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Review Paper

Diseases affecting gerbera cultivation and their control measures

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Abstract

Gerbera (*Gerbera jamesonii*) is cultivated all over the world and is widely popular as an ornamental plant. It is grown in beds or pots and used as cut-flower, in making bouquets and for decoration in ceremonial functions. According to the USDA NASS Census of Horticulture 2014, gerbera has a value of over $41.2 million dollars annually. It 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 area under gerbera cultivation in India is increasing day by day due to its large scale uses and the market demands. Gerbera production has been hampered by numerous diseases that affect its flower quality and quantity. Along with fungal, bacterial and insect diseases, viral and phytoplasma diseases causes considerable loss in gerbera cultivation and marketing. In this review, we describe about disease symptoms, virus and phytoplasma detection methods and identification of causal pathogens of important viral diseases affecting gerbera production worldwide, based on their biological, serological and molecular characteristics, and their effective disease management strategies.

Key worlds: *Gerbera jamesonii*, virus diseases, disease management, virus-free plants, virus resistant transgenic plants.

INTRODUCTION

Gerbera (*Gerbera jamesonii*) is a popular ornamental plant of family *Asteraceae*, named after Gerber, a German naturalist. It is native to South Africa and commonly known as Barberton Daisy or African daisy. The genus *Gerbera* consists of about 40 species. Out of the recorded species, only one species *G. jamesonii* is under cultivation. Subtropical and Mediterranean climate is suitable for its growth and cut flower production around the world. These climatic zones pass through Israel, Italy, Spain, Portugal, Morocco, Colombia, Japan, South Africa, Australia and Southern India. The plants are stem-less, tender and perennial herbs. They are dwarf, 30-45 cm tall & hairy. Leaves are leathery, narrower at the base and wider at the top and are arranged in rosette at the base. The flowers are daisy like, 7-15 cm across. The flower may be single or double and available in various self colored cultivar as well as bicolor. They are of wide range colors include yellow, orange, cream, white, pink red, scarlet, salmon, maroon, terracotta and various other intermediate shades. It is considered as the fifth most used cut flower in the world (after rose, carnation, chrysanthemum and tulip) (http://en.wikipedia.org/wiki/Gerbera). It 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), Silvester (white), Salvadore (yellow), Rosaline (pink), Davaellen, Goliath, Cream Clementine (creamy white), Maroon Clementine (orange), Flamingo (Pale rose), Delphi (white), Vista (red), Uranus (yellow), Fredenking (yellow), Terra queen (Pink), Dustty (red), Valentine (pink), Labalga (lilac), Fredaisy (pink), Fredorella (red) etc. There are many other cultivars which are cultivated commercially in India are: Cream Clementine, Maroon Clementine, Delphi, Vista, Uranus, Terraqueen, Dusty, Valentine, Diablo, Mariso, Pascal, Winter Queen, Jaffa, Sangria, Diana, Paganini, Rosetta, Gloria, Pricilla, Sunway, Zingaro, Balance, Dune and Monique etc. (https://vikaspedia.in/agriculture/cropproduction/package-of-practices/flowers/gerbera-cultivation).

Gerbera is a very attractive, commercial cut flower crop and marketed in the International florists' trade in huge quantities. These plants are grown throughout the world in a wide range of climatic conditions. The most important production areas are: the Netherlands, Italy, Germany,

France, and California. The Netherlands produces 420 million stems of gerbera per year which is valued at 145 million Netherlands guilders (Sudhagar, 2013). The production of gerbera was approximately US$ 220 million in 2001 representing 70 million stems sold in US alone (Broek *et al.*, 2004). About 7 species were recorded in India, distributed in the temperate Himalayas from Kashmir to Nepal at altitudes of 1,300 to 3,200 meters. Gerbera production is maximum (2016 MT in 70 ha area) among the others cultivated flowers such as carnation, gladiolus, marigold, rose, tuberose in Uttrakhand. Gerbera contributes with a production of 17,840 MT and stands fourth important cut flower in India. The total area of gerbera in India is 820 ha with a cut flower production of 17, 840 MT and loose flower of 3,960 MT. However, maximum production of gerbera comes from Uttarakhand (7, 200 MT) while, maximum area coverage is from Assam (600 ha). The share of gerbera cut flower production in Karnataka is 200 MT and loose flower production is 580 MT (Anonymous, 2015). According to DBT and Small Farmer's Agribusiness Consortium, India, gerbera ranks as 2nd most domestic tissue culture flower crops in India after carnation, anthurium, orchids in floriculture industry. The cut flower sticks of gerbera are been sold in market with the variable rate depends on the flower quality and size. According to recent survey of floriculture today, gerbera farmers have recorded earnings of Rs. 25,000 - 30,000/- from an area of 134 sqm. The average income per unit area perhaps is the highest in floriculture, ranging from Rs. 100 to Rs. 200 per sqm. According to National Horticulture Board (NHB) of India 2015-16 major gerbera producing states are Assam (12.42 Tones), Uttarakhand (5.14 Tones), Telangana (3.14 Tones), Karnataka (2.37 Tones), Meghalaya (0.92 Tones), Maharashtra (0.66 Tones), Tamil Nadu (0.52 Tones), Himachal Pradesh (0.30 Tones) Nagaland (0.04 Tones) and Mizoram (0.03 Tones). Gerbera is grown commercially in India for export and domestic market. In India, we produce very high quality cut flower of the crop and millions of tissue cultured plants are produced. Tropical Floritech Pvt. Ltd. in Bangalore is the leading player in commercial cultivation in India (Chaudhary and Prasad, 2000). The area under gerbera cultivation in Karnataka is estimated at 25 ha with production of 53 lakhs cut flowers at an estimated value of Rs. 15 lakh.

