PHYTOPLASMA

Dr. Shankar Hemanta Gogoi
SMS Plant protection
KVK Dimahasao

Introduction

Phytoplasmas are prokaryotic plant pathogens belonging to the class Mollicutes, a group of wall-less microorganisms phylogenetically related to low G+C Gram-positive bacteria. Phytoplasmas were discovered in 1967 and were named mycoplasma-like organisms (MLOs), due to their morphological and ultrastructural similarity to mycoplasmas, already known as aetiologic agents in animal and human diseases. Following the application of molecular technologies MLOs were designed as a coherent, genus-level taxon, named "*Candidatus* Phytoplasma". In this new clade, groups and subgroups have been defined and many of them are now considered species.

Phytoplasmas are small sized (0.3-1.2μm), single-celled polymorphic mollicutes characterized by possessing small genomes ranging from 530 to 1350 kb (among the smallest known for any self-replicating organisms), a low G+C content in their DNA (23.0–29.5 mol %) and living a trans-kingdom parasitic lifestyle. They could survive and multiply only in hysotonic habitats such as plant phloem or insect haemolymph. So, they are strictly host-dependent. They could multiply in insect vectors and also infect their eggs. Phytoplasmas were known to be pathogenic to more than a thousand plant species. The phytoplasmas continuously cycle between plants and insects and require both organisms for survival and dispersal in nature. This requirement necessitates the adaptation to a broad range of environments, including the phloem of their plant hosts and the gut lumen, haemolymph, saliva and endocellular niches in various organs of their insect hosts.

Since it has not been possible to culture phytoplasma, much of the information about its morphology is derived from the study of serial thin sections of phloem sieve tubes of infected plants under an electron microscope. Cell wall degrading enzymes such as cellulase and macerase can be used to separate phytoplasmas from intact phloem sieve tubes. The presence of phytoplasmas in sieve tubes of infected plants can be detected using dark field light microscopy. Thus, the morphology of phytoplasmas can be studied at various stages of crop development. In the early stages of yellow diseases such as pear decline, aster yellows, and tomato big bud disease, branched filamentous bodies are the predominant forms. In an electron

microscope, various pleomorphic phytoplasmas are observed. Small spherical (60-100 nm dia.), large globular (150 to 1100 nm dia.), and globular and branched filamentous (1-2 m to several m) are examples. Small round to large globular forms predominates in the late season or in advanced pathological stages. Simple round forms are observed in thin sections, which may be filamentous or branched bodies visible in thick ultramicroscope sections or serial sections of sieve elements. The ultrastructure of phytoplasmas reveals that they are held together by a trilamellar unit membrane measuring 7.5 to 10 nm in width. Ribosomes and nuclear material in the form of fine fibrillar stands of DNA are found in the cytoplasm. All phytoplasmas studied thus far have a single immune-dominant protein (of known function) that accounts for the majority of the cell membrane's protein content. This protein has been shown to interact with insect microfilament complexes and is thought to play a role in the insect-phytoplasmas interaction.

