SRI VENKATESWARA INTERNSHIP PROGRAM
FOR RESEARCH IN ACADEMICS
(SRI-VIPRA)

SRI-VIPRA

Project Report of 2024: SVP-2418

"Exploring the Diversity and Distribution of Phytoplasma (a phytopathogenic mollicute affecting diverse plant species) associated diseases causing significant yield losses globally"

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SRIVIPRA PROJECT 2024

Title: Exploring the Diversity and Distribution of Phytoplasma (a phytopathogenic mollicute affecting diverse plant species) associated diseases causing significant yield losses globally

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List of students under the SRIVIPRA Project

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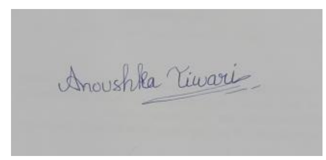
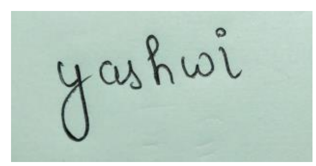
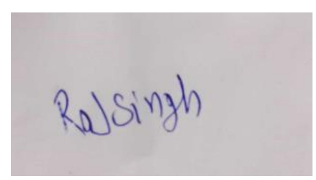
1		Anoushka Tiwari	1422016	B.Sc. [H] Botany-Semester V	
2		Yashwi Prajapati	1222018	B. Sc.[H] Biochemistry-Semester V	
3		Raj Singh	1122115	B.Sc.[P] Life sciences-Semester V	

Signature of Mentor (Dr. Amrita Singh)

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2418 titled "**Exploring the Diversity and Distribution of Phytoplasma (a phytopathogenic mollicute affecting diverse plant species) associated diseases causing significant yield losses globally"**. The participants have carried out the research project work under my guidance and supervision from 1st July, 2024 to 30th September 2024. The work carried out is original and carried out in an online/offline/hybrid mode.

Signature of Mentor (Dr. Amrita Singh)

Acknowledgement

First and foremost, we are incredibly thankful to our respected Principal Prof. Vajala Ravi for providing us this opportunity to work under SRI-VIPRA internship 2024.

We would also like to extend our sincere thanks to our mentor Dr. Amrita Singh who assigned us the project "Exploring the Diversity and Distribution of Phytoplasma (a phytopathogenic mollicute affecting diverse plant species) associated diseases causing significant yield losses globally". We would like to extend our gratitude to her as her assistance throughout the project was crucial for the success of this project. Her guidance, support and valuable insights helped steer this project to fruition.

Additionally, we would like to acknowledge the support and encouragement of our family and friends whose belief in us kept us motivated throughout the journey.

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Introduction

'Candidatus Phytoplasma', generally referred to as Phytoplasma are organisms of the domain Bacteria belonging to the class Mollicutes. Genus *'Candidatus* Phytoplasma' are found in various distinct forms i.e. they are pleiomorphic. They do not possess any cell wall but are surrounded by a two-layered limiting membrane (EPPO GLOBAL DATABASE, https://gd.eppo.int/taxon/PHYP64/datasheet). They reside within the sieve elements of plants. These bacteria are associated with more than 600 plant diseases worldwide significantly impacting agricultural economics (Bertaccini et al., 2014). They are transmitted by phloem-sucking leafhopper species, primarily from the Cicadellidae, Cixiidae, and Psyllidae families (Weintraub and Beanland, 2006). Phytoplasmas have lost many genes that play crucial roles in various metabolic pathways due to reductive evolution. Thus, they are dependent on the host for their nutrition and that is why they haven't been cultured axenically. Phytoplasma are intracellular bacteria that can transfer via insect vectors and grafts in the plants and produce various symptoms such as flower malformation, yellowing, dwarfing, witches' broom, phloem necrosis etc. [Wei and Zhao (2022); Stone and Putnam (2004)]. Phloem feeding insect families such as *Cicadellidae* (leafhoppers), *Cixiidae* (planthoppers) and *Psyllidae* (psyllids) are the major vectors for phytoplasmas (Montano et al., 2024).

Discovery, History and Background of Classification

In 1967, Doi et. al. in their research on Hachijojima mulberry seedlings affected with mulberry dwarf disease, potatoes showing symptoms of witches' broom and petunias affected with Aster yellows found some abnormal particles. In their electron microscopic observation, they found numerous spherical to asymmetric oval Mycoplasma-like or PLT-like (Psittacosis-Lymphogranuloma-Trachoma) particles of various sizes (80 to 800 mu in diameter) in the phloem parenchyma. These particles contained ribosome-like granules and nucleoplasm-like materials. Thus, with observations under various parameters, they were kept under MLOs due to their morphological resemblance to the Mycoplasma that affects humans and animals (EPPO GLOBAL DATABASE, https://gd.eppo.int/taxon/PHYP64/datasheet, Doi et al., 1967).

In 1989, based on 16S rRNA sequencing, MLOs were identified as distinct from Mycoplasma (Wei and Zhao, 2022). 16S rRNA gene was utilized for this purpose as all bacteria have at least one copy of 16S rRNA which is highly conserved and evolves slowly making it the most widespread target for studying bacterial phylogeny (Bertaccini et.al, 2022). In 1993, under the proposal of the International Committee on Systematic Bacteriology (ICSB) subcommittee on Taxonomy of Mollicutes, the earlier name MLO was replaced with Phytoplasma. Further, with the advent of DNA sequencing, 16S rRNA gene sequencing was used to classify organisms such as unculturable bacteria. In 1994, the term *Candidatus* was introduced for non-culturable bacteria giving appropriate status to the potential taxa based on 16S rRNA gene sequences. Presently, all phytoplasma strains are kept in the provisional *Candidatus* phytoplasma genus (Wei and Zhao, 2022).

The classification of *Phytoplasma* species, specifically *'Candidatus Phytoplasma'* species is primarily based on the analysis of the 16S rRNA gene. This gene serves as a highly conserved molecular marker making it ideal for taxonomic studies. Strains sharing >98.65 % sequence identity to the reference strain were considered members of the respective *'Candidatus Phytoplasma'* species. Strains showing identity <98.65 % to the reference strain, but showing >98.65 %with other strains of the same *'Candidatus Phytoplasma'* species will be considered related to this *'Candidatus Phytoplasma'* species. For the 16Sr RNA gene identity threshold, standard three identity thresholds (97.50%, 98.00%, 98.65%) were evaluated based on deposited 16S rRNA gene sequences (Table 1). The 98.65% threshold was found to be more accurate, hence increasing species diversity and reducing misclassifications (Bertaccini et.al., 2022). Based on RFLP analysis (Restriction Fragment Length Polymorphism) of the F2nR2 region of 16S rRNA gene, the group/subgroup classification has been done (Wei and Zhao, 2022).