PATHOGENS AND DISEASES AFFECTING GERBERA CULTIVATION

Gerbera production is challenged by numerous diseases caused by insect, fungal, bacterial, nematodes, viral and phytoplasma pathogens that affect its flower quality and quantity. The major pests (whitefly, aphid, leaf miner, thrips, mites), diseases (powdery mildew, collar rot, root rot, stem rot, leaf spot), nematodes (root-knot, spiral), and their symptoms, biology, spread, and management have been discussed by Reddy, 2016. The details of diseases caused by insect, fungal, bacterial, nematodes, viral and phytoplasma pathogens are described as under:

Insect-Pest Diseases

Insect-Pest incidence is the major factor responsible for yield reduction in gerberas. Gerberas have a wide variety of pests such as aphids (*Myzus persicae* and *Aphis jabae*) (which transmit *Cucumber mosaic virus* and Potyviruses in nature), Leaf miner (*Liriomyza trifolii* and *L. soncho*), Mites (*Steneotarsonemus pallidus* and *Polyphagotarsonemus latus*), Western flower thrips and caterpillars. The whiteflies (*Bemisia tabaci* Genn., Hemiptera: Aleyrodidae) are major potential insect pests of greenhouse gerberas (Table. 1) (Shalini *et al.*, 2019). These insect pests affect plant health by sucking of their sap as well as by transmitting many diseases from infected to healthy plants.

Nematode Problems

Although a multitude of plant parasitic nematodes are found associated with gerbera elsewhere in the world (Lamberti et al., 1987), root knot nematodes belonging to *Meloidogyne* spp. are predominant in India (Nagesh and Parvath Reddy, 2001). In India, yield loss in gerbera due to *Meloidogyne incognita* was reported to be 31.1% (Nagesh and Parvatha Reddy, 2000). A survey was conducted in the different districts of Tamil Nadu in the year of 2013 in order to determine the most important plant parasitic nematodes species associated with gerbera. The analysis of soil and root samples collected from the rhizosphere of gerbera in each district revealed the presence of only five species of plant parasitic nematodes. These are *Meloidogyne incognita, Helicotylenchus multicinctus, Pratylenchus coffeae, Tylenchorhynchus* spp. and *Rotylenchulus reniformis*. The present investigation revealed that *M. incognita* is one of the serious limiting factors in commercial cultivation of gerbera under polyhouse conditions present in Tamil Nadu (Manju and Subramanian, 2015). Nematodes also transmit some diseases caused by viruses such as: *Tomato bushy stunt virus* and other *Nepoviruses* which also affect gerbera and other plants grown in polyhouse/glasshouse conditions.

Fungal Diseases

Gerberas have several fungal disease problems such as: root rots (by *Pythium irregulare, Rhizoctonia solani*); crown and root rot (by *Phytophthora cryptogea, P. drechsleri*); Sclerotium rot (by *Sclerotium rolfsii*); Botrytis blight (by *Botrytis cinerea*); powdery mildews (by *Erysiphe cichoracearum, Oidium*

crysiphoides); leaf spots (by *Alternaria alternate*) (Farhood and Hadian, 2012), (by *Corynespora cassiicola*) (Shi *et al*., 2012) and by (*Phyllosticta gerberae, Alternaria spp.).* The downy mildews, yellow discoloration on leaf, later turning light to dark brown on gerberas are caused by *Bremia luctucae* (Wolcan *et al*., 2010), and White rust (white erumpent sori) is caused by *Albugo tragopogonis* (Vazquez *et al*., 1997). The oval, circular or irregular, brown to black lesions with

Table 1: Insect pest, fungal, bacterial and nematodes diseases on Gerbera and their disease management

Insect-Pest	
Pest	**Managements (Reddy, 2016)**
Whitefly (*Trialewiodes vaporariorum*)	Spray Dimethoate (Rogor), Endosulphon (2 ml/l water)
Aphids (*Myzus persicae* and *Aphis jabae*)	Spray Dimethoate 2 ml/l of water
Leaf Miner (*Liriomyza trifolii* and *L. soncho*)	Spray Chloropyriphos, Diclorovous (Nuvan) (1 ml/l)
Thrips	Spray Rogor, Nuvacron (2 ml/litter)
Mites (*Steneotarsonemus pallidus*)	Spray Dicofol (Kelthane), Wettable Sulpher (1.5 g/l)
Catterpiller	Apply Thimet (Phorete) 2 g/plant apply around the base of the plant
Western Flower Thrips	Spray imidacloprid 0.4 ml/l followed by Pongamia or Neem oil 10 ml/l in case of severe incidence

Bacterial Diseases			
Pathogen/Cause	**Disease & Symptoms**	**Management**	**Reference**
Pseudomonas cichorii	**Bacterial Leaf Spot:** Small to large spots are circular at first, and then become irregular and dark brown to black. May have a concentric ring pattern.	Maintain low relative humidity. Avoid overhead watering.	Miller and Knauss, 1973, Alivizatos, 1986, Marques *et al*., 2016
Nematodes Disease			
Pathogen/Cause	**Disease & Symptoms**	**Management**	**Reference**
Meloidogyne incognita	**Nematodes Root knot** Stunting of plants, yellowing of leaves, wilting of plants, and heavy galling on roots.	Pre plant treatment of beds with dazomet followed by the application of neem cake (1 kg/m 2, 15 days later) along with *Paecilomyces lilacinus* reduced populations of *M. incognita*.	Manju & Subramanian, 2015; Reddy, 2016
Fungal Diseases			
Pathogen/Cause	**Disease & Symptoms**	**Management**	**Reference**
Alternaria	**Alternaria Leaf Spot:** Brown specks form on florets and the leaf centers become white on the leaf spots.	Maintain low relative Humidity and do not wet leaves when watering. Apply Fungicides: Dithane M-45 (Mancozeb) spray (1.5 g/lit.) to protect the plants	Farhood and Hadian (2012), Shi *et al*., (2012) and Reddy, 2016
Botrytis cinerea	**Botrytis Blight:** Petioles have long brown spots. Leaves turn yellow and die. Petals have tan spots. Stems at soil level are killed. Infected tissues become covered with gray fungal growth.	Space plants to insure good air circulation. Maintain low humidity. Avoid watering late in the day. Remove crop debris. Apply a fungicide to protect plants.	Zhang, 2006
Fusarium solani	**Fusarium Stem Rot:** Petiole of leaves blacken at the base as the plant collapses.	Plant in pasteurized potting media. Discard infected plants.	Li *et al*., 2008
Phytophthora cryptogea	**Phytophthora Crown Rot:** Plants wilt suddenly. Leaves turn brown. Roots are rotted and a crown rot develops.	Plant in pasteurized potting media. Avoid overhead watering. Apply fungicide to protect plants.	Martin *et al*., 2014
Golovinomyces cichoracearum (formerly Erysiphe)	**Powdery Mildew:** White fungal growth develops on the surface of leaves.	Apply fungicide: Aliette, Topsin-M (2 g/lit), to protect plants.	Wolcan *et al*., 2010 and Troisi *et al*., 2010
Pythium	**Pythium Root Rot:** Plants wilt and die as roots rot.	Plant in pasteurized potting media. Apply a fungicide: Captan, Benlate, Aliette drench to soil (2 g/lit) to protect plants.	Suzuki *et al*., 2009
Rhizoctonia solani	**Rhizoctonia Crown Rot:** Stems at the soil level have a brown lesion. Plants wilt and die.	Plant in pasteurized media. Apply a fungicide: Wettable Sulphur spray (1.5 g/lit) to protect plants.	Reddy, 2016
Thielaviopsis basicola	**Thielaviopsis Root Rot:** Plants turn yellow, wilt, and die. Roots are dark brown to black.	Plant in pasteurized media. Apply fungicide to protect plants.	Reddy, 2016

