Sri Venkateswara College

Innovative Teaching

Dr. Manoj Thakur
Assistant Professor
Department of Botany

Title: Basic skills and aspects of presentations related to science topics.

The Students of the second semester of Botany (Hons.) showed interest to learn about the basic skills and aspects needed to present any science topics. Considering this curiosity of the students, they were addressed in the classroom for the same. Basic learning procedure related to the PowerPoint presentations (font style size, colour themes, animations etc.) and the topic of interest (Phytoplasmas) were discussed. Moreover, 7 groups of students were made and each student within a group was assigned with different subtopic from the main topic "Phytoplasmas". General instructions were given to cover the topics during preparing and presenting PowerPoints. Students were made familiar with the plagiarism and citations or referencing. Two weeks of time was given and then the One-day students' seminar (presentations) was conducted on 25.5. 2023 in the Honours Lab of Botany Department on the title, 'Molecular and biological properties of Phytoplasma', related to their DSC- 4 (Microbiology and plant body interaction) subject. The pdf of the individual slides by the students has attached to this report at the end.

Figure 1: Group 1 participants- Kashish, Insha, Deepali, and Kritika while presenting.

GROUP 1 presented about the introduction, history, discovery and molecular detection of Phytoplasma. This group discussed that Phytoplasmas are minute, unicellular prokaryotes which are pathogenic to plants specifically. They are also known as Mycoplasma like organisms due to various similarities. They were first seen in China, around 1000 years ago, followed by their discovery in Japan in 1967. With the advancement in microscopy and technologies, scientists around the world were able to detect them through various technologies such as: Polymerase Chain Reaction, Real -time PCR, Loop- mediated isothermal amplification, DNA microarrays, Next- generation sequencing and digital PCR. Kashish, Insha, Deepali, and Kritika were present in this group.

Group 2 participants (Mansi, Shreyasri, Nikita, Rupam, and Manisha) discussed about the classification of Phytoplasma. Phytoplasma are classified into class Mollicutes, Order Acholeplasmatales, Family Acholeplasmataceae and Genus *Candidatus*. Phytoplasma were explained in detail. A major molecular characteristic for the definition and identification (sequences in the 16srRNA genes) were discussed in great detail. It was also concluded that the classification can be redefined as further discovery of different genera and strains takes place.

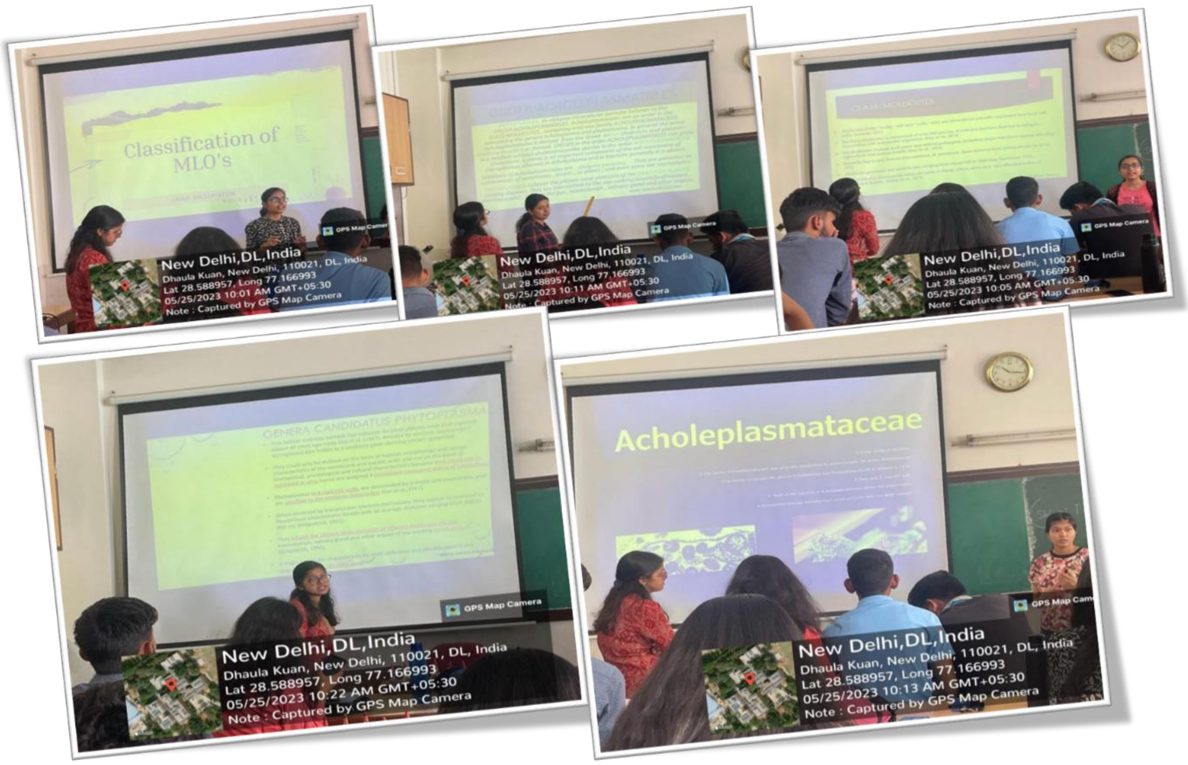


Figure 2: Group 2 participants, Mansi, Shreyasri, Nikita, Rupam, and Manisha in action.

Group 3 (Mohit, Priyanka, Anoushka, Sushant) participants discussed about the maintenance and mutations of phytoplasma. They highlighted the fact that since the late nineteenth century, microbiology has relied on the ability to grow pure cultures of microorganism, propagation and mutagenesis of microorganisms on artificial media which enabled the isolation, maintenance, propagation and mutagenesis of microbes. There are different aspects for the maintenance of phytoplasmas such as disease surveillance, sanitation and hygiene, insect control. They further added that mutations can occur in the genome of phytoplasma which leads to genetic variations that helped scientists to understand the evolution, pathogenicity, and epidermiology of phytoplasmas. Mutation can occur through various ways such as Horizontal gene transfer and through insertions and deletions in genetic makeup of phytoplasmas.

Group 4 participants such as Yamini, Poorva, Mahak, and Shreya discussed about the genomics of phytoplasmas. Genomics is the study of structural, functional, evolution and mapping of the genome. The genomics of phytoplasma and mycoplasma are different as

Figure 3: Group 3 participants while presenting. Please refer text for more details

Figure 4: Group 4 participants

mycoplasma stops codon encodes for amino acid while phytoplasma genomics doesn't code for amino acid similar to all bacteria, plants and animals. Phytoplasma metabolic pathways are essential for growth, development, and survival, and are linked to the transport of nutrients within the phytoplasma cells. Membrane protein - encoding gene (ORF3) which is present in plasmid of OY-M is absent in insect transmission - deficient mutant OY-NIM implying, gene may be involved in insect-mediated transmission.

Group 5 (Samridhi, Adyasha, Aditya, Khushboo, and Kritika) discussed about the mechanisms to infect plants and insects. Phytoplasma enters insects during feeding on infected plants. It replicates within the insect's gut, then migrates from the gut to the insect's salivary glands and when the insect feeds on a healthy plant, phytoplasma is transmitted through saliva. Phytoplasma enters the plant's phloem disturbs it's regularity, causing infection and disease.

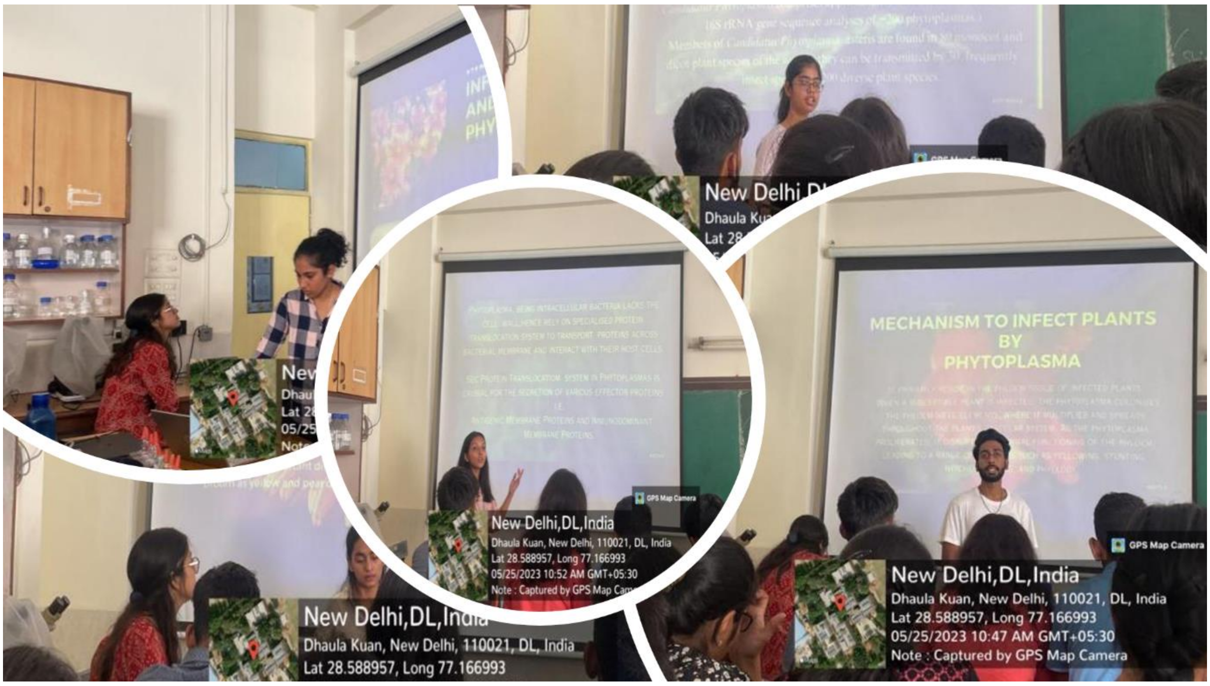


Figure 5: Group 5 (Samridhi, Adyasha, Aditya, Khushboo, Kritika) participants while presenting.

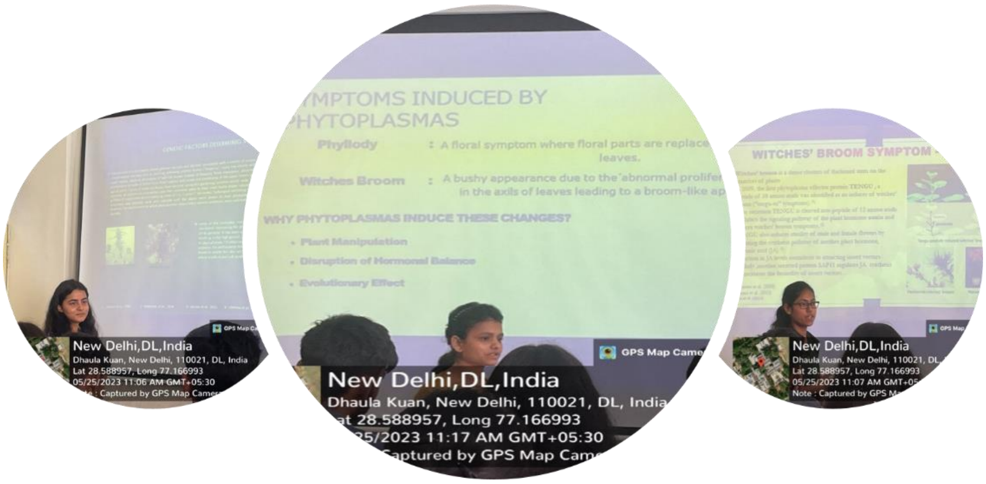


Figure 6 : Group 6 students

Group 6 (Pooja, Pankaj, Prachi, and Priyanshi) discussed about the genetic factors determining symptoms as phytoplasma infected plants exhibit various symptoms such as witches' broom, phyllody, purple top, floral virescence, phloem necrosis, etc. **Witches' broom** is abnormal proliferation of axial buds leading to enormous branching in plants. Witches' broom is induced by an effector protein called **TENGU** which inhibits auxin synthesis. Phyllody is formation of leaf like tissues instead of flowers i.e floral parts are replaced by small green leaves. Purple top is reddening of axial parts or young leaves which develops due to accumulation of anthocyanin.

Rathendra, Grace, and Chandrashekhar made the Group 7 and I (Dr. Manoj Thakur) was impressed with the excellent presentation skills of this group. They reinforced the facts about detection of phytoplasmas which includes methods such as tetracycline recovery of plant, TEM scanning, DAPI staining, DFD method, PCR Amplification and LAMP. They also highlighted the role of antibiotics for controlling phytoplasma.

Figure 7: Group 7 students presenting in Honours lab of Botany Department.

Conclusions and learning outcomes.

The session was concluded by Kashish (one of the student) where she stated that Phytoplasma are minute, unicellular prokaryotic bacteria's which are pathogenic to plants. It was first seen in China followed by its discovery in Japan by a Japanese scientist in 1967. These causes a variety of symptoms in plants such as virescense, phyllody, proliferation of auxiliary roots and many more. This pathogen mainly resides in phloem tissue of a plant. Phytoplamsa is also known as mycoplasma like organism due to its various similarities with them, like prokaryotic, parasitic and pleomorphic. Earlier it was not easy to detect these bacterias but with advancement in microscopy and technology, scientists were able to detect these via various methods such as: Polymerase chain reaction, Real-time PCR, Loop- mediated isothermal amplification, DNA microarray and Next - generation sequencing. The technological and microscopic advancement has also led to their classification which is based on: genetic analysis, phylogenetic, host-plant range and symptomatology. To maintain and prevent plants from the infection we need to keep in mind some of the maintenance aspects like disease surveillance, sanitation and hygiene, insect control and vector control. With their more detailed study, we have also come across with their mutations which could result from various factors such as external factors or interactions with other organisms , the ways are horizontal gene transfer or through insertions and deletion in the genetic makeup , via recombination, point mutations and gene duplication. The detection of phytoplasms can be done through various methods which includes tetracycline recovery of plants, TEM scanning, DAPI staining, DFD method, PCR amplification and LAMP. For controlling their growth, tetracycline and rifampicin bases antibiotics are used.