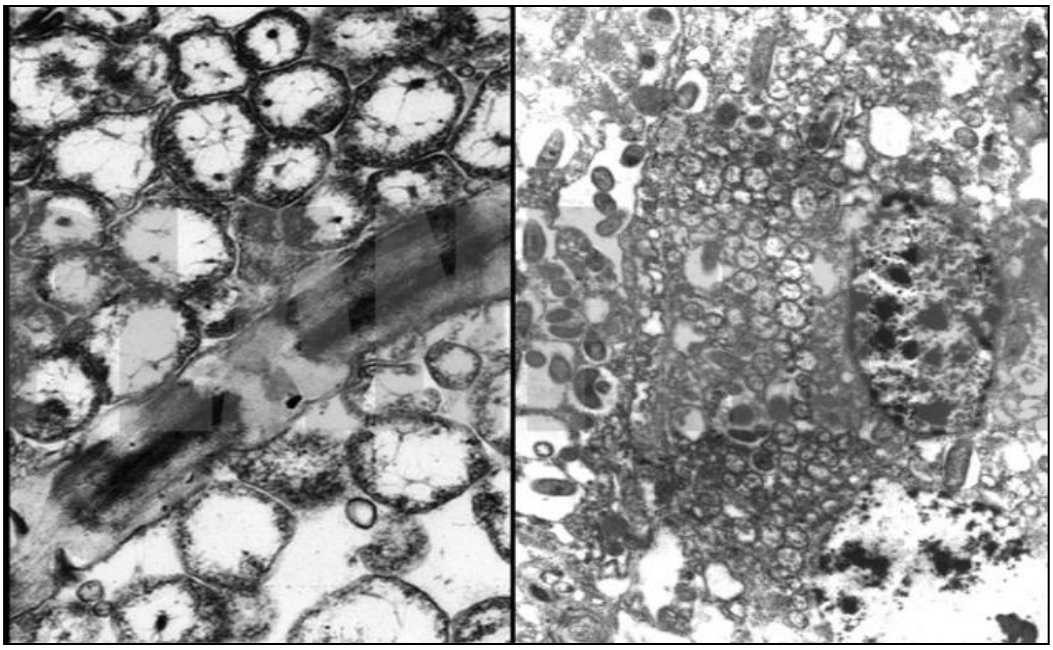

Fig : Transmission electron micrograph showing Phytoplasma bodies present in phloem sieve plates of plant. (Courtesy - www.costphytoplasma.ipwgnet.org)

Taxonomy

The true nature of phytoplasmas, as well as their taxonomic position among lower organisms, remain unknown. The Subcommittee on the Taxonomy of Mollicutes proposed in 1992 that the term phytoplasma be used instead of MLOs to refer to phytopathogenic mollicutes. The genus phytoplasma was established in 2004 and is currently at Candidatus status, which is reserved for bacteria that cannot be cultured. Its taxonomy is complicated by

the fact that it cannot be cultured and thus cannot be classified using methods commonly used for prokaryote classification.

However, based on the current knowledge their taxonomic position is as follows:

Kingdom : Prokaryote

Division : Firmicutes

Class : Mollicutes

Order : Acholeplasmatales

Family : Acholeplasmataceae

Genus : *Candidatus* phytoplasma

Genes encoding 16S ribosomal RNAs were highly conserved across the phytoplasma clade and therefore have served as a primary molecular tool for phytoplasma identification, genotyping, taxonomic assignment and group / subgroup classification. Phytoplasma taxonomic groups are based on differences in the fragment sizes produced by the restriction digest of the 16Sr RNA gene sequence called RFLP (Restriction fragment length polymorphism) or by comparison of DNA sequences from the 16s/23s spacer regions. The table () shows the phytoplasma groups, subgroups and the *Candidatus* species belonging to each group.

Table. Phytoplasma 16S ribosomal RNA RFLP groups and given *Ca*. phytoplasma species

Group	**No. of Subgroup**	***Candidatus* Phytoplasma species**
16SrI: aster yellows	22	*'Ca.* P. asteris' '*Ca*. P. lycopersici'
16SrII: Peanut witches'-broom	6	*'Ca*. P. aurantifolia' '*Ca.* P. australasia'
16SrIII: X-disease	19	*'Ca.* P. pruni'
16SrIV: Coconut lethal yellows	3	*
16SrV: Elm yellows	6	*'Ca.* P. ulmi' '*Ca*. P. ziziphi' '*Ca*. P. rubi"

Group	**No. of Subgroup**	***Candidatus* Phytoplasma species**
		'*Ca*. P. balanitae'
16SrVI: Clover proliferation	8	*'Ca.* P. trifolii' '*Ca*. P. sudamericanum'
16SrVII: Ash yellows	3	*'Ca.* P. fraxini'
16SrVIII: Loofahwitches'-broom	1	*'Ca.* P. luffae'
16SrIX: Pigeon pea witches'-broom group	7	*'Ca.* P. phoenicium
16SrX: Apple proliferation	5	*'Ca.* P. mali' 'Ca. P. prunorum' 'Ca. P. pyri' 'Ca. P. spartii'
16SrXI: Rice yellows dwarf	3	*'Ca.*P. oryzae'
16SrXII: Stolbur group	8	*'Ca.* P. japonicum' '*Ca*. P. solani' '*Ca*. P. australiense' '*Ca*. P. fragariae' '*Ca*. P. convolvuli'
16SrXIII: Mexican periwinkle virescence	2	*
16SrXIV: Bermuda grass white leaf	2	*'Ca.* P. cynodontis'
16SrXV: Hibiscus witches'-broom	2	*'Ca.* P. brasiliense'
16SrXVI: Sugarcane yellows leaf	1	*'Ca.* P. graminis'
16SrXVII: Papaya bunchy top	1	*'Ca.* P. caricae'
16SrXVIII: American potato purple top wilt	1	*'Ca.* P. americanum'
16SrXIX: Chestnut witches'-broom	1	*'Ca.* Phytoplasma castaneae'
16SrXX: Rhamnus witches' broom	1	*'Ca.*P. rhamni'