Table 1: Strain composition of '*Ca.* Phytoplasma' species at diverse 16S rRNA gene sequence identity thresholds, analysis performed with BLASTn (Basic Local Alignment Search Tool for nucleotides) (Bertaccini et.al., 2022) with the following settings: Query coverage 95–100 %; percentage of identity 97.5–100.

'*Ca*. Phytoplasma' species	**Min/Max sequence identity (%) vs reference strain**		**No. of member strains**		**No. of related strains**	
		≥97.5%	**≥98 %**	**≥98.65 %**	**≥98 %**	**≥98.65 %**
'*Ca*. P. allocasuarina'	98.52	1	1	0	0	1
'*Ca*. P. americanum'	99.67/99.87	4	4	4	0	0

'*Ca*. P. asteris'	97.77/100	**374**	**372**	**366**	**2**	**8**
'*Ca*. P. aurantifolia'	97.50/99.73	**293**	**265**	**97**	**28**	**196**
'*Ca*. P. australasia'	97.51/100	**236**	**229**	**175**	**7**	**61**
'*Ca*. P. australiense'	98.68/99.93	12	12	12	0	0
'*Ca*. P. balanitae'	99.41/99.80	13	13	13	0	0
'*Ca*. P. brasiliense'	98.73/99.93	10	10	10	0	0
'*Ca*. P. caricae'*						
'*Ca*. P. castaneae'*						
'*Ca*. P. cirsii'	99.93/100	2	2	2	0	0
'*Ca*. P. cocostanzaniae'	99.33/100	17	17	17	0	0
'*Ca*. P. convolvuli'	99.93/100	9	9	9	0	0
'*Ca*. P. costaricanum'	99.15/99.61	17	17	17	0	0
'*Ca*. P. cynodontis'	98.37/100	**36**	**36**	**35**	**0**	**1**
'*Ca*. P. dypsidis'	99.83/99.88	6	6	6	0	0
'*Ca*. P. fragariae'	97.67/99.93	**15**	**14**	**10**	**1**	**5**
'*Ca*. P. fraxini'	97.61/99.93	**22**	**17**	**9**	**5**	**13**
'*Ca*. P. graminis'	98.34/99.74	**5**	**5**	**4**	**0**	**1**
'*Ca*. P. hispanicum'	98.53/99.47	**7**	**7**	**6**	**0**	**1**
'*Ca*. P. japonicum'*						
'*Ca*. P. luffae'	99.87/99.93	13	13	13	0	0
'*Ca*. P. lycopersici'*						
'*Ca*. P. malaysianum'	99.54	1	1	1	0	0
'*Ca*. P. mali'	99.74/100	20	20	20	0	0
'*Ca*. P. meliae'	99.45/99.86	5	5	5	0	0
'*Ca*. P. noviguineense'	99.66/100	26	26	26	0	0
'*Ca*. P. omanense'	99.58	1	1	1	0	0
'*Ca*. P. oryzae'*						
'*Ca*. P. palmae'	98.05/100	**80**	**80**	**66**	**0**	**14**
'*Ca*. P. palmicola'	99.28/100	24	24	24	0	0
'*Ca*. P. phoenicium'	97.54/99.93	**78**	**73**	**51**	**5**	**27**
'*Ca*. P. pini'	99.74/99.93	3	3	3	0	0

'*Ca*. P. pruni'	97.87/100	**207**	**205**	**203**	**2**	**4**
'*Ca*. P. prunorum'	99.50/100	35	35	35	0	0
'*Ca*. P. pyri'	99.14/100	34	34	34	0	0
'*Ca*. P. rhamni'	100	1	1	1	0	0
'*Ca*. P. rubi'	99.35/99.77	7	7	7	0	0
'*Ca*. P. sacchari'	98.68/99.93	30	30	30	0	0
'*Ca*. P. solani'	98.17/99.93	**73**	**72**	**72**	**1**	**1**
'*Ca*. P. spartii'*						
'*Ca*. P. stylosanthis'	99.94	1	1	1	0	0
'*Ca*. P. sudamericanum'*						
'*Ca*. P. tamaricis'*						
'*Ca*. P. trifolii'	97.98/100	**80**	**79**	**76**	**1**	**4**
'*Ca*. P. tritici'	100	1	1	1	0	0
'*Ca*. P. ulmi'	99.53/99.93	14	14	14	0	0
'*Ca*. P. woydetiae'	98.48	1	1	0	0	1
'*Ca*. P. ziziphi'	99.54/100	33	33	33	0	0

*Only one strain available for comparison; in bold, number of 'Ca. Phytoplasma' species in which reassignment is needed to follow the revised guidelines. [**Source:** Bertaccini, A., et al. (2022)]

Global scenario: Distribution and Diversity of Phytoplasma strains across different countries

1. South America

Bolivia

This country has a majority of climatic variations from humid tropics in the east to snowfall in the west. These variations in climatic conditions give this agriculture driven country a potential to grow a vast number of crops. Some of the staple crops of the region are potatoes, wheat, oats, alfalfa, corn, sugarcane, grapes, rice etc., along with several vegetables (https://www.g-fras.org/en/world-wide-extension-study/south-america/south-america/bolivia.html). Strains of Aster yellows group (16SrI) phytoplasmas were majorly responsible for witches' brooms in alfalfa (*Medicago sativa L)*, "brotes grandes" of potato (*Solanum tuberosum* L.). *'Ca P. australasiaticum'* of the subgroup 16SrII-D were found infecting peach plants (*Prunus persica*). In Yew Plum Pine (*Podocarpus macrophyllus* Kusamaki), plants with reduced leaves and shortened internodes were found to be infected with strains of *'Ca. P. citri'.* Phytoplasma strains belonging to ribosomal group 16SrIII were found to infect Bell peppers (*Capsicum annum*) producing reduced leaves and shortened internodes, strawberries producing small fruits and rosettes; pink peppercorns (*Schinus molle)* showing symptoms of witches' broom and yellowing, Peruvian carrot (*Arracacia xanthorrhiza)* producing little leaf and yellowing symptoms. China tree plants showing yellowing was found to be infected with 16SrXIII-C strain CbY1 (Bertaccini et al., 2024).

