PREVALENCE OF SESAMUM PHYLLODY IN ONATTUKARA TRACT AND EVALUATION OF FUNGAL ROOT ENDOPHYTE *Piriformospora indica* FOR ITS MANAGEMENT

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2019-11-133

DEPARTMENT OF PLANT PATHOLOGY

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by

GIFTY K. J.

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THESIS

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2022

DECLARATION

I hereby declare that this thesis entitled **"Prevalence of sesamum phyllody in Onattukara tract and evaluation of fungal root endophyte *Piriformospora indica* for its management"** is a bona fide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
^{0}C	Degree Celsius
AMF	Arbuscular mycorrhizal fungi
cm	Centimeter
CD	Critical difference
DAG	Days after germination
DAG'	Days after grafting
D.I	Disease incidence
et al.	and other co-workers
g	Gram
h	Hour
L	Litre
µl	Microlitre
µg	Microgram
ml	Millilitre
mg	Milligram
min.	Minute
nm	Nanometer
ng	Nanogram
OD	Optical Density
PDA	Potato Dextrose Agar
PDB	Potato dextrose Broth
rpm	Rotations per minute
sp.	Species
Sec.	Seconds
V.I	Vulnerability index
viz.	Namely

Introduction

1. INTRODUCTION

Sesamum (*Sesamum indicum* L.) also known as till or gingely is an erect, herbaceous annual plant which is one of the ancient cultivated oilseed crops. It is known as 'Queen of oilseeds' in view of its high-quality oil and protein content. Although Africa hosts most of the wild relatives, the genetic arguments support its Indian origin (Bedigian, 2003). Sesamum belongs to the family Pedaliaceae, which comprises of 16 genus and 60 species, wherein 40 species were identified and 36 were mentioned in Index Kewensis. 18 species were from Africa, 8 species were from India-Srilanka region and wild species were prevalent in Africa. *Sesamum indicum* (2n=26) is mostly considered as a domesticated crop (Ashri and Singh, 2007).

Sesamum is an erect, herbaceous annual plant with both indeterminate and determinate growth habits, but most varieties have an indeterminate growth habit, where new leaves, flowers and capsules are produced continuously as long as the environment is suitable for growth, allowing the plants to reach a height of 200 cm. Based on climatic circumstances and variety, sesame plant takes around 70 to 150 days to complete their life cycle (Ashri, 1998).

Sesamum seed has high food value as it is rich source of oil and protein. The presence of antioxidants (sesamin, seasminol and sesamolin) increases its medicinal value to many folds (Bramway, 2010). The seeds contain 48-55 per cent oil, 17-32 per cent protein, 14-16 per cent sugar and 6-8 per cent fiber. Oil consists of about 80 per cent unsaturated fatty acids (oleic and linoleic acids). Sesame protein is rich in methionine and tryptophan, but deficient in lysine. Other constituents include minerals (4-7 per cent) and vitamins *viz.* niacin, folic acid and tocopherols (Hedge, 2012). It lowers the blood pressure, decreases bad cholesterol, increases good cholesterol and maintains normal blood pressure levels. Seeds contain 'phytate', a natural anti-cancer substance inhibiting the growth of cancer cells. Iron and calcium content of sesamum seeds prevents anaemia and osteoporosis. It reduces tension and stress, nourishes the nervous system, and relieves fatigue and insomnia (Shivhare and Satsangee, 2012). It is also a good source of vitamins, antioxidants,

omega-6 fatty acids and dietary fiber with potential medicinal effects. It is having anti-pyretic, anti-oxidant, anti-hypertensive, anti-microbial, anti-cancer and anti-inflammatory properties (Shasmitha, 2015).

In India, sesamum is ranked sixth among edible oil crops, after groundnut, rapeseed, mustard, sunflower and soybean (Iqbal *et al.*, 2018). Nearly 97 per cent of the world's sesamum comes from Asia and Africa. Myanmar, India, China, Tanzania, Ethiopia, Uganda and Nigeria are among the top sesamum-producing countries. India occupies an area of 14 lakh ha with production of 6.89 lakh tonnes and productivity 485.4kgha^{-1} (FAO STAT, 2019). Sesame is primarily grown in India during two seasons: kharif (June–November) and rabi (November–April), with the kharif season accounting for about 75 per cent of annual production. The major sesame growing states in India are Gujarat, Uttar Pradesh, Rajasthan and Madhya Pradesh together accounted for 85 per cent of total acreage, while other states serve the remaining 15 per cent (IOPEPC, 2019). The crop is best suited for cultivation in tropical climates, sandy, well-drained soil with hot climate and moderate rainfall (Mohanty *et al.*, 2021).

