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PHYTOPLASMAS, THE FAST SPREADING VECTOR BORNE PATHOGENS OF FLOWER CROPS: INDIAN SCENARIO#

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ABSTRACT

Floriculture trade in India is one of the most lucrative fields, earning valuable forex to the national exchequer. Exchange of planting material via different modes plays a major role in spreading of the pathogen. Floriculture being an international industry, the transmission of pests or pathogens through live plants, seeds, cuttings and flowers etc., is very high. In recent years, the occurrence of phytoplasmal diseases in flower crops is increasing due to extensive exchange amongst many groups. The prominent symptoms of phytoplasma infection are the flowers turning into leafy structures called phyllody and the formation of sterile green flowers called virescence. In floricultural crops, these two symptoms are of major disadvantages as the flowers are the main economic product. The phyllody symptoms are most often mistaken for novel plant type and are multiplied unwittingly. Phytoplasmas are switch between plant and animal kingdoms. Phytoplasma spread in the field through leaf hoppers by inhabiting in their gut, haemolymph, salivary glands etc. Among the ornamental plants and flower crops, various groups of phytoplasma have been reported from India. More than 40 per cent of the infections are reported from the members of the Asteraceae family with 16SrI-B group, '*Candidatus* Phytoplasma asteris'.

Key words: Floriculture, disease, diagnosis, genomics, management

The floriculture sector is making rapid progress in India with 3.13 lakh ha of land under floriculture, producing 2059000 tonnes of cut flowers. Although, the mainstay of Indian floriculture is cultivating traditional flowers in an open field, the production of cut flowers under polyhouses, production of potted plants, plug plants, landscape plants and lawn grass is increasing. Due to lack of awareness among the stakeholders, often the affected plants are further propagated with a premise that the unique visible symptoms are that of novel variants. This practice is responsible for the spread of phytoplasmas among floricultural crops. Though the efforts to identify phytoplasmal diseases have been evident over the past three decades, their accurate detection and etiology became possible only after the advent of molecular biology tools. The remedial measures advocated so far focused mostly on vector management and prophylactic measures rather than curative.

In an attempt to introduce new plant types and cultivars from other nations or continents, there is greater threat for the entry of invasive plants, weeds, pests and pathogens. The plants infected with viruses, viroids and fastidious prokaryotes are of major concern as they are easily vectored by insect pests and are very difficult to manage once they are established. Ornamentals can act as reservoirs of potential pathogens that spread to other crops. While awareness on virus infection has increased among farmers, the knowledge about the vascular inhabiting insect vectored fastidious prokaryotes is meager. Due to lack of awareness about pathogen and propagation of infected mother plants rapidly spread the phytoplasma diseases.

Symptomatology

Phenotypic expression of phytoplasma infections is the result of alterations in plant morphology due to the imbalance in plant hormones. The common morphological symptoms due to phytoplasma infections can be classified as those occurring during the vegetative phase and flowering phase. Vegetative parts of the phytoplasma infected plants show little leaves or witches' broom symptoms. "Witches broom' symptoms occur as a result of proliferation of axillary buds, short internodes and loss of apical dominance. The reduced size of leaves and their occurrence in clusters is called "little leaf". Other non-specific symptoms include abnormal internode elongation, generalized stunting, abnormal yellowing of the plant called as yellows, excessive pigmentation, loss of the pinnation in leaves, leaf malformations, necrosis of vascular

bundles, dieback of twigs/ branches and decline in trees. During flowering, infected plants develop "phyllody" in which green leaf-like structures are produced instead of flowers, as the phytoplasma infections deregulate the genes involved in flowering (Fig. 1). Virescence is another symptom where the coloured parts in flowers turn green due to loss of pigments. Inhibition of photosystem II and biosynthesis of chlorophyll and carotenoid is reported in phytoplasma infected plants. Sterility of flowers, flower malformations, rudimentary reproductive structures, lack of differentiation of sexual organs are the other symptoms that are observed. The symptom expression varies with respect to host plant and the strain of phytoplasma.

Phytoplasma disease diagnosis

Japanese group of plant pathologists and entomologists discovered that phytoplasma cause dwarf disease in mulberry through Transmission Electron Microscopy (TEM) (Doi et al., 1967). Phytoplasma formally called mycoplasma-like organisms (MLOs) are plant pathogenic bacteria in the class mollicutes, lack rigid cell walls, surrounded by single membrane and are pleomorphic with a size of 80 to 800nm. Until the early 1980s, phytoplasma diseases were diagnosed by transmission electron microscopy (TEM) because of their sub-cellular nature and the difficulty in culturing them on artificial media. In the 1980s, fluorescent microscopy techniques such as direct fluorescence detection (DFD) and DAPI staining were developed where DFD detects auto fluorescence of necrotic phloem cells and DAPI detect phytoplasmal DNA. ELISA, the popular diagnostic technique for viruses couldn't become popular for phytoplasma diagnostics as antibody preparation was difficult. Around 1990, advances in molecular biology enabled

Fig. 1. Typical symptoms of phytoplasma infection. a. Phyllody (Chrysanthemum); b. Virescence (Gerbera); c. Little leaf (Periwinkle); and d. Yellows (Aster)

direct detection of phytoplasmal DNA by nucleic acid hybridization and the polymerase chain reaction (PCR). The 16S rRNA gene based PCR has become the gold standard in phytoplasma diagnostics (Gundersen et al., 1996). Recently, Loop-mediated Isothermal Amplification (LAMP) technique gained importance in diagnosing phytoplasma diseases (Dickinson, 2015). Invention of next generation sequencing enabled in drawing the picture of complete genome of phytoplasma. Recently two draft full genomes of phytoplasma strains associated with sugarcane grassy shoot (SCGS) and bermuda grass white leaf (BGWL) diseases are available from India (Kirdat et al., 2020).