Chile

Chile's zona central is its agricultural heartland possessing mediterranean climatic condition thus supporting the country's largest wine production from grapevines along with various other fruit production. The vast agricultural produce was also hit by several plant diseases which led to decline in their production. Grapevine plants were reported with downward rolling and reddening of leaves and yellowing of leaves in red and white varieties respectively. The major causative ribosomal subgroups were identified as 16SrI-B species *'Ca. P. asteris'* and 16Srl-C species '*Ca. P. tritici'.* 16SrIII-J subgroup of phytoplasmas has been found to cause similar symptoms in the red and white Grapevine varieties. Due to widespread presence of vectors, seven reservoir species (*Convolvulus arvensis L., Galega officinalis L., Polygonum aviculare L., Rosa spp., Brassica rapa L., Erodium spp., Malva spp., and Rubus ulmifolius Schott)* of 16SrIII-J phytoplasmas have been reported. This subgroup has also been reported to cause deformities and malformations in potato, prickly pear (*Opuntia ficus -indica)*, lettuce (*Lactuca sativa*), Swiss chard (*Beta vulgaris),* cherry *(Prunus avium*), sugar beet (*Beta vulgaris subsp. vulgaris Altissima Group).* In Grapevine plants showing GY symptoms; citrus plants such as navel oranges (*Citrus sinensis),* mandarins (*Citrus reticulata)* with leaf yellowing, infection of '*Ca. P. ulmi'* belonging to 16SrV-A have been reported *(*Bertaccini et.al., 2024).

Colombia

In Colombia, several phytoplasma species have been reported. Some major species are *'Candidatus* phytoplasma asteris', '*Ca*. P. pruni'*, 'Ca*. P. ziziphi'*, 'Ca. P.* fraxini'*, 'Ca.* P. phoenicium*, 'Ca.* P. Solani'. *'Ca.* P. asteris' of ribosomal subgroup 16SrI-B has been reported to cause oil palm lethal wilt (OPLW). Some other identified hosts of *'Ca.* P. asteris' 16SrI-B were *Liquidambar styraciflua L., Fraxinus uhdei (Wenz.) Lingelsh., Populus nigra L., Pittosporum undulatum Vent*., and *Croton spp*. The phytoplasma species '*Ca*.P. pruni' of subgroup 16SrIII was reported to cause Cassava frogskin disease (CFSD). Phytoplasmas of ribosomal subgroup 16SrV were reported to cause severe degenerative diseases in liquidambar trees and *S. tuberosum*. '*Ca.* P. fraxini' *o*f ribosomal subgroup 16SrVII-A were also reported to infect liquidambar (Bertaccini et.al.,2024).

Ecuador

Various species in Ecuador were reported to cause diseases in different plant species. Some of the identified species were *'Candidatus* P. asteris'*, 'Ca.* P. aurantifolia'*, 'Ca.* P. ulmi'. '*Ca.* P. asteris' of the ribosomal subgroup 16SrI-F has been reported to cause potato purple top symptoms in potatoes. Similar symptoms were also reported by '*Ca.* P. aurantifolia' and '*Ca*. P. ulmi' *(*Bertaccini et.al., 2024).

Paraguay

In Paraguay, the presence of phytoplasma belonging to ribosomal group 16SrIII and 16SrXIII were predominantly reported. Mixed infection of these groups of phytoplasma were reported in *Melia azedarach* (China tree). The symptoms observed were yellowing, decline and little leaf (Bertaccini et.al., 2024).

Peru

The reported ribosomal groups of phytoplasma in Peru mainly belonged to 16SrI (Aster yellow group).'*Ca.* P. asteris', *'Ca.* P. pruni' and related strains of 16SrI group were reported to cause midriff chlorosis, leaf reddening, short internodes and plant growth reduction in corn plants (*Zea mays L.).* Strains from this group were also reported to cause phytoplasma associated symptoms in alfalfa, carrots, coconut, clover, maize, papaya, oats and dandelions. Potatoes from Huayao were reported to be infected by phytoplasma strains belonging to 16SrII group ('*Ca.* P. australasie'). The phytoplasma subgroup 16SrIII-J was reported to cause yellowing, dwarfism, shoot proliferation, internode shortening, lack of seed and pod production in Faba beans (*Vicia faba).* The variants of ribosomal subgroup 16SrXV-B were reported to cause Grapevine yellow

(GY) disease in vineyards of Piura along with papaya bunchy top being reported from the same region (Bertaccini et.al., 2024).

Argentina

The major ribosomal groups found were 16SrIII and 16SrX. The subgroup 16SrX-C was reported to cause reddening of leaves and branch phloem necrosis in pear plants (*Pyrus sp.).* The same subgroup also caused chlorotic leaves, ridges and thickening of central veins in peach plant (*Prunus persica sp.).* The subgroups 16SrIII-B, 16SrIII-J, 16SrIII-X, 16SrIII-W of ribosomal group 16SrIII were reported in the region. 16SrIII-B was reported to affect China tree leading to branch size reduction, leaf yellowing, and witches' broom. Peach plants affected with phytoplasma belonging to 16SrIII-B sub-group showed reddish and curled leaves with necrotic leaf area. Plum plants affected with it showed witches' broom and yellowing. The key plants infected by 16SrIII-J are garlic, tomato, summer squash, sunflower, cassava, and sugar beets (Bertaccini et.al., 2024).

2. Middle East

The Middle East is an intercontinental region consisting of countries from Asia and Africa. It includes Syria, Lebanon, Jordan, Iran, Israel, Kuwait, Bahrain, Qatar, U.A.E, Saudi Arabia, Oman and Yemen. Till date, fourteen 16Sr groups of phytoplasma have been discovered associated with 164 plant species in the region (Hemmati et al., 2021).

Iran

The diverse range of temperature and climatic conditions make it possible to cultivate a diverse variety of crops in Iran. Iran is a major producer of berries, pistachios, stone fruits, apricots, figs, almonds, walnuts, cherries, apples, melons etc. along with cereals, olives, cotton and spices (https://www.britannica.com/place/Iran). The strains belonging to the phytoplasma group 16SrI-B, 16SrII (subgroup II-B, II-C), 16SrVI (subgroup VI-A and VI-D), 16SrIX (IX-B, IX-C, IX-D), 16SrX-F and 16SrXII-A have been reported to cause economic losses in the *Prunus* species. Witches' broom in almond (AlmWB) caused by the phytoplasma strain *'Ca.* P. phoencium ' of the group 16SrIX-B has caused severe loss in production (Hemmati et al., 2021).

Saudi Arabia

Although Saudi Arabia is widely thought of as a desert, it has a region of 173 million hectares suitable for agriculture. Thus, it becomes utterly important to analyze the disease-causing agents in the plants which cause loss of crops. The reported phytoplasma ribosomal subgroups in the region were 16SrII and 16SrXIV. 16SrII were found to infect date palms (*Phoenix dactylifera L.*). *'Ca.* P. aurantifolia' of ribosomal subgroup 16SrII-B has been reported to cause witches' broom disease of lime (WBDL) in lime plants producing large numbers of small branches and leaves. The leaves of infected plants become smaller and yellow in color, they do not produce fruits and usually die within four to eight years. Various strains of 16SrII-D subgroup were reported to cause alfalfa witches' broom and tomato witches' broom. Phytoplasma belonging to 16SrII-X were found to cause deformities in potato and eggplant within the region. Eggplants were also identified as the hosts for 16SrII-D sub-group of phytoplasma. In Saudi Arabia, 16SrII-D was also reported to cause deformations in cabbage. The relation between 16SrII-D and *Hibiscus rosa-sinensis, Calendula officinalis, Z. Magellan, Plectranthus scutellarioides, Conocarpus lanceolatus* and *Washingtonia robusta* have also been reported in the region. '*Ca*. P. cynodontis' of ribosomal subgroup 16SrXIV was reported to infect *'Cynodon dactylon L.'* and cause Bermuda grass white leaf disease (BGWL). The infected plants showed bushy growth, whitening of leaves, little leaves and plant death (Hemmati et al., 2021).