concentric rings on gerbera leaves are also reported to be caused by *Alternaria* spp (Mirkova and Konstantantinova, 2003) (Table.1).

Bacterial Diseases

Bacterial leaf spot disease on gerbera caused by *Pseudomonas cichorii* also is reported. The symptoms of this disease were: small to large spots, circular at first and then became irregular and dark brown to black spots (Miller and Knauss, 1973; Alivizatos, 1986; Marques *et al.,* 2016) (Table. 1).

PHYTOPLASMA DISEASES OF GERBERA

Phytoplasmas are intracellular obligate prokaryotes which lack cell wall, a pleiomorphic of filamentous shape, a diameter less than 1 micro meter, have a small genome and are mainly transmitted by leafhoppers. 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 *et al.,* 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 that of previously described '*Ca.* Phytoplasma' taxon (IRPCM, 2004).

Gerbera production are also reported to be affected by phytoplasma from different part of the world (Table. 2) such as: Phytoplasma, 16SrII from Australia and Phytoplasma 16SrI from Italy (Siddique, 2005); *Candidatus* Phytoplasma asteris' from Southern Italy (Spano *et al.,* 2011); and '*Candidatus* Phytoplasma asteris' (16SrI group) associated with yellows disease of gerbera from India (Gautam *et al.,* 2015a). The details of gerbera diseases caused by phytoplasma have been described as under:

Phyllody and green flower disease on gerbera

In 2005, A. B. M. Siddique, first time reported phytoplasma association with gerbera phyllody in Australia based on symptoms, TEM study 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 transmission electron microscopy (TEM) and the presence of pleomorphic bodies similar to phytoplasma was observed exclusively in diseased plants (Siddique, 2005).

The presence of phytoplasma DNA in the infected plants was also confirmed by Polymerase chain reaction (PCR) with phytoplasma specific primers. Further DNA 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.,* 1998. The results of TEM, PCR and sequencing analysis clearly indicated phytoplasml association with phyllody disease of gerbera (Siddique, 2005).

Virescence and abnormal flower colour disease

In January 2010, Spanò and coworker reported '*Candidatus* phytoplasma asteris' infection in *Gerbera jamesoniim* apulia from Southern Italy. The infected plants had phytoplasma like symptoms: virescence, phyllody and abnormal flower colour) and disease incidence was nearly 100% in cv. Maxima. The phytoplasma infection was detected in total DNA extracted from the leaves of three symptomatic by nested PCR with primers P1/P7 (Schneider *et al.,* 1995) and R16F2/R2 (Lee *et al.,* 1993). DNA fragments of 1.2 kb, corresponding to 16S rDNA, were amplified only from DNA of the three symptomatic samples. The amplified fragments showed identical RFLP patterns, which were indistinguishable from those produced by the European aster yellows strain of the "*Candidatus* Phytoplasma asteris" (Spanò *et al.,* 2011).

Further, the 1,803bp amplicon from P1/P7 PCR amplification was cloned and sequenced (Accession No. JF795864). The ClustalW2 alignment confirmed that 16S rDNA of gerbera phytoplasma shared 99.9% identity with *Oenothera* phytoplasma (accession No. M30790; Lim and Sears (1989) and 99.0% identity to several '*Ca*. P. asteris' isolates of different ribosomal subgroups: A, B, D, E, F, K, and P. *Ca*. P. asteris was widely spread in Italian cut flower crops but this was the first report from gerbera in Apulia (Spanò *et al.,* 2011).

Leaf yellows, shortening and flower deformation disease

In India, Gautam and co-worker first time reported '*Candidatus* Phytoplasma asteris' (16SrI group) associated with yellows disease of gerbera (*Gerbera jamesonii*) in 2014. The severe leaf yellows, shortening of whole plant and flower deformation symptoms were observed on gerbera plants growing in a polyhouse at Lucknow, Uttar Pradesh, India in 2012. The disease incidence was about 15-20% and symptoms exhibited by the diseased gerbera were suggestive of the presence of phytoplasma. Total DNA was extracted from leaf samples collected from symptom-bearing plants (Ahrens and Seemüller, 1992) and PCRs were performed using P1/

P6 universal primers (Deng and Hiruki, 1991) followed by nested PCR using primers R16F2n/R16R2 (Gundersen and Lee, 1996). Products of ~1.2 kb were amplified for four out of four samples from symptom-bearing plants, but not from the symptomless plants, demonstrating the association of a phytoplasma with the disease (Gautam *et al.,* 2015a).

