 'White Burley', *C. amaranticolor* and *Datura stramonium* developed symptoms typical for TRV. Inoculation of healthy gerbera seedlings with sap from the infected *N. tabacum* caused line patterns and ring spots and the virus could be re-isolated from these plants. This was the first time that TRV has been detected in gerbera in The Netherlands (Hakkaart, 1968). The Netherlands produces 420 million healthy stems of gerbera per year which is valued at 145 million Netherlands guilders.

Diseases caused by INSV in gerbera

The INSV of family *Tospoviridae*, genus *Orthotospovirus* is an important pathogen in a broad host range of ornamental plants. INSV is easily mechanically transmissible, often causes severe damage on infected plants, and spread rapidly through insect vector (*Thysanopthera*). INSV has been reported to infect more than 300 plant species. Stunting ringspots, brown to purple spots on leaves or stems, stem browning (cankers), flower breaking symptoms may be present on a plant infected with INSV.

The infection of INSV was detected by ELISA and RT-PCR in gerbera along with cyclamen, gardenia and hibiscus collected from an Auckland site in 2006. The analysis of the partial nucleotide sequences obtained by RT-PCR product from gerbera (Accession number: FJ495172) was found to be closely related to the published sequences of INSV, hence it considered as the new hosts for INSV in New Zealand (Elliott et al., 2009).

Phytoplasma pathogens affecting gerbera

Phytoplasmas are phloem-limited, insect-transmitted, plant pathogenic bacterial parasites that are responsible for hundreds of diseases world-wide. They are non-cultivable degenerate grampositive prokaryotes in the class *Mollicutes*.They are associated with typical yellowing, stunting of whole plant, virescence, phyllody, proliferation of axillary buds, witches' broom and die back symptoms (Al-Saady and Khan, 2006; Bertaccini, 2007; Harrison and Helmick, 2008). They are associated with severe yield losses in a variety of plant species of horticultural, agricultural and ornamental importance (Chaturvedi et al., 2010). Several '*Candidatus* Phytoplasma' taxon have been described and specifications for novel species designation are based on less than 97.5% of 16S rDNA sequence identity with previously described '*Ca*. Phytoplasma' taxon (Anonymous, 2004). Gerbera production are also reported to be affected by phytoplasma from different part of the world (Table 1) such as Phytoplasma 16SrII from Australia and Phytoplasma 16SrI from Italy (Siddique, 2005); *Candidatus* Phytoplasma asteris' from Southern Italy (Spanò et al., 2011a); and '*Candidatus* Phytoplasma asteris' (16SrI group) associated with yellows disease of gerbera from India (Gautam et al., 2015).

In 2005, A. B. M. Siddique, first time reported phytoplasma association with gerbera phyllody in Australia based on symptoms, TEM and molecular study. During a survey, he observed the phyllody symptoms (green flower) on gerbera plants in Central Queensland, Australia. Leaves and flowers from both symptomatic and asymptomatic healthy plants were examined by TEM and the presence of pleomorphic bodies similar to phytoplasma was observed exclusively in diseased plants. The presence of phytoplasma DNA in the infected plants was also confirmed by PCR with phytoplasma specific primers

(Siddique, 2005). Further sequence analysis of the PCR product revealed high homology with other phytoplasma DNA in the database. Based on phylogenetic analysis of 16S rRNA the gerbera phyllody phytoplasma was grouped under Peanut witches broom as described by Lee et al. (1993). The results of TEM, PCR and sequencing analysis clearly indicated phytoplasml association with phyllody disease of gerbera (Siddique, 2005).

In January 2011, Spanò and coworker reported *'Candidatus* phytoplasma asteris*'* infection in *G. jamesonii* in Apulia from Southern Italy. The infected plants had phytoplasma like symptoms of virescence, phyllody and abnormal flower colour and disease incidence was nearly 100% in cv. Maxima. The phytoplasma infection detected by nested PCR and found similar as "*Candidatus* Phytoplasma asteris" based on RFLP patterns. This was the first report from gerbera in Apulia (Spanò et al., 2011a).