Egypt

In Egypt, phytoplasma belonging to ribosomal groups 16SrI (aster yellows) and 16SrII (peanut witches' broom) were reported to affect important crops such as tomatoes (*Solanum lycopersicum*), potatoes (*Solanum tuberosum*) and date palms (*Phoenix dactylifera*). Symptoms observed were stunted growth, leaf yellowing and poor fruit production. Phytoplasmas have also been reported in ornamental plants like hibiscus (*Hibiscus rosa-sinensis*) making it a widespread issue in both agriculture and horticulture (Hemmati et al., 2021).

Iraq

In Iraq, phytoplasma infection has been reported in date palms (*Phoenix dactylifera*) and citrus plants such as lime (*Citrus aurantiifolia*) and lemon (*Citrus limon*). The ribosomal groups 16SrI and 16SrII have been reported to cause stunting, witches' broom and leaf discoloration which has led to substantial crop losses. Vegetables like tomatoes (*Solanum lycopersicum*) and ornamental plants were also reported to be affected by these pathogens, thus increasing the urgency of managing these diseases (Hemmati et al., 2021).

Oman

In Oman, lime trees (*Citrus aurantiifolia*) were reported to be heavily affected by witches' broom disease caused by 16SrII-B phytoplasmas leading to excessive branching, reduced leaf size, and eventual plant death. Date palms (*Phoenix dactylifera*) and vegetable crops like tomatoes (*Solanum lycopersicum*) also suffer from phytoplasma infections making this a critical issue for the country's agriculture, particularly citrus and date farming (Hemmati et al., 2021).

Turkey

The phytoplasma 16SrI (aster yellows) and 16SrX (apple proliferation) groups were reported to cause symptoms such as yellowing of leaves, stunted growth and malformed fruits in key crops such as grapevines (*Vitis vinifera*), peaches (*Prunus persica*) and apricots (*Prunus armeniaca*). "Bois noir" disease caused by 16SrXII phytoplasmas were reported to severely affect grapevine industry in the region (Hemmati et al., 2021).

3. South East Asia

Thailand

Phytoplasmas from the ribosomal group 16SrI (Aster yellows) and 16SrII (Peanut witches' broom) group have been reported in Thailand. '*Ca.* P. asteris' of 16SrI group was found to affect crops like lettuce and other vegetables leading to symptoms such as yellowing and stunted growth. Further, peanut witches' broom disease caused by 16SrII phytoplasma leads to distorted growth in peanuts resulting in severe yield loss. Rice yellow dwarf disease caused by 16SrXI phytoplasma has also been reported to affect one of the country's staple crops (Pierro et.al., 2019).

Vietnam

In Vietnam, phytoplasmas belonging to 16SrII and 16SrXI groups were reported to be prevalent. The group 16SrII has been reported to affect crops like peanuts, tomatoes and sesame. '*Ca.* P. sacchari' of 16SrXI group was reported to cause sugarcane white leaf disease which severely impacts sugarcane plantations by inducing leaf whitening, stunted growth and eventual plant death. This disease is devastating particularly to Vietnam's sugarcane industry (Pierro et.al., 2019).

Malaysia

In Malaysia, the phytoplasma ribosomal group 16SrI (Aster yellows) and 16SrII were reported to be significant agricultural pathogens. Aster yellows phytoplasmas were reported to affect vegetables like lettuce and celery. Additionally, outbreaks of tomato and eggplant witches' broom caused by 16SrII phytoplasmas have been reported. The diversity of hosts in Malaysia highlights the need for continuous monitoring and control measures (Pierro et.al., 2019).

Indonesia

Indonesia faces challenges with phytoplasmas belonging to 16SrXI group especially affecting rice which is the country's primary staple crop. Rice yellow dwarf disease caused by 16SrXI phytoplasma results in leaf yellowing, stunted plant growth and reduced grain production. Additionally, sugarcane white leaf disease caused by 16SrXI group phytoplasma is another major disease leading to significant sugarcane yield loss in the country (Pierro et.al., 2019).

Philippines

Philippines has reported severe cases of sugarcane white leaf disease caused by '*Ca.* P. sacchari' of ribosomal group 16SrXI which leads to bushy growth, white leaves and plant death. The disease has become a major threat to the country's sugarcane industry. Other phytoplasma diseases such as infection caused by 16SrI (Aster yellows) phytoplasmas are also reported in vegetable crops leading to reduced productivity in lettuce and other high-value vegetables (Pierro et.al., 2019).

Myanmar

Phytoplasmas belonging to groups 16SrII and 16SrXI have been reported in Myanmar particularly affecting staple crops like rice and peanuts. Rice yellow dwarf disease caused by 16SrXI group phytoplasma resulted in severe yield losses. Peanut witches' broom (16SrII) phytoplasma has also been reported and has impacted peanut farming in the region (Pierro et.al., 2019).

Cambodia and Laos

In both Cambodia and Laos, rice yellow dwarf disease caused by 16SrXI phytoplasmas is a prominent concern due to the heavy reliance on rice as a staple crop. Additionally, 16SrII phytoplasmas affecting tomatoes and peanuts have also been reported with severe negative economic consequences for local farmers (Pierro et.al., 2019).

4. Europe

Phytoplasma diseases have a significant impact on European agriculture due to their widespread distribution and host diversity. In Europe, several ribosomal subgroups of phytoplasma have been identified including 16SrI (aster yellows group), 16SrX (apple proliferation group), and 16SrXII (stolbur group) which infect a variety of crops. The 16SrI group is commonly associated with diseases in vegetables like lettuce and celery while 16SrX is notorious for causing economic losses in apple orchards by inducing symptoms like proliferation of small, misshapen fruits. The 16SrXII subgroup affects solanaceous plants such as tomatoes and potatoes leading to stunted growth and leaf yellowing. In vineyards, the '*Ca.* P. solani' 16SrXII-A strain is responsible for Bois noir, a major grapevine disease across Southern and Central Europe. Additionally, diverse strains of phytoplasma affect other crops like peach, pear and stone fruits leading to significant economic losses. Ongoing research aims to better understand the distribution and ecological factors driving phytoplasma diversity in Europe, as well as to develop effective management strategies to mitigate their impact on agriculture (Jarausch et al. (2013), Davies and Adams (2000); Lee et al. (2000)].