Onattukara is a traditional sesamum cultivating area of Kerala practicing an intensive cropping system with cropping sequence of paddy-paddy-sesamum. Sesamum is the sole crop in summer rice fallows of Onattukara tract. The crop thrives in this area because of the residual moisture in rice fields. Sesamum had a low stability index (0.31) in this tract due to its persistently poor performance over the years. Excessive weed growth, uneven maturation and significant shedding result in extremely low yield (average of 202 kg ha^{-1}) (John *et al.*, 2013). Onattukara is an important agro-ecological zone spreading over Karunagapally taluk of Kollam district and Karthikapally and Mavelikara taluks of Alappuzha district (Bindhu *et al.*, 2014).

The pests and diseases affecting the seedling, foliage, flowers, pods, and stem cause an average yield loss of 25 per cent of worldwide production. Leaf spots, stem and leaf blotch, charcoal rot, anthracnose, mildew and phyllody are the major diseases. Similarly, insect pests such as capsule borer, leaf roller, sphinx moth, aphids, and gall midge hinder the crop (Weiss, 2000). Abiotic factors like waterlogging,

salinity and chilling temperature also limits the sustainability of the crop (Tripathy *et al.*, 2019). Sesame phyllody disease caused by phytoplasmas is considered as one of the most important diseases, causing yield loss of about 5-15 per cent in the world (Phookan *et al.*, 2019).

Doi *et al.* (1967) described the presence of small pleomorphic bodies resembling mycoplasmas in ultrathin sections of the phloem sieve elements of plants infected with mulberry dwarf. In 1994, the trivial name "phytoplasma" was adopted replacing the term MLO and classified as a new genus '*Candidatus* Phytoplasma' based on the 16S rDNA sequences (Lee *et al.*, 1998).

Symptoms of sesamum phyllody are phyllody, virescence, floral proliferation, and witches' broom. Fasciation of shoots was also observed in some of the infected plants (Akthar *et al.*, 2009). Phylogenetic analysis revealed the association of distinct phytoplasmas with sesamum grown in different parts of the world. Four phytoplasma groups 16SrI, 16SrII, 16SrVI and 16SrIX are reported to be associated with sesamum phyllody disease (Rao *et al.*, 2015).

Conventional management practices rely upon the application of insecticides, rouging and production of phytoplasma-free propagation material. However, these could have undesirable environmental impacts and are costly. Till date, no effective management practices have been evolved against phytoplasma diseases (Valarmathi and Ladhalakshmi, 2020). Developing biological control measures has thus become an important component for the sustainable sesamum production. In this respect, beneficial fungal endophytes can be a promising biocontrol strategy against sesamum phyllody.

The relationship between *Candidatus* Phytoplasma mali and the apple endophyte *Epicoccum nigrum* in the experimental host *Catharanthus roseus* were investigated by Musetti *et al.* (2011). Endophyte-treated apple proliferation grafted plants had showed only leaf yellowing, whereas untreated grafted plants had showed deformed leaves, enlarged veins, leaf discolorations, diffuse yellowing, and little

flowers, revealing the effect of *E. nigrum* colonization on apple proliferation phytoplasma.

P. indica, a wide host range root colonizing endophytic fungus has been isolated from the sandy desert soils of Rajasthan, India. Similar to the mycorrhizal fungi, it promotes plant growth, yield, early flowering and resistance of the colonized plants against biotic and abiotic stresses (Oelmuller *et al.*, 2009). It has been widely used for many roots and foliar fungal disease management (Johnson *et al.*, 2014). Its effectiveness against *Blackeye cowpea mosaic virus* in *Chenopodium amaranticolor* showed a significant reduction in local lesions with per cent inhibition of 60-73 percent over control (Chandran *et al.*, 2021). Hence, it is relevant to explore the possibility of beneficial fungal root endophyte *P. indica* against sesamum phyllody phytoplasma.