The severe leaf yellows, shortening of whole plant and flower deformation symptoms were observed on *G. jamesonii* plants growing in a polyhouse at Lucknow, India. The association of '*Candidatus* Phytoplasma asteris' (of 16SrI group) with the disease of gerbera was detected by PCRs using P1/P6 phytoplasma universal primers (Deng and Hiruki, 1991) followed by nested PCR using primers R16F2n/R16R2 (Gundersen and Lee, 1996) primers. The obtained amplicons were cloned and sequenced (accession numbers: JX674049 and KC880350). Based on their high sequence identities (99%) and close phylogenetic relationships with Italian gerbera phytoplasma strain of '*Ca.* P. asteris', the gerbera phytoplasmas from India were identified as '*Ca.* P. asteris'-related strains (Gautam et al., 2015).

Management of phytoplasma disease in gerbera

The management of diseases caused by various phytoplasma pathogens in gerbera has been reported by Bertaccini, 2007; Chaturvedi et al., 2010; Gautam et al., 2015; and Tanno et al., 2018. They suggested that the control of epidemic outbreak of phytoplasma diseases can be done either by controlling the vector or by eliminating the pathogen from the infected plants by meristem tip culture; antibiotics or other chemical such as tetracycline etc. (Bertaccini, 2007; Chaturvedi et al., 2010). It is also necessary to keep the low number of phytoplasma vectors (leafhoppers and planthoppers) by use of some insecticides. Therefore, the holistic approaches may be adopted for efficient control of the phytoplasma diseases.

Traditional vector control methods are insufficient to control the disease (Weintraub and Beanland, 2006). The most reliable means of controlling vectors is by covering the crop with insect-proof screening. Walsh et al., (2006) demonstrated that the pathogen vectors could be 100% controlled by covering the plants with insect exclusion netting. Screening is the only method to attain excellent vector control; however, its applicability is so severely limited due to the logistics of large scale agriculture in major crops. On the other hand, conventional insecticides, even when frequently used (e.g. Wally et al., 2004), will not control the appearance of disease because pathogen transmission occurs faster than insecticides can act, and there is often a constant influx of new vectors from surrounding habitats. At best, use of insecticides might help control vector populations, and thus reduce intra-crop transmission.

Because transmission occurs quickly, plants become infected before insecticides can act on the vector. The single most effective means of controlling the vector is to

cover plants with insect exclusion netting; however, this is not practical for most commercial crops. Because of these limitations, researchers are turning to genetic manipulation of plants to affect vector populations and pathogen transmission. These novel control schemes include symbiont control (SyBaP), plant lectins, and systemic acquired resistance (SAR) (Weintraub, 2007).

Since gerbera is propagated through vegetative means and mass multiplication through tissue culture, Gautam et al. (2015) suggested the detection of phytoplasma in gerbera at early stages of its development and removal of infected plants from the cultivated field may be the most importance tool for development of its disease management practices. Moreover, minimizing the population of the phytoplasma transmitting insect vectors (leafhoppers, planthoppers) is also necessary by use of an effective insecticide or by some eco-friendly bio-pesticides.

It is well known that the only antimicrobials being used to control phytoplasmas are tetracycline-class antibiotics. Tanno et al. (2018) performed the comprehensive screening of antimicrobials to control phytoplasma diseases using an in vitro plant-phytoplasma co-culture system and developed an accurate and efficient screening method to evaluate the effects of antimicrobials. In this study, they tested 40 antimicrobials, in addition to tetracycline, and four of these (Doxycycline, Chloramphenicol, Thiamphenicol and Rifampicin) decreased the accumulation of '*Candidatus* (Ca.) Phytoplasma asteris'. The phytoplasma was eliminated from infected plants by the application of both Tetracycline and Rifampicin (Tanno et al., 2018).

According to Kumari et al. (2019), several approaches were suggested for the management of phytoplasma diseases and their insect vectors. Control of insect vectors through pesticides is a plausible way to limit the spreading of phytoplasma diseases infecting crops. Developing cultivars resistant to either phytoplasmas or their insect vectors would be a long-lasting tool for the control of phytoplasma diseases. However, the limited work has been done on the development of resistant genotypes of the crops. Other management strategies such as rouging of infected plants, adjustment of date in sowing, use of clean propagating material, rotation with non-host crops, and removal of weeds coupled with vector control are effective methods for the containment of phytoplasma-associated diseases. The dependency of the phytoplasmas on a living host for their survival makes it impossible their management with a single chemical and is quite different from the management carried out for fungi or bacteria. Hence, an integrated approach may be the most viable and sustainable option by integrating components of cultural, physical, biological, resistance and chemical applications (Kumari et al., 2019).