Indian scenario

History of Phytoplasma diseases in India

The history of phytoplasma diseases in India dates back more than a century. The root (wilt) disease of coconut was first reported in the former state of Travancore (South Kerala) around 1874 and became increasingly evident by 1882. This disease was later found to be present in 410 thousand hectares in Kerala. Subsequently, the sandal spike disease was described in the Coorg district of Karnataka in the early 19th century and since then, it has been reported to occur in the states of Karnataka, Kerala, and Tamil Nadu. The yellow leaf disease of the areca nut was first reported in 1914 from the central Kerala regions of Muvattupuzha, Meenachal, and Chalakudy. The disease later spread throughout Kerala. Thomas and Krishnaswamy (1939) reported a brinjal little leaf disease suspected to be viral, which was later confirmed to be caused by phytoplasma. The occurrence of sesame phyllody in South India in the early 19th century was also confirmed to be caused by phytoplasma. The sugarcane grassy shoot disease (SCGS) was identified as the most important phytoplasma disease in the Indian subcontinent, after it was first reported in the Ahmednagar district of Bombay in 1949. Many plant diseases that were initially described as viral or of unknown origin have since been attributed to phytoplasma, and now 129 plant species are reported to be infected with phytoplasma diseases in India.

Distribution and Diversity of Phytoplasma strains affecting different Plants across India

SPICES AND MEDICINAL CROPS

Medicinal herbs and spices are a class of economically valuable industrial crops that are utilised both for local use and export. Significant financial losses are brought on by phytoplasma infections in a number of species of medicinal plants and spices (Table 2). So far, thirteen distinct kinds of medicinal plants in India have been shown to harbor phytoplasmas from four groups (16SrI, 16SrII, 16SrVI, and 16SrXIV).

Black pepper (*Piper nigrum*) phyllody was linked to "*Ca*. P. asteris" (16SrI group) from Calicut, India. According to Adkar-Purushothama et al. (2009), black pepper yellows is a disease linked to phytoplasma in the South Indian state of Karnataka's Coorg district. The infected leaves curl and become yellow. Little leaf disease of fennel (*Foeniculum vulgare*) linked to phytoplasmas has been reported from Lucknow. The disease's characteristic symptoms include growth retardation, an excessive number of axillary shoots and development of thin, narrow leaves leading to symptoms of witch's broom. Disease incidence of 5-12% was observed in commercial fields. The seriously afflicted plants lose all of their color and are unable to develop an inflorescence. By using electron microscope observations, the linked phytoplasma was studied and PCR assays were used to characterize it as a strain related to "*Ca.* P. aurantifolia". *Catharanthus roseus* infected with strains belonging to the 16SrI group showed little leaf and phyllody symptoms (Fig.1a). Moss rose purslane, or *Portulaca grandiflora* is used medicinally because it contains a number of vitamins and has cytotoxic and antibacterial properties (Fig.1b). Ajayakumar et al. (2007) reported that the presence of a strain similar to "*Ca*. P. trifolii" was linked to little leaf disease of *P. grandiflora*. More omega-3 fatty acids than any other green vegetable plants are found in the herbaceous annual *P. oleracea*, often known as common purslane. In sugarcane fields in Gorakhpur, Uttar Pradesh, little leaves and yellowing symptoms has been recently noticed in *P. oleracea*. The disease was linked to a phytoplasma strain related to "Ca. P. asteris". Cultivated for its roots' alkaloids, *Withania somnifera* is a significant therapeutic crop. Numerous findings describe the existence of phytoplasmas belonging to the 16SrVI group in *Withania*. The symptoms of witches' broom were present in the infected plants along with phyllody, little leaves and dense clusters of rapidly multiplying branches with short internodes.

SUGARCANE

The sugar industry is one of the largest agriculture-based industries in India and a crucial driver of rural development contributing significantly to the country's economic growth. Among the various biotic and abiotic stresses affecting sugarcane, the sugarcane grassy shoot disease is the most economically significant disease (Viswanathan and Rao, 2011; Rao et al., 2012).

Phytoplasmas have been found to be associated with two major sugarcane diseases: **grassy shoot (SCGS)** (Fig.1c) and **white leaf (SCWL)** (Rao et al., 2012). Additionally, **sugarcane yellow leaf syndrome (YLS)** has been reported to be linked with a Luteovirus and a phytoplasma (Gaur et al., 2008). YLS has also been found to be associated with phytoplasmas in Africa, Australia, India, Cuba, and Mauritius (Rao et al., 2012). The SCGS disease can cause yield losses ranging from 70 to 100% in susceptible varieties posing a significant economic concern for both farmers and the sugar (Viswanathan and Rao, 2011; Rao et al., 2012). The yield losses caused by SCGS are even higher in ratoon crops when the primary infection appears early in the crop cycle. The disease was first observed near Belapur in the Ahmednagar district of Maharashtra in 1949 but it has now been recorded in all the sugarcane-growing states of India (Rao et al., 2012). The most common symptoms associated with the SCGS disease are **grassy shoots, chlorotic leaves and shoot proliferation**. The disease is also characterized by the **production of a large number of thin, slender, adventitious tillers from the base of the affected plants.**

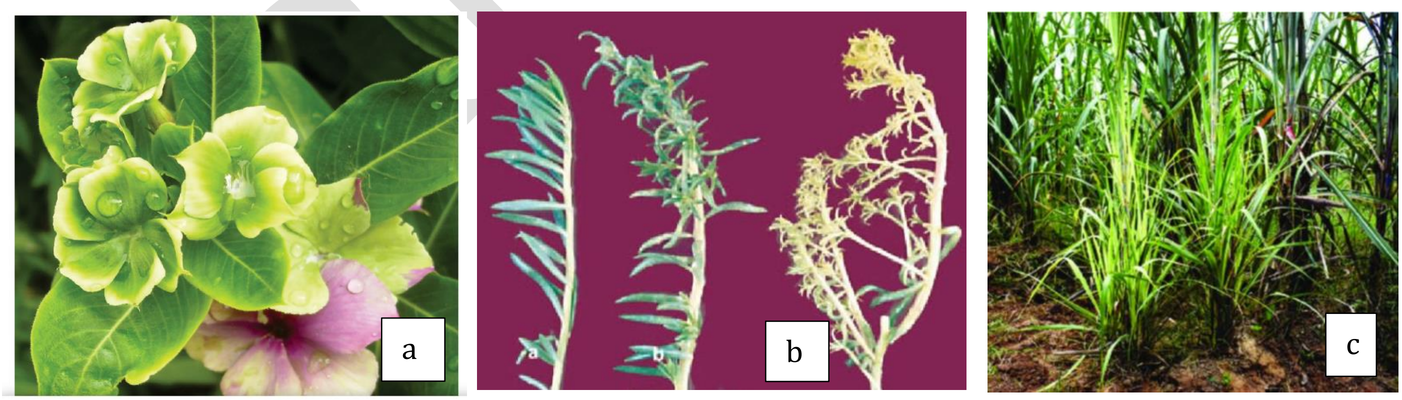

Fig.1. a. *Catharanthus roseus* showing virescence (Barbosa et al., 2012) b. *Portulaca grandiflora* showing little leaf symptom (Ajaykumar et. al., 2007) c. Sugarcane showing symptom of grassy shoot disease (Singh et al., 2021)