In this context, the present study has been undertaken with the following objectives.

- Studies on symptomatology
- Molecular detection and characterization of phytoplasma inciting sesamum phyllody disease in Onattukara tract
- Evaluation of fungal root endophyte *Piriformospora indica* for its management.

Review of Literature

2. REVIEW OF LITERATURE

Sesamum (*Sesamum indicum* L.) is one of the oldest and important oilseed crops in the world preferred for its high-quality edible oil content, nutritious protein and sufficient carbohydrate. Sesame seeds hold significance in medicinal forms due to rich source of linoleic acid, Vitamin A, B1, B2 and E (Brar and Ahuja, 1980). Among various biotic stresses, the economically important and most destructive disease affecting sesamum is phyllody. Earlier virus was considered as its causal organism that was then proved as mycoplasma like organisms (MLO), and termed recently as phytoplasma (Das and Mitra, 1998a). It is found to be managed using resistant cultivars, early sowing or by controlling insect vectors. Use of beneficial endophytic microbes can be a better alternative for the management of the disease. In this chapter, a review of the studies undertaken has been presented.

3.1 COLLECTION OF PHYTOPLASMA INFECTED SESAMUM PLANTS, AND WEED HOSTS SHOWING THE SYMPTOMS AND ASSOCIATED INSECTS FROM AEU 3

Sesamum indicum Linn. (Pedaliaceae) is an annual shrub with white bell-shaped flowers with a tinge of red, yellow or blue. It's grown for its oil-rich seeds. It can be found in tropical, subtropical, and southern temperate climates around the world, with an emphasis on India, China, South America, and Africa. Sesamum thrives on sandy, well-drained soil, hot weather, and moderate rainfall. It is propagated via seed and ripens in four months. Each plant has the potential to produce 15-20 fruits with 70-100 seeds each (Chakraborthy *et al*., 2008). India's major sesame-growing states include Gujarat, Uttar Pradesh, Rajasthan and Madhya Pradesh, together accounted for 85 per cent of total acreage, with the remaining 15 per cent covered by other states (IOPEPC, 2019).

In Kerala, Sesamum is primarily grown as a single crop in the summer rice fallows of Onattukara tract. Sesamum thrives in this area because of the residual moisture in rice fields. The crop establishment is usually weak, resulting in low yields. The soils are porous, acidic, and deficient in all major plant nutrients.

Karthikapally and Mavelikara taluks of Alappuzha and Karunagapally taluk of Kollam districts of Kerala make up Onattukara sandy loam tract, a significant agro-ecological zone (Bindhu *et al*., 2014).

Sesamum phyllody was first discovered in Indo-Pakistan subcontinent in 1908 (Vasudeva and Sahambi, 1955) and has now increased dramatically in recent decades in India, particularly in the peninsular regions, with a prevalence of around 75 per cent (Manjunatha *et al*., 2012). Many research works have been studied the incidence of sesamum phyllody in different sesamum growing areas and revealed the phytoplasmal group and subgroup associated.

Madhupriya *et al*. (2015) found a 7-55 per cent incidence of sesame phyllody in sesame-growing regions of nine Indian states viz., West Bengal, Chhattisgarh, Madhya Pradesh, Uttar Pradesh, Delhi, Gujarat, Tamil Nadu, Rajasthan and Maharashtra during 2011 - 2013. In sesame fields through-out the nine Indian states surveyed, both phyllody (conversion of floral components into leaf-like structure) and witches' broom symptoms were observed.

From the sesamum fields of Delhi (NBPGR), Singh *et al*. (2016) recorded 3 per cent of plants with fasciation symptoms with normal flower and fruit development. A false capitulum-like structure with whorled leaves and flowers was noted at the shoot apical region in such plants. The size of leaves and flowers were severely reduced indicating little leaf symptoms. Almost 10 per cent of the plants had displayed these symptoms. The leaves were one-third of the size of a healthy leaf, and height was reduced by about 60 per cent. Flowers and leaves showed signs of early abscission. The flowers were sterile and unable to bear fruit. In 2 per cent of plants, internode shortening and significant stunting were also found with nearly 90 per cent drop in plant height.