Genome size and diversity

The genome sizes of many phytoplasma are larger than many culturable mollicutes as determined through pulse field gel electrophoresis. A wide range of genome variation from 530kb to 1350kb has been observed among phytoplasma (Fig. 2 & 3). Similarly, in Aster yellows group itself the genome size is widely variable from 660kb to 1130kb and in the X

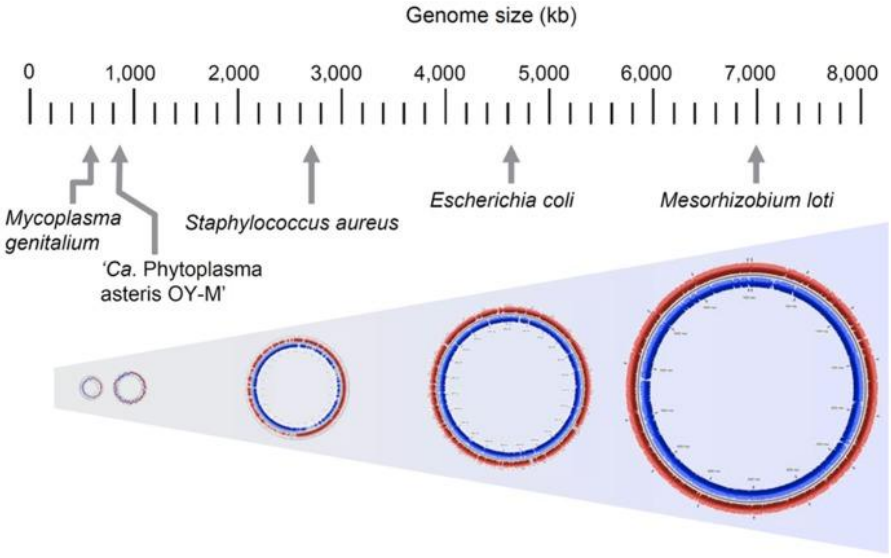

Fig. 2. Size of phytoplasma genome in comparison to other bacteria (Source: Oshima et al., 2013)

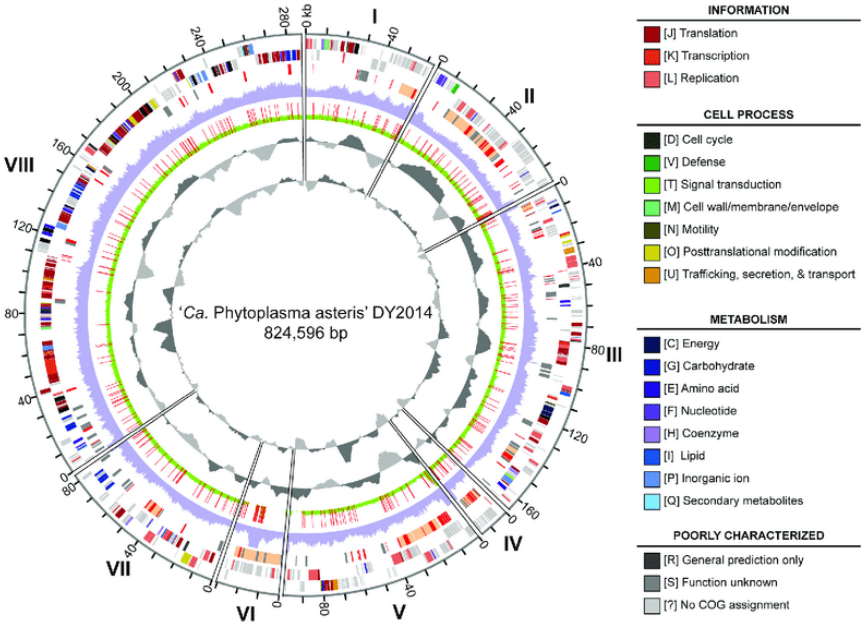

Fig. 3. Genome map of *Ca.* Phytoplasma asteris causing periwinkle yellowing (Source: Cho et al., 2019)

disease group the chromosome size varied from 670 to 1075kbs. The smallest genome so far is of bermuda grass white leaf phytoplasma *i.e.* '*Ca*. P. cynodontis' which is about 530kb. The complete genome sequence of four phytoplasmas and one mutant is available so far. This includes onion yellows mutant, Aster yellows-witches broom strain, '*Ca*. P. australiense' an Australian strain and a New Zealand strain and apple proliferation phytoplasma. '*Ca*. P. australiense'. A number of draft genome sequences of X disease, stolbur phytoplasma, Pigeon pea witches broom etc. are also available. *Ca*. P. asteris and *Ca*. P. australiense have circular chromosomes while *Ca*. P. mali has a linear chromosome. *Ca*. P. asteris and *Ca*. P. australiense reported to contain extra-chromosomal elements (Plasmids) which may be playing an essential role in insect vector transmission.

Among the sequenced genomes, *Ca*. P. australiense has the largest chromosome (960kb) and *Ca*. P. mali is the smallest genome with 602kb. All the genomes have GC content in the range 27-28 per cent with *Ca*. P. mali, 22 per cent. The number of protein coding genes predicted from genomes ranges from 497 to 1126. Phytoplasma genomes have reported to harbor repeated sequences in large clusters approximately of 20kb size that are termed as Potential Mobile Units (PMUs), which appear to be unique to phytoplasma. In genome, different islands of PMU like genetic elements but lacking many segments of the PMUs are also observed and are termed as Sequence Variable Mosaics (SVMs). These PMUs, SVMs and plasmids are responsible for the adaptation of phytoplasma to diverse plant hosts, environments and insect hosts. Phytoplasmas have limited number of genes, thus the biosynthetic capabilities are also limited. In phytoplasma genomes, many genes coding for amino acid, fatty acids and nucleotides biosynthesis are missing. The genome lacks the genes involved in oxidative phosphorylation, tricarboxylic acid cycle, pentose phosphate pathway and the phosphotransferase system. Phytoplasma genomes lack genes coding for F-type ATPases responsible for transmembrane potential and in turn ATP synthesis (Oshima et al., 2013). The genomes largely encode for efficient transporter systems for the uptake of nutrients, which include ATP binding cassette indicating the phytoplasma is dependent on host for most of its metabolism. An exploration into the ability of phytoplasmas to adapt to two diverse environments that of plant and insects through microarray analysis of OY-M strain revealed that approximately 33 per cent of gene expression changes while shifting the host and the PMUs are highly expressed in insects than plants. Phytoplasma membrane proteins and secreted proteins play an important role in host cell interaction and its virulence (Bai et al., 2006). An altered level of oxygen and carbon dioxide has been observed to affect the population of phytoplasma in Oenothera leaf tip cultures.