Figure 8: Picture taken during conclusive remarks given to everybody

Reference

Namba S. Molecular and biological properties of phytoplasmas. Proc Jpn Acad Ser B Phys Biol Sci. 2019;95(7):401-418. doi: 10.2183/pjab.95.028. PMID: 31406061; PMCID: PMC6766451

DSC 4 PRESENTATION BY BOTANY HONS. 1ST YEAR

Group 1

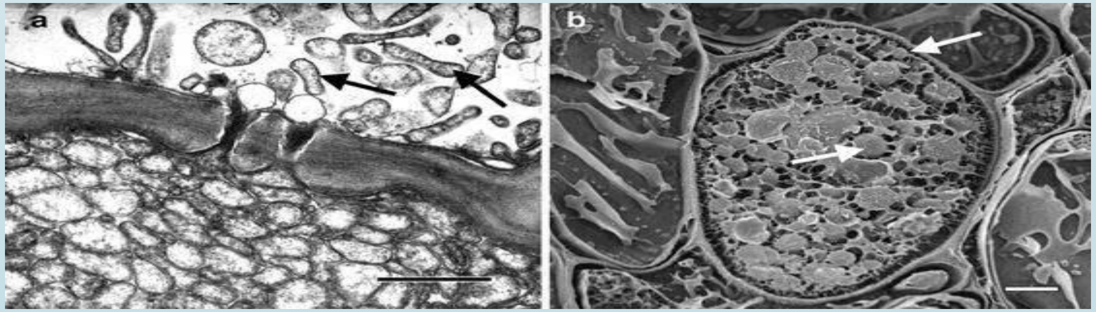
PHYTOPLASMA:BRIEF INTRODUCTION, HISTORY AND DISCOVERY OF PHYTOPLASMA AND MOLECULAR DETECTION OF MLOs.

KASHISH 1422003
INSHA 1422004
DEEPALI 1422007
KRITIKA 1422002

SUBMITTED TO
MANOJ THAKUR

BRIEF INTRODUCTION ON PHYTOPLASMA

-KASHISH
-1422003

- Phytoplasma , initially termed as mycoplasma – like organisms (MLO) ,is an obligate parasite of plants which are actually phloem- inhibiting bacteria and were discovered by a Japanese scientists in 1967.
- (Furch ACU, et al.,2021)
- They belong to the class mollicutes. They are very minute unicellular prokaryotic organisms that have a size ranging from 200 – 800nm .
- (Satta E , et al.)
- They are pleomorphic since they don't have a rigid cell wall and is having a single membrane.
- They are known to have the smallest genome.
- 

-KASHISH

- They live in plant phloem tissues, and their plant- to – plant transmission occurs via insect vectors such as psyllids and planthoppers , grafting and dodder plants.
- It thus infects phloem tissue by moving through the phloem Sap to congregate in mature leaves.
- The symptoms include virescence (loss of normal flower colour) ,phyllody (development of floral parts into leaf – like structures) , proliferation of auxiliary shoots resulting in 'witches broom' , sterility of flowers , yellowing and phloem necrosis.
- (Ishiie, 1965)

KASHISH

DIFFERENCE BETWEEN MYCOPLASMA AND PHYTOPLASMA

MYCOPLASMA

- Mycoplasma is a group of small typically parasitic bacteria that mainly infects animals but are pathogenic to plants as well.
- Type size of mycoplasma ranges between 150-250nm.
- It has various transmission modes.
- It has a unique cell membrane containing sterols.

PHYTOPLASMA

- Phytoplasma is a group of obligate bacterial parasites of plant phloem tissues mainly.
- It's size ranges between 200-800nm.
- It transmits mainly through insect vectors like leaf hoppers and psyllids.
- They have a three layered lipoprotein membrane.

-KASHISH

Similarities between mycoplasma and phytolasma

- They both are small prokaryotic microorganisms.
- Both of them lack cell wall.
- Both these groups are pleomorphic.
- Both of them are parasitic.

-KASHISH

HISTORY OF MYSTERIOUS PLANT DISEASES

- The earliest record of phytoplasma diseases was 1000 years ago ,Phytoplasma-infected tree peonies in Song, China.
- Another phytoplasma disease was found in Japan i.e, mulberry dwarf disease which was first observed about 200 years ago. This disorder is highly destructive to the production of mulberry leaves .
- Mulberry dwarf disease thus causes considerable damage to the silk manufacturing industry, which was a major export industry and important in the modernization of Japan.

(Ishiie,1965)

INSHA NAZIR

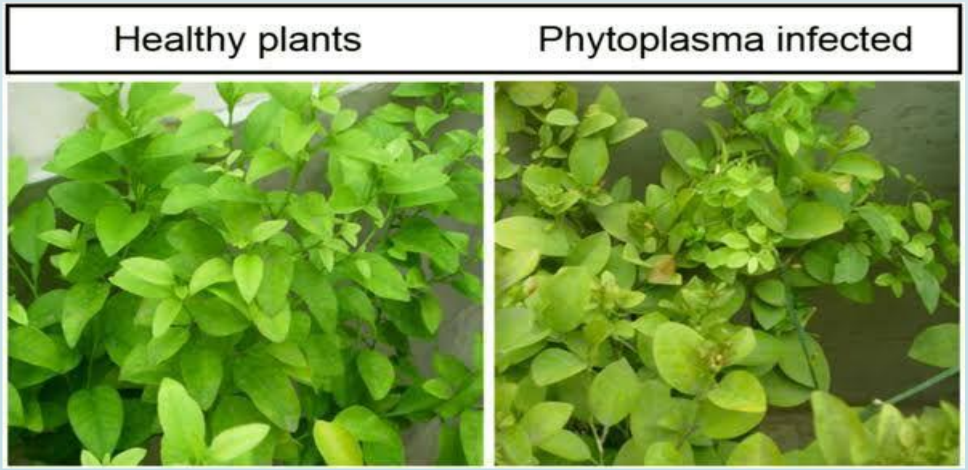
Fig. 1. The earliest record of a phytoplasma disease is evident in peonies, attributed to Zhao Chang, a court painter of the Song Dynasty of China. The two flowers on the middle-right exhibit floral virescence symptoms. Printed with permission of the Museum of the Imperial Collections, Sannomaru-Shozokan.

- In 1897, the National Diet set up the first national research committee to determine the cause of the disease, but the committee failed after researching the problem for 7 years. Later, the discovery of its transmissibility by insects and by grafting led to the hypothesis that mulberry dwarf disease was caused by a virus, although the pathogen remained undiscovered.
- After many years the history of phytoplasma research was traced back in the mid-1960s when scientists began to observe certain plant diseases that could not be attributed to any known viruses, bacteria, or fungi. These diseases, which affected a wide range of plant species, displayed symptoms such as yellowing, stunting, and deformations.
- It was not until the 1970s that researchers began to consider the possibility of a bacterial cause.

INSHA NAZIR

- In 1970, the term "phytoplasma" was coined by Japanese researchers who were studying the causal agent of a disease affecting rice plants known as "rice yellow dwarf." They proposed that these pathogens were bacterial in nature but lacked a cell wall, similar to mycoplasmas. But it was all hypothetical .

(Evelyn strauss , 2009)

- Throughout the 1970s and 1980s, researchers made progress in understanding phytoplasmas and their association with plant diseases. They developed culturing techniques and electron microscopy methods to visualize these organisms.
- 

INSHA NAZIR

- In the 1990s, molecular techniques such as polymerase chain reaction (PCR) became widely available and revolutionized phytoplasma research. PCR allowed researchers to detect and identify phytoplasmas directly from plant tissues, providing a more efficient and sensitive diagnostic tool. This led to the identification of numerous phytoplasma species associated with various plant diseases. Such as leafhoppers and psyllids, which play a crucial role in spreading phytoplasma-infected plants.

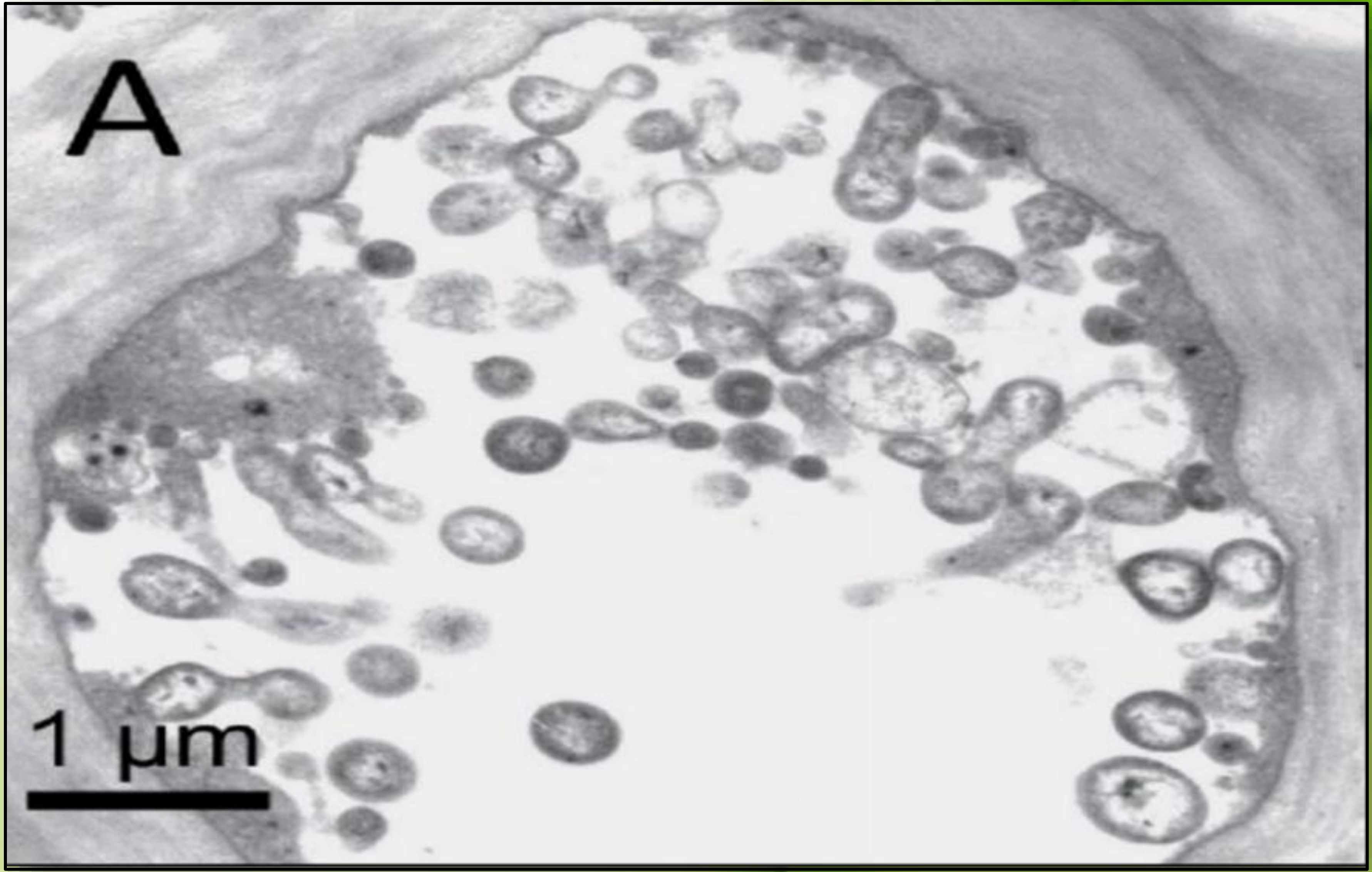
In 2001 Researchers have also identified various plant hosts including yellows, witches' brooms, and stolbur diseases etc.

(Davis, M.R and Raid , 2002)

INSHA NAZIR

Discovery of Mycoplasma-Like Organisms

Deepali Kumari
1422007
B.Sc.(H)Botany



Deepali Kumari
1422007
B.Sc.(H)Botany

Doi et al In 1967 using electron microscopy first described the consistent presence of small pleomorphic bodies resembling mycoplasma.

(By Shigetou Namba)

In the section phloem siene element of plants infected with mulberry dwarf and other typical yellow disease but not in healthy plant.

These microbes resembled mycoplasma in their cell size and sensitivity tetracyclic which inhibit protein synthesis with 30s ribosomes in prokaryotic.

Deepali Kumari
1422007
B.Sc.(H)Botany

These similarities in biological and morphological properties named mycoplasma like organisms.

The discovery *MLOs* stimulated worldwide re-investigations numerous yellow diseases that assumed to be caused by virus .

- *Tetracycline – Mediated Recovery Of Mulberry Dwarf Symptoms*

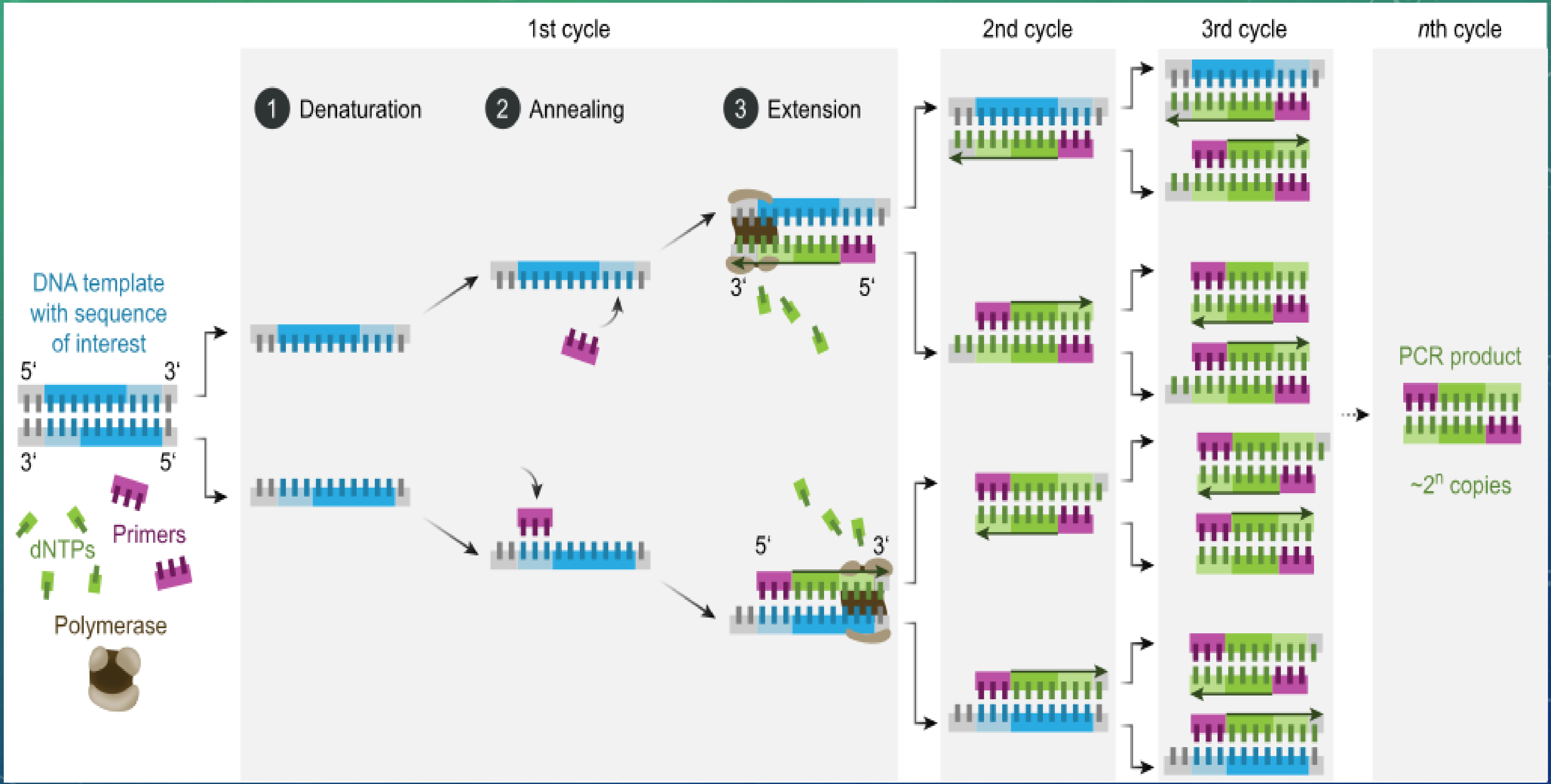
Deepali Kumari
1422007
B.Sc.(H)Botany

By several reports initially by electron microscopy revealed consistent association between MLOs and yellow diseases.