Amplicons from two samples, *Gerbera jamesonii* yellows phytoplasma (GYLu1) and *Gerbera jamesonii* yellows phytoplasma (GYLu2), were cloned, sequenced and sequence data was deposited under Accession Nos.: JX674049 (GYLu1) and KC880350 (GYLu2). Sequence comparison using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed 99% homology between GYLu1 and GYLu2. GYLu1 and GYLu2 also shared 99% sequence identity with several strains of '*Candidatus* Phytoplasma asteris' reported worldwide. A similarity of 98% was verified between GYLu1 and GYLu2 when compared with the sequence of '*G. jamesonii*' phytoplasma (JF795864), a member of '*Ca*. P. asteris' from Italy. To find out phylogenetic relationships of the gerbera phytoplasma strains under study and 16S rDNA sequences of '*Ca.* P. asteris' strains and other phytoplasmas available in GenBank, a phylogenetic tree was generated using the neighbour-joining method. The results demonstrated that GYLu1 and GYLu2 clustered with the strains of '*Ca.* P. asteris'. Based on 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.,* 2015a).

VIRAL DISEASES AFFECTING GERBERA

Like other viruses, plant viruses are acellular, submicroscopic, obligate parasite and made up of nucleoprotein (DNA or RNA). 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: *Impatiens necrotic spot virus* (INSV), *Tobacco mosaic virus* (TMV), *Tomato black ring virus* (TBRV), *Cucumber mosaic virus* (CMV), *Tobacco rattle virus* (TRV) are reported but a very few report are of single stranded negative sense RNA viruses are reported and *Tomato spotted wilt virus* (TSWV) is one among them.

Many viral diseases on gerbera are reported worldwide. Some of them are: Concentric rings and distortion of leaves caused by TSWV and Necrotic spot on leaf caused by TSWV and INSV are reported from Siberia (Stankovic *et al*., 2011) and from New Zealand (Elliott *et al*., 2009), respectively. Gerbera is also host for TMV and TBRV reported from China (Zhang *et al*., 2009). Color break on the petals, and deformed flowers on gerbera reported is due to infecting *Cucumber mosaic*

Table 2: Virus and phytoplasma diseases on 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	Ranvikar *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, 1967
			USA	Stouffer *et al.,* 1965
			Germany	Schmelzer *et al.,* 1966
Tomato black ring virus		Black ring on leaf	China	Zang, 2009
Tobacco mosaic virus	Aphids and leafhoppers	Mosaic on leaf	China	Zang, 2009
Candidatus Phytoplasma Asteris'	Leafhoppers	Virescence, phyllody and abnormal flower colour	Southern Italy	Spano *et al.,* 2011
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 and Bellardi, 1998
Ca. Phytoplasma aurantifolia' (16SrII),	Leafhoppers	Phyllody symptoms (green flower) on Gerbera	Australia	Siddique *et al.,* 2005

virus on gerbera from India (Verma *et al.,* 2004a), *Tobacco rattle virus* (TRV) are also reported on gerbera from Netherland (Schmelzer *et al.,* 1966; Stouffer *et al.,*1965). In 2002, Slovenia, *Chrysanthemum stem necrosis virus* (CSNV) was also detected in gerbera. The identity of CSNV was confirmed by ELISA and PCR (Ravnikar *et al.,* 2003) (Table 2).

Diseases Caused by *Tomato Spotted Wilt Virus* (TSWV)

Tomato spotted wilt virus (TSWV) belongs to the genus *Tospovirus*, family *Bunyaviridae,* is a spherical negative-sense RNA virus that has a diameter between 80-110nm. TSWV is transmitted by thrips. The western flower thrips (*Frankliniella occidentalis*) is the vector that predominantly transmits TSWV globally and in greenhouses. TSWV infects over 1000 plant species and causes significant economic damage to many agronomic and horticultural corps. In some areas the virus has been found to be ubiquitous in the environment as it can infect many weeds, landscape plants, and native plants. Symptoms of tomato spotted wilt differ among hosts and can be variable in a single host species. Stunting is a common symptom of TSWV infection, and is generally more severe when young plants are infected. Chlorotic or necrotic rings form on the leaves of many infected hosts.

In May 2009, Stankoviæ and co-worker first time reported TSWV on infected gerbera having chlorotic oak-leaf patterns followed by necrosis and distortion symptoms of leaves in green house of Vranjska Banja (Pèinj District) in Serbia based on disease incidence, disease transmission, serological and molecular study. During a survey approximately 30% gerbera plants were found to be symptomatic within a greenhouse. Leaf sap of infected gerbera were mechanically inoculated on healthy *Petunia* × *hybrida* resulted local necrotic spots symptoms on leaf suggested the presence of a *Tospovirus*. For the serological study, symptomatic leaves were tested for the presence of TSWV by commercial double-antibody sandwich (DAS)-ELISA diagnostic kits (Loewe Biochemica, Sauerlach, Germany). TSWV was detected serologically in 18 of 20 gerbera samples. The presence of TSWV in ELISA-positive symptomatic gerbera plants was further confirmed by conventional reverse transcription (RT)-PCR. Total RNAs were extracted with an RNeasy Plant Mini Kit and RT-PCR was conducted with the One Step RT-PCR Kit (Qiagen) using Serbian tobacco TSWV isolate (GQ279731) and RNA extract from healthy gerbera as positive and negative controls, respectively. Two different sets of TSWV-specific primers, L1 TSWVR/L2 TSWVF and M962/M66, for a 276-bp fragment of the RNA-dependent RNA polymerase (RdRp) gene and a 897 bp fragment of the NSm gene, respectively, were used for both amplification and sequencing. RT-PCR analyses of each tested plant detected the presence of amplification fragments of expected size. The amplified products corresponding to part of the RdRp and NSm genes derived from the isolate 158-Gerb were purified and sequenced in both directions (Accession Nos. HQ246452 and HQ246453). Sequence analysis of the partial RdRp gene, conducted using MEGA4 software, revealed 91.1 to 98% nt identity (95.1 to 98.8% amino acid [aa] identities) with corresponding sequences of TSWV L RNA. The highest identity was found with an isolate from globe artichoke (AM940436) in Greece, and isolates from tomato (GQ279732), impatiens (GQ132190), and tobacco isolates (GQ279731, FJ189392, and FJ189393) found within Serbia. Analysis of the NSm sequence of isolate 158-Gerb demonstrated nucleotide identities varying between 90.6 and 99.6% (80.9 and 99.6% aa identities) with those of previously reported TSWV isolates. The highest identity was with tobacco isolate (GQ373174) from Serbia. This was the first report infecting gerbera in Serbia, which may have a devastating influence on its production. (Stankoviæ *et al.,* 2011).