Management of viral diseases in gerbera

Management of viral diseases is much more difficult than that of diseases caused by other pathogens (Varma et al., 2002) because of the viral diseases have a complex disease cycle, efficient vector transmission and no effective virucides available. Integration of various approaches like the avoidance of sources of infection, control of vectors, cultural practices (conventional) and use of resistant host plants (non conventional) have been used for the management of diseases caused by plant viruses (Fig. 2).

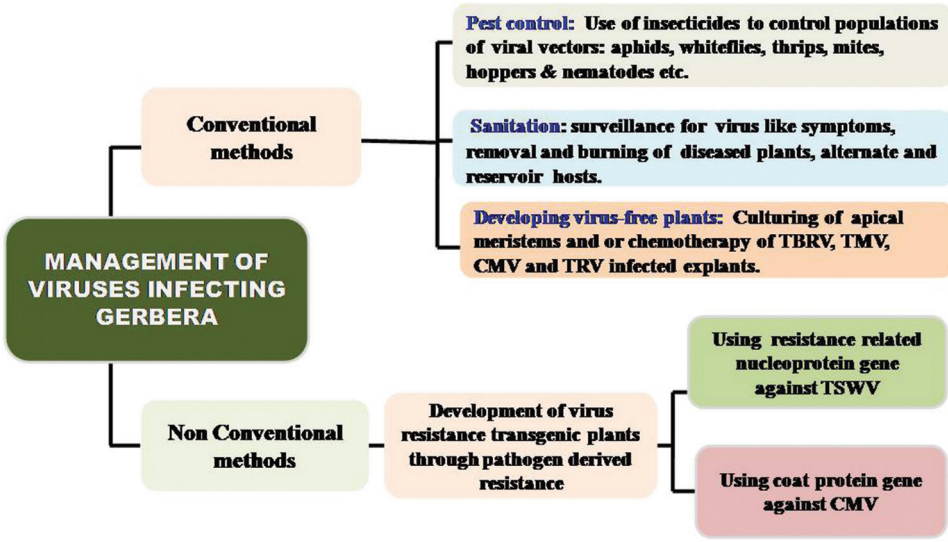

Fig. 2. Summary of management of viruses infecting Gerbera (based on current literature: Zhang, 2009; Gautam et al., 2017; Korbin et al., 2002; Brisco-McCann and Hausbeck (2016)

By cultural practices

Prevention is the key for managing the viral diseases because virus-infected plants cannot be cured. If viral infection is suspected in gerbera plants, samples should be sent to testing facilities to confirm the presence of the virus. Once the disease has been identified, the only management option is to discard infected plants (Whipker, 2014). However, managing the vector of the virus, the spread of western flower thrips can be minimized. This can be done using strategies to physically exclude the pests such as installing fine mesh screens (mesh size < 135 nm) on external openings to prevent entry of thrips vectors into the greenhouse. Monitoring using indicator plants, such as petunia, or sticky cards can be helpful to provide early warnings of the presence of *F. occidentalis* (Allen and Matteoni, 1991). It is worthwhile to mention that western flower thrips can acquire virus at larval stage and it can transmit the TSWV and if we prevent adults from developing, transmission of the virus may be prevented.

By sanitation

It is well kwon fact that sanitation of the cultivation fields enhances crop production by many folds. Remove all plant debris as well as weeds and flowering plants growing nearby production areas as these can be sources of new infections and infestations. It was suggested that soil sterilization can also eliminate the developmental stages of vector species (Brisco-McCann and Hausbeck, 2016).

By biological controls

Biological controls can be effective for controlling of thrips species when their populations are low. Some predator species have been identified for control of western flower thrips. These are *Euseius stipulatus*, *Metaseiulus occidentalis* (Nesbitt), *Amblyseius andersoni* (Chant), *Amblyseius scutalis* (Athias-Henriot), and *Amblyseius* (Euseius) *tularensis* (Congdon) (Brisco-McCann and Hausbeck, 2016).