Deepali Kumari
1422007
B.Sc.(H)Botany

MOLECULAR DETECTION OF MLOS

- THE MOLECULAR DETECTION OF MLOS (MYCOPLASMA-LIKE ORGANISMS) TYPICALLY INVOLVES VARIOUS TECHNIQUES AIMED AT IDENTIFYING AND CONFIRMING THE PRESENCE OF THESE MICROORGANISMS. MLOS ARE A GROUP OF BACTERIA THAT LACK A CELL WALL AND CAN CAUSE DISEASES IN PLANTS, SUCH AS YELLOWS DISEASES IN CROPS. HERE ARE SOME COMMONLY USED MOLECULAR DETECTION METHODS FOR MLOS:
- 1. POLYMERASE CHAIN REACTION (PCR): PCR IS A WIDELY USED TECHNIQUE FOR DETECTING AND AMPLIFYING SPECIFIC DNA SEQUENCES. IN THE CASE OF MLO DETECTION, SPECIFIC PRIMERS TARGETING CONSERVED REGIONS OF MLO GENOMES CAN BE DESIGNED. IF MLO DNA IS PRESENT IN A SAMPLE, PCR AMPLIFICATION WILL GENERATE A SPECIFIC DNA FRAGMENT THAT CAN BE VISUALIZED AND CONFIRMED.
- 2. REAL-TIME PCR: REAL-TIME PCR, ALSO KNOWN AS QUANTITATIVE PCR (QPCR), ALLOWS FOR THE QUANTIFICATION OF THE MLO DNA PRESENT IN A SAMPLE. IT PROVIDES INFORMATION ON THE AMOUNT OF MLO DNA, WHICH CAN BE USEFUL FOR DISEASE MANAGEMENT AND MONITORING.
- 3. LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP): LAMP IS A SENSITIVE AND RAPID DNA AMPLIFICATION TECHNIQUE THAT CAN BE USED TO DETECT MLOS. IT OPERATES UNDER ISOTHERMAL CONDITIONS AND AMPLIFIES TARGET DNA WITH HIGH SPECIFICITY, MAKING IT SUITABLE FOR FIELD-BASED DIAGNOSTICS.
- ~ KRITIKA


1st cycle
2nd cycle
3rd cycle
nth cycle
1 Denaturation
2 Annealing
3 Extension
DNA template with sequence of interest
5‘
3‘
3‘
5‘
dNTPs
Primers
Polymerase
3‘
5‘
5‘
3‘
PCR product
~2n copies

~ Kritika

4. DNA microarrays: DNA microarrays or DNA chips consist of an array of probes that can detect the presence of specific MLO DNA sequences. The probes are designed to hybridize with complementary DNA fragments present in the sample, enabling the identification of MLOs.

5. Next-generation sequencing (NGS): NGS technologies, such as Illumina sequencing, enable high-throughput sequencing of DNA samples. This approach can be used to identify and characterize MLOs by sequencing their genomes or specific regions of interest. It provides detailed information about the genetic composition of MLOs.

6. Digital PCR: Digital PCR is a precise method for absolute quantification of target DNA. It partitions the PCR reaction into thousands of individual reactions and provides accurate quantification of the MLO DNA present in a sample.

These molecular detection techniques offer high sensitivity and specificity, enabling the reliable identification and quantification of MLOs in plant samples. The choice of method depends on factors such as the available resources, time constraints, and the specific research or diagnostic requirements.

Author- Sambade, A., & Oliveira, M. (2016). Molecular detection and characterization of phytoplasmas.

- Bertaccini, A., Duduk, B., & Paltrinieri, S. (2014). Molecular detection of phytoplasmas. Marcone, C. (2019). Molecular detection and identification of phytoplasmas

Kritika

GROUP 2

Classification of MLO's

GROUP PRESENTATION

Group 2

Members
MANSI
NIKITA
RUPAM
MANISHA
SHREYASHRI

~ microbes ~ microbesmicrobes

Introduction

What are MLO's

Mansi mavi 1422008

Basis of classification

- Genetic analysis
- phylogenetic
- Host plant range
- Symptomatology

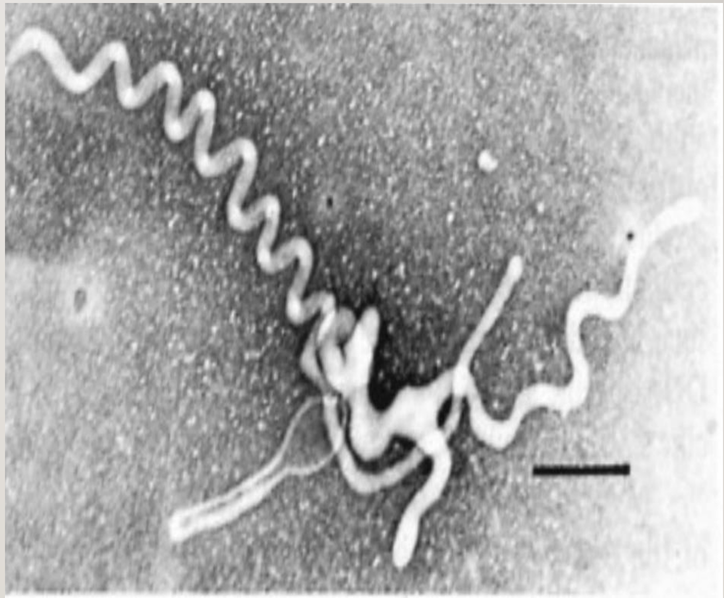
Ref: Namba *el .at 1993*

Mansi Mavi 1422008

CLASS: MOLLICUTES

- ***Mollicutes*** **(Latin "mollis," soft and "cutis," skin) are intracellular parasitic organisms that lack cell walls. Berman, 2012**
- **The Class *Mollicutes* is composed of over 200 species of wall-less bacteria that live in obligate association with eukaryotic organisms. May *et al*., 2014.**
- **The Mollicutes include both plant and animal pathogens, including major infectious agents affecting agriculture and human health. May *et al*., 2014.**
- **Recently has a new human mycoplasma, *M. penetrans*, been isolated from AIDS patients. Lo *et al*., 1992**
- **Mollicute genomes are small in size, ranging from about 600 to 2500 kbp. Herrmann, 1992.**
- **Most mollicutes incorporate large amounts of sterols, which serve as a very effective buffer of membrane fluidity. Stulke *et al*., 2019.**

- ***Spiroplasmas*** **are helical *Mollicutes*. So far, *spiroplasmas* are known to cause the stubborn disease in citrus plants and the brittle root disease in horseradish (*Spiroplasma citri*), stunt disease in corn plants, and a disease in periwinkle. Agrios *et al*., 2005.**
- ***Spiroplasmas*** **are resistant to penicillin but are inhibited by tetracycline. Agrios *et al*., 2005.**



Typical helical morphology of spiroplasma

Photographs courtesy of T.A. Chen, Rutgers

Brittle root disease in *Horseradish*

Periwinkle DISEASE

Stubborn disease in *Citrus* plants

Stunt disease in *Corn* plant

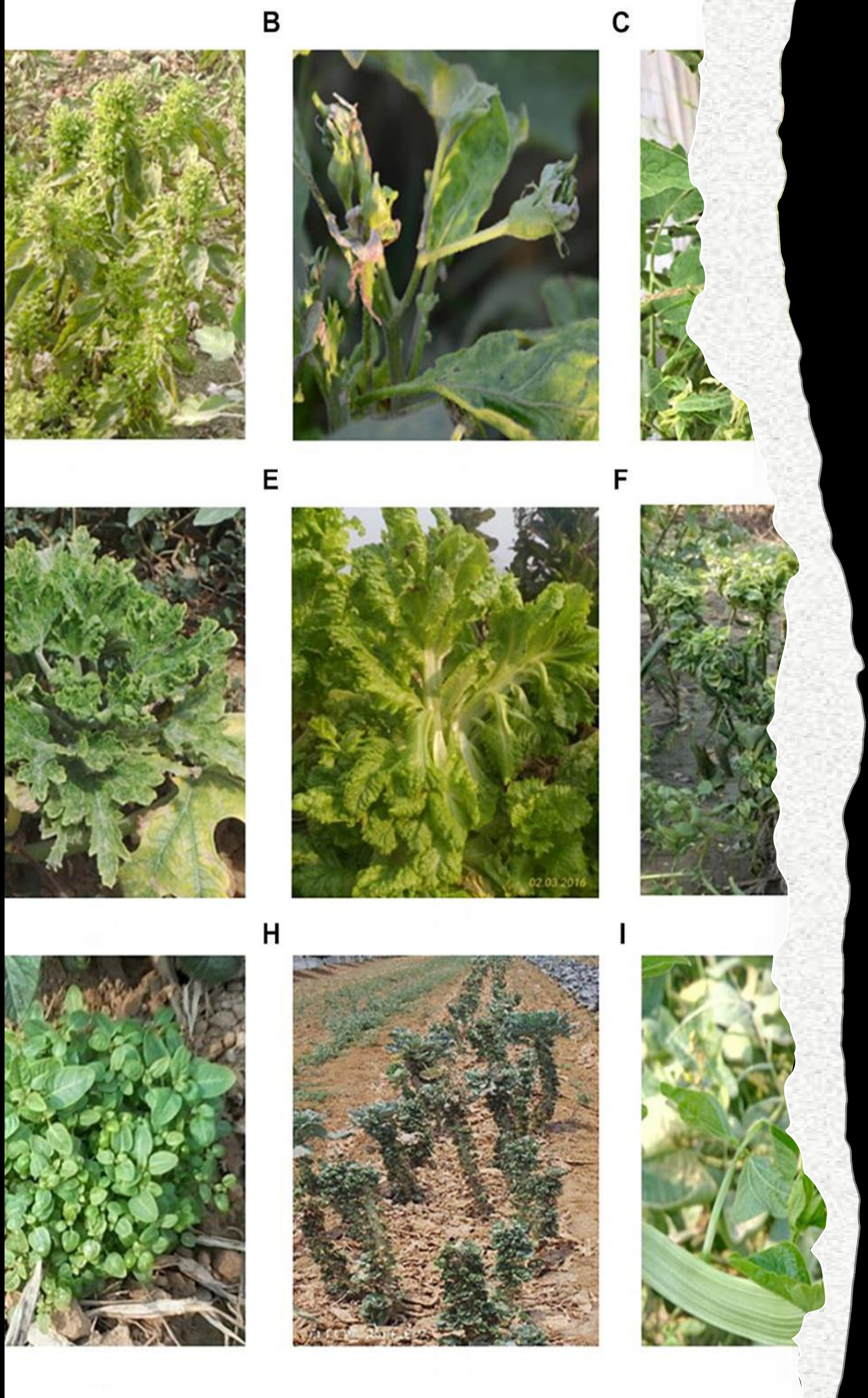
ORDER:ACHOLEPLASMATALES

PHYTOPLASMA, an obligate intracellular parasite belongs to the ORDER:ACHOLEPLAMATALES. Acholeplasmatales are an order in the CLASS:MOLLICUTES , containing only one family ACHOLEPlASMATACEAE, comprising the genera acholeplasma and phytoplasma. In general the word acholeplasmatales is derived from the Greek a=not chole=bile and plasma= anything moulded or formed. SPECIES in the order ACHOLEPLASMATALES can grow in a medium without cholesterol,unlike species in the order MYCOPLASMATALES. Cholesterol, a sterol, is an important component of the cell membrane of mycoplasmas,whereas in acholeplasma and in bacteria generally it is absent.

Members of Acholeplasmatales are Obligate parasites . They are parasites or commensals of vertebrates , insects , or plants ; and even some are saprophytes.

PHYTOPLASMAS colonize the phloem sieve elements of the VASCULAR PLANTS, causing diseases. They are transmitted by the sap-sucking insects{leafhoppers , planthopppers} , living in the gut , haemolymph , salivary gland and other organs.

NAME- SHREYA SRI { 1422009}



Acholeplasmataceae

.It is the family of Bacteria.

2.The family Acholeplasmataceae was originally established to accommodate the genus *Acholeplasma.*

3The family comprises the genera *Acholeplasma* and *Phytoplasma* found by Edward in 1970.

4.They lack of true cell wall.

5. Most of the bacteria of Acholeplasmataceae family are pleomorphic.

6.Acholeplasmataceae bacteria have small genomes With low **G+C** content.

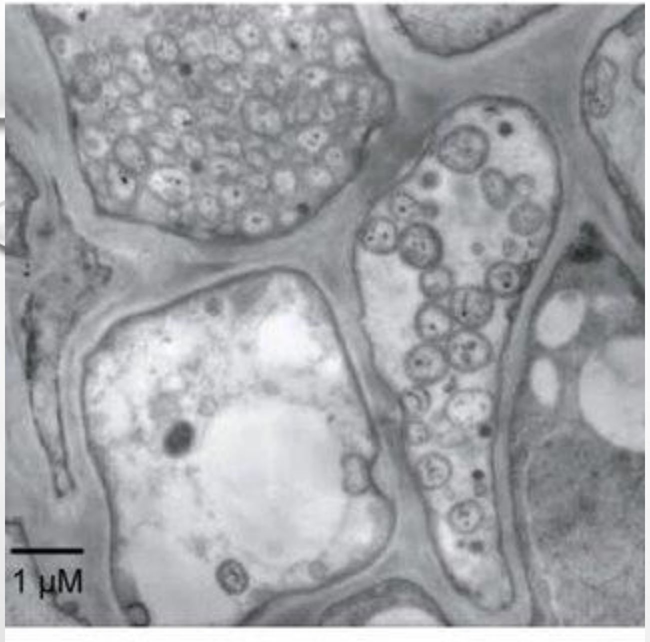

Electron micrograph of ***Phytoplasma***

DISEASE CAUSED BY ***Phytoplasma***

Manisha

Author :Abad Ja,Randall C,Moyer JW(1997)

GENERA *CANDIDATUS PHYTOPLASMA*



Electron microphotograph of *Candidatus Phytoplasma asteris* attacking plant phloem.