In 2010, Spanol and co-worker reported of a resistance breaking strain of *Tomato spotted wilt virus* (TSWV) from *G. jamesonii* Apulia in Southern Italy. During survey, they notice that greenhouse-grown *G. jamesonii* in Apulia were showing severe malformations of die flowers and necrotic spots on the leaves. Estimated disease incidence in the gerbera plants grown in the greenhouse was 50% in cvs Sporza and Dune, 20% in cv. Lancaster and 10% in cv. Poseidon. TSWV was detected in all samples tested by dot blot hybridization. Two viral isolates obtained from cvs 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. Tomato cvs UC82 and Faino were systemically infected by the two virus isolates. These results show that the TSWV isolates Sporza and Dune are of the resistance-breaking (RB) type (Ciuffo *et al.,* 2005). This was the first report of a RB strain of TSWV in gerbera in Italy (Spanol *et al.,* 2010).

In 2014, Marys and co-worker reported of *Tomato spotted wilt virus* (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. Total RNA was extracted with the RNeasy Plant Mini kit (QIAGEN, Hilden, Germany) from two gerbera and two chrysanthemum ELISA-positive samples. The TSWV coat protein gene was amplified by conventional RT-PCR using primers CP1 TSWV (TTAACTTACAGCTGCTTT) and CP2 TSWV (CAAAGCATATAAGAACTT). A single DNA product of ~823 bp was amplified from all samples. RT-PCR products were directly sequenced (Accession Nos. KF146700 and KF146701 derived from chrysanthemum, KF146702 and KF146703 derived from gerbera). The resulting sequences showed over 99% identity with each other and were found to be closely related (over 99%) with TSWV isolates deposited in GenBank originating from different hosts from France (FR693058, FR693055), Montenegro (GU339506, GU339508, GU355940), Italy (HQ830187), New Zealand (KC494501), South Korea (KC261967), and the United States (AY744476). This was the first confirmed report of TSWV infecting gerbera and chrysanthemum in Venezuela. The relatively widespread occurrence of TSWV in Miranda State underscores the need for systematic surveys to assess its incidence and impact on ornamental crops so that appropriate management tactics can be developed (Marys *et al.*, 2014).

Diseases Caused by *Cucumber Mosaic Virus* (CMV)

Cucumber mosaic virus (CMV) is the type member of genus *Cucumovirus* in the family *Bromoviridae*. It is reported to infect 1287 plant species in 518 genera belonging to 100 families (Edwardson, Christie, 1987) worldwide. The most common symptom induced by CMV is mosaic but the virus also causes fern leaf, stunting of vegetable crops and malformation of their fruits. It is transmitted by numerous species of aphid, through the sap, the seeds and dodder (Francki *et al.*, 1979; Kaper and Waterworth, 1981; Dijkstra and Khan, 2006). CMV particles are icosahedral in shape and 29 nm in diameter (Fig. 1A), each consisting of 180 subunits of a single CP of ~24 kDa and one of the genomic RNAs. Based on their nucleic acid sequence similarity, CMV strains can be divided broadly into two major subgroups (I and II), with subgroup I strains divided into two (A and B) subgroups. The CMV genome contains five genes, expressed from either the three genomic RNAs or two subgenomic RNAs (Fig. 1B). The 1a and 2a proteins are involved in virus replication, whereas the 2b protein is an RNA silencing suppressor, an antagonist of other host defense mechanisms. The 3a protein and CP are essential for both cell-to-cell and long-distance movement processes. Protein 2b and CP are expressed from subgenomic

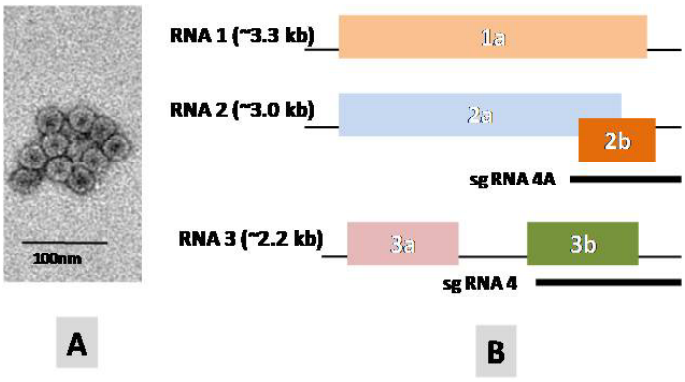

Fig. 1. *Cucumber mosaic virus* (A) Particle structure under TEM and their (B) Genome organization.

RNAs, designated RNA 4A and RNA 4, respectively. RNA 4 is packaged together with RNA 3, whereas the packaging arrangements for RNA 4A are not known, except that it is only packaged by subgroup II CMV strains. CMV causes significant losses to most of the major crops, around the world, therefore are the bottlenecks to the crop production (Hull and Davies 1992; Valkonen 1998; Raj *et al.*, 2008) including gerbera (Gautam *et al.*, 2014).