Phytoplasma vector transmission

Vector-host plant interaction plays a crucial role in limiting or expanding the spread of phytoplasma. Polyphagous vector species are potential to inoculate the phytoplasma among wide range of plant species, depending on susceptibility of host plant. Phytoplasma, like plant pathogenic viruses and symbiotic prokaryotes can be trans-ovarially transmitted. When phytoplasma infected *Scaphoideus titanus* Ball (vector of grape yellows in Europe) females were allowed to lay eggs on healthy host plants, the hatched nymphs of all developmental stages including adults were found to be infected and capable of transmitting phytoplasma (Alma et al., 1997). Kawakita et al. (2000) used electron microscopy to observe phytoplasma in the ovaries and other tissues of *Hishimonoides sellatiformis.* The phytoplasma-insect relationship can be beneficial, deleterious, or neutral in terms of its impact on the fitness of the insect host. Beanland et al. (2000) determined that exposure to one strain of AY increases both the lifespan and fecundity of female *Macrosteles quadrilineatus*; however, exposure to another strain of AY increases the lifespan of test insects but not the number of offspring produced. The corn leafhopper, *Dalbulus maidis*, a specialist, cannot live on unrelated hosts such as healthy aster (*Callistephus chinensis* Nees) when reared on several strains of AY-infected aster, its lifespan is increased and it fed and survived on healthy aster as well (Purcell, 1988). The effects of phytoplasma infection on the insect hosts have implications for the incidence and spread of disease. Vectors that live longer have the opportunity to infect more plants and produce more offspring.

Disease management strategies

Phytoplasmas are obligate parasites which solely depend on the planting material or insect to spread from one location to another. Thus the right intervention at these two epidemiological factors can contain the disease spread to newer locations, but it is very difficult to achieve. Strategies to cure the already infected plant are very rare and with those available, success

rate is very low. The possible solutions to manage phytoplasmal diseases are as follows.

- Clean planting material is the preventive or prophylactic measure where the elimination of phytoplasma and vectors in planting material.
- Insect control can manage the phytoplasma diseases to some extent.

Introduction of infected planting material to a new area where the potential vectors are present can damage the entire plantation of the crop in the area as exemplified in the case of Limonium by Weintraub et al. (2004). To achieve clean propagating material, certification of mother stocks that are phytoplasma free and maintaining the mother plant under insect proof net throughout the year is a must. Production of phytoplasma free planting materials through meristem tip culture and their certification is inevitable to maintain the propagating material disease free. In case of grafting or budding buds/scions and root stock has to be phytoplasma free. In case phytoplasma free mother stock is not available, clean propagation material can be obtained through thermotherapy and meristem tip culture. Since these treatments also do not completely eliminate the pathogen from the whole lot, further certification of the planting material through molecular technique is required to certify it as disease free. Thermotherapy of cuttings and scions followed by the maintenance of grafts in insect proof conditions has been found a good option to produce phytoplasma free grafts. Hot water treatment of propagating material is also found promising. In grapevine hot water treatment of scions at 50^0C for 40 minutes was found effective in reducing phytoplasmal load. Such strategies can be adopted in woody ornamentals (Linck et al., 2019).

Indian Scenario

Sandal spike disease was the first phytoplasmal disease reported in India (Nayar, 1977). Thereafter a large number of phytoplasma diseases have been described, which included brinjal little leaf disease, grassy shoot disease of sugarcane, rice yellow dwarf disease, sesamum phyllody, white leaf disease of *Cynodon dactylon*, little leaf disease of *Acanthospermum hispidium* and yellowing disease of *Urtochloapa nicoides*). More than 129 phytoplasma diseases are attributed to one of six '*Ca*. Phytoplasma' species in India (Rao et al., 2017). The number has been increasing as new reports arise and that species is *Ca*. P. asteris.

Phytoplasma diseases of ornamental plants and flower crops

Due to the development of molecular tools, phytoplasmas have been characterized from as many as 60 plant species including ornamentals in India (Table 1). Aster yellows phytoplasma group (16SrI) are the most common group of phytoplasmas associated with more than 31 diseases of ornamentals, tree species, vegetables, sugarcane, fruit crops and pulses in India. Even though phytoplasma diseases are of common occurrence in India, only few of them have been fully characterized, particularly in North-Eastern and Southern parts of the country. Further studies of these phytoplasma diseases are prompted including their characterization, incidence, transmission and vector identification. Since 2011 there are reports of occurrence of phytoplasma in more than 10 ornamental species. Association of '*Candidatus* P. asteris' with leaf yellows and witches' broom symptoms of brachycome species in India, association of rigeon pea witches' broom phytoplasma (16Sr IX) infecting *Phlox drummondii* in India, rice yellow dwarf phytoplasma (16Sr XI-B subgroup) infecting *Jasminum sambac*, association of pigeon pea witches' broom phytoplasma infecting *Carpobrotus edulis* (L.) N.E. Br, association of '*Ca.* P. asteris' (16SrI group) with flattened stem and witches' broom symptoms of *Petunia hybrida*, characterization of an isolate of '*Ca.* P. asteris' infecting *Zinnia elegans*, detection and identification of phytoplasmas associated with little leaf and witches' broom disease of marigold (*Tagetes erecta* L.) in India are some of the recent reports of phytoplasma in India (Rao et al., 2017).