LEGUMES

Legumes are farmed mainly for their grain seed, cattle fodder and silage and as green manure that improves soil quality. They are an important source of protein, dietary fibres, carbs and minerals. Reports of phytoplasma diseases in soybean, cowpea, mung bean, chickpea and pigeon pea in India are scarce (Fig.2a). During a field study, Thorat et al. (2016) noted symptoms of little leaf, phyllody, stunting and branch proliferation in *Phaseolus vulgaris* (French bean) and *Vigna unguiculata* (cowpea) in Akola and Pune regions of Maharashtra. According to reports (Raj et al., 2006; Ragimekula et al., 2014; Thorat et al., 2016), the phytoplasmas 16SrI-B, 16SrII-D, and 16SrII-C were found in cowpea, mungbean, and *Crotalaria pallida* (smooth rattle pods).

Ninety mung-bean breeding lines in Tirupati, Andhra Pradesh displayed symptoms of phytoplasma infection in September 2013. These symptoms included stunting, extensive branch proliferation, reduction in leaf size, phyllody and longitudinal splitting of green pods followed by germination of green seeds (Fig.2b). At ICRISAT, Patencheru (Hyderabad), Reddy (1987) identified symptoms of phyllody and witches' broom in a number of pigeon pea cultivars affected by phytoplasma. Furthermore, strains linked to '*Ca.* P. asteris' and '*Ca.* P. phoenicium' has been reported to infect pigeon pea plants in Lucknow, Uttar Pradesh leading to stunting, shortening of internodes and petioles producing a bunchy look (Fig.3a) and reduction in number of leaves (Rao et al., 2017).

The 16SrII-D subgroup 'Ca. P. aurantifolia' was identified as the related phytoplasma (Ragimekula et al., 2014). Rao et al. (2017) observed the existence of strains related to '*Ca.* P. phoenicium' (16SrIX-C), '*Ca.* P. trifolii' (16SrVI-D) in lentils, and 'Ca. P. cynodontis' (16SrXIV-A) in cowpea.

FRUITS

The popular fruit papaya (*Carica papaya*) is extensively grown in India. Two phytoplasmas were found to be associated with papaya. '*Ca*. P. asteris' has been reported to produce dieback symptoms in papaya in Uttar Pradesh (Rao et al., 2011) and a strain related to '*Ca.* P. aurantifolia' has been reported from Pune which showed growth of axillary shoots in infected papaya plants. (Fig.3b). *Ziziphus jujuba* (Indian ber) is an important plant which is being cultivated for its fleshy fruits across the sub-continent. Khan et al. (2008) reported cases of *Z. jujube* and *Z. nummularia* infected by *Ca.* P. ziziphi in Payagpur, Bahraich district of Uttar Pradesh. Severe rosetting and little leaves has been reported to be the main symptoms exhibited by them (Fig. 3c). The16Sr DNA sequences found in phytoplasma strains infecting both the species were 98% identical to those of phytoplasmas in the 16SrV group and 99% identical to each other.

Research on pomegranates from Turkey and Iran revealed phytoplasmas from the 16SrI-B and 16SrXII-A subgroups. Rao et al. (2020a) provided the first report of pomegranate infection by phytoplasma in India. '*Ca*. P. australasia' (16SrII-D subgroup) related strain has been reported to infect pomegranate from Pusa and rice yellow dwarf group (16SrXI-B) strain infected pomegranate from Baramati.

The long-lived evergreen fruit tree Sapota (*Manilkara zapota*) often referred to as chikoo in India, is a member of the Sapotaceae family and is endemic to southern Mexico. The sapota fruit is a good supply of vitamins, lipids, protein, fiber, calcium, phosphorus, iron, carotene, and carbohydrates with a digestible sugar content of 12 to 18%. Karnataka, Gujarat, Tamil Nadu, Maharashtra, Andhra Pradesh, West Bengal, Telangana, Orissa, and Tripura are the main states in India where sapota is being grown. Sapota is grown on 156 thousand hectares of land yielding an annual production of 11.76 lakhs MT and 12.12 MT/ha of productivity. The top producers of sapota are India, Venezuela, Guatemala and Mexico. Various fungal infections have been documented to affect sapota plants. Phytoplasmas belonging to 16SrI-B, 16SrVI-D and 16SrXIV-A subgroups have been reported to infect sapota trees in Indian states of Karnataka, Kerala and Tripura (Rao et al; 2020b)

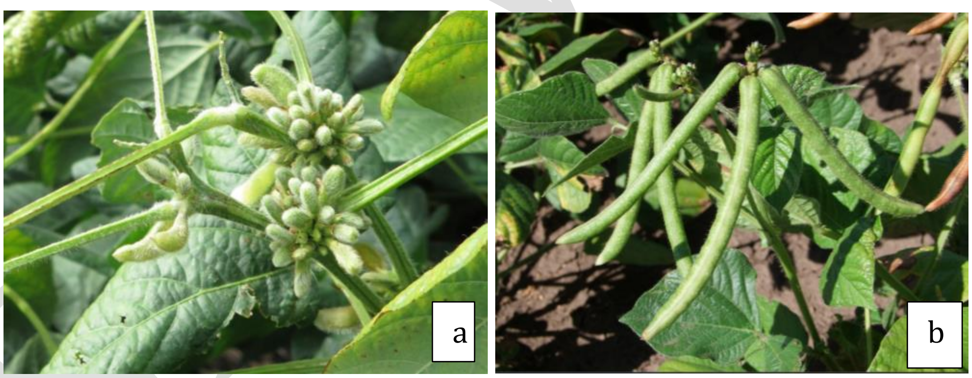

Fig.2. a. Little pod symptoms in soybeans (www.thebeatsheet.com.au), b. Puffy pod in mung-beans caused by late phytoplasma infections (www.thebeatsheet.com.au).