Group	**No. of Subgroup**	***Candidatus* Phytoplasma species**
16SrXXI: Pinus phytoplasmas	1	*'Ca.*P. pini'
16SrXXII	2	'*Ca*. P. palmicola'
16SrXXIII	1	*
16SrXXIV	1	*
16SrXXV	1	*
16SrXXVI	1	*
16SrXXVII	1	*
16SrXXVIII	1	*
16SrXXIX: Cassia witches' broom	1	'*Ca*. P. omanense'
16SrXXX: Salt cedar witches' broom	1	'*Ca*. P. tamaricis'
16SrXXXI: Soybean stunt	1	'*Ca*. P. costaricanum'
16SrXXXII: Malaysian periwinkle virescence and phyllody	3	'*Ca*. P. malaysianum'
16SrXXXIII: Allocasuarina muelleriana phytoplasma	1	'*Ca*. P. allocasuarinae'

*-No type species name

Disease cycle

Phytoplasmas are transmitted by circulative and persistent transmission by phloem feeding leafhoppers (Cicadellidae), planthoppers (Fulgoridae), and psyllids (Psyllidae). The phytoplasma is acquired by an insect feeding on the phloem of an infected plant (Fig. 1). The source plant can affect leafhopper phytoplasma acquisition from the host. Vectors' inability to acquire from a particular plant species may be due to plant metabolites that disrupt insect feeding, or the insect's feeding behaviour may differ depending on the host plant. Alternatively, the titre of the phytoplasma in the host plant may affect acquisition.

After acquisition, phytoplasmas pass the insect's gut wall and multiply in the haemolymph. They then go into the salivary glands, where they multiply further (Fig. 1). The processes behind phytoplasma migration through insect membranes are poorly known. Surface

adhesions may play a role in phytoplasma transmission. The presence of receptors shows that the phytoplasma carries the genetic material essential for insect vector penetration.

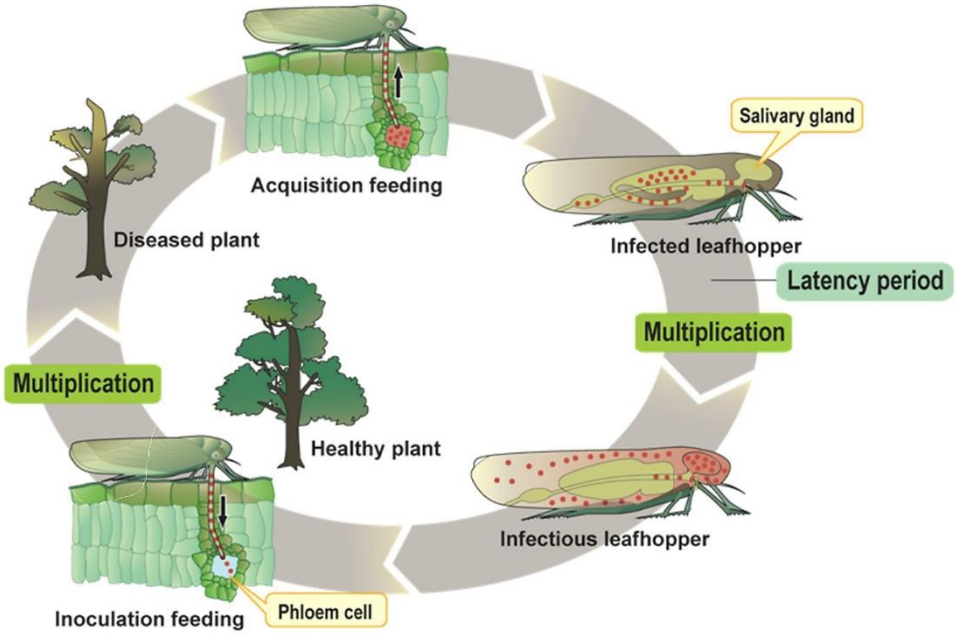

Fig 1. Disease cycle of phytoplasmas.