Lady beetles (Coleoptera: Coccinellidae), ladybugs, or ladybird beetles are among the most visible and best known beneficial predatory insects. Over 450 species are found in North America. Most lady beetles in North America are beneficial as both adults and larvae, feeding primarily on aphids. They also feed on mites, small insects, and insect eggs (https://biocontrol.entomology.cornell.edu/predators/ladybeetles.php).

Biological control of aphid vectors of CMV by ladybird (*Coccinella transversalis*) has also been studied by Kumar, 2009 during the Ph D dissertation. The biological control of aphid vectors population (capable of transmitting CMV and TAV and potyviruses in several plant species) has been attempted by ladybird (*C. transversalis*), a predator of aphids. Though, the feeding behavior of *C. transversalis* has been observed on chrysanthemums. Different larval stages, as well as adult lady bird predators have been explored for minimizing aphid population. The larval stage is found to be most efficient for feeding of aphid population as compared to adults (Fig. 3). It feeds approximately 20 aphids per minute. It was also observed that aphids quickly migrate from the ladybirds. These observations may be utilized for minimizing the aphid population, indirectly minimizing the load of the virus in nature (Kumar, 2009). It is suggested that such eco-friendly approaches of virus-disease management are needed to be developed which neither has adverse effect on human health, nor possesses hazards to the environment.

Predation has immediate consequences for prey fitness and early assessment of predation risk may be advantageous for prey. Ninkovic et al. (2013) investigated the ability of the bird cherry-oat aphid, *Rhopalosiphum padi*, to detect one of its important predators, seven spot ladybird, *Coccinella septempunctata*, via chemicals in the predator's walking

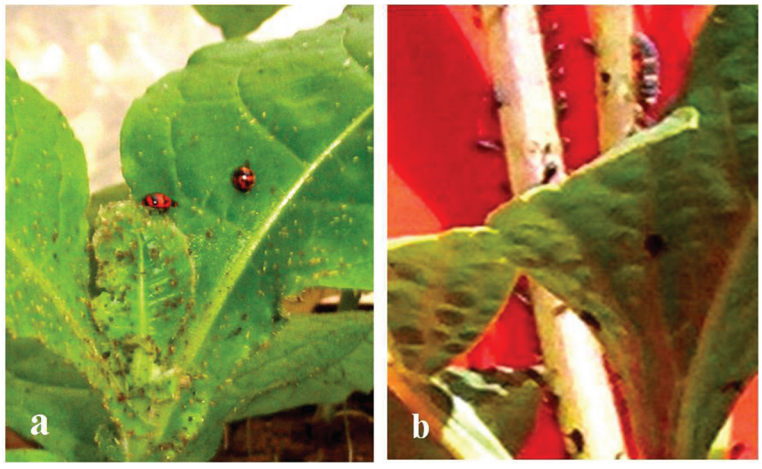

Fig. 3. Adult and larvae stages of ladybird (*Coceinella transversalis*) feeding on aphids on Tobacco and Dahlia plants

track. This avoidance mechanism may play an important role in the biological control exerted by predatory ladybirds on aphid populations (Ninkovic et al., 2013).

By use of virus-free gerbera planting material

Viruses spread from mother plant to their progenies through planting of infected cuttings, tubers and other vegetative plant materials that have great possibility of virus transmission. Consequently, population of plants may become infected by the virus if not protected timely and hence reliable early diagnosis of viruses is essential for designing their efficient disease management. Use of virus-free planting material and their transplantation in greenhouses has been suggested for better crop production (Agrios, 2005).

Literature survey revealed the only record of a post graduated dissertation by Zhang, 2009, who attempted development of virus-free plant*s* o*f G. jamesonii* cv. Bolus for management of four viruses: TBRV, TMV, CMV and TRV. He inoculated these viruses on *G. jamesonii* and used its three different explants (tip, leaf and torus) for their elimination through heat treatment followed by in-vitro tissue culture. He found that tip and torus culturing was the best method for obtaining virus-free gerbera plants. He verified the virus-free plants by multiplex RT-PCR and real-time RT-PCR and found that the rates of virus-free by tip cultures were 75.0% and 66.7%, respectively, while rates were 52.0% and 54.5%, respectively, by torus cultures. In this study the highest 75.0% and 54.5% virus-free gerbera plants were obtain by tip culture and torus culture, respectively in combination of thermotherapy. The success of virus-free for *G. jamesonii* Bolus by tip culture and torus culture was the first report from China (Zhang, 2009).