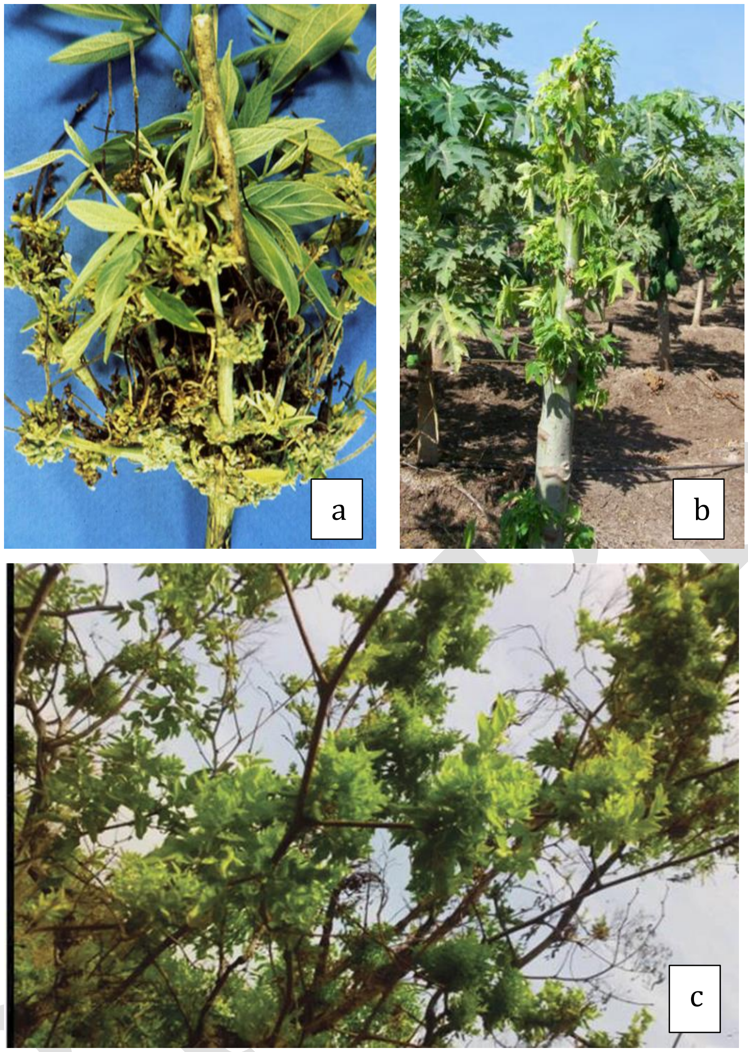

Fig.3. a. Pigeon pea showing symptoms of witches'-broom (Source: https://www.plantdiseases.org/witches-broom-pigeon-pea), b. *Carica papaya* showing axillary shoot proliferation symptoms (Verma et al.,2012). c. *Ziziphus jujuba* showing witches' broom symptoms (Shao et al., 2016).

VEGETABLES

Vegetables are low-yield crops that can be produced throughout the year and yield greater financial rewards. About 40 varieties of vegetables are grown in India and are raised from a variety of species including cruciferous, leguminous, cucurbitaceous and solanaceous green vegetables and root crops. Numerous vegetables have been reported to be linked to distinct ribosomal groups of phytoplasma like 16SrI in potato

(Tiwari et al., 2013), chili (Khan and Raj, 2006), ladies' finger and 16SrII in lettuce, carrot and beans (Table 2). Brinjal, lettuce, celery and cabbage have been reported to be infected with16SrVI-D strains of phytoplasmas. Garg et al. (1989) highlighted the existence of creatures resembling mollic bugs in potatoes afflicted with marginal leaf chlorosis. Reports of sick potatoes with phytoplasmas came from Shimla. Numerous phytoplasma-associated diseases can cause up to 40% yield losses in eggplant. The symptoms of eggplant manifest 1-2 months post-sprouting exhibiting severe stunting, reduced leaf size and phyllody, small leaf, shortened internodes and branch proliferation. Sometimes yield loss can even reach up to 100% in several plant species (Rao and Kumar, 2017). Egg plants are known to be infected with phytoplasmas belonging to 16SrI, 16SrII-D and 16SrVI-D sub-groups with the 16SrVI-D subgroup being the most common (Kumar et al., 2012; Azadvar and Baranwal 2012; Kumar et al., 2017).

PALMS

Palm family comprises twenty genera and roughly 96 species, 24 of which are indigenous to India and belong to nine genera (Kulkarni & Mulani, 2004). The eight states that grow coconuts (*Cocos nucifera*), areca nuts (*Areca catechu*), and oil palms (*Elaeis guineensis*) for commercial purposes are the Andamans, Andhra Pradesh, Goa, Karnataka, Kerala, Maharashtra, Tamil Nadu and Tripura. Oil palm was introduced later, in 1971 in Kerala and 1988 in other states. Over 150 years ago, coconuts and areca nuts were farmed extensively in Kerala. The root (wilt) disease (RWD) and tatipaka disease (TD) of coconut, yellow leaf disease (YLD) of areca nut, spear rot disease (SRD) of oil palm (OPS) and oil palm stunting (OPS) are the phytoplasma diseases that have been identified in these crops. The dates and locations of the first reports of these diseases are as follows: TD in 1949 in Andhra Pradesh (Rao et al., 1956); OPS in 2010 in Andhra Pradesh; RWD in Kerala in 1874–1882, in 1882–1883 and YLD in 1914 in Kerala (Rao 2017).

BAMBOO

Solid bamboo, also known as Calcutta bamboo (*Dendrocalamus strictus*, Poaceae) is found in China, Southeast Asia, and the tropical regions of the Indian subcontinent. *D. strictus* has a 20–65year flowering cycle and is semelparous. One of the hardest bamboos in India, it is widely utilized to make pulp, rayon, paper mills, lightweight house construction and furniture making it a plant species of commercial significance. Its leaves are used as fodder and its young shoots are consumed. It is also utilized in traditional medicine. '*Ca.* P. aurantifolia' was linked to *D. strictus* witches' broom disease by Yadav et al. (2016).

SANDALWOOD

The medium-sized *Santalum album* is a priceless tree. Both the oil and the heartwood are widely utilized in the fragrance and medicinal sectors. Sandalwood is widely used in religious rites and ceremonies. Sandalwood trees are very vulnerable to the disease known as **sandal spike phytoplasma**. The impacted plants' shortened internodes and packed leaves give them a spike-like look. The disease also produces symptoms of phyllody. Within two or three years, the afflicted tree dies (Sen Sarma, 1981). Sandal spike disease can be identified by a number of methods, including nested PCR assay (Khan et al., 2004), light microscopy using DAPI (Thomas and Balasundaran, 1998), Dienes' stain (Ananthapadmanabha et al., 1973), transmission electron microscopy (Ghosh et al., 1985), and the manifestation of symptoms. By comparing the 16Sr DNA sequence and using phylogenetic analysis, it was shown that the aster yellows subgroup 16SrI-B was linked to the sandal spike.