At ICAR-DFR, an attempt has been made to map the distribution of phytoplasma infecting ornamental plants and flower crops (Fig. 4 and Table 1). Phytoplasma infecting major flower crops has been diagnosed and characterized based on 16SrRNA from the following crops (Table 2).

At ICAR-DFR, 16SrRNA based identification of phytoplasma infecting various members of *Asteraceae* family has been undertaken and two groups of phytoplasma 16Sr-IB and 16Sr-IID have been found to infect marigold (16Sr-I), chrysanthemum and aster (16SrIID). Germplasm of aster and chrysanthemum have been screened for incidence of phytoplasma. In vegetative propagated marigold locally known as Calcutta, incidence of *Ca*. P. asteris has been observed and has become a major concern of spread through the cuttings to different parts of the country. The year

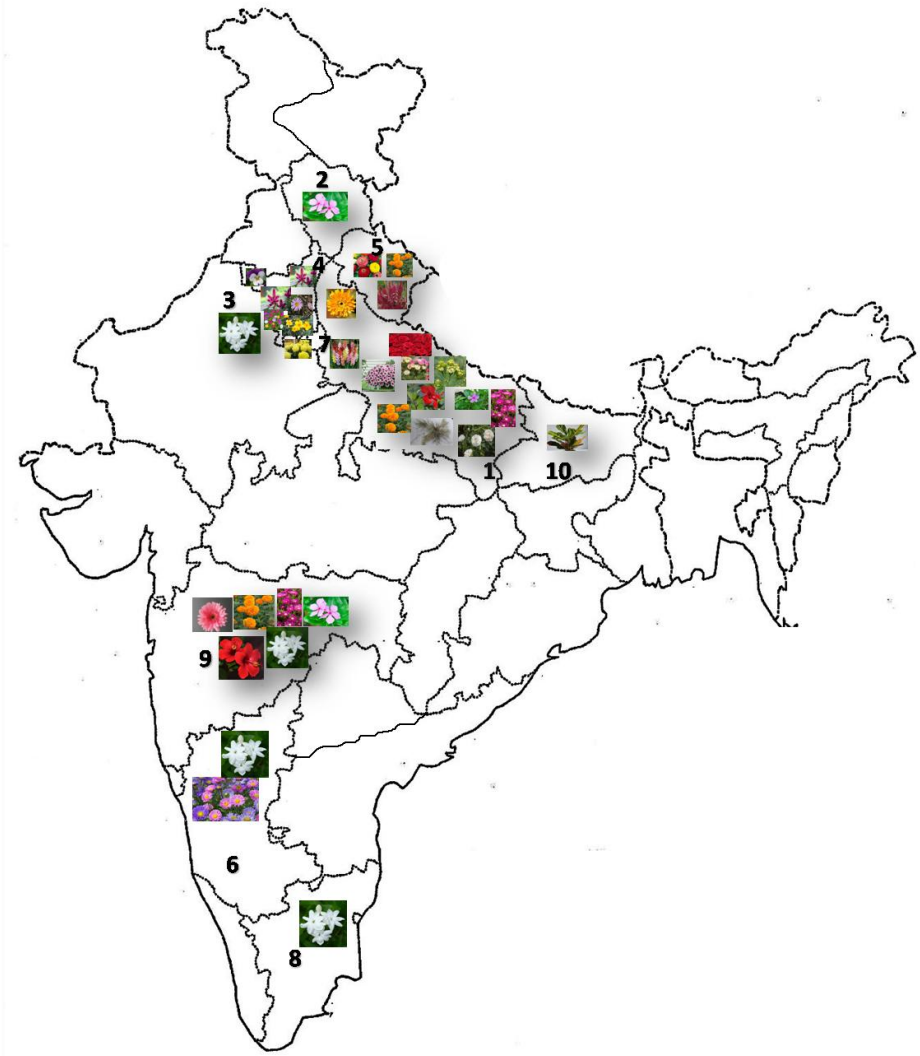

Fig. 4. State wise disease distribution map of phytoplasma on ornamental and flower crops in India (Map not to scale). Based on the surveys and reports from various geographical regions.

round dynamics of leafhopper population and incidence of phytoplasma has been evaluated. The putative leafhopper vectors have been identified. Multi locus genotyping for the right identification of phytoplasma up to species level has been initiated. Parthenium has been identified as a major collateral host in field for year round occurrence of phytoplasma. Various chemicals, botanicals and physical methods for management are being studied to reduce phytoplasmal incidence. Programmes for right identification of phytoplasma infections and management have been organized from time to time for the stakeholders.

Phytoplasma diagnostics

The most common method used for diagnosis of phytoplasma in India is nested PCR based on universal primers pairs P1 and P7 and R16F2n/R16R2 (Table 3). In the recent times, international concern on accuracy of using single gene i.e. 16SrRNA for identification has lead to initiation of identification based on Multiple Loci like the genes SecA, Tuf and Immunomembrane proteins (Imp) has been initiated. MLST has been employed for phytoplasma characterization in Marigold and Impatiens recently (Panda et al., 2021a; Panda et al., 2021b).

Genomics and taxonomy

Phytoplasma are categorically placed into 33 ribosomal groups with a number of sub-groups each (Bertaccini and Lee, 2018). The taxon '*Candidatus* Phytoplasma' species has been identified based on unique 16S rRNA gene sequence (>1200 bp), if the 16S rRNA gene sequence has more than 97.5 per cent similarity to that of any of the previously described species, it has

Table 1. Distribution of phytoplasmal diseases in ornamental and flower crops with respect to various locations in different states based on the reports. (Source: Rao et al., 2017)