The phytoplasma-infected plants become stunted, and the floral parts are changed into green leaf-like structures, followed by profuse vegetative development, resulting in yield losses of up to 34 per cent or even 100 per cent in the cases of severe incidence (Sarwar and Haq, 2006). Incidence of sesamum phyllody was

studied in districts of Gujarat during 2008-2009. In Kharif season the incidence ranged from 0-1.5 per cent and 0-4.5 per cent during summer season. The shortened incubation period of pathogen both in host plant and insect vector is the reason for increased incidence during summer period (Pathak *et al*., 2013). The phyllody incidence had a positive correlation with the vector population and temperature in the summer season, whereas sunshine hours and diurnal variation was negatively correlated. In kharif season, the incidence had a positive correlation with the vector population, temperature and the diurnal variation; whereas negative correlation with the total rainfall, windspeed and the number of rainy days (Phookan *et al*., 2020).

Devanna *et al*. (2020) reported the sesamum phyllody incidence at different locations of Karnataka, where average disease incidence of 18-31.6 per cent was observed in summer season (April to June) crop, whereas an incidence of 24.8-38.0 per cent was recorded in the same fields during the followed kharif season (June to September). The difference in the incidence of sesamum phyllody in two subsequent growing seasons might be due to increased vector population as the crop is cultivated continuously, which could carry the inoculum from the preceding season crop.

Sesamum leafhopper *Orosius albicinctus* was collected from sesame fields and their role in transmission of phyllody has been confirmed by subjecting it to nested PCR using phytoplasma specific primers. The sequencing of amplicons showed the presence of sesame phyllody causing phytoplasma *Candidatus* phytoplasma australasiae related strain belonging to 16Sr11 subgroup (ICAR-CPCRI, 2018).

Akhtar *et al*. (2013) observed the presence of leafhoppers *O. albicinctus* on yellow sticky traps placed in sesame fields approximately three weeks after germination. Leafhoppers counting 10-15 were recorded per week in a trap and the population was found to be rising during the growth period of the crop. The symptoms first appeared in few genotypes as reduction in leaf size after four weeks of germination, and later it developed into twisted and reduced leaves with shortened internodes, leaves arranged closely on the top of the stem and plants become stunted with no capsule formation.

Weed plants could act as a natural reservoir of plant pathogens. Merin *et al*. (2015) observed *Pedalium murex*, a weed showing symptoms of shortened internodes, reduced leaf size, phyllody and stunted growth from coconut plantations in South India. The association of *Candidatus* phytoplasma australasiae with the infected samples was recorded. Rao *et al*. (2017a) reported *Ocimum canum* from infected fields of Gorakhpur district as a reservoir of sesame phyllody phytoplasmas. 16 Sr1 group phytoplasma were associated with the *O. canum* little leaf disease and the same phytoplasma were reported from infected sesame plants. This was the first report of association of 16Sr1-B subgroup phytoplasma with *O. canum* little leaf disease.

Phytoplasma suspected witches' broom symptoms were observed in a natural weed *Cannabis sativa* subsp. *sativa* from the sesame fields in Jammu with an incidence ranging from 5-12 per cent. Phylogenetic analysis and RFLP analysis revealed the association of '*Ca.* Phytoplasma asteris' (16Srl-B subgroup) with the infected samples (Singh *et al*., 2018). Chen *et al*. (2017) reported association of 16Sr I-B phytoplasma with *Melochia corchorifolia* showing virescence, witches' broom and phyllody symptoms from China. 16Sr II-V subgroup was also found to be associated with witches' broom disease of *M. corchorifolia* in China (Yu *et al*., 2021).