Insect vectors are only able to transmit the phytoplasma after their salivary glands become infected, resulting in a 2-6 week latent period between acquisition and transmission (Fig. 1). Once an insect vector becomes infective, it is inoculative for life. When the insect's latency period is over, it can spread the phytoplasma to a plant host by feeding on phloem tissue. The phytoplasma is transported from the insect into the phloem via saliva, which is needed for mouthpart lubrication during feeding. Not all leafhoppers who consume the phytoplasma during acquisition may transfer it. Transmission efficiency can be affected by the insect's gender and life cycle, as well as temperature, host plant age, and host plant type. Because phytoplasmas multiply in the phloem of the plant host before causing symptoms, there is a latent period between insect vector infection and symptom expression (Fig. 1). The phytoplasmas multiply in the 'infected plant's phloem tissue and can spread throughout the plant, including the roots.

Phytoplasmas can also be transferred from host plant to host plant by cleft grafting, dodder (parasitic vine, Cuscuta spp.), or vegetative propagation such as cuttings or rhizomes. Phytoplasmas have been found in the embryo tissues of coconut palms with lethal yellowing disease, raising the possibility of phytoplasma seed transfer.

Symptoms of Phytoplasma Diseases

Phytoplasma infected plants exhibit a wide range of specific and non-specific symptoms suggesting profound disturbances in the normal balance of plant metabolism, including yellowing, chlorosis, or bronzing of foliage, stunting (reduction of internodes and leaf size), phyllody (the development of floral parts into leafy structures), virescence (the development of green flowers and the loss of normal pigments), proliferation of secondary auxiliary buds often resulting in a witches' broom effect, abnormal elongation of internodes leading to slender shoots, proliferation of secondary roots, big bud, enlarged stipules, inhibition of flowering, flower proliferation and other flower abnormalities, abnormal fruits and seeds, off-season growth and brown discoloration of phloem tissues. The symptoms may vary with the phytoplasma, host plant, age of the plant at the time of infection, stage of the disease and environmental conditions.

Phyllody: It is most common in sesamum and appears during the blooming season. Floral structures are transformed into green leafy structures. The sepals become leaf-like structures, and the corolla, stamens, and carpels become green and leafy. The ovary is also malformed, resulting in an elongated structure. The leaves are shortened, the plant is stunted, and the branching is abnormal, resulting in a plant that is malformed beyond recognition.

Spikes: Spike disease affects all ages of sandal trees. It comes in two types: rosette and pendular. Rosette spike is more common and is also known as spike or spike disease. The entire shoot has the appearance of a spike with four rows of spiked bristles. The plant's size has been drastically reduced.

Little leaf: Little leaf is widespread in brinjal. The plants' leaves and nodes have shrunk dramatically, giving them a bushy appearance. Many axillary buds are stimulated to develop into short branches with small leaves.

Grassy shoot disease: It is a serious sugarcane disease. It is distinguished by the development of numerous, thin tillers from the base of affected plants. Premature and excessive tillering causes the clump to look crowded and grass-like. Tillers have pale yellow, thin, narrow leaves that resemble grasses. The affected clumps are stunted and have varying degrees of chlorophyll loss ranging from total green to white.

Phytoplasma infection causes foliar yellowing and reddening, small leaves, vein clearing, vein enlargement, vein necrosis, leaf roll, leaf curl, premature defoliation, undersized fruits, poor terminal growth, sparse foliage, dieback, stunting of overall plant growth, and decline in woody plants. It is unlikely that phytoplasmas consumed nutrients, caused deficiency

in plants, and displayed all of these symptoms. In rare cases, phytoplasma-infected plants do not exhibit any symptoms during their lifetime.

Plate: A) Sesamum phyllody B) Sugarcane grassy shoot C) Brinjal Little leaf

Transmission of phytoplasma

Transmission of phytoplasma from plant to plant occurs primarily during the feeding activity of inoculative vector insects, by the vegetative propagation of infected plant material or by graft inoculation. In nature, phytoplasma mainly spread by phloem-feeding insects, reside in phloem sieve tubes and persistently colonize their hosts. The geographical distribution and impact of phytoplasma diseases depends on the host range of the phytoplasma as well as the feeding behaviour of the insect vector. Insect vectors can acquire more than one phytoplasma species/strain, either by feeding on multiple-infected source plants or by feeding sequentially on different plants infected by different phytoplasmas. Vector specificity varies from high, when phytoplasmas are transmitted by only one or two vectors, to extremely low, when a specific phytoplasma can be transmitted, mostly by polyphagous leafhopper species.