Place /State	Plant Host	Botanical name	Associated disease symptoms	Phytoplasma species
Gorakhpur /UP	Ice Plant	*Carpobrotus edulis*	Little leaf and leaf yellow	'*Ca*. P. phoenicium'
	Rose Periwinkle	*Catharanthus roseus*	Phyllody	'*Ca*. P. asteris'
	Golden Dew Drop	*Duranta erecta*	Yellows	'*Ca*. P. asteris'
	Hibiscus	*Hibiscus rosa-sinensis*	Yellow leaf	'*Ca*. P. asteris'
	White rose	*Rosa alba*	Little leaf phyllody, witches' broom, virescence, bud proliferation	'*Ca*. P. asteris'
	Bermuda grass	*Cynodon dactylon*	White leaf	'*Ca* Phytoplasma
Lucknow /UP	Chrysanthemum	*Chrysanthemum* sp.	Little leaf, Virescence,	'*Ca*. P. asteris'
	Gladiolus	*Gladiolus* sp.	Yellowing and malformation of flowers	'*Ca*. P. asteris'
	Damask rose	*Rosa damascena*	Rose witches broom	-
	Moss rose	*Portulacagrandiflora*	Portulaca Little Leaf	-
	Petunia	*Petunia* sp.	Witches' broom	'*Ca*. P. asteris'
	Mexican Marigold	*Tageteserecta*	Little leaf	'*Ca*. P. asteris'
	Southern Cone Marigold	*Tagetesminuta*	Phyllody	'*Ca*. P. cynodontis'
Shahajahanpur /UP	Madagascar Periwinkle	*Catharanthus roseus*	Little leaf, Phyllody, Yellows	-
Sitamadhi/ BR	Garden Croton	*Codiaeum variegatum*	Leaf yellow, witches' broom	'*Ca*. P. asteris'
Himachal Pradesh	Indian Periwinkle	*Catharanthus roseus*	Little leaf, Phyllody	'*Ca*. P. asteris'
Mukteshwar /UK	New Zealand Christmas Bell	*Alstroemeria psittacina*	Little leaf	'*Ca*. P. asteris'
	Zinnia	*Zinnia elegans* Jacq.	Phyllody	'*Ca*. P. asteris'
Pantnagar /UK	Amaranthus	*Amaranthus* spp.	Phyllody	'*Ca*. P. aurantifolia'
	Mexican Marigold	*Tagetes erecta*	Witches' broom	'*Ca*. P. aurantifolia'
New Delhi /DL	Yellow Allamanda	*Allamanda cathartica*	Little leaf	'*Ca*. P. trifoli'
	Daisy	*Brachyscome* sp.	Little leaf, witches' broom	'*Ca*. P. asteris'
	Silver Cock's Comb	*Celosia argentea*	Flat stem, witches' broom	'*Ca*. P. asteris'
	Chrysanthemum	*Chrysanthemum morifolium*	Phyllody	'*Ca*. P. aurantifolia'
	Four o' clock Flower	*Mirabilis jalapa*	Little leaf	'*Ca*. P. aurantifolia'
	Garden Petunia	*Petunia hybrida*	Flattened stem, witches'	'*Ca*. P. asteris'
	Phlox	*Phlox drummondii Hook.*	Witches' broom	'*Ca*. P. phoenicium'
	Hybrid Tea Rose	*Rosa hybrid*	Little leaf, Multiple buds	'*Ca*. P. aurantifolia'
	Chrysanthemum	*Chrysanthemum* sp.	Little Leaf	*'Ca*. Phytoplasma'
	Saponaria	*Saponaria* sp.	Little leaf	'*Ca*. P. trifoli'
	Wild Pansy	*Viola tricolor*	Witches' broom	'*Ca*. P. asteris'
Jaipur /RJ	Jasmine	*Jasminum sambac*	Witches' broom	'*Ca*. P. cynodontis'
Karnal /HR	Silver Cock's Comb	*Celosia argentea*	Fasciation	'*Ca*. P. australasia'
Punjab	China Pink	*Dianthus chinensis*	Leaf Yellowing	-
Pune /MH	Hibiscus	*Hibiscus rosa-sinensis*	Yellow leaf	'*Ca*. P. trifoli'
	Annual Chrysanthemum	*Chrysanthemum* sp.	Phyllody, Little leaf	'*Ca*. P. asteris'
	Gerbera	*Gerbera jamesonii*	Phyllody	-
	Marigold	*Tagetes erecta*	Phyllody, Stunting	-
	Periwinkle	*Catharanthus roseus*	Little leaf, Virescence	-
	Aster	*Callistephus chinensis*	Phyllody, Yellows	'*Ca.* P. australasiae'
	Jasmine	*Jasminum sambac* L. Aiton	Phyllody, Little leaf	-
Devanahalli/ Bengaluru/ KA	Jasmine	*Jasminum sambac* L.	Little leaf	-
Mysuru/ KA	Aster	*Callistephus chinensis*	Virescence and phyllody	'*Ca*. P. aurantifolia'
Tamil Nadu	Jasmine	*Jasminum* sp.	Phyllody	-

Table 2. Flower crops exhibiting natural incidence of phytoplasma symptoms

Sl. No.	Crop	Varieties showing natural incidence of phytoplasma
1.	Chrysanthemum	Pusa Anmol, Pusa Chitraksha, PAU-D-11, PAU-35, Preet Shringar, White Prolific, Aprajita, Mahatma Gandhi, Naughty White, Gulmohar, Fireball, Royal Princess, Bidhan Gold
2.	Aster	Phule Ganesh Pink and White, Arka Kamini, Arka Archana
3.	Marigold	Calcutta, Arka Bangara

Table 3. *Candidatus* phytoplasma species associated with ornamental plants of India based on PCR diagnosis