Phytoplasma associated with sesame phyllody and a weed *Sclerocarpus africanus Jacq* showing little leaf and witches broom symptoms was confirmed by direct and nested PCR. Among the four predominant leafhopper species identified, *Hishimonas phycitis* was found to be associated. BLAST analysis of nested PCR products from infected sesamum plants, *S. africanus* and *H. phycitis* revealed about 99 per cent sequence identity. This sequence identity of vector and weed revealed its role in acting as a potential natural source of sesame phyllody phytoplasma resulting in the secondary spread (Nabi *et al*., 2015a). Phookan *et al*. (2019) identified sesamum phyllody transmitting insect vectors in Assam. The highest population of *H. phycitis* and *Exitianus indicus* were observed in infected fields. *H. phycitis* was reported to transmit sesame phyllody phytoplasma to healthy plants to an extent of 83.33 percent in transmission trials conducted with a seven-day acquisition and inoculation feeding time each. This was validated by PCR

and nested PCR assays followed by sequencing. The relationship of *H. phycitis* with the aster yellows phytoplasmas was confirmed by BLAST analysis of 16S rDNA sequences.

Chattopadhyay *et al*. (2015) reported that an increase in disease incidence by 1 per cent reduced the yield by 8.36 $kgha^{-1}$ in Coimbatore, Tamil Nadu. Gogoi *et al*. (2017) studied the effect of sesamum phyllody in yield parameters. Average number of capsules per plant was observed to be 15.2 to 21.6 in different varieties of healthy plants grown in different locations in Assam which was found to be reduced drastically (0.0 to 6.8 number per plant) in diseased plant. Considering the seed yield in healthy plant, it was observed to be 1.84 to 2.40g per plant which also found to be reduced (0.0 to 0.92g per plant) in diseased plant. Hence, per cent loss of 58.54 to 100 per cent in the number of capsules per plant and 57.20 to 100 per cent in the seed weight were observed in diseased plant compared to the healthy plant.

Singh *et al*. (2007) found that seven cultigens, as well as two wild species, *Sesamum alatum* and *Sesamum mulayanum*, were resistant to phytoplasma with a mean incidence of less than 5 per cent. It was evident that in cultivated cultivars (KMR14 and Pragati), resistance is governed by a single recessive gene, but wild species have a single dominant gene imparting resistance to phytoplasma. Akhtar *et al*. (2013) ranked three genotypes *viz.* NS 98002-04, NS 99005-01 and NS 98003-04 as highly resistant, as they didn't show any symptoms till the crop harvest; whereas eleven others, NS97001-04, NS96019-04, NS01004-04, NS940051-04, Sumboonkkae, NS20005-04, Ahnsankkac, NS 11704, NS 11504, NS990, and Hansumkkae were grouped as resistant. Other genotypes with PDI values ranging from 10.71 to 65.12 per cent, were classified as moderately resistant to highly susceptible. Vamshi *et al*. (2018) screened twenty genotypes for per cent disease incidence and variation in type of symptom in the field conditions of Telangana state. Out of twenty genotypes eighteen were found susceptible to the disease while two genotypes were found resistant. Maximum disease incidence of 66 per cent was recorded in S1-1687-1 followed by DS-5, Swetha and JLT-408; whereas two genotypes G-TIL-10 and Thilothama showed 50 per cent disease incidence. Genotypes expressed variation in symptom development.

Climate change has significant effect on the occurrence of sesame phyllody. By comparing local climate changes in West Mediterranean Region of Turkey, researchers discovered non-occurrence of phyllody infection. According to the findings, high rainfall, low temperature and high humidity could all be factors strongly related to non-occurrence of the disease (Cagirgan *et al*., 2013).

2.2 SYMPTOMATOLOGY

One of the earliest and reliable methods for differentiating the expression of phytoplasma is symptomatology. Previously, symptom expression was used to detect phytoplasma diseases, but due to the absence of specific symptoms on infected plants throughout the life cycle of the crop, it could be difficult to identify. In case of phytoplasma infected sesamum plants, Akhtar *et al*. (2009) observed different types of symptoms including floral virescence, phyllody and proliferation which could be accompanied by seed capsule cracking, yellowing, formation of dark exudates on foliage and floral parts. Shoot apex fasciation observed as flattening of shoot apex, shortened internodes, and intense proliferation of leaf and flower buds. Flowers of these plants appeared normal in shape, but were small and aggregated as clusters at the apex. Leaves on the lower parts of infected plants, stems, and roots usually did not exhibit symptoms. Vivipary was also recorded where germination of seeds in the capsule occurs. Successful transmission by grafting, dodder (*Cuscuta compestris*) and leaf hoppers (*O. albinctus*) were reported.

