Plants and Phytoplasmas

Subjects: Agriculture, Dairy & Animal Science

Contributor: Assunta Bertaccini

Plant pathogen presence is very dangerous for agricultural ecosystems and causes huge economic losses. Phytoplasmas are insect-transmitted wall-less bacteria living in plants, only in the phloem tissues and in the emolymph of their insect vectors. They are able to manipulate several metabolic pathways of their hosts, very often without impairing their life. The molecular diversity described (49 '*Candidatus* Phytoplasma' species and about 300 ribosomal subgroups) is only in some cases related to their associated symptomatology.

Keywords: plant diseases ; pathogenicity ; molecular classification

1. Introduction

Plant pathogen presence is usually known as a very dangerous component of agricultural ecosystems and is associated with huge economic losses. The world history was also often shaped by dangerous plant epidemics or pandemics such as the wheat rust that was among the main causes of the Roman empire failure, the potato late blight by *Phythophthora infestans* producing the Irish migration to America due to the famine, and the coffee rust obliging to stop the coffee cultivation is several areas, mainly in islands. Recently plant pathogenic bacteria have played an important role in reducing kiwi cultivation, due to the canker by *Pseudomonas syringae* pv. *actinidiae* [1], and citrus, through the greening ('*Candidatus* Liberibacter' species) [2] diseases. Moreover, there are bacteria hosted by plants and insects that are both associated with severe epidemic or with useful changes in plant behavior. While their presence in apple trees causes severe losses in production and kills millions of coconut palm trees in the Caribbean, the presence of a poinsettia branching bacterium is allowing its commercial production as pot plant (**Figure 1**).

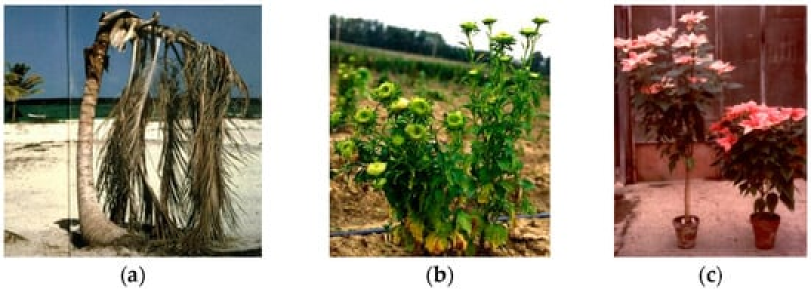

Figure 1. Coconut (**a**), aster (**b**), and poinsettia (**c**) infected by phytoplasmas.

Phytoplasmas are insect-transmitted wall-less bacteria provisionally classified to be the '*Candidatus* Phytoplasma' species [3][4]. They live only in the plant phloem tissues and in the emolymph of their insect vectors, especially concentrated in the salivary glands. Their relationship with both plants and insects is very intimate and they are able to manipulate several metabolic pathways, very often without impairing the host's life [5].

2. Phytoplasma Discovery

The phytoplasma presence in plants historically dates back about 1000 years, when special tree peonies exhibiting green flowers were given to the Chinese court during the Song dynasty (900 BC) as the most precious and beautiful flower of the empire. However, scientific records of phytoplasma-associated plant diseases started when, in 1967, mulberry dwarf, rice yellow dwarf, and sweet potato witches' broom, long considered to be caused by viruses, using electron microscopy, were found to be colonized by small pleomorphic bodies (80–800 nm in diameter) resembling mycoplasmas (bacterial pathogens of humans and animals) and were named mycoplasma-like organisms (MLOs) [6]. Their discovery stimulated worldwide investigation and numerous plant diseases were associated with the consistent presence of MLOs. These bacteria were long considered unculturable, but about 10 years ago, colonies containing molecularly different phytoplasmas began to be obtained in artificial media from different infected plant species (**Figure 2**) [7][8][9][10][11].