'*Candidatus* Phytoplasma' species	Primers used	Acronym/group-sub-group	GeneBank accession number	References
'*Ca*. P. asteris'	P1/ P6 and R16F2n/ R16R2	16SrI-B	EU694109	Chaturvedi et al. (2009a)
	P1/ P6 and R16F2n/ R16R2	16SrI	HM230686	Singh et al. (2011)
	P1/ P6 and R16F2n/ R16R2	16SrI	DQ431842	Raj et al. (2007)
	P1/ P6 and R16F2n/ R16R2	16SrI	HM230688	Singh et al. (2011)
	P1/ P6 and R16F2n/ R16R2	16SrI-I	FJ491455	Raj et al. (2009)
	P1/ P7 and R16F2n/ R2	16SrI-F	FJ939287	Chaturvedi et al. (2010)
	P1/ P6 and R16F2n/ R16R2	16SrI	GU594056	Singh et al. (2011)
	P1/ P6 and R16F2n/ R16R2	16SrI-D	FJ429364, KP096315	Chaturvedi et al. (2009b)
	P1/ P6 and R16F2n/ R16R2	16SrI-B	HM230685	Singh et al. (2011)
	P1/ P6 and R16F2n/ R16R2	16SrI-B	HM230685	Raj et al. (2011)
	P1/ P7 and R16F2n/ R16R2	16SrI-B	MN832584	Rihne et al.2020)
'*Ca*. P. aurantifolia'	R16mF2/ R1 and fU5/ rU3	PWB/16SrII	EU362627	Arocha- Rosete et al. (2008)
		PWB/16SrII-D	KX013259	Kumar et al. (2016)
	R16mF2/R1 and fU5/rU3	PWB/16SrII-G	EU362631	Arocha-Rosete et al. (2008)
Ca. P. australasia	P1/ P7 and R16F2n/ R16R2	16SrII-D	-	Sumashri et al. 2020 Prabha K et al. 2019
'*Ca*. P. cynodontis'	P1/ P7 and 3Far/ 3Rev and R16F2n/ R16R2	RYD/ 16SrXI-B	KF728950	Madhupriya et al. (2015)
'*Ca*. P. phoenicium'	P1/ P7 and R16F2n/ R16R2	AlmWB/ 16SrIX-C	KJ782631	Shukla et al. (2014)
'*Ca*. P. trifoli'	P1/ P7 and 3Far/ 3Rev	CP/ 16SrVI-D	KX641023	Khasa et al. (2016)
	P1/ P7 and R16F2n/ R16R2	16SrVI-D	-	Rihne et al. 2019

been placed under a novel '*Ca*. Phytoplasma' species (IRPCM, 2004). Diversity analysis was undertaken based on the 16SrRNA sequences reported from India. The sequences of 16SrRNA region were retrieved from public database and aligned using clustalW and phylogenetic and diversity analysis was undertaken based on Maximum likelihood method using MEGA 6 software. Results indicate that they belong to three different taxonomic groups and sub-groups like 16Sr I (*Ca.* Phytoplasma asteris), 16SrVI (Clover proliferation group), 16Sr XI-B (Rice yellow dwarf phytoplasma), 16SrIX-C sub-group (*Ca.* P. phoenicium -pigeon pea witches broom), dominated by 16Sr I group. Almost all the phytoplasma species reported exhibited close relation to those reported in countries from North America and East Asia. An elaborative analysis in this direction is required to reach any particular conclusion. Two draft genomes of phytoplasmas associated with sugarcane grassy shoot (SCGS) and Bermuda grass white leaf diseases '*Ca.* P. cynodontis' are submitted from India. SCGS genome is 95.43 per cent complete with 333.98× coverage 404 protein-coding genes, 12 transfer RNA (tRNA), and two rRNA genes. Bermuda grass white leaf phytoplasma genome annotated is 91.32 per cent complete with 373.6× coverage and have 425 protein-coding genes, 13 tRNA, and three rRNA genes (Kirdat et al., 2020).

Phytoplasma transmission

Phytoplasma transmission occurs through sap-sucking insect vectors belonging to families Cicadellidae (leafhoppers) and Fulgoridae (plant hoppers). The study conducted by Tiwari et al. (2016a) shows *Pyrilla perpusilla* (Walker) (Lophopidae: Hemiptera) as a potential vector in transmission of 16SrXI-B sub-group phytoplasmas in sugarcane crops infected with sugarcane grassy shoot (SCGS) disease showing white leaf symptoms at Shahjahanpur district of Uttar Pradesh. In the transmission study conducted by Tiwari et al. (2016b), three leafhopper species *Cofanauni maculata*

(Signoret) (Cicadellidae: Hemiptera), *Exitianus indicus* (Distant) (Cicadellidae: Hemiptera) and *Maiestas portica* (Melichar) (Cicadellidae: Hemiptera) were found to be putative vectors of Sugarcane grassy stunt phytoplasma caused by *Ca.* P. oryzae (16SrXI group). The identification of new vectors of Sugarcane grassy stunt phytoplasma suggested that these leafhopper species may be responsible for secondary spread of SCGS phytoplasma. By PCR detection of sugarcane grassy stunt phytoplasma molecules Srivastava et al. (2006) confirmed the presence of sugarcane grassy stunt phytoplasma molecules in *Deltocephalus vulgaris* (Cicadellidae: Hemiptera) a potential vector of the phytoplasma. PCR assay has successfully detected the presence of sugarcane grassy shoot phytoplasma (16Sr XI) in the leafhopper vector *E. indicus* tested positive for the phytoplasma molecule 16Sr XI (Rao et al., 2014). *E. indicus* was a putative vector for '*Ca.* P. cynodontis' and play a role in transmitting 16SrXIV group phytoplasmas in *Cynodon dactylon*. *Ca*. P. cynodontis causes chlorotic little leaves, witches' broom and shortened stolons /rhizomes on *Cynodon dactylon* observed in Shahjahanpur, Uttar Pradesh, India. In the study conducted by Taloh et al., 2020, the leafhopper, *Hishimonus phycitis* (Distant) (Cicadellidae: Hemiptera) was found positive to 16SrVI group phytoplasmas infecting chrysanthemum, which confirms *H. phycitis* as a putative vector for the transmission of 16SrVI group of phytoplasma strain in chrysanthemum. In the molecular detection of phytoplasma molecules in leafhopper vectors, *H. phycitis* was identified positive for 16SrI-B and 16SrII-D sub-groups of phytoplasmas from chrysanthemum fields at Delhi and jasmine fields at Bengaluru, respectively. This study suggested that *H. phycitis* may acts as potential natural source for secondary spread of the identified phytoplasma strains (Gopala, 2018). In the transmission studies conducted by Pathak et al. (2012), *Orosius albicinctus* (Distant) (Cicadellidae: Hemiptera) successfully transmitted the phytoplasma molecules from infected plants to the healthy plants. In the transmission study conducted by Sajad un Nabiet al. (2015), *H. phycitis* (Distant) was found to be positively associated with the transmission of sesame phyllody caused by *Ca* .P. asteris' sub-group I-B. In the transmission study conducted by Manish Kumar et al. (2017), *H. phycitis* was identified as carrier and natural vector of 16SrVI-D sub-group of phytoplasmas causing brinjal little leaf (Fig. 5-6).