- Non helical wall-less bacteria that colonize the plant phloem were first reported almost 40 years ago when Doi et al. (1967) detected by electron microscopy mycoplasma-like bodies in a mulberry plant showing yellow symptoms.

- They could only be defined on the basis of habitat, morphology and certain characteristics of the membrane and nucleic acids and not on the basis of biochemical, physiological and cultural characteristics because they could not be cultivated in vitro hence are assigned a provisional taxonomic status of *Candidatus*.

- Phytoplasmas lack rigid cell walls, are surrounded by a single unit membrane, and are sensitive to the antibiotic tetracycline (Doi et al.,1967)

- When observed by transmission electron microscopy, they appear as rounded to filamentous pleiomorphic bodies with an average diameter ranging from 200 to 800 nm (Kirkpatrick, 1991).

- They inhabit the phloem sieve elements of infected plants and the gut, haemolymph, salivary gland and other organs of sap-sucking insect vectors (Kirkpatrick, 1991).

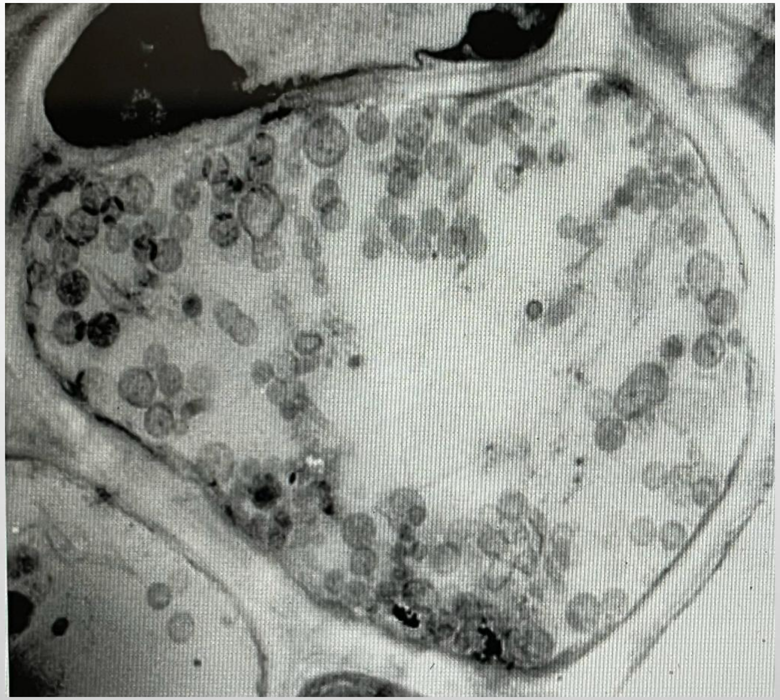
- A major molecular characteristic for their definition and identification is the sequence of their 16S rRNA gene.

NIKITA GHOSH (1422010)

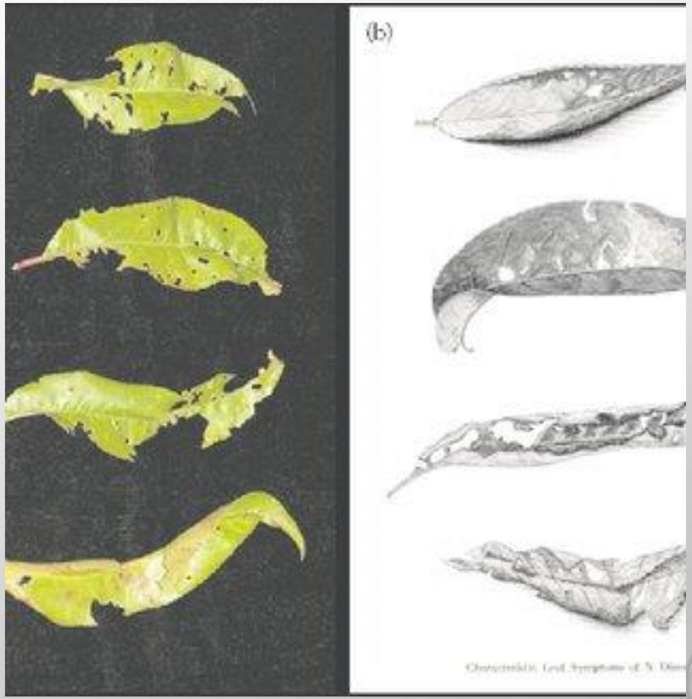
- Due to the distinctive sequences in their 16S rRNA gene, several phytoplasma specific PCR assays have been developed (Ahrens and Seemüller, 1992; Firrao et al., 1993; Lee et al., 1993;Namba et al., 1993; Gibb et al., 1995; Lorenz et al.,1995; Padovan et al., 1995; Smart et al., 1996).

- The genera includes phytoplasmas associated with other perennial fruit tree diseases, including European stone fruit yellow (‘*Candidatus Phytoplasma prunorum*’), peach yellow leaf roll, and pear decline (‘*Candidatus Phytoplasma pyri*’) (Seemüller et al., 1994). In contrast, most phytoplasmas infecting stone fruit in North America (X-diseases), including ‘*Candidatus Phytoplasma pruni*’ (Davis et al., 2013), are members of the Western-X disease group (Poggi Pollini et al., 2001).

Peach yellow leaf roll caused by *Candidatus Phytoplasma pyri*



Candidatus Phytoplasma aurantifolia



Candidatus Phytoplasma pruni causing X disease

NIKITA GHOSH(1422010)

Conclusion

Discovery of the undiscovered

Mansi Mavi 1422008

GROUP 3

MAINTANTENCE & MUTATIONS OF PHYTOPLASM

DSc-4
Group[3]:

-Anoushka

-Mohit

-Shreyanshu

-Sushant

MAINTENANCE OF PHYTOPLASMS

Phytoplasmas are specialized bacteria that infect plants and cause a variety of diseases. While it is not possible to directly maintain phytoplasmas outside of their plant hosts, there are several approaches and practices that can help manage and prevent the spread of phytoplasmal diseases. (Austin,B.,2017)

Since the late nineteenth century, microbiology has relied on the ability to grow pure cultures of microorganisms on artificial media. Such culture enables the isolation, maintenance, propagation, and mutagenesis of microorganisms of interest, and plays central roles in many studies of microbial taxonomyand plant pathology. However, it is difficult to culture phytoplasmas. Hence the importance in studying on how to maintain and propogate the phytoplasma (Shigouto,N.,2019)

PRIYANKA YADAV

FACTORS IN MAINTAINING PHYTOPLASMS

-

HOST PLANTS

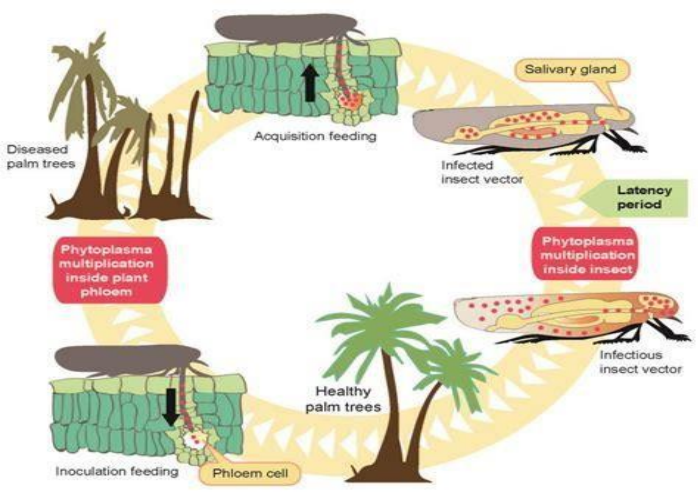
- Phytoplasms rely on host plants for survival and reproduction. They infect the phloem tissue of plants and colonize the sieve tubes, where they multiply and spread throughout the plant. Some phytoplasmas have a narrow host range and can only infect specific plant species, while others have a broad host range. This is why it is always important to maintain the host plants when using them for growth of phytoplasms

Phyllody induced by phytoplasma infection on a coneflower (*Echincea purpurea*)

Priyanka yadav

INSECT VECTORS

Phytoplasms are obligate parasites that require insect vectors for transmission. Infected insects acquire the phytoplasma while feeding on infected plants and transmit it to healthy plants during subsequent feeding. The phytoplasma replicates in the insect's body and moves to the salivary glands, from where it is injected into plants during feeding. Hence due this its important regulating the conditions appropriately when using a incest vector in order to propagate the phytoplasm



Phytoplasma life cycle

PRIYANKA YADAV

ASPECTS OF MAINTENANCE

ASPECTS OF MAINTENANCE

- Disease surveillance: Regular monitoring and surveillance of plants for symptoms associated with phytoplasm diseases are crucial. Early detection allows for timely intervention and control measures to minimize the spread of the pathogen.
- Sanitation and hygiene: Good agricultural practices, including proper sanitation and hygiene, are essential for disease management. Infected plants, plant debris, and weed hosts should be removed and destroyed to prevent the spread of phytoplasmas. This includes pruning and destroying infected plant parts, uprooting and disposing of severely infected plants, and controlling weed hosts that can serve as reservoirs for the bacteria.
- Insect control: Since phytoplasms are transmitted by insect vectors, managing the insect population is important for disease control. Integrated pest management (IPM) strategies that involve cultural, mechanical, and chemical control methods can help reduce the population of insect vectors. This includes the use of insecticides, barriers, traps, and cultural practices such as crop rotation and planting resistant varieties.

Mohit

ASPECTS OF MAINTENANCE

- Vector control: Targeting the insect vectors that transmit phytoplasmas can help break the disease cycle. Insecticides and biological control agents can be used to manage the population of vector insects. Timing of insecticide application should coincide with the activity period of the vectors to achieve maximum effectiveness.
- Quarantine measures: Implementing quarantine measures can help prevent the introduction and spread of phytoplasms to new areas. This includes restricting the movement of infected plant material, establishing quarantine zones, and enforcing regulations on the transportation and trade of plants.
- Research and education: Continuous research and education are important for understanding phytoplasm biology, improving diagnostic techniques, and developing effective management strategies. Scientists and extension services play a vital role in disseminating information, conducting outreach programs, and providing guidance to farmers and stakeholders regarding the identification, prevention, and control of phytoplasmal diseases.

SUSHANT

MUTATIONS OF PHYTOPLASMS

MUTATIONS IN PHYTOPLASMS

Mutations can occur in the genomes of phytoplasmas, leading to genetic variation among different strains. These mutations can arise spontaneously or be induced by external factors such as environmental stress or interactions with other organisms. The study of these mutations helps scientists understand the evolution, pathogenicity, and epidemiology of phytoplasmas.

The isolation of phytoplasma mutant lines was attempted to identify the determinants of differences in their phenotypes. OY-M (mildly pathogenic line) was isolated from OY-W, which has been maintained with the aid of plant and insect hosts for 20 years the mutant exhibits almost no plant pathogenicity.

(Shimoi T., et al, 1998)

Anoushka

HOW DO MUTATIONS OCCUR

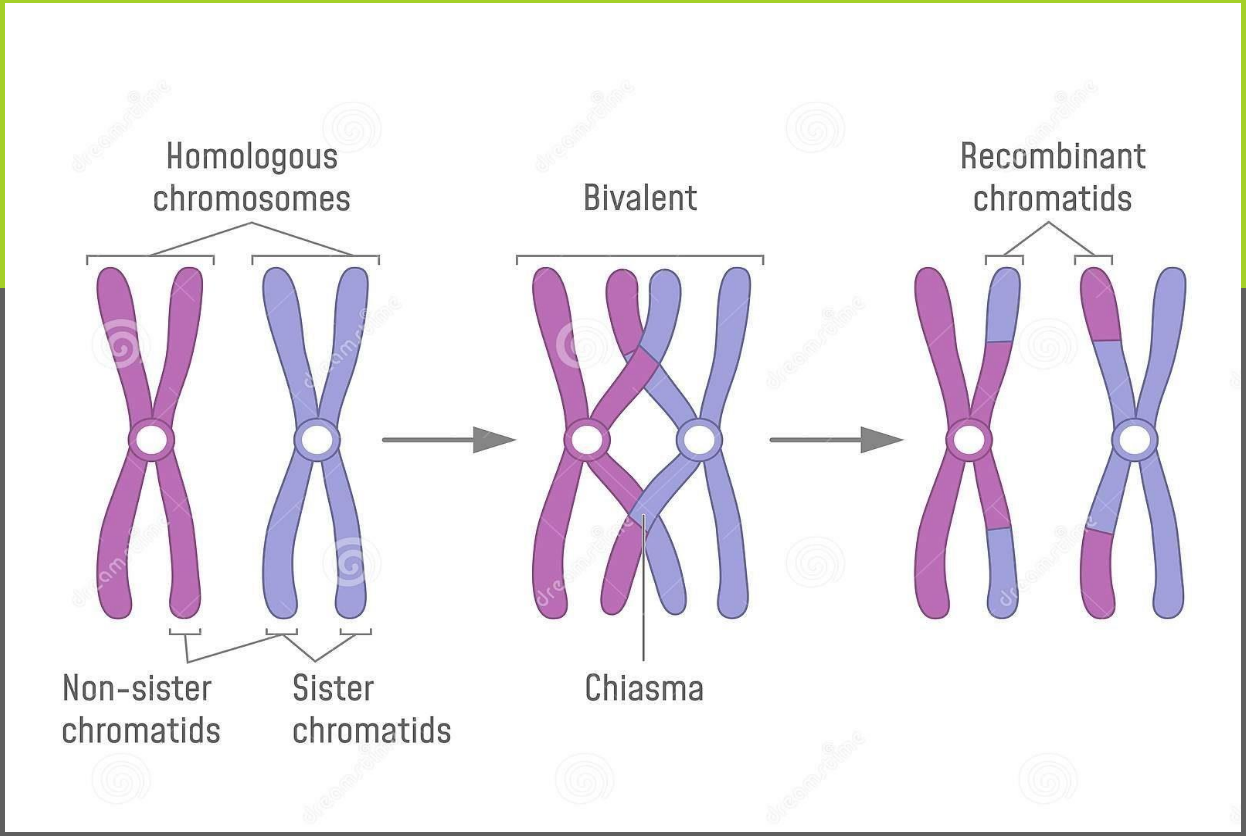
- Horizontal Gene transfer :Phytoplasms can acquire genes from other organisms through horizontal gene transfer (HGT). This process involves the transfer of genetic material between phytoplasms and other bacteria, including non-phytoplasma species. HGT can contribute to the evolution and adaptation of phytoplasmas by providing them with new genes or functions
- Insertions and deletions: These mutations involve the addition or removal of one or more nucleotides in the DNA sequence. They can cause frameshift mutations, which disrupt the reading frame and can lead to non-functional or truncated proteins.