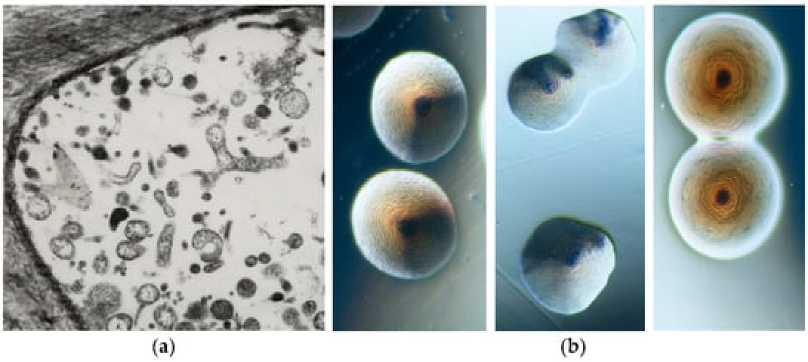

Figure 2. Transmission electron microscopy picture of a thin section in the phloematic tissue of a phytoplasma infected gladiolus plant showing the presence of strong pleomorphism (×8000) (**a**). Tree morphotypes of colonies containing phytoplasmas under binocular microscope (×40) (**b**).

3. Phytoplasma Classification

The '*Candidatus* Phytoplasma' genus provisional classification is highly relevant due to its application in epidemiological and ecological studies, mainly aimed at keeping the severe phytoplasma plant diseases under control worldwide. The updated proposed guidelines accommodate those '*Ca*. Phytoplasma' species strains sharing > 98.65% sequence identity of their full or nearly full 16S rRNA gene sequences, obtained with at least 2-fold coverage of the sequence, compared with those of the reference strain of such species [4]. The officially published '*Candidatus* Phytoplasma' species are 49; however, they do not cover all the relevant biodiversity, especially in reference to differential geographic distribution and/or host species. Therefore, the differentiation in ribosomal groups and subgroups [12] is still valuable and should be used to be able to work on their epidemiology and prevention in the different areas of the world. The main distribution of strains is tightly related to the geographic areas and to the dissemination performed by propagation materials, such as cuttings and seeds, that are also infected, even if only in low percentages (1–3%).

4. Relationship between Phytoplasma Symptomatology and Classification

Together with the study on the diseases associated with the presence of these bacteria, the first step was to give them a name according to the diverse disease in which the association was detected with specific phytoplasmas. Today, 30 years after this exercise started it appears clear that the molecular diversity described in phytoplasmas (49 '*Candidatus* Phytoplasma' species and about 200 ribosomal subgroups) using the 16S ribosomal gene as basic standard is only in some cases related to a differential symptomatology. Identical symptoms are associated with different phytoplasmas and vice versa. Moreover, phytoplasmas associated with decline symptoms in some species could be associated with phyllody/virescence in others, such as '*Ca*. P. solani' infecting potatoes, tomatoes, and grapevine. Therefore, contrary to the other plant pathogens it is necessary to verify the pathogen identity by molecular tools on a case-by-case basis; however, at the same time, it is of utmost importance to also recognize the symptoms associated with the presence of the phytoplasmas in order to appropriately manage the disease. These content of the main symptoms and several associated phytoplasmas worldwide is aimed at helping the recognition of the presence of these bacteria in plants, further clarifying their relationship with the host plants. This feature is, however, not stable over time also in the same plant species, considering the never-ending mechanism of patho-adaption that is part of life also in microorganisms; pathogens are special microorganisms that are simply looking for new ecological niches to ensure their survival and do not aim to destroy or kill the hosts.

4.1. Shoot Proliferation and Witches' Broom

Diseases with symptoms of witches' broom can be caused by basidiomycetes but could also be associated with the presence of phytoplasmas. In both cases they are economically important in a number of crop plant species, including the cocoa tree, jujube, citrus, and apple and timber trees, such as poplar, *Melia azedarach*, and paulownia (**Figure 3**). Among woody species, this malformation is almost always associated with the presence of specific phytoplasmas, such as in apple ('*Ca*. P. mali), lime ('*Ca*. P. aurantifolia'), lilac ('*Ca*. P. fraxini'), paulownia ('*Ca*. P. asteris'), almond ('*Ca*. P. phoenicium'), *Juniperus* (16SrIX-E), walnut (16SrIII-G), *Balanites triflora* ('Ca. P. balanites'), spartium ('*Ca*. P. spartii'), black alder (16SrX-E), hibiscus ('*Ca*. P. brasiliense'), *Guazuma* spp. (16SrXV-B), chestnut ('*Ca*. P. castaneae'), *Cassia italica* ('*Ca*. P. omanense'), and salt cedar ('*Ca*. P. tamaricis'). In herbaceous host plants, the presence of witches' broom was reported in diverse species, some of them as hosts of new phytoplasma strains (**Table 1**), such as strawberry, peanut, cactus, tabebuja, tomatillo, chayote, black raspberry, erigeron, alfalfa, and pigeon pea.