Survey and field evaluation of flower growing areas have found the incidence of leaf hopper vector *Hishimonas phycitis* in marigold and aster. Year round study of seasonal dynamics of leafhopper vector and phytoplasma incidence has been studied. It has been observed that increase in phytoplasma incidence percentage was directly correlated to increase in leaf hopper population in the field (Data not shown). Evaluation of plant germplasms have been undertaken for the natural incidence of phytoplasma symptoms in the field. In case of aster no significant effect of varieties have been observed as both white and purple varieties have been found to be susceptible to phytoplasma. In chrysanthemum percentage incidence varied from 1 to 2 maximum 10%.

Phytoplasma management

As phytoplasmas are very difficult to cultivate on artificial media very limited studies have been done in India to manage them. Tetracycline treatment has been found to reduce the load of phytoplasmas in the affected plants but did not completely eliminate the disease symptoms. As early as in 1978, Verma and Dubey demonstrated the influence of treatment method, duration and concentration of tetracycline on little leaf symptoms in brinjal. Treatment with tetracycline antibiotics at weekly intervals reduced the symptoms of phytoplasma in petunia (Ajayakumar et al., 2007). *In vitro* treatment with oxytetracycline (75 mg/l) was found to be effective in eliminating phytoplasma in plant tissues with 50 per cent efficiency (Singh et al., 2007) Production of disease free planting material of sugarcane by eliminating Sugarcane grassy shoot (SGGS) phytoplasma through apical meristem (2-3 mm) culture has been achieved (Tiwari et al., 2011). Various immune priming chemicals have been evaluated for the recovery of phytoplasma infections in chrysanthemum at ICAR-DFR. The following chemicals, benzoic acid 0.006g/ L, salicylic acid 0.001mM,BTH0.1mM (Acibenzolar S Methyl), IBA100ppm,$GA_3$150mg/ l, chitosan 1.5ml/ l, tetracycline 500ppm, oxytetracycline500ppm, grape seed extract 1g/ l and Alliette 0.1 per cent have been evaluated and there has been no promising effect of these treatments for symptom reversal. To prevent the transmission and spread of the pathogen through nursery plants, creating awareness about phytoplasma is imperative. As a part of awareness about phytoplasma diseases among the nurserymen and farmers, workshops have been organized at Kadiyam, Andhra Pradesh, Junnar Taluka, Pune district etc. by DFR. An integrated approach employing cultural, chemical, biological and nutrient management is essential for reducing the advance of phytoplasmal diseases.

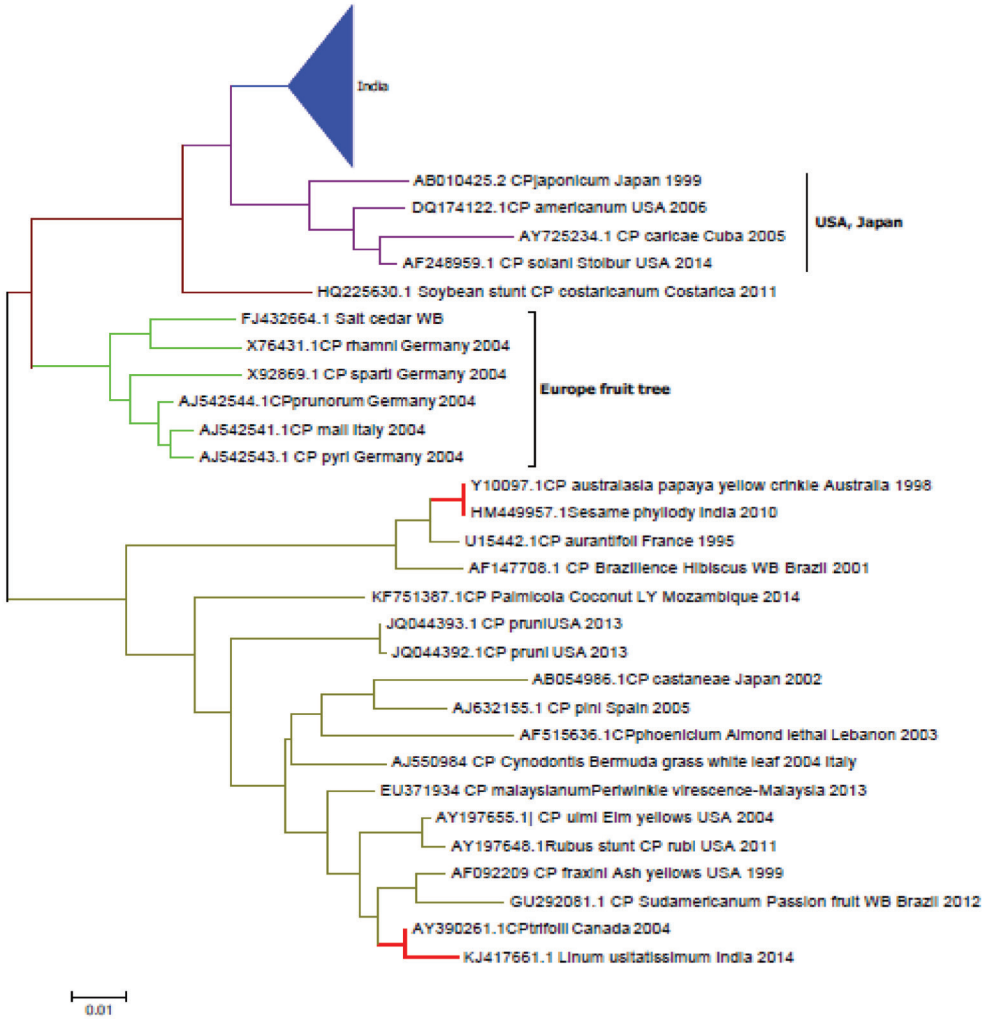

Fig. 5. Phylogenetic tree depicting the relationship of Indian phytoplasma strains with phytoplasmas of other parts of the world based on 16SrRNA gene sequences