Anoushka

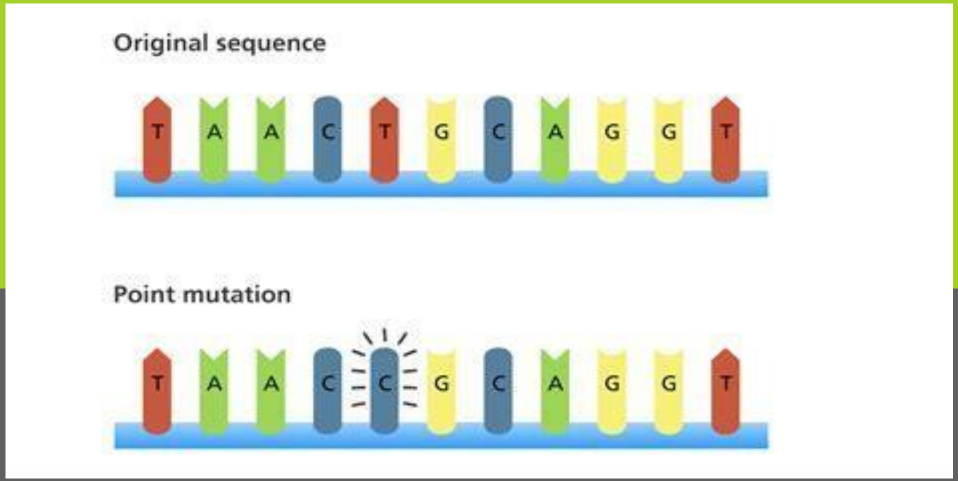
HOW DO MUTATIONS OCCUR?

- Recombination: Phytoplasms can undergo genetic recombination, which involves the exchange of genetic material between different phytoplasma strains. This can occur when two phytoplasmas infect the same plant or when a single plant is infected by multiple strains. Recombination can lead to the generation of novel phytoplasma variants with unique characteristics.
- Genetic Variation : Phytoplasms have a relatively small genome size and a high rate of genetic variation. Mutations occur naturally in the phytoplasma genome during replication, resulting in genetic diversity. These mutations can lead to changes in the virulence, pathogenicity, or transmissibility of the phytoplasma
- Point mutations: These are single-base changes in the DNA sequence. Point mutations can lead to amino acid substitutions in the proteins encoded by the phytoplasma genome, potentially altering their structure or function.
- Gene duplications: Phytoplasmas can acquire duplicate copies of genes through genomic rearrangements or horizontal gene transfer. Gene duplications can provide a source of genetic variation and contribute to the evolution of new traits in phytoplasmas.

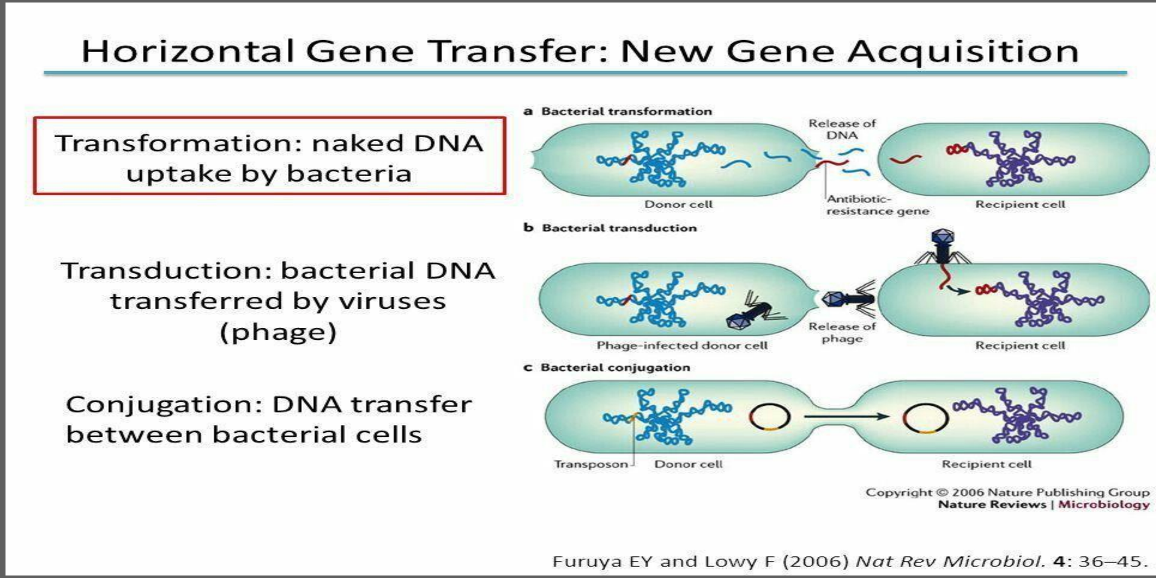
Shreyanshu



Recombination



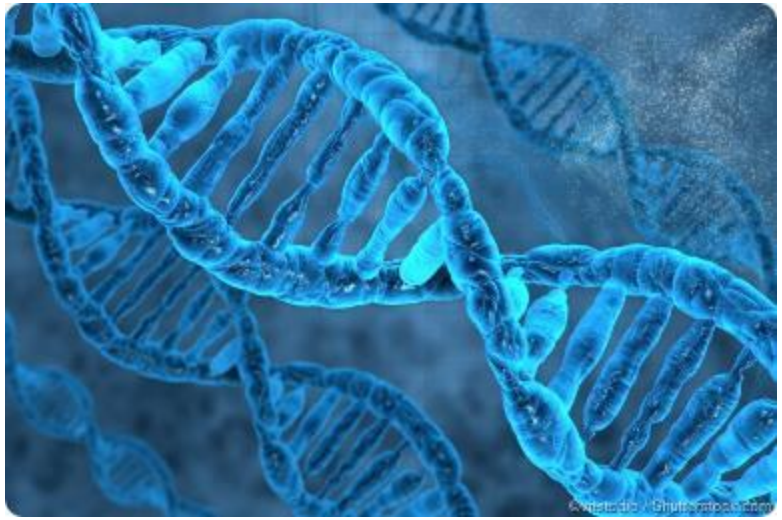
Point mutation



Horizontal Gene Transfer

GROUP 4

PHYTOPLASMA GENOMICS



GENOMICS

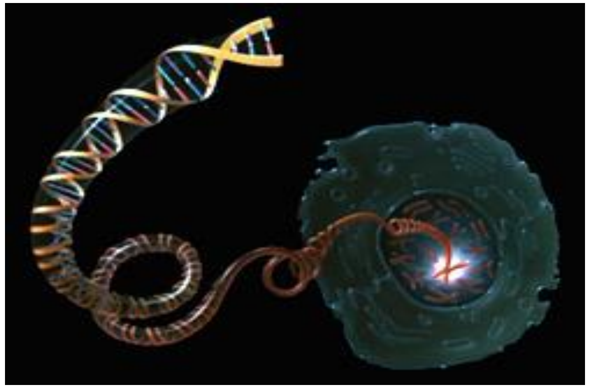
- Genomics is the study of structure, function, evolution and mapping of genomes.
- It involves analysing the complete DNA sequence and the associated genes in an organism.

(Willey, Sherwood and Woolverton,2009)

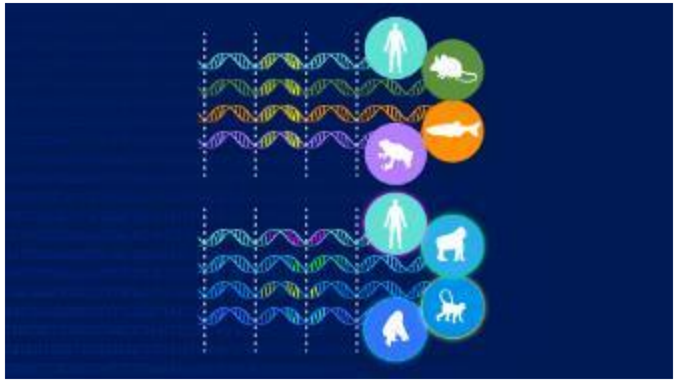
SHREYA
ROLL NO- 1422041

TYPES OF GENOMICS

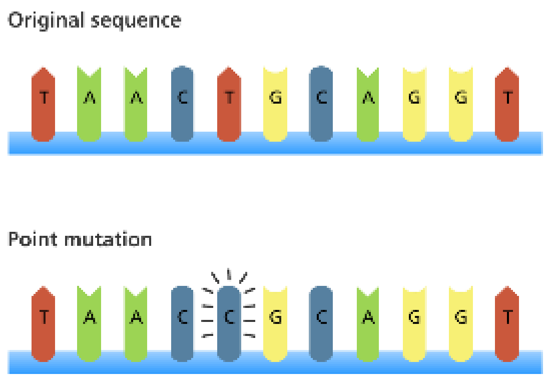
STRUCTURAL GENOMICS



FUNCTIONAL GENOMICS



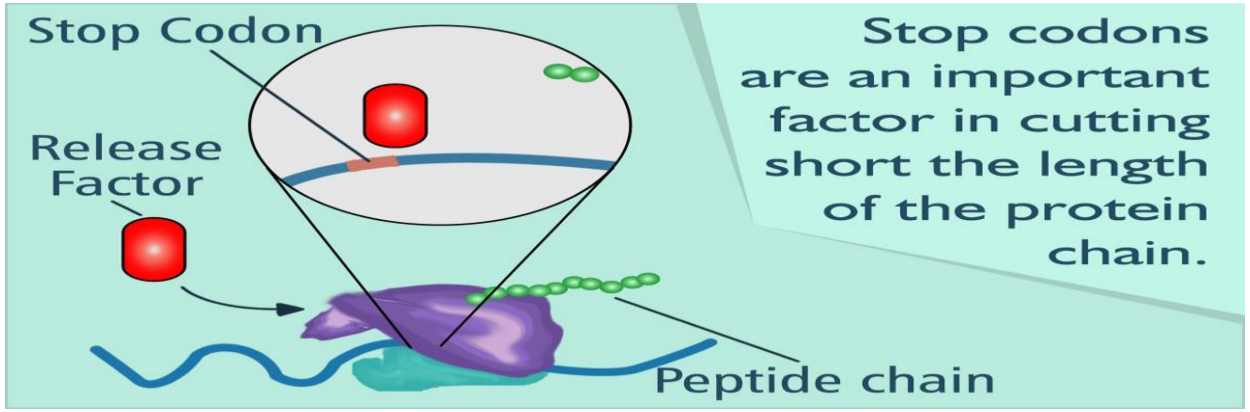
COMPARATIVE GENOMICS



MUTATIVE GENOMICS

SHREYA
ROLL NO- 1422041

- A stop codon is a sequence of three nucleotides (a trinucleotide) in DNA or messenger RNA (mRNA) that signals a halt to protein synthesis in the cell. There are 3 type of stop codons (i.e., UAA, UAG and UGA).



- Usage of UGA stop codon as a tryptophan-encoding codon by mycoplasma .
- Burdensome to overcome this experimental barrier by replacing UGA with the general tryptophan codon UGG.
- However, when several phytoplasma operons were analyzed to determine the numbers of presumed tryptophan UGA codons, it was found that, unlike mycoplasmas, every UGA was a functional stop codon (Furuki *et*.al.,2002).
- Moreover, a gene encoding peptide chain release factor 2 that recognizes UGA as a termination codon is present in the phytoplasma genome. This, combined with several other genetic features shared between phytoplasmas and common bacteria such as E. coli, prompted us to commence advanced molecular and genetic analyses of phytoplasms .

Mahak singh

Onion Yellow : OY-M

- 1994 - *Phytoplasma* whole-genome sequencing started,Japan.
- Onion yellows *Phytoplasma* OY-W and OY-M used.
- 2002 – Draft genome sequence : OY-W (Oshima et al.,2002)
- 2004 - First complete genome sequence : OY-M .(Oshima et al., 2004)
  - 860,631 bp
  - Circular
  - G+C = 28%
  - 754 ORFs (73%)
  - Metabolic genes and transporter genes
  - "A little life without work"

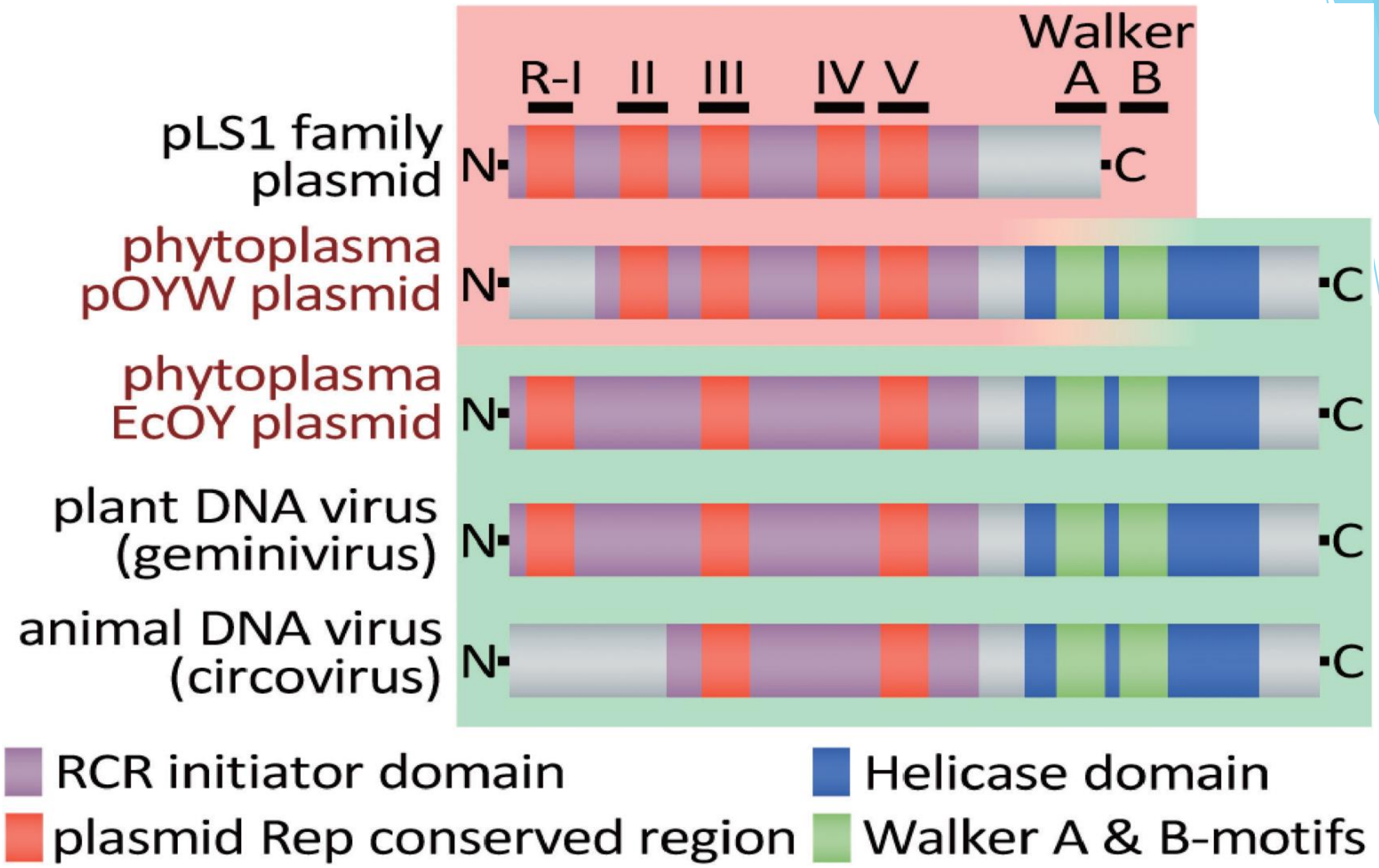
Yamini Raina

Phytoplasma genome

- ❑Duplicated genes
- ❑Potential mobile units (Bai et al.,2006)
- ❑Conservative manner organization (Arashida et al.,2008)
- ❑Gene expression regulation (Toruño et al.,2010)
- ❑Evolution in insect symbiosis and plant parasitism (Oshima et al.,2007)
- ❑Plasmids
- ❑Chimeric replication proteins (Reps)

Membrane protein-encoding gene (ORF3) : Lack in OY-NIM plasmid.