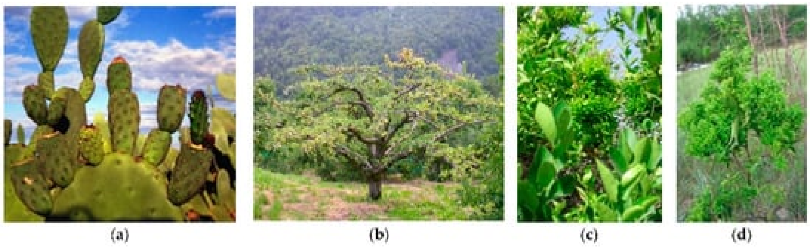

Figure 3. Cactus pear (*Opuntia ficus-indica*) proliferation (**a**), apple proliferation (**b**), citrus witches' broom (**c**), and jujube witches' broom (**d**) are associated with the presence of phytoplasmas in diverse areas of the world.

Table 1. Molecular diversity and geographic distribution of selected phytoplasmas belonging to different ribosomal groups/'*Candidatus* Phytoplasma' species (marked by different color) associated with witches' broom symptoms.

Disease (Acronym)	Continent	16Sr Subgroups	'*Candidatus* Phytoplasma' Species	GenBank Accession Number	References
Aster yellows w. b. (AY-WB)	America	16SrI-A	'*Ca.* P. asteris'	NC_007716	[13]
Paulownia w. b. (PaWB)	Asia	16SrI-D		AY265206	[14]
Strawberry witches' broom (STRAWB1), (STRAWB2)	America	16SrI-I / -K		U96614, U96616	[15]
Peach rosette-like (PRU0382)	America	16SrI-W		HQ450211	[16]
Peanut witches' broom (PnWB)	America	16SrII-A		L33765	[17]
Lime witches' broom (WBDL)	Asia	16SrII-B	'*Ca.* P. aurantifolia'	U15442	[18]
Cactus witches' broom (CWB)	Asia	16SrII-G to -L		EU099568, EU099552, EU099569, EU099572, EU099551, EU099546, EF647744	[19]
Tabebuia witches' broom	America	16SrII-O			[20]
Tomatillo witches' broom	America	16SrII-T		U125185	[21]
Walnut witches' broom (WWB)	America	16SrIII-G		AF190226, AF190227	[22]
Poinsettia branch-inducing (PoiBI)	Europe, America	16SrIII-H		AF190223	[22]
Chayote w. b. (ChWBIII)	America	16SrIII-J		AF147706	[23]
Black raspberry w. b. (BRWB7)	America	16SrIII-Q		AF302841	[24]
Conyza witches' broom	America	16SrIII-X		KC412026	[25]
Jujube witches' broom (JWB-G1)	Asia	16SrV-B	'*Ca.* P. ziziphi'	AB052876	[26]
Balanites triflora w. b. (BltWB)	Asia	16SrV-F	*Ca.* P. balanitae'	AB689678	[27]
Korean jujube witches' broom	Asia	16SrV-G		AB052879	[26]
Bischofia polycarpa witches' broom	Asia	16SrV-H		KJ452547	[28]
Blackberry witches' broom	Europe	16SrV-I		KR233473	[29]
Clover proliferation (CP)	America	16SrVI-A	'*Ca.* P. trifolii'	AY390261	[30]
Erigeron witches' broom (ErWB)	America	16SrVII-B		AY034608	[31]
Argentinian alfalfa w.b. (ArAWB)	America	16SrVII-C		AY147038	[32]