Vectors responsible for phytoplasma transmission in nature are insects of the order Hemiptera, primarily phloem feeding leafhoppers (Cicadellidae) and psyllids in a persistent, propagative manner. In their natural insect vectors, phytoplasmas traverse the intestinal wall, circulate in haemolymph, and multiply in tissues including salivary glands, where phytoplasma cells are incorporated into saliva injected into plants during inoculation. Transmission of phytoplasmas through propagation material can occur and lead to their long-distance dispersal and introduction into regions where they have not previously been found. Recent reports on the detection of phytoplasma in both the seed and seedling progeny of alfalfa, canola, corn, tomato and oilseed rape plants indicate that seed transmission in certain plant-host phytoplasma

pathosystems is possible. In addition, all phytoplasmas can be transmitted experimentally by plant parasitic dodder (*Cuscuta spp.*) and by grafting infected plant material onto healthy plants.

Conclusion

None of the PLO‟s has so far been cultured on synthetic media and persistant type of insect vector relationship, it is very difficult to distinguishing naturally occurring variants of this important group of pathogens and understand their epidemiology and interactions with the host. However, within the last decade, molecular biology has considerably improved our understanding of plant pathogenic mollicutes. Different methodologies may now be utilised to examine mollicute interactions with their plant and insect hosts, and transgenosis can now be employed to manage mollicute-induced disease.

References

C. Streten and K. S. Gibb (2006). Phytoplasma diseases in sub-tropical and tropical Australia. *Australas. Plant Pathol*., **35**:129–146

Christensen, N. M.; Axelsen, K.B.; Nicolaisen, M. and Schulz, A. (2005). Phytoplasmas and their interaction with their hosts. *Trends Plant Sci*., **10**:526-535

Doi, Y.; Teranaka, M.;You, K. and Asuyama, H. (1967). Mycoplasma-or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows or paulownia witches' broom. *Ann. Phytopathol. Soc. Japan*, **33**:259-266.

Gogoi, S. H. (2021). Biochemical, histopathological and molecular characterization of sesamum phyllody disease in Assam. Ph. D. Thesis Assam Agricultural University, Assam, India.

Hodgetts, J.; Harrison, N.; Mumford, R.; Boonham, N. and Dickinson, M. (2007). Taxonomic grouping based on analysis of the 16s/ 23s spacer regions which shows greater variation then the normally used 16Sr RNA gene results in classification similar to that derived from 16Sr RNA data but with more detailed subdivision. *Plant Pathol*., **56**:357-365.

Lee, I. M.; Davis, R. E. and Gundersen-Rindal, D. E. (2000). Phytoplasmas: Phytopathogenic mollicutes. *Annu. Rev. Microbiol*., **54**:221-225.

Nabi, S.; Madhupriya; Dubey, D.; Rao, G.P.; Baranwal, V.K. and Sharma, P. (2015). Characterization of phytoplasmas associated with sesame (*Sesamum indicum*)

phyllody disease in North India utilizing multilocus genes and RFLP analysis. *Indian Phytopathol*. **68**(1): 112-119.

Pathak, D. M.; Parakhia, A. M. and Akbari, L. F. (2012). Symptomatology and transmission of sesame phyllody disease caused by phytoplasma. *J. Mycol. Plant Pathol.*, **42**: 479-484.

Rao, G. P.; Nabi, S. U. and Madhupriya (2015). Overview on a century progress in research on sesame phyllody disease. *Phytopathogenic Mollicutes*, **5**(2): 74-83.

The IRPCM. (2004). Phytoplasmas/Spiroplasma working Team: Phytoplasmas Taxonomy group: Candidatus phytoplasma, a taxon for the wall less, non-helical prokaryotes that colonize plant phloem and insects. *Int. J. Syst. Evol. Microbiol*., **54**: 1243-1255.

Wei, W.; Davis, R. E.; Lee, I. and Zhao, Y. (2007). Computer Simulated RFLP analysis of 16Sr RNA genes: identification of ten new phytoplasmas groups. *Int. J. Syst. Evol. Microbiol*. **57**: 1855-1867.