Yamini Raina



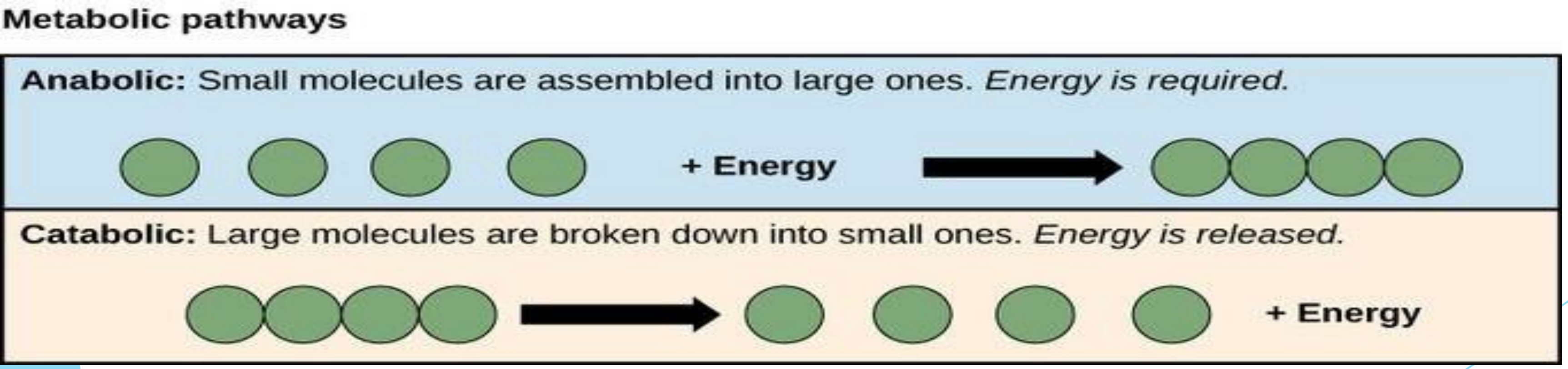
The chimeric “Rep” proteins of *Phytoplasma* plasmids may be the missing link between bacterial plasmids and DNA viruses.

Yamini Raina

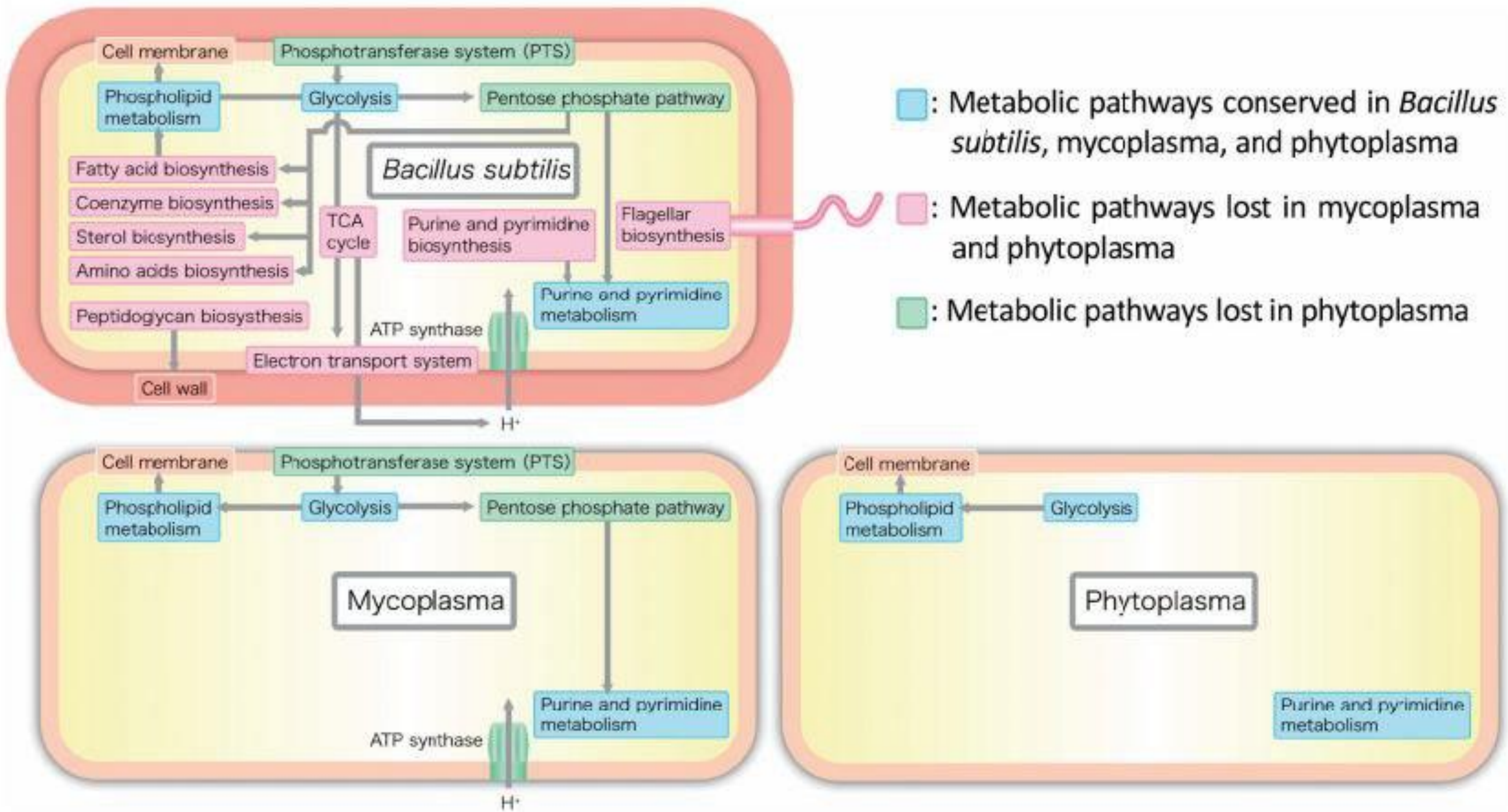
Metabolic pathway in *Phytoplasma*

- A metabolic pathway is a series of chemical reactions in a cell that build and breakdown molecules for cellular processes

- There are two types of metabolic pathway
- 1.Anabolic pathways synthesize molecules and require energy.
- 2.Catabolic pathways break down molecules and produce energy.



POORVA


Cell membrane
Phosphotransferase system (PTS)
Phospholipid metabolism
Glycolysis
Pentose phosphate pathway
Fatty acid biosynthesis
Coenzyme biosynthesis
Sterol biosynthesis
Amino acids biosynthesis
Bacillus subtilis
TCA cycle
Purine and pyrimidine biosynthesis
Flagellar biosynthesis
Purine and pyrimidine metabolism
Peptidoglycan biosysthesis
ATP synthase
Electron transport system
Cell wall
H+
: Metabolic pathways conserved in Bacillus subtilis, mycoplasma, and phytoplasma
: Metabolic pathways lost in mycoplasma and phytoplasma
: Metabolic pathways lost in phytoplasma
Cell membrane
Phosphotransferase system (PTS)
Phospholipid metabolism
Glycolysis
Pentose phosphate pathway
Mycoplasma
Purine and pyrimidine metabolism
ATP synthase
H+
Cell membrane
Phospholipid metabolism
Glycolysis
Phytoplasma
Purine and pyrimidine metabolism

POORVA

Phytoplasma metabolic pathways

- Essential for the growth, development, and survival
- Help the phytoplasm obtain energy and nutrients
- Involved in the production and regulation of certain enzymes and proteins
- Closely linked to the transport of nutrients within the *phytoplasmic* cells.
- Can adapt to changes in the host plant's environment

POORVA

THANK YOU!!

GROUP 5

MECHANISM TO INFECT PLANTS AND INSECTS BY PHYTOPLASMA.

PRESENTED BY

SAMRIDHI 1422043
ADYASHA 1422044
ADITYA 1422045
KHUSHBOO 1422046
KRITIKA 1422047

Submitted to
Manoj Thakur Sir

INTRODUCTION

- Phytoplasmas are bacterial pathogens that cause diseases in plants and insects. They are transmitted by insect vectors such as leafhoppers, planthoppers.

- The mechanism of phytoplasma infection involves the attachment of the bacteria to the insect vector's gut epithelium and eventual transmission to the plant host.

- The mechanism by which phytoplasmas infect plants and insects involves a complex interplay between the pathogen, the host plant, and the insect vector. Here's a general overview of the process

SAMRIDHI

Transmission to plants: Phytoplasmas are primarily transmitted to plants by insect vectors, such as leafhoppers, planthoppers, and psyllids.

Activities within insects: Once inside an insect vector, phytoplasmas colonize the insect's digestive system, salivary glands, or other tissues.

Acquisition and inoculation: When the infected insect feeds on a healthy plant, phytoplasmas are released into the plant's phloem tissue through the insect's saliva during feeding.

Phloem colonization: They invade the phloem cells, disrupting their normal functioning and altering the plant's physiology.

MECHANISM TO INFECT PLANTS BY PHYTOPLASMA

It primarily reside in the phloem tissue of infected plants. When a susceptible plant is infected, the phytoplasma colonizes the phloem sieve elements, where it multiplies and spreads throughout the plant's vascular system. As the phytoplasma proliferates, it disrupts the normal functioning of the phloem, leading to a range of symptoms such as yellowing, stunting, witches' brooms, and phyllody.

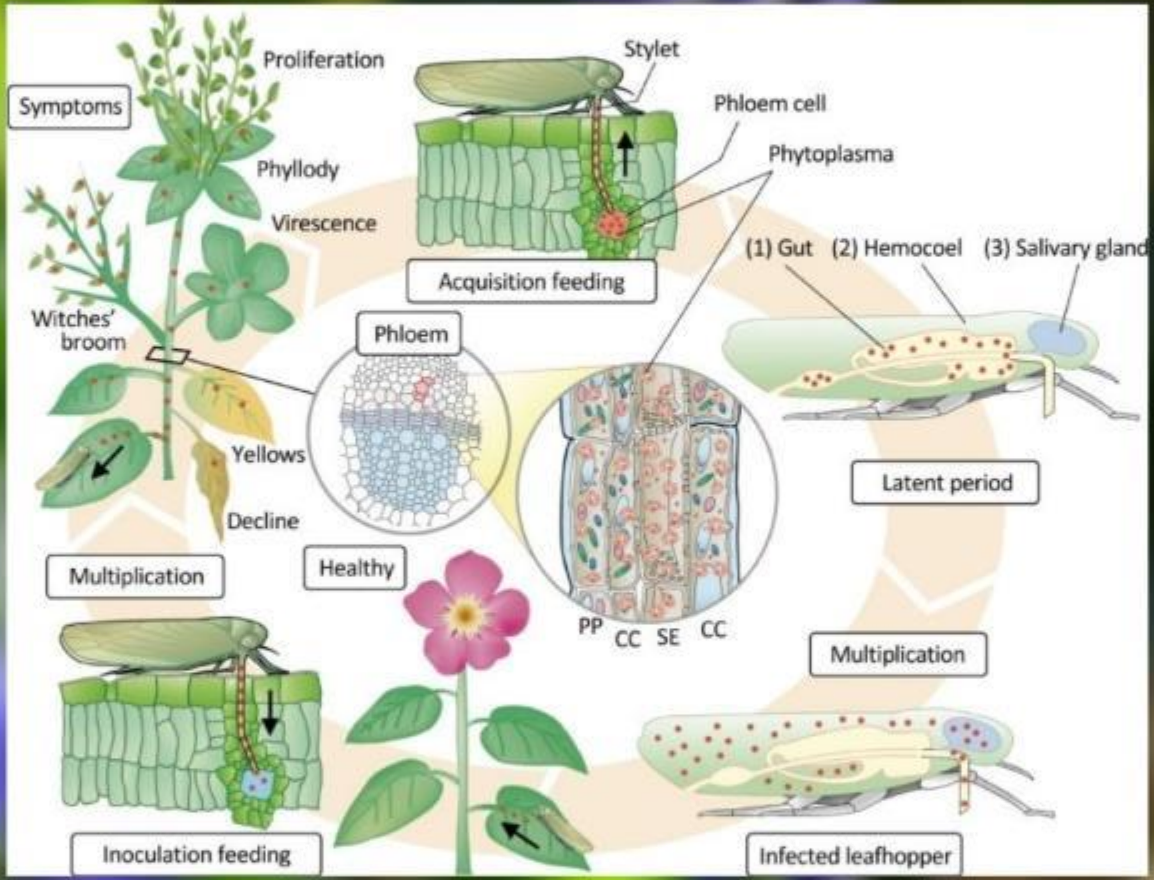
ADITYA

MECHANISM TO INFECT ANIMAL BY PHYTOPLASMA

Phytoplasma enters insects during feeding on infected plants.
It colonizes and replicates within the insect's gut.
Phytoplasma migrates from the gut to the insect's salivary glands.
When the insect feeds on a healthy plant, phytoplasma is transmitted through saliva.
Phytoplasma enters the plant's phloem, causing infection and disease.