Disease (Acronym)	Continent	16Sr Subgroups	'*Candidatus* Phytoplasma' Species	GenBank Accession Number	References
Erigeron w. b. (EboWB-Br0)	**America**	**16SrVII-D**		**KJ831066**	[33]
Loofah witches' broom (LufWB)	**Asia**	**16Sr VIII-A**	**'*Ca*. P. luffae'**	**AF086621**	[34]
Pigeon pea w. b. (PPWB)	**America**	**16SrIX-A**		**AF248957**	[35]
Almond witches' broom (AlWB)	**Asia**	**16SrIX-B/-D**	**'*Ca*. P. phoenicium'**	**AF515636, AF515637**	[36]
Juniperus witches' broom	**America**	**16SrIX-E**		**GQ925918**	[37]
Almond and stone fruit witches' broom (N27-2), (A1-1)	**Asia**	**16SrIX-F/-G**	**'*Ca*. P. phoenicium'**	**HQ407532, HQ407514**	[38]
Apple proliferation (AP)	**Europe, Asia**	**16SrX-A**	**'*Ca*. P. mali'**	**AJ542541**	[39]
Spartium witches' broom (SpaWB)	**Europe**	**16SrX-D**	**'*Ca*. P. spartii'**	**X92869**	[40]
Black alder w. b. (BAWB, BWB)	**Europe**	**16SrX-E**		**X76431**	[41]
Hibiscus witches' broom (HibWB)	**America, Asia**	**16SrXV-A**	**'*Ca*. P. brasiliense'**	**AF147708**	[42]
Guazuma w. b. (GWB)	**America**	**16SrXV-B**		**HQ258882**	[43]
Chestnut witches' broom	**Asia**	**16SrXIX-A**	**'*Ca*. P. castaneae'**	**AB054986**	[44]
Rhamnus witches' broom	**Europe**	**16SrXX-A**	**'*Ca*. P. rhamni'**	**AJ583009**	[40]
Weeping tea witches' broom	**Oceania**	**16SrXXV-A ***		**AF521672**	[45]
Cassia w. b. (CaWB)	**Asia**	**16SrXXIX-A**	**'*Ca*. P. omanense'**	**EF666051**	[46]
Bindweed witches' broom (RBiWB)	**Asia**	**16SrXXIX-B**		**KY047493**	[47]
Salt cedar witches' broom	**Asia**	**16SrXXX-A**	**'*Ca*. P. tamaricis'**	**FJ432664**	[48]

w. b., witches' broom; *, described as sequence deposited in GenBank only.

The excessive shoot proliferation results in poor or no fruit production and severely reduces the cultivation of some of these crops. Citrus in the Arabian Peninsula, jujube in China, and apple proliferation in Europe are some of the most severe cases that greatly reduce the possibility to produce and commercialize popular fruits. This modification is due to the loss of apical dominance of the shoots linked to disorders in the hormone balance.

4.2. Stunting and Little Leaf

Stunting in plants could be due to virus or phytoplasma presence; however, it must also be verified that glyphosate or similar pesticides were not applied in the area in which these malformations are present in plants in the past years, since this can produce indistinguishable symptoms (**Figure 4**). The presence of phytoplasmas is reported in several plant species enclosing small fruits, vegetables, corn, and soybean; in some cases, these bacteria were associated with the presence of little leaf or stunting also in trees, such as cherry, eucalyptus, and *Sophora japonica* [49] (**Table 2**). In strawberries the case *Fragaria multicipita* was discovered to be not a true species, but just a cloned phytoplasma-infected genotype [15]. The hormone imbalance, according with the diverse infected species, is usually present and the transportation of starch and other metabolites for the appropriate development is very often impaired.

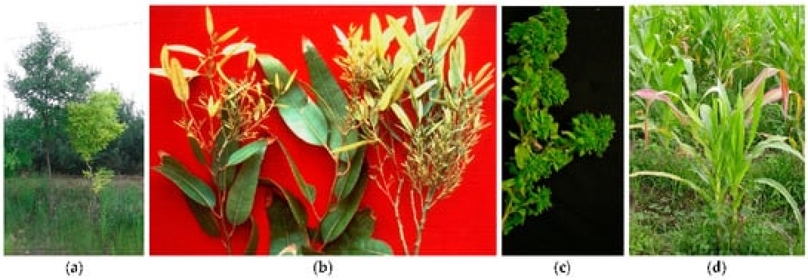

Figure 4. *Sophora japonica* stunting and yellows (**a**); *Eucalyptus* little leaf (**b**), periwinkle little leaf (**c**), and corn stunting (**d**).

Table 2. Molecular diversity and geographic distribution of selected phytoplasmas belonging to different ribosomal groups/'*Candidatus* Phytoplasma' species (marked by different color) associated with little leaf and stunting symptoms.