CMV-free gerbera plants have been developed through *in vitro* chemotherapy using 30 mg/L virazole by Gautam and co-workers in 2017. The total 38 developed plants (Fig. 4) when screened by RT-PCR using coat protein specific primers of CMV showed absence of CMV in 81.6% (31/38) plants. The 31 CMV-free plants showed better plant growth and better blooming performance as compared to the control ones. Elimination of CMV by *in vitro* chemotherapy (using virazole) of capitulum explants of gerbera cv. Zingaro has been reported for the first time from India (Gautam et al., 2017).

Development of virus resistance transgenic gerbera plants

Pathogen-derived resistance has been observed to be mediated either by the protein encoded by the transgene (protein-mediated) or by the transcript produced from the transgene (RNA-mediated) also known as post transcriptional gene silencing (PTGS) or both (Varma et al., 2002). In Literature, several virus resistance transgenic plants have been develop in various plants but in gerbera first reported from Poland for TSWV (Korbin et al., 2002) and then after from India for CMV (Gautam et al., 2019).

Nucleoprotein gene based viral resistance for TSWV

Nucleoprotein gene based viral resistance in G. hybrid cv. Prince', 'Paul', 'Alaska' and 'Zuzanna for TSWV has successfully been introduced by Korbin and co-worker in 2002 through Agrobacterium-mediated transformation. The integration of a resistance-related nucleoprotein gene of TSWV into gerbera explants successfully demonstrated that