It has been found that diseased flowers are having six stamens whereas the normal flower having five stamens. The pistils fused at the margins and transformed into a pseudosyncarpous ovary. Instead of ovules, there were small petiole-like outgrowths inside the ovary which later grew and burst through the wall of the false ovary producing small shoots. It produced more phylloid flowers at the axillae as the shoot grows. Thus, phyllody infected plants were sterile, resulting in total loss of yield (Nakashima *et al*., 1999). The infection caused ‘pin type’ flowers, by preventing the function of filaments (Cagirgan *et al*., 2013). Sepals and petals were converted to green leaf-like structures with thick veins and remain smaller in size. Most of the stamens retained their normal shape, but some of the anthers became green leaf-like

structures and become flattened (Gogoi *et al*., 2017). Such physiological conditions appeared to be due to the phytoplasmal effects on hormonal, nutritional, developmental and stress signaling pathways, and their interactions (Dermastia, 2019).

The intensity of the conversion of floral parts into deformed structures was shown to be linked to infection time. The plants that were infected before flowering showed severe symptoms throughout the plant and were completely sterile. Plants infected during flowering, on the other hand, had severe symptoms on the upper half of the plant, which were sometimes followed by rudimentary flowers that produced extremely small capsules with deformed seeds (Akhtar *et al*., 2009). Madhupriya *et al*. (2015) recorded two significant symptoms, phyllody and witches' broom, in all nine surveyed states of India with an incidence of 7-55 per cent. In sesame fields throughout nine Indian states, both phyllody (conversion of floral components into leaf-like structure) and witches' broom (dense mass of younger shoots growing from a single point) symptoms were observed.

The phytoplasma-specific floral symptoms of phyllody are induced by the ubiquitin-proteasome-mediated degradation of floral homeotic MADS domain proteins by PHYL1, a phytoplasma-secreted protein. In the genus Phytoplasma, homologs of the PHYL1 gene are well conserved. The ABCE class MADS domain proteins and their functions are generally conserved in flowering plants. PHYL1 is a protein that targets class A and class E proteins, which play a key role in the determinacy of floral organs and floral meristems. PHYL1 homologs in phytoplasmas and ABCE class MADS domain proteins in flowering plants are therefore highly linked, implying that PHYL1 and its homologs are determinants of phyllody symptoms (Maejima *et al*., 2014).

Nakashima *et al*. (1999) suggested that the symptoms could be induced by phytoplasma-produced compounds inhibiting floral organ development rather than phloem flow disruption. Ji *et al*. (2009) observed that the infection with phytoplasma was generally associated with increased soluble carbohydrates, starch content and decreased photosynthetic rate, carboxylation efficiency and pigment content of leaves.

The infected plant parts show an increase in phenol content, dry matter and transpiration rate while decrease in chlorophyll content, moisture content, total nitrogen and crude protein. It adversely affects the yield contributing characters which includes branches per plant, plant height, number of capsules per plant and test weight. Loss in yield depends on the infection time, with the highest decline occurring in plants infected early rather than later in their growth cycle (Pathak, 2011).

In both plant and insect hosts, phytoplasma could be detected by biological, histological, immunological and molecular approaches. Biological testing is time-consuming, and the results might be harder to decipher. Histological technique utilizes fluorescence or electron microscopy to examine phloem tissues after staining the phytoplasma DNA. Microscopy techniques such as Transmission Electron Microscopy (TEM), light microscopy and DAPI (DNA-specific-6-diaminido-2-phenylindole) fluorescence microscopy technique were previously used in India to diagnose phytoplasma disease (Deeley *et al*., 1979). Phytoplasmas in living tissues have also been identified using bioimaging approaches that need sensitive, selective, and non-toxic fluorescent dyes as well as confocal or multiphoton microscopy (Christensen *et al*., 2005). Diene staining under light microscopy and DAPI staining under fluorescent microscopy were used for the detection (Musetti and Favali, 2004). The fluorochrome dye DAPI (4'6 diamidino-2-pheilindole) binds to AT-rich dsDNA in phytoplasma cells. Young tissues (petioles of young leaves, phloem tissues, shoots, branches, and roots) are stained with DAPI solution and examined under a fluorescence microscope in thin sections (20–30 µm). The presence of phytoplasmas is indicated by accumulations of bluish-white fluorescence in sieve elements (Rao *et al*., 2011). Moreover, DNA-based technology has allowed scientists to differentiate diverse molecular clusters within these organisms.