Disease (Acronym)	Continent	16Sr Subgroups	'*Candidatus* Phytoplasma' Species	GenBank Accession Number	References
Blue dwarf wheat (BDW)	**Asia**	**16SrI-C**	**'*Ca*. P. tritici'**	**DQ078304**	[50]
Blueberry stunt (BBS3)	**America**	**16SrI-E**		**AY265213**	[14]
Cherry little leaf (ChLL)	**Europe**	**16SrI-Q**		**AY034089**	[51]
Pepper little leaf (PeLL)	**America**	**16SrI-S**		**DQ092321**	[52]
Tomato little leaf (ToLL)	**America**	**16SrI-T**		**DQ375238**	[52]
***Vasconcellea cundinamarcensis* little leaf**	**China**	**16SrII-U**		**KP057205**	[53]
Spiraea stunt (SP1)	**America**	**16SrIII-E**		**AF190228**	[23]
***Heterothalamus* little leaf (HetLL)**	**America**	**16SrIII-W**		**KC412029**	[26]
Broccoli stunt (BSP-21)	**America**	**16SrIII-Z**		**JX626327**	[22]
Rubus stunt (RuS)	**Europe**	**16SrV-E**	**'*Ca*. P. rubi'**	**AY197648**	[54]
***Fragaria multicipita*, multiplier disease**	**America**	**16SrVI-B**		**AF190224**	[15]
Periwinkle little leaf (PLL-Bd)	**Asia**	**16SrVI-D**		**AF228053**	[55]
Portulaca little leaf (PLL-Ind)	**Asia**	**16SrVI-H**		**EF651786**	[56]
Soybean stunt (SoyST1c1)	**America**	**16SrXXXI-A**	**'*Ca*. P. costaricanum'**	**HQ225630**	[57]

4.3. Phyllody and Virescence

The transformation of different plant organs into leaves is a very relevant symptom among those associated with phytoplasma presence and is known as phyllody; this type of malformation could also be due to the application of pesticides based on hormone-like molecules. The virescence is the change of the color of flowers to green, which is due to phytoplasma presence, but in some cases the diagnostics can be tricked by the existence of flowers that are green and the presence of genetic factors modifying the anthocian distribution in the plant, as can be seen in a Chinese variety of rose and in some special clones of periwinkle (**Figure 5**). The most relevant phytoplasma-associated diseases are reported in flowering species for commercialization; however, virescence is also present in horticultural and seed crops, such as tomatoes, cabbages, strawberries, and clover, among several other species (**Table 3**).

Figure 5. Virescence in gladiolus (**a**) and in periwinkle (**b**); phyllody in echinaea (**c**), rose (**d**), and strawberry (**e**). The rose flowers are showing virescence and phyllody due to genetics, rather than the phytoplasma presence in all the others.

Table 3. Molecular diversity and geographic distribution of selected phytoplasmas belonging to different ribosomal groups/‘*Candidatus* Phytoplasma’ species (marked by different color) and associated with phyllody and virescence symptoms.

Disease (Acronym)	Continent	16Sr Subgroups	‘*Candidatus* Phytoplasma’ Species	GenBank Accession Number	References
Clover phyllody (CPh)	**America**	**16SrI-C**		**AF222065**	[15]
Faba bean phyllody (FBP)	**Asia, Africa**	**16SrII-C**		**X83432**	[58]
***Pichris echioides* phyllody (PEY)**	**Europe**	**16SrII-E**		**Y16393**	[58]
Cotton phyllody (CoP)	**Africa**	**16SrII-F**		**EF186827**	[59]
Strawberry leafy fruit (SLF)	**America**	**16SrIII-K**		**AF274876**	[15]
Dandelion virescence (DanVir)	**Europe**	**16SrIII-O/-P**		**AF370120, AF370119**	[60]
***Heterothalamus* little leaf (HetLL)**	**America**	**16SrIII-W**		**KC412029**	[26]
***Centarurea solstitialis* virescence (CSVI)**	**Europe**	**16SrVI-E**		**AY270156**	[61]
Catharanthus phyllody (CPS)	**Africa**	**16SrVI-F**		**EF186819**	[59]
Naxos periwinkle virescence (NAXOS)	**Europe, Asia, America**	**16SrIX-C**		**HQ589191**	[62]
Sarsoon phyllody	**Asia**	**16SrIX-H**		**KU892213**	[63]
Japanese hydrangea phyllody	**Asia**	**16SrXII-D**	**‘*Ca*. P. japonicum’**	**AB010425**	[64]
Mexican periwinkle virescence (MPV)	**America**	**16SrXIII-A**	**‘*Ca*. P. hispanicum’**	**AF248960**	[65]
Strawberry green petal (STRAWB2)	**America**	**16SrXIII-B**		**U96616**	[15]
Malaysian periwinkle virescence (MaPV)	**Asia**	**16SrXXXII-A**	**‘*Ca*. P. malaysianum’**	**EU371934**	[66]

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