ADITYA

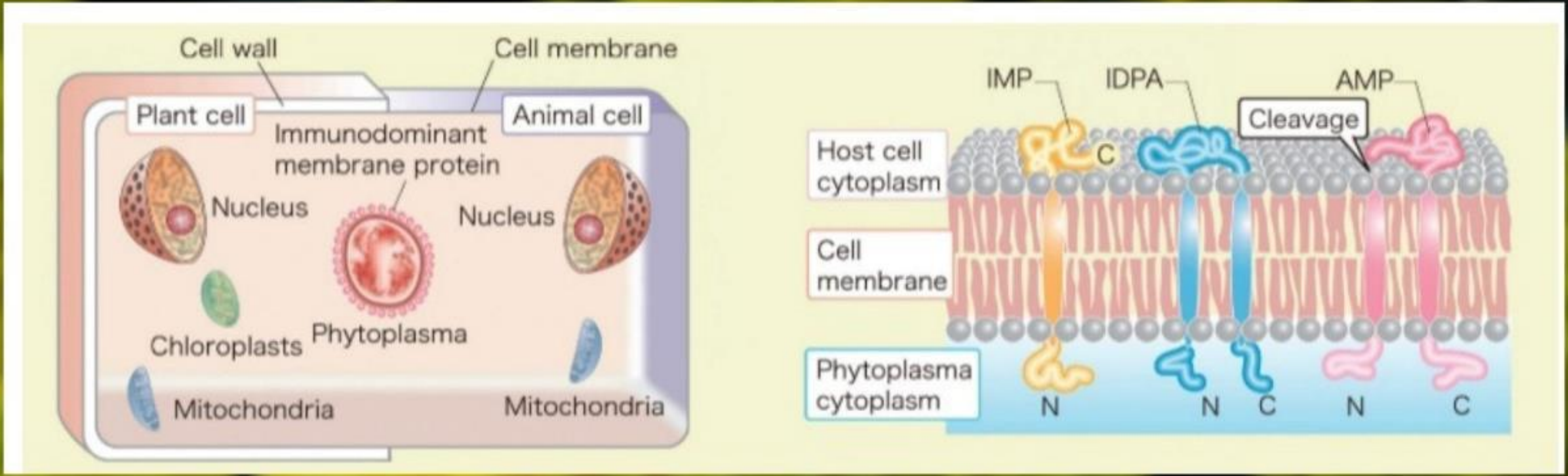
MECHANISM TO INFECT PLANTS AND INSECTS BY PHYTOPLASMA.



Phytoplasma movement pathway

Phloem sap of infected plant > stylet > Gut > salivary gland > Stylet > Phloem sap of not infected plant

ADITYA AND KRITIKA



The immunodorminant membrane proteins of phytoplasmas and their localisation. As most of the phytoplasma cell surface is covered with three membrane proteins termed immunodorminant membrane proteins (IMP,AMP and IDPA),which are thought to be involved in interactions with both insect and plant host.

KRITIKA

Phytoplasma, being intracellular bacteria lacks the cell wall,hence rely on specialised protein translocation system to transport proteins across bacterial membrane and interact with their host cells.

Sec Protein Translocatiom system in Phytoplasmas is crusial for the secretion of various effector proteins i.e,
Antigenic Membrane Proteins and Immunodominant Membrane Proteins.

KRITIKA

AMP participates in ATP synthesis.

In the context of phytoplasma metabolism, nucleotides like AMP and IMP are likely to be essential for various cellular processes, including DNA and RNA synthesis, energy production, and signaling pathway.

Phytoplasmas, being intracellular bacteria, require nucleotides for their own metabolic needs and to interact with their host plants.

author -suzuki and jung;2004

KRITIKA

PHYTOPLASMA HAVE A BROAD HOST RANGE WHICH INCLUDES PLANT HOST AND VECTORS(INSECT HOST)

THEY ARE TRANSMITTED FROM PLANT TO PLANT VIA VECTORS I.E INSECTS BUT THEY ARE NOT TRANSMITTED TRANSOVARIALLY(VIA INFECTED EGG)

FEW FACTORS LIKE AVAILABILITY OF VECTOR AND CERTAIN HOST PROTEIN(AMP, IMP, IDPA) ENSURES THE SPREAD OF PHYTOPLASMA (PLANT ACTIN ALSO PLAYS AN IMP ROLE IN SPREAD OF PYTOPLASMA)

Candidatus Phytoplasma comprises approximately 30 distinct clades (based on 16S rRNA gene sequence analyses of ~200 phytoplasmas.)

Members of *Candidatus Phytoplasma* asteris are found in 80 monocot and dicot plant species of the world . they can be transmitted by 30, frequently insect species, to 200 diverse plant species.

ADYASHA

Members of Candidatus Phytoplasma asteris are found in 80 monocot and dicot plant species of the world . they can be transmitted by 30, frequently insect species, to 200 diverse plant species.

Aster Yellows Phytoplasma ,subclade Candidatus Phytoplasma asteris is the largest among the Candidatus Phytoplasma subclades

ADYASHA

Examples-

- Aster Yellows phytoplasma strain Witches' Broom (AY-WB) can be transmitted by the polyphagous Macrosteles quadrilineatus to China aster and lettuce
- Flavescence dorée phytoplasma has 2 types both of which can be transmitted by the same leafhopper vector Scaphoideus titanus
- The solanaceous vegetable species are infected by various groups of phytoplasmas
- In potatoes, five phytoplasma groups (16SrI, 16SrII, 16SrIII, 16SrVI, and 16SrXII) and seven subgroups (16SrI-B, 16SrI-C, 16SrII-A, 16SrIII-B, 16SrVI-A, 16SrVI-C, and 16SrXII-A)

Phytoplasma vectors -

Most known vectors are leafhoppers (Cicadellidae) ; fewer are known among the planthoppers (Fulgoroidea) and only two genera of jumping plant lice (Sternorrhyncha: Psyllidae).

ADYASHA

CONCLUSION

Phytoplasma are bacterial plant patogen consisting of more than 50 phylogenetic group that cause devastating diseases in various crop worldwide. They are obligate parasite restricted to the phloem tissue of the host plant and are transmitted from plant to plant mostly by leaf hoppers. They reproduce within the tissue of their insect vector and are transfered in the salivary secretion to new host plant during feeding phytoplasma apidemiology involves a tritrophic relationship between the pathogen and several host and vector.

The interaction between phytoplasma and their vector hosts are complex and influence by insect vectoring abilities and the consequences of infection for vector Aster yellows, the primary vector of which is the leaf hopper (forvs) is the most common and widespread . Clover proliferation also transmitted by leafhopper is the casual agent of important diseases such as Clover proliferation and alfalfa which is broom as yellow and pear decline have caused economic problems for sevral decades

KHUSHBOO

THANK YOU.

GROUP 6

GENETIC FACTORS DETERMINIG SYMPTOM DEVELOPMENT

- Phytoplasma-infected plants exhibit phloem necrosis and decline[1] associated with a variety of unique morphological changes such as stunting, yellowing witches broom ("tengu-su", many tiny shoots with small leaves), phyllody (formation of leaf-like tissues instead of flowers), floral virescence; abnormal proliferation (growth of shorts from floral organs), and purple top (reddening of the upper leaves and apical parts).[2] Some of these attributes have become successful gardening varieties world- wide. For example, all commercial poinsettias owe their economic value to their small bushy shape induced by grafting of a poinsettia branch-inducing strain of 'Ca. P. pruni'.[3] Moreover, hydrangeas exhibiting floral virescence and phyllody were very valuable until the plants were shown to host phytoplasmas. However, the mechanisms by which phytoplasmas induce these various symptoms were unknown until recently

Floral Virescence

Abnormal Proliferation

Stunting

Phyllody

Witches Broom

- Some of the molecular mechanisms by which phytoplasmas induce their typical symptoms have gradually been elucidated. Comparing the genome sequence of OY-Q and OY-M revealed duplication of glycolytic gene clusters in the OY-W genome. It has been suggested that this difference is responsible for the high consumption of carbon sources, resulting in the high growth rates and severe symptoms, such as yellowing, dwarfism and decline associated with OY-W phytoplasmas.[4] Furthermore, the mechanisms of purple top symptoms have been revealed. Phytoplasma infection activates the anthocyanin biosynthetic pathway. Increased accumulation of anthocyanin not only changes the color of leaves to purple but also act as antioxidant that protects plant cells from damage caused by reactive oxygen species, which results in leaf cell death.[5]

1 - Uehara et al., 1999 *2 - Maejima et al., 2014* *3 - Neriya et al., 2011* *4 - Oshima et al. , 2007* *5 - Himeni et al., 2014*

Presented By - Priyanshi Singh

Bamboo phyllody

Brassica phyllody

Aster yellow

Authors- Bertaccini, Bojan Duduk, Samanta

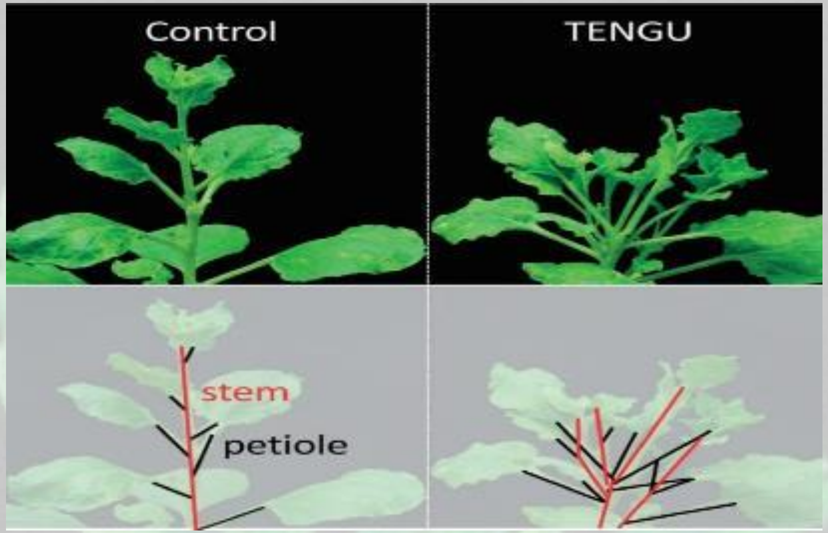
Genetic factors determining symptoms development

- Phytoplasmas are associated with diseases in several hundreds of plant species, including many economically important food, vegetable, and fruis. Typical symptoms include virescence/phyllody (development of green leaf like structures instead of flowers) ,sterility of flowers, proliferation of axillary buds resulting in a witches' broom growth, abnormal internodes elongation and generalized stunting. Phytoplasmas may induce many other nonspecific symptoms resulting from stress . Hovewer not all infections are necessarily deleterious. For example, the free branching form of poinsettia has been widely used in commercial production of this popular seasonal ornamental.
- Phytoplasmas substantially undistinguishable on 16S rDNA gene can be associated with diseases inducing different symptoms and/or affecting different plant species, but different phytoplasmas can be associated with similar symptoms in the same or in different plant host(s).

Pankaj Singh

WITCHES' BROOM SYMPTOM - TENGU

- **Witches' broom** is a dense clusters of thickened stem on the branches of plants .
- In 2009, the first phytoplasma effector protein **TENGU** , a peptide of 38 amino acids was identified as an inducer of witches' broom ("tengu-su" symptoms).[1]
- After secretion TENGU is cleaved into peptide of 12 amino acids.
- It inhibits the signaling pathway of the plant hormone **auxin** and induces witches' broom symptoms.[2]
- TENGU also induces sterility of male and female flowers by inhibiting the synthesis pathway of another plant hormone, jasmonic acid (JA).[3]
- Reduction in JA levels contribute to attracting insect vectors .
- Similarly ,another secreted protein SAP11 regulates JA synthesis and increases the fecundity of insect vectors .



Tengu-peptide induced witches' broom symptom

Paulownia witches' broom

Poinsettia witches' broom

1) Hashimoto et al. (2009)
2) Sugawara et al. (2013)
3) Minato et al. (2014)

POOJA SINGH

Phytoplasmas: Unlocking the Mysteries Behind Witches Broom and Phyllody

Phytoplasmas are microscopic organisms that can cause a range of symptoms in plants. Two of the most unique and fascinating symptoms they induce are witches broom and phyllody. In this presentation, we'll explore what causes these changes and their impact on plant health and yield.

PRACHI KUMARI

SYMPTOMS INDUCED BY PHYTOPLASMAS

Phyllody : A floral symptom where floral parts are replaced by small green leaves.

Witches Broom : A bushy appearance due to the abnormal proliferation of buds in the axils of leaves leading to a broom-like appearance.

WHY PHYTOPLASMAS INDUCE THESE CHANGES?