In 1975, J.R. Finlay first time reported CMV on gerbera (*Gerbera jamesonii* Bolus) plants from two nurseries at Bundaberg and from a home garden at Brisbane Australia based on mottled on leaf and distorted petals with colour break symptoms on flower, virus transmission by mechanically and by aphid *Myzus persicae*, transmission electron microscopic (TEM) and serological study. 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 the TEM study, Polyhedral particles 25 nm in diameter were observed in negatively stained preparations obtained from the sap of infected *Nicotiana clevelandii* by butanol clarification and two cycles of differential centrifugation. 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 transmitted by over 150 *M. persicae* in 6 tests, and it rarely produced systemic infection in cucumber. 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 not been recorded previously as the

cause of a disease in gerbera (Finlay, J. R., 1975)

In 2004, Verma and co-worker first time reported CMV on gerbera (*G. jamesonii*) in India based on virus transmission, enzyme-linked immune-sorbent assay (ELISA), TEM study of virus particle and its molecular characteristics. 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 Institute of Himalayan Bioresource Technology, Palampur and nearby nurseries. During host range or transmission study, the virus evoked chlorotic local lesions and systemic mosaic on many test species. The virus was also transmitted in non-persist manner by *Myzus persicae* and *Aphis gossypii* and was identified as CMV using ELISA with CMV-specific antibodies. Polyhedral particles approximately 29nm were observed with electron microscopy of leaf dips from symptomatic gerbera leaves. For molecular study, total RNA was isolated from the infected gerbera and *N. glutinosa* plant. CMV-specific primers were used to detect the virus with reverse transcription-polymerase chain reaction (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). This gene sequence was submitted to GenBank database (Accession no. AJ634532) as RNA3 of 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-worker reported CMV on Gerbera *(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. 2). The disease incidence was 16.27% in cv. Zingaro followed by 11.57% in cv. Silvester. The transmission of causal virus was attempted using the leaf sap of naturally infected gerbera (*G. jamesonii)* plants of cultivars (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, suggesting the Koch's postulates. 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.

Fig.2. A view of naturally infected gerbera plants exhibiting chlorotic mosaic symptom in polyhouse conditions (a), a close view of infected leaf showing severe chlorotic mosaic and greening of vein symptoms (b), and severe color breaking and flower deformation symptoms (c) as compared to healthy flower (d).

White Burley and *N. rustica* at 30-35 dpi. For molecular detection of the virus, the total RNA was extracted from 100 mg leaf tissue of symptomatic plants of gerbera cv. Zingaro and Silvester using TRIzol (Sigma, USA) and reverse transcription-polymerase chain reaction (RT-PCR) was performed using CMV-CP gene specific primers. Electrophoresis of RT-PCR products resulted in amplification of expected size ~650 bp bands in naturally infected gerbera samples of cv. Zingaro and Silverster, similar to as in CMV-Banana strain taken as positive control, confirming the presence of CMV. Further, the complete RNA3 genome of CMV was amplified by RT-PCR using CMV-RNA3 primer from three infected gerbera leaf samples. The amplicons obtained were cloned sequenced and deposited in GenBank under the accessions JN692495, JX913531 (from cv. Zingaro) and JX888093 (from cv. Silvester). These sequences shared 98-99 % identities to each other and with a strain of CMV-Banana reported from India, and 90-95 % identities with various strains of CMV reported worldwide. Phylogenetic analysis revealed their closest affinity with CMV-Banana strain, and close relationships with several other strains of CMV of subgroup IB. This study provided evidence of subgroup IB CMV causing severe chlorosis and flower deformation in two cultivars (Zingaro and Silvester) of *G. jamesonii* in India (Gautam *et al.*, 2017).

Diseases Caused by *Tobacco Rattle Virus* (TRV)

Tobacco rattle virus (TRV) is an important plant pathogenic virus of family: *Virgaviridae* 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). Its particles are tubular with helical symmetry, 23 nm in diameter; Over 400 species of plants from 50 families of vegetables, ornamentals, and weed are susceptible to infection. It causes the disease corky ringspot in potatoes.

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 FA, 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: *Nicotiana tabacum* 'White Burley', *Chenopodium 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, 1967). It is unlikely that the disease will become of economic importance in The Netherlands since soil steaming before the beginning of the culture is already common practice as a measure of control of foot rot caused by fungus species.

Diseases Caused by *Impatiens Necrotic Spot Virus* (INSV)

Impatiens necrotic spot virus (INSV) of family: *Tospoviridae*, genus: *Orthotospovirus* is an economically 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.

INSV was first detected in New Zealand in August 2003 in *Freesia refracta* plants growing in a nursery on the South Island of New Zealand. However, in June 2006 a begonia (Begonia × tuberhybrida) specimen from a fourth North Island nursery was received which was found to be infected with INSV. Other species confirmed by RT-PCR to be infected at this site include: cyclamen (*Cyclamen persicum*), gardenia (*Gardenia jasminoides*), gerbera (*Gerbera jamesonii*), as well as hibiscus (*Hibiscus rosa-sinensis*). These hosts were considered as the new hosts for INSV. Partial nucleotide sequences obtained by RT-PCR were found to be closely related to the published sequences (Elliott *et al.,* 2009).

MANAGEMENTS OF GERBERA DISEASES

The management of diseases caused by insect pests, fungal, bacterial, nematode, phytoplasma and various viral pathogens in gerbera are being described in details below.

Management of Insect-Pest Infecting Gerbera

Gerbera infecting insect-pest may be controlled by insecticides such as: Dimethoate (Rogor), Endosulphon (2ml/Litter water) for Whitefly; Chloropyriphos, Diclorovous (Nuvan) (1 ml/litter) for Leaf Miner; Rogor, Nuvacron (2ml/litter) for Thrips; Dicofol (Kelthane), Wettable Sulpher (1.5g/litter) for Mites and Thimet (Phorete) 2g/plant may be applied around the base of the plant for leaf eating caterpillar etc. (Table 1).

Fungal, Bacterial and Nematodes Disease Management

Common fungal diseases infecting gerbera can be controlled by fungicides such as: Captan, Benlate, Aliette drench to soil (2g/lit) for Root rot; Aliette, Topsin-M (2 g/lit) for Powdery mildew; Wettable Sulphur spray (1.5g/lit) for Crown rot; Dithane M-45 (Mancozeb) spray (1.5g/lit.) for Alternaria leaf spot (https://ccari.res.in/gerbera.pdf). While, bacterial diseases may be controlled by spraying of antibiotic such as: streptocycline at 0.2 g/l or Kasugamycin at 1.25 ml/l (Table 1).