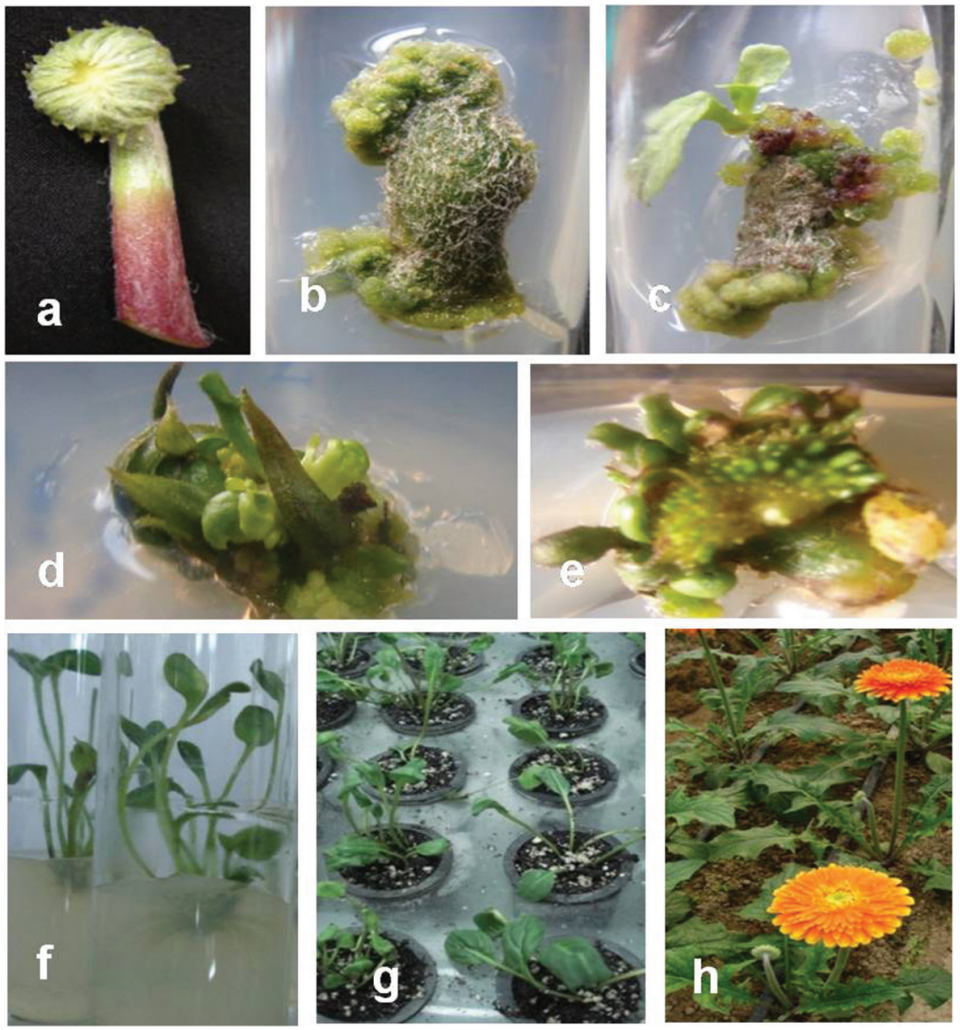

Fig. 4. Various regeneration stages during development of virus-free gerbera plants from floral bud explants: callus initiation, shoot proliferation, rooting of shootlets, hardening, establishment of rooted plants and blooming stage of plants in glasshouse

transgenic plants were resistant when challenged by mechanical inoculation of TSWV (Korbin et al., 2002).

The transformed plants of four gerbera cultivars with nucleocapsid N-gene of TSWV were evaluated for resistance to the virus and several phenotypical traits. Sixteen out of 33 transformed genotypes (transgenic plants) were confirmed by PCR with specific primers for N and *npt* II genes. After mechanical inoculation with TSWV, typical symptoms of viral infection appeared in the control plants after two to four weeks but no disease symptoms were observed in any of the infected transgenic plants. Assessment of other phenotypical traits of gerbera confirmed lack of significant differences between transformed and control plants. Except for one genotype of 'Prince' and one genotype of

'Zuzanna', all of the transformed plants were recommended as the potentially good breeding material (Korbin, 2006).

Coat protein based viral resistance in gerbera for CMV

Keeping in view of trait improvement and development of in built resistance against CMV in gerbera, Gautam and co-worker in 2019 attempted the genetic transformation using CMV-CP gene and petiole base explants of gerbera. For screening of resistance, the developed transgenic plants were challenged with leaf sap inoculums of CMV. The results showed virus resistance in 53% and virus tolerance (delayed and mild symptoms) in 33% plants while rest of the plant showed severe disease symptoms for virus infection after challenged with mechanical inoculation of CMV. The developed protocol may be adopted for transferring any other gene of agronomic or economic interest in gerbera plants (Gautam et al., 2019).

Virus-induced gene silencing (VIGS) for natural defense mechanism in plants for TRV

VIGS is a natural defense mechanism in plants which leads to sequence-specific degradation of viral RNA. For identifying gene functions, TRV-based VIGS has been applied for silencing of endogenous genes in many plant species. *Gerbera hybrida* (Asteraceae) has emerged as a novel model for studies in flower development and secondary metabolism. For this highly heterozygous species, functional studies have been conducted through reverse genetic methods by producing stable transgenic lines, which, however, is labour-intensive and time-consuming (Deng et al, 2012).

For the development of TRV-based VIGS system for gerbera, and for the first time for an Asteraceaeous species, Deng et al. (2012) screened several gerbera cultivars and optimized the agroinfiltration methods for efficient silencing. Gene fragments for gerbera phytoene desaturase (GPDS) and Mg-chelatase subunits (GChl-H and GChl-I), expressed from a TRV vector, induced silencing phenotypes in leaves, scapes, and involucral bracts indicating their feasibility as markers for green tissues. In addition, robust silencing symptoms were achieved in gerbera floral tissues by silencing the anthocyanin pathway gene for chalcone synthase (GCHS1) and a gerbera B-type MADS-box gene globosa (GGLO1), confirming the phenotypes previously observed in stable transgenic lines. Unexpectedly, photobleaching induced by GPDS and GChl-H or GChl-I silencing, or by the herbicide norflurazon, resulted in silencing of the polyketide synthase gene G2PS1, which has no apparent connections to carotenoid or chlorophyll biosynthesis. They have shown feasibility of VIGS for functional studies in gerbera, but their results also show that selection of the marker gene for silencing must be critically evaluated (Deng et al., 2012).