- **Plant Manipulation**
- **Disruption of Hormonal Balance**
- **Evolutionary Effect**

PRACHI KUMARI

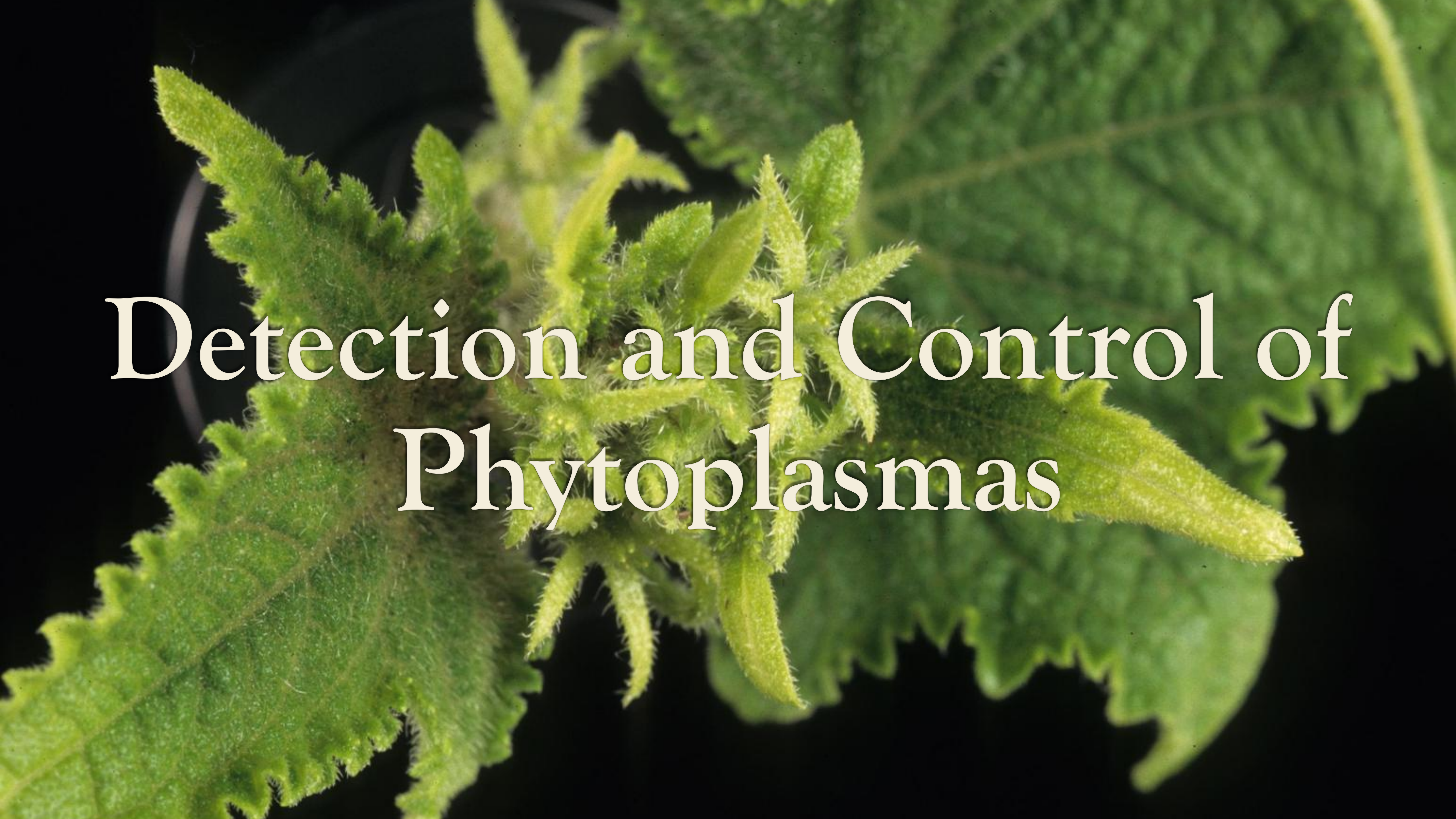
Witches Broom

Phyllody

PRACHI KUMARI

GROUP 7

Detection and Control of Phytoplasmas



Detection of Phytoplasmas

- Phytoplasmas are difficult to culture. Thus, different diagnostic methods were used.
- Early diagnostic methods included : electron microscopy observation using ultra-thin sections of sieve elements and plant recovery after tetracycline treatment.
- In ultra-thin sections of fasciated stems from symptomatic samples, many phytoplasma-like bodies (PLBs) were identified based on the spherical and dumbbell-shaped structures under TEM. The PLBs ranged from 330 to 700 nm in diameter and contained ribosome-like bodies and a central region of fibrillar material, presumed to contain DNA.
- Tetracycline works by acting on phytoplasmas and mycoplasmas both causing there decrease or elimination to drastic levels.

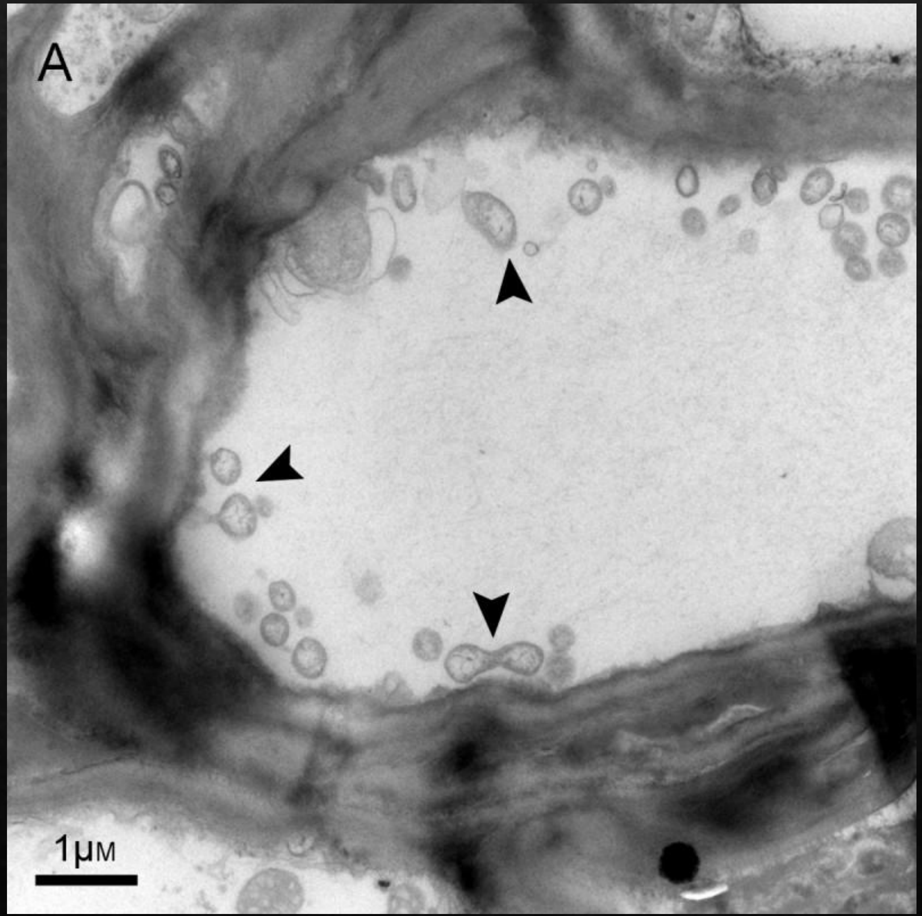


Figure: Transmission electron micrograph of phytoplasma-like bodies (indicated by black narrow) in stem tissues of symptomatic *China ixeris*.

Ratendra Pratap Singh

Detection of Phytoplasmas

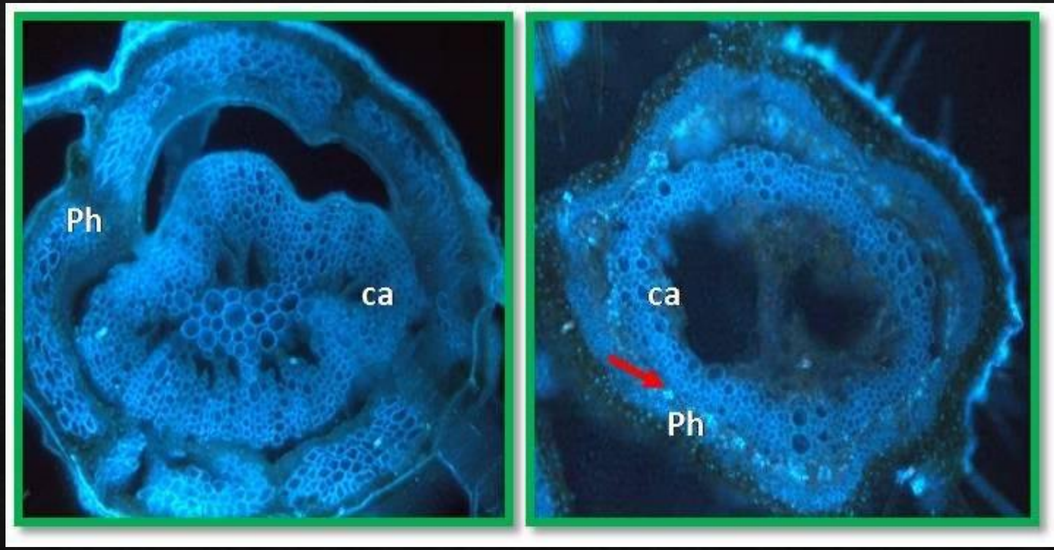
- Due to which, plant shows signs of recovery thus confirming that it was a victim to phytoplasmas.
- Subsequent methods DFD Method, fluorescent staining (DAPI) of phytoplasma DNA in phloem tissue, an antiserum-based method to detect the conserved membrane protein (Antisera raised against phloem-limited phytoplasmas generally react only with the phytoplasma strain used to produce the antigen.) *[Wei et al., 2004]* and PCR to amplify conserved gene regions.
- **Direct Fluorescence Detection (DFD) Method:** Thin sections (0.5-8.0 mm thick) were prepared from fresh tissues of plants infected with phloem-limited viruses (yellows-type viruses) or mycoplasma-like organisms (MLOs). When observed under a reflecting fluorescence microscope (RFM) (Type: Olympus BHS-RF-A), they distinctly showed a yellow fluorescence in their phloem tissues. Such a fluorescence was never found in those of healthy plants. By electron microscopy of the same materials, the fluorescence was proved to originate in necrotic phloem cells. From these results, the direct detection of fluorescing cells in phloem tissues under RFM is concluded to be suitable for the diagnosis of infections by phloem-limited viruses or MLOs.*[Namba et al., 1981]*

Symptoms of phytoplasma-infected *China ixeris*. The symptoms are curving stem (A); flat stem, shorting stalk (C); narrowing leaves (B) and clustering of multi-inflorescence (D), compared to healthy one (E).

Ratendra Pratap Singh

Detection of Phytoplasmas

- **<u>DAPI Staining:</u>** The 4',6-diamidino-2-phenylindole (DAPI) stain technique is a simple method that was developed for confirming the presence of phytoplasmas in hand-cut or freezing microtome sections of infected tissues. DAPI binds AT-rich DNA preferentially, so that phytoplasmas, localized among phloem cells, can be visualized in a fluorescence microscope. Infected tissues generally show bright phytoplasma-like spots in the phloem sieve tubes that are not present in the healthy tissues.*[Andrade & Arismendi, Purdue University, Hiruki & da Rocha, 1986]*
- **<u>PCR:</u>** In the early 1990's PCR coupled with RFLP analysis allowed the accurate identification of different strains and species of phytoplasma. It can be divided into three phases: total DNA extraction from symptomatic tissue or insects; PCR amplification of phytoplasma-specific DNA; characterization of the amplified DNA by sequencing, RFLP analysis or nested PCR with group-specific primers.*[Marzachi C., 2004]*



Fluorescent micrograph of healthy and phytoplasma affected stem sections of crotalaria stained with DAPI

Grace

CONCLUSION

PRESENTED BY KASHISH

Characteristics of Phytoplasmas

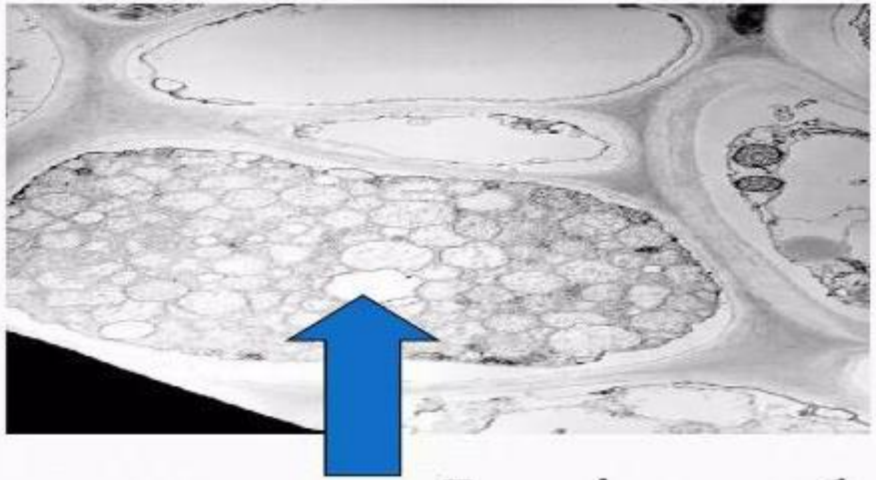
- **Single celled, wall-less prokaryotes, resembling mycoplasmas in morphology** (Doi *et al.,*1967)
- **Obligate parasites, can't be grown in *in-vitro* cell free culture** (Lee *et al.,* 1986)
- **Transmitted by phloem feeding insects (leafhoppers, plant hoppers, psyllids) and *Cuscuta*** (Tsai *et al*., 1979)
- **Sensitive to tetracycline but resistant to penicillin** (Ishii *et al.* 1967)
- **Descended from low G+C gram positive bacterium** (Woese *et al.,* 1987)

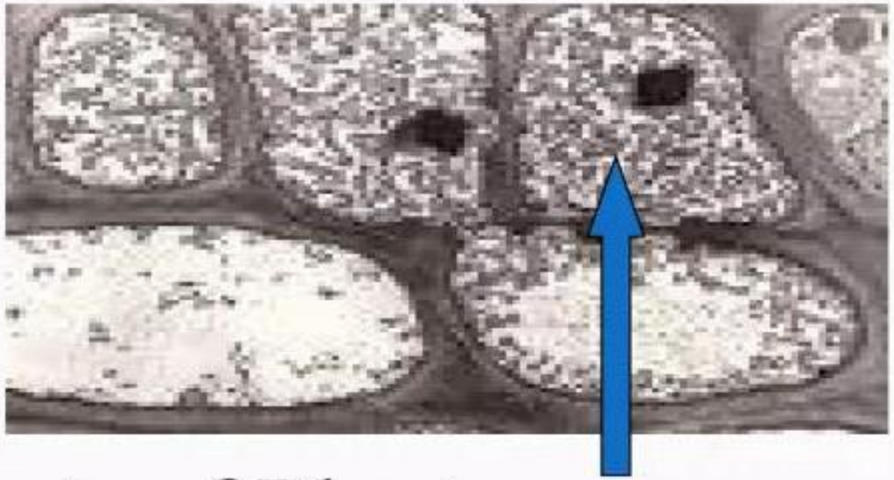
Lee *et al*. (2000) *Annu. Rev. Microbiol*

Cont..

- **Round to filamentous** (Kirkpatrick,1982)
- **Size 200-800 nm**

***Phytos*- plant + *plasma*- thing moulded (Greek)**





In sieve elements of Plants

Symptoms of some Phytoplasma diseases

Carrot yellow

Sesame phyllody

Little leaf of brinjal

lethal yellowing

Vitches’ broom lime

Palm wilt

Brassica phyllody

Grape vine yellow

Phytoplasmas Classification

"After a time the growth of and accumulation of specimens or phenomena forces people to try to classify" - **Pirie (1995)**

Hurdles to definite description and classification:

- Obligately parasitic habit
- Structural fragility
- Presence in low numbers in infected plants
- Intimate association with host tissues

Firraro *et al.* (2005) *J. Pl. Pathol*

- Detection and control of phytoplasmas
- Several technologies to detect phytoplasmas have been developed such as auto florosecnce
- These methods are expensive so diagnosis of diseases is difficult in laboratories.
- For control only tetracycline class antibiotics have exhibited transient suppresion of phytoplasma propagation and symptoms.
-

- In the last quarter century, although there have been many
- barriers to the study of phytoplasmas, such as the
- difficulty of culturing and transforming them and
- the necessity of producing plant or insect hosts to
- maintain them, their molecular and biological properties have been elucidated.