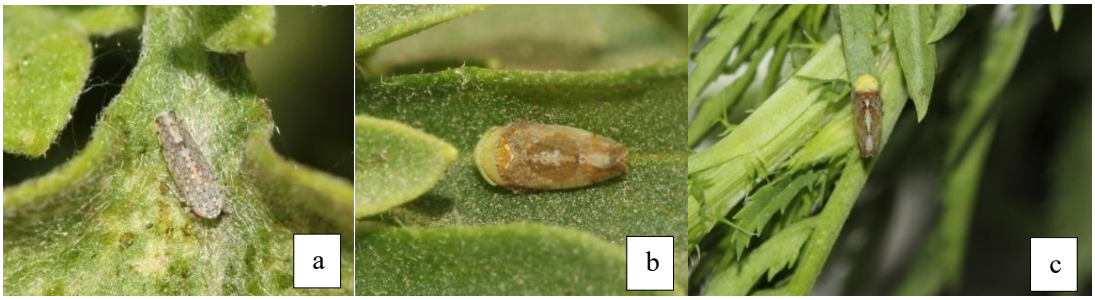

Fig. 6. Leaf hoppers vectors of phytoplasma. a. *Orosius albicinctus* on chrysanthemum; *Hishimonus phycitis* on b. Aster; c. Marigold

Phytoplasma and biosecurity concerns

Indian floriculture industry has grown widely and has established itself across the continents through import and exports. Exporting 4308 MT of floriculture products worth Rs.11200 lakhs are imported in our country every year with fresh cut flowers, foliages and branches, live plants and bulbs having a major share. The boom in the nursery industry along with the real estate, has augmented the import of exotic plants and variants from across the globe. The entry of plants bring along the microbial parasites of the host plants as well and if the new location is favorable, they flourish quickly and start conquering the other members of the family. Even though unknowingly, such imports

have introduced and established many weeds and pathogens in our country in the past like parthenium, eichhornia, michania, lantana, salvinia etc and many pests and diseases like Banana bunchy top, African cassava mosaic disease and a long list of introduced insect pests. Phytoplasma cannot be an exception. Some of the morphological manifestations of phytoplasma infections like greening in gerbera, chrysanthemum, colour breaking in tulip are of ornamental value and are subjected for rapid propagation purely due to lack of awareness. This practice is posing a major threat to our country's biosecurity and this is going to affect the global floriculture trade in future.

The prevalence of a pathogen in our country can be a trade barrier for the export of that planting material to another country. Countries like Australia, New Zealand, Canada, Europe and USA have already developed a system to screen invasion of pathogens. Our major trading partners with respect to export of floricultural products are USA, Germany, Netherland, UK and UAE and top five countries from where India imports are Thailand, Netherland, China, Italy and USA (APEDA Import export statistics 2019-20). The Plant quarantine order 2003 and its amendments in India also regulate and restrict imports of phytoplasma infected plants and planting materials and other flower products imported from other countries. Even though a system is in place, it is not foolproof and lot of escapes occur in India which is evident from the variety of symptom expressions of phytoplasma infections in wide variety of plants and the increasing number of reports of new species of phytoplasma from our country (Prabha et al., 2017). Towards ensuring quality planting material National Horticulture Board has made an initiative by developing guidelines for recognizing horticultural nurseries with a slogan 'Our quality planting material is our Property. We care' where nurseries are ranked from satisfactory to excellent by evaluating source of parent material, propagation in disease free condition by adopting technically prescribed method, adoption of good nursery management practices, reliable record keeping and training of staff etc. If the evaluation is strictly followed and every nursery tries to achieve excellent ranking, issues related to the disease spread will be automatically resolved. Awareness on phyto-biosecurity threats posed by phytoplasma is a must among the first hand users or stakeholders involved in import, inspection, propagation, cultivation, export and certification to secure the floral wealth of our country from exotic strains of phytoplasma and other pathogens. A major hurdle with respect to the floriculture and phytoplasma in India is the ineffective domestic quarantine due to free flow of planting materials from one location to another. India is blessed with wide range of agroclimatic conditions making it favourable for the establishment and spread of phytoplasma. A sense of responsibility and sincere effort from each and every one associated directly or indirectly with floriculture or horticulture as a whole is required to conserve our biosecuirty.

CONCLUSIONS

Phytoplasma is a major threat to the quality and quantity of flower production due to phyllody and virescence. Its occurrence in flower crops poses a major threat to food crops as well. Leaf hoppers in the field contribute to the spread of the phytoplasma from infected plants to healthy ones. Presence of collateral host weeds like parthenium helps year round survival of the phytoplasma in Indian fields. Recent trends in vegetative propagation of seed propagated flower crops like Marigold have resulted in high rate of incidence of phytoplasma in flower crops. This necessitates the need for certification of mother plants and cuttings of vegetative propagated flower crops and ornamentals as phytoplasma free to prevent further spread to the different parts of the country. So far tetracycline is the only effective bacteriostat found effective against phytoplasma but due to its rampant use in the poulty industry, which is posing a threat to the environment, is being considered for its ban. Management of this pathogen is a priority and alternatives to antibiotics and eco-friendly strategies for management of insect vectors are imperative to keep a check on the ever expanding pathogen.

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