Phtoplasma Disease Management

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). Since gerbera is propagated through vegetative means and mass multiplication through tissue culture, Gautam *et al.,* 2015a suggested 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.

It is well known that the only antimicrobials being used to control phytoplasmas are tetracycline-class antibiotics. Recently, 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).

Management of Viral Diseases

Management of viral diseases is much more difficult than that of diseases caused by other pathogens (Verma *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. 3).

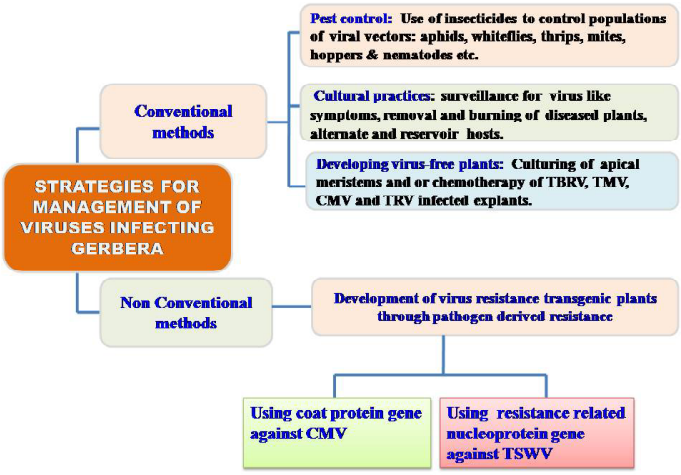

Fig. 3. Summary of management of viruses infecting Gerbera (based on literature by Korbin 2002; Zang, 2009; Gautam *et al.*, 2017 and Elizzabet *et al.*, 20016).

By Cultural Practices - Prevention is the key for managing *Tospovirus* diseases because *Tospovirus*-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).

By Sanitation - It is well kwon fact that sanitation of the cultivation fields enhances crop production by many folds.

Fig. 4. Different regeneration stages in the development of virus-free plants. Floral bud showing callus initiation after one month (a-b), shoot proliferation (c-d) after 60 day on MS medium supplemented with BA (1.0 mg/l), IAA (0.5 mg/l) and Ads (0.5 mg/l) hormones, rooting of shootlets in Gerbera rooting media after 20 days (e), hardening of plants at culture room conditions for one week (f) and in glasshouse condition (g). A panoramic view of glasshouse grown virus-free gerbera cv. Zingaro plants in comparison with CMV infected gerbera plants at blooming stage (h).

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 (Elizabeth *et al.*, 2016).

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) (Elizabeth *et al.*, 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 (*Coceinella 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. 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. 5). 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.

Fig. 5. Aphid feeding by ladybird (*C. transversalis*) in its adult (A & B) and larvae (C & D) stage on Chrysanthemum (A), Tobacco (B) and Dahlia plants (C & D).

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

Use of Virus-free Gerbera Planting Material - Viruses spread from mother plant to their progenies through infected cuttings, tubers and other vegetative plant materials that have great possibility of virus transmission. Use of virus-free planting material and their transplantation in greenhouses that isolates crop from other plants which harbor or may harbor viral diseases has been suggested for better crop production yield (Agrios, 1978).

Literature survey revealed the only record of a post graduated dissertation by Zhang, 2009, who attempted development of virus-free plants of *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).

Then after, Gautam and co-workers in 2017 attempted successful elimination of CMV through *in vitro* chemotherapy (using 30 mg/L virazole) of ~4 x 8 mm^2 capitulum explants of infected gerbera cv. Zingaro for its quality improvement. A total of 38 plants were developed from 57 explants on Murashige and Skoog (MS) medium supplemented with 1 mg/L 6-benzylaminopurine (BAP), 0.5 mg/L indole-3-acetic acid (IAA) and 0.5 mg/L adenine sulphate (Fig. 4). The developed plants showed absence of CMV in 81.6% (31/38) plants when screened by RT-PCR using coat protein specific primers of CMV. The CMV-free plants showed better plant growth: increase of 53.7% in length of leaf lamina and 59.2% in leaf width as well as better blooming performance: increase of 62.6% in flower size (diameter in cm) and 69.1% in number of flowers per pot having intense red flower colour as compared to the control ones. Elimination of CMV by in vitro chemotherapy (using virazole) of capitulum explants of gerbera cv. Zingaro is being reported for the first time from India (Gautam *et al.*, 2017).

Development of Virus Resistance Transgenic Gerbera - 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 *Tomato Spotted Wilt Virus* (TSWV)

Nucleoprotein gene based viral resistance in *G. hybrid* cv. Prince', 'Paul', 'Alaska' and 'Zuzanna for *Tomato spotted wilt virus* (TSWV) has been developed by Korbin and co-worker (Korbin *et al.,* 2002 and Korbin, 2006). They successfully introduced resistance to TSWV using *Agrobacterium*-mediated transformation. They integrated a resistance-related nucleoprotein gene from the virus into Gerbera explants and found that these transgenic plants did demonstrate resistance when mechanically inoculated. They used shoots, bases of shoot clumps and leaf explants; rooting hormones: TDZ = 1-1.5, IAA = 0.2-0.5 and Kinetin = 1, IAA = 0.2-0.5/IAA = 5 (mg/l); Agrobacterum strain LBA 4404 harboring pBin19 plasmid and cefotaxime and Kanamycin 250 and 70 (mg/l) for genetic transformation. Resulted transformation efficiency was 5.4 % and transgenic plants did demonstrate resistance when mechanically inoculated by TSWV (Korbin *et al.,* 2002).

Plants of four gerbera cultivars were transformed with nucleocapsid N-gene of *Tomato spotted wilt virus* were evaluated in terms of 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 can be potentially good breeding material (Korbin, 2006).