WEEDS

Three weed species namely, *D. ciliaris, E. indica, and P. minor* infected by phytoplasma strains belonging to 16SrI and 16SrXIV groups have been reported as new phytoplasma hosts in India. *P. hysterophorus* and *O. burmannii* have been identified as hosts for a novel phytoplasma strain belonging to 16SrII group (Mall, S., 2015).

Table 2: Diversity and Distribution of different phytoplasma strains affecting diverse plant species across India

Group	Sub-group	Species	Crops affected	Diseases caused	Symptoms	Distribution
16Sr1	B	*Ca.* P. asteris	Aster nuts (leaves)	Yellow leaf of aster	Yellowing of aster nuts leaves	J&K, Himachal Pradesh, UP, Uttarakhand, Punjab, Haryana
16Sr1	B	*Ca.* P. asteris	Sugarcane	Sugarcane Grassy Shoot disease	orange, yellow discolored leaves distributed sporadically in the field but usually more concentrated on the borders	West Bengal, Tamil Nadu
16SrVI	D		Brinjal, lettuce, celery, and cabbage	Marginal leaf chlorosis	Chlorosis on leaf margins	Shimla (Himachal Pradesh) and khushinagar
16SrIII	E		Papaya plant	Little leaf of papaya (PaLL)	Papaya's canopy starts to take on the shape of flower petals, with small bunches of leaves arranged in a radius.	Pune, Maharashtra
16SrII	D	*Ca.* P. aurantifolia	*Callistephus chinensis* (China aster), *Rose*	Peanut Witches' Broom Group	Virescence and phyllody in China aster	

			(35 cultivars) Mung bean (Vigna radiata)			
16SrV		*Ca.* P. ulmi	***Ulmus*** **spp.** (Elm trees)	Elm Yellows (also known as Elm Phloem Necrosis)	Yellowing of leaves (chlorosis) Premature leaf drop Necrosis of phloem tissue Stunted growth	
16SrVI	D	*Ca*. P. trifolii	Lentil	Clover Proliferation Group		Uttar Pradesh, Madhya Pradesh
16SrIX	C	*Ca*. P. phoenicium	*Phlox drummondii, Carpobrotus edulis*		Early flowering and development of all the buds of the infected branches. Extensive yellowing, stunting, proliferation of shoots, and little leaves	Uttar Pradesh, Delhi, Pune
16SrXI		*Ca*. P. oryzae	-	-	-	
16SrXIV		*Ca*. P. cynodontis	-	Bermuda grass white leaf disease (BGWL)	Stunting, phyllody, floral malformation, and flattening of stem	
16SrI, 16SrII-D,	16SrVI-D	*Ca.* P. trifolii	Brinjal leaves	Brinjal little leaf (BLL)	Stunted growth in plants Reduced leaf	Widespread in India

and 16SrVI-D					size (hence the name "little leaf")	
16SrXI	16SrXI-B	*Ca.* P. oryzae	Durum wheat Bread wheat	Phytoplasma-associated disease in wheat	No tassel formation or formation of small, deformed tassels without grains	Regional Station of ICAR-Indian Agricultural Research Institute, Indore, India

CONCLUSION

The global distribution and diversity of phytoplasmas highlight the widespread impact of the phyto-pathogen on agriculture affecting a variety of crops across continents. From South America to Southeast Asia and the Middle East and further, distinct phytoplasma strains have particularly caused significant agricultural losses by infecting staple crops such as potatoes, rice and sugarcane. Besides agricultural crops, phytoplasmas have also severely impacted ornamental plants. Advances in molecular diagnostics, especially the use of 16S rRNA gene sequencing have greatly enhanced the identification and classification of these unculturable bacteria, yet challenges remain in their management. As vectors continue to spread, the risk of further outbreaks rises, making phytoplasma research vital for improving disease control and safeguarding global food security. Effective monitoring and integrated pest management strategies are essential in minimizing the impact of these diseases and ensuring sustainable agricultural productivity. Extensive research studies are being conducted in India to study the spread and effectiveness of phytoplasma diseases so that disease control methods can be devised. There is a pervasive threat posed by phytoplasma infections across a wide range of crops in India. The identification of phytoplasmas in 129 plant species including economically and medicinally important ones, reflects their adaptability and ability to affect diverse agricultural systems. The substantial economic losses documented in spices, medicinal plants and sugarcane crops in India highlight the urgency of addressing these infections. In India, there is significant yield losses in crops such as black pepper (*Piper nigrum*), fennel (*Foeniculum vulgare*) and sugarcane, which can reach up to 100% in severe cases. The symptoms associated with these infections such as phyllody, stunted growth, and chlorotic leaves indicate the detrimental effects on crop health and productivity. Furthermore, the identification of specific phytoplasma strains linked to these diseases, such as *Candidatus Phytoplasma asteris* and *Candidatus Phytoplasma phoenicium*, allows for targeted research and management approaches. The widespread occurrence of phytoplasma-associated diseases in both major agricultural and horticultural crops signifies the need for enhanced surveillance and management practices. The role of insect vectors, particularly in the transmission of these pathogens, emphasizes the complexity of controlling phytoplasma diseases and the importance of integrated pest management strategies.

FUTURE PROSPECTS

The following key areas warrant attention:

Research and Surveillance: Ongoing research into the biology, ecology and epidemiology of phytoplasmas will enhance our understanding of their transmission dynamics and interactions with plant hosts. This knowledge will facilitate the development of targeted management strategies.

Molecular Characterization: Continued advancement in molecular techniques, such as PCR and RFLP analysis will provide robust tools for rapid identification and classification of phytoplasma strains. The updated classification system, with 89 subgroups and 29 groups, will be invaluable for researchers and agricultural practitioners in diagnosing and managing phytoplasma diseases.

Integrated Management Strategies: Effective management strategies should integrate molecular diagnostics, resistant crop varieties and cultural practices to mitigate the impact of phytoplasma infections. Education and training of farmers about the identification of symptoms and pest management will also be critical in controlling disease spread.

Policy and Extension Services: Development of policy frameworks to support research and extension services will enhance the dissemination of knowledge regarding phytoplasma management. This approach will empower farmers and stakeholders to adopt best practices in crop management.

Focus on Biodiversity: Protecting biodiversity through the conservation of native plants and natural enemies of phytoplasma vectors can help in the natural control of these diseases, thereby safeguarding agricultural productivity and ecosystem health.

Overall, addressing the challenges posed by phytoplasmas requires a multifaceted approach involving research, diagnostics and community engagement. By prioritizing these areas, the agricultural sector can manage the impact of phytoplasma diseases effectively, ensuring the sustainability of crop production and the protection of biodiversity in India as well as across the world.

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