Development of quick and reliable virus diagnostics for gerbera viruses

The development of quick and reliable virus diagnostic protocols for detection of viruses in gerbera is the prerequisite for indexing of gerbera materials in bulk, and to identify virus/disease free materials to be used for large scale gerbera propagation and its mass multiplication through tissue culture industry. In this direction, Gautam (2015) attempted for standardization and development of two protocols: Western blot immuno-

assay and RT-PCR using antiserum of CMV and CMV-CP specific primers, respectively, for successful detection of CMV in two varieties of gerbera being cultivated in India. Though these developed tests were successful for efficient and reliable detection of CMV infection in gerbera, however, Western blot immunoassay is not very suitable for mass testing as compared to ELISA tests.

Molecular detection of CMV isolates of gerbera by RT-PCR was developed by standardization of conditions most suitable for cDNA synthesis and PCR cycles: denaturation, primer annealing, extension and the final extension at the end for successful amplification of an expected size ~650 bp band of the coat protein region of CMV using CMV-CP gene specific primers: CMV-CP-Forward: 5'-GCATTCTAGATGGACAAATCTGAATC-3' and CMV-CP-Reverse: 5'-GCATGGTACCTCAAACTGGGAGCAC-3' (Gautam, 2015). The RT-PCR performed with the total genomic RNA of the infected gerbera and other host plants and CMV-CP gene specific primers resulted in amplification of ~650 bp bands in naturally infected gerbera samples of cvs. Zingaro and Silverster, which was similar as in CMV-Banana infected sample taken as positive control. The sap inoculated gerbera (cvs. Zingaro and Silvester), *C. sativus* and *N. tabaccum* cv. White Burley plants also showed the ~650 bp amplicon when tested by RT-PCR, confirming the presence of CMV in sap inoculated plants (Gautam, 2015).

Zhang and coworkers in 2009, developed multiplex RT-PCR for detecting Tomato black ring virus (TBRV), Tobacco mosaic virus (TMV) and CMV, simultaneously for detecting TSWV, TRV and TMV; and real-time -PCR for detecting 4 viruses: TBRV, TMV, CMV and TSWV, respectively from *G. jamesonii* cv. Bolus. The multiplex RT-PCR for detecting TBRV, TMV and CMV could detect virus as low as in 1.0 μg the three leaf tissues. While the multiplex RT-PCR for detecting TSWV, TRV and TMV could detect as low as 1.0 mg the three leaf tissues. The real-time -PCR could detect as low as 1.0 ng, 1.0 ng, 100 pg and 1.0 μg of the leaf tissues with TBRV, TMV, CMV and TSWV, respectively (Zhang et al., 2009). They have used several virus-free plants by tissue culture for the study on *G. jamesonii* infected by these viruses for the first time, verified the effect of virus-free by multiplex RT-PCR and real time-PCR, found that the virus-free rates of tip culture were 75.0% and 66.7% respectively, the virus-free rates of torus culture were 52.0% and 54.5% respectively (Zhang et al., 2009).

Conclusion

Many factors such as Insect-pest, fungal, bacteria, phytoplasma and viruses are considered as the bottleneck for gerbera production. Control of these pathogens can be done using insecticide, fungicide and antibiotic etc. The control of viruses in gerbera plant is slightly difficult because of non availability of effective viricide. However, several conventional and non-conventional methods *viz.* sanitation, bio-control, development of virus-free plants and transgenic gerbera plants are available for effective management of gerbera viruses. Present review particularly focuses on disease symptoms of infected gerbera as well as biological, serological and molecular detection of viruses and phytoplasma infecting gerbera worldwide. This review indicated that viruses until reported on gerbera have single stranded RNA genome. Therefore, RNA based pathogen derived resistance against gerbera viruses would be useful for gerbera virus management. The protocol de-

veloped for elimination of CMV and production of virus-free elite varieties of gerbera cv. Zingaro plants may be utilized to save the germplasm from virus infection. The technique may also be used for the mass propagation of virus-free elite varieties of gerbera, which may of importance to the floriculture industry.

The information summarized in this review will be useful for gerbera growers worldwide that ultimately would benefit in uplifting the economic and social status of the gerbera related farmers. Moreover, an eco-friendly approach like biological control of virus transmitting vectors in nature has also been suggested for virus-disease management which neither has adverse effect on human health, nor possesses hazards to the environment. The developed diagnostic protocols may be used for quick and reliable detection of viruses in gerbera, and for indexing of gerbera materials in bulk, and to identify virus/disease free materials to be used for large scale gerbera propagation for farmers and its mass multiplication through tissue culture industry.

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