Coat Protein Based Viral Resistance in Gerbera for *Cucumber Mosaic Virus* (CMV)

Keeping in view of trait improvement and development of in built resistance against viruses in gerbera, Gautam and co-worker attempted the genetic transformation using CMV-CP gene and leaf, petiole and petiole base explants of gerbera. When differentiate hormones combination were added to MS basal medium, base petiole of explant has shown higher regeneration efficiency as compared to petiole and leaf explants. The 7-25 mg/l concentrations of Kanamycin were used in this study for *Agrobacterium* mediated transformation of base petiole explants of gerbera. The use of 15 mg/l concentration of Kanamycin was found optimum during transformation experiments.

Conclusively, a total of 310 base petiole explants of gerbera cv. Zingaro were co-cultivated for transformation. Out of them 97 explants were successfully regenerated on selection medium. All shoots were transferred to rooting medium where 52% plants had developed a well organized branched root system. The developed transgenic plants were placed under greenhouse conditions for acclimatization from where the survived 88% plants were chosen for molecular validation. The results of PCR, Southern and Northern blot analysis confirmed that total 89% plants had CP gene integration in their genome. The transgenic plants when 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, unpublished data submitted to IJEB).

Development Quick and Reliable Virus Diagnostic Protocols for Gerbera Viruses

The development quick and reliable virus diagnostic protocols for detection of viruses in gerbera were 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, 2015b attempted for standardization and development of two protocols: Western blot immunoassay and RT-PCR using antiserum of CMV and CMV specific primers, respectively, for successful detection of *Cucumber mosaic virus* (CMV) in two varieties of gerbera being cultivated in India.

Western blot immunoassay for CMV detection in gerbera was developed by (Gautam, 2015b). During serological detection by western blot immunoassay, the crude protein preparations from naturally infected gerbera cvs. Zingaro and Silvester samples reacted positively with the antiserum of CMV (PVAS242a, ATCC, USA), raised against the capsid protein, and showed two bands of 26 and 52 kDa, similar as in case of CMV-Banana (Vishnoi 2012) taken as positive control (Fig. 6); however, no such band was observed in a sample of healthy gerbera. The 26 kDa band was of coat protein of CMV and 52 kDa band seems to be a dimmer of 26 kDa protein as reported in case of many CMV strains (Francki *et al.,* 1979; Raj *et al.,* 1997). These results of western blot immunoassay indicated the CMV infection in Gerbera plants (Gautam, 2015b).

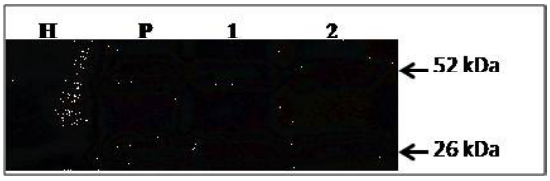

Fig. 6. Western blot immunoassay, using CMV-CP antiserum, showing 26 and 52 kDa bands in naturally infected gerbera cvs. Zingaro and Silvester samples (lane 1- 2) confirming CMV infection as compared to CMV-Banana (P) positive control. Lane H is healthy gerbera.

Molecular detection of *Cucumber mosaic virus* isolates in gerbera by RT-PCR was also developed by (Gautam, 2015b). The RT-PCR performed with CMV-CP gene specific primers resulted in amplification of expected size ~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 (Fig. 26). 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 (Fig. 7), confirming the presence of CMV in sap inoculated plants (Gautam, 2015b).

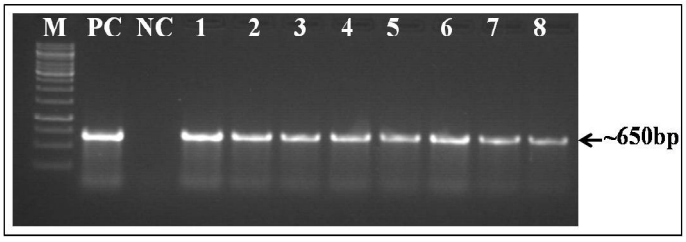

Fig. 7. RT-PCR amplification of CP gene from naturally infected and sap inoculated plants using CMV-CP gene specific primers confirming CMV infection. Lanes: PC = CMV-Banana infected tobacco plant (as positive control); NC = healthy gerbera plants (as negative control); 1-4 = infected gerbera samples; 5-8 sap-inoculated test plants of *N. tabacum* cv. White Burley, *N. glutinosa*, *N. rustica* and *C. sativus*; M = DNA marker.

Zang and coworkers in 2009, developed multiplex RT-PCR for detecting *Tomato black ring virus* (TBRV), *Tobacco mosaic virus* (TMV) and *Cucumber mosaic virus* (CMV), simultaneously; for detecting *Tomato spotted wilt virus* (TSWV), *Tobacco rattle virus* (TRV) and *Tobacco mosaic virus* (TMV); and real-time RT-PCR for detecting 4 viruses: TBRV, TMV, CMV and TSWV, respectively from *G. jamesonii* Bolus. The multiplex RT-PCR for detecting TBRV, TMV and CMV could detect as low as 1ìg the three leaf tissues. While the multiplex RT-PCR for detecting TSWV, TRV and TMV could detect as low as 1mg the three leaf tissues. The real-time RT-PCR could detect as low as 1ng, 1ng, 100pg and 1ìg the leaf tissues with TBRV, TMV, CMV and TSWV, respectively (Zang *et al.,* 2009).

CONCLUSIONS

G. jamesonii is an important, commercial ornamental plant. Its popularity increases day by day worldwide. In India gerbera industry suppose to be sunrise industry in export point of view. It takes important role in India as well as world economy. However several factor responsible for bottleneck of gerbera production, Insect-pest, fungal, bacteria, phytoplasma and viruses have important role. Control of insect, fungal, bacteria and phytoplasma pathogens can be done using insecticide, fungicide and antibiotic etc. The control of gerbera plant viruses is slightly difficult because of non availability of effective viricide for viruses. 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 developed 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.

This 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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