2.3 HORMONAL VARIATON IN DISEASED AND HEALTHY PLANTS

Phytoplasmal infection alters the normal patterns of hormonal translocation resulting in a drastic morphological and anatomical change. Phytoplasmal infection alters the normal patterns of hormonal translocation resulting in a drastic morphological and anatomical change. TENGU, a single phytoplasma virulence

factor that causes witches' broom and dwarfism, is a small secreted protein. When TENGU was expressed in *Nicotiana benthamiana* plants, the plants exhibited witches' broom and dwarfism symptoms, which are common in phytoplasma infections. In TENGU-transgenic plants, the expression levels of two pin-formed (PIN) genes were around 2-3 times lower. Polar auxin transport is mediated by PIN genes that encode components of the auxin efflux mechanism. The enoyl-CoA hydratase enzyme, which is involved in the conversion of IAA conjugated forms to free IAA, was shown to be downregulated in infected plants. As a result, the conjugated forms of IAAs found in phloem saps of diseased plants are typically thought to be inactive storage forms (Hoshi *et al*., 2009).

Treatment of '*Candidatus* phytoplasma asteris' and *'Candidatus* phytoplasma solani' infected shoots with added IAA in subsequent sub cultures resulted in symptom remission, improved elongation, and increased fresh weight in treated shoots (Perica, 2008). The levels of jasmonic acid and auxin were lower in TENGU-transgenic plants, implying that TENGU suppresses ARF6 and ARF8, reducing endogenous phytohormone levels and impairing flower formation. It may also act as an inducer of sterility (Minato *et al*., 2014).

The hormonal analysis showed a significant increase in auxin (IAA), gibberellin and cytokinin while reduction in abscisic acid in phyllody infected sesamum samples compared to the healthy samples (Youssef *et al*., 2018).

Little leaf disease of brinjal increased the IAA content in all tissues compared to that in healthy ones. The increase was 35 per cent in severely infected leaf tissues, 47.7 per cent in severely infected stem tissues and 28 per cent in floral tissues that show severe symptoms. The root inhibition test and chlorophyll retention test for cytokinin content found that the little leaf infected stem and floral tissues exhibited higher cytokinin activity than the healthy tissues. However, infected leaves showed lower amount of cytokinin than the healthy ones. Maximum cytokinin was observed in severely infected floral tissues and was 47 ppm. Cytokinins act along with endogenous IAA to promote plant growth and development. As a result, any imbalance in IAA content or cytokinin activity can cause infected plants to grow

abnormally. The altered IAA-cytokinin may play an important role in the development of 'little leaf syndrome' (Das and Mitra, 1998b).

In the mulberry yellow dwarf infected leaves, the mRNA level of the cytokinin biosynthetic gene encoding isopentyl transferase increased whereas the cytokinin biodegradation gene encoding cytokinin oxidase declined, possibly resulting in an increase in cytokinin in the infected leaves (Gai *et al*., 2014). Enhanced cytokinin biosynthesis initiates meristematic activity resulting in axillary bud growth (Bhatla and Lal, 2018). Here, the ratio of cytokinin and auxin may alter, and this might be partly responsible for mulberry yellow dwarf symptoms such as dwarfism and witches' broom. Lime Witches' Broom (LWB) phytoplasma infected Mexican lime treated with a resistance inducer were analysed for phytohormones. Variations in levels of cytokinin in infected plant might be the reason for LWBD symptoms such as witches' broom. Treatment has propelled the levels of these phytohormones to their levels in healthy plant (Rastegar *et al*., 2020).

Infection of tomato with the potato purple top phytoplasma resulted in a considerable decrease in endogenous levels of gibberellic acid (GA3), which was linked to down-regulation of genes encoding important enzymes involved in the biosynthesis of bioactive GAs and their precursors. Exogenous application of GA3 to infected tomato plants could partially restore pathologically-induced GA deficiency and reduce disease symptoms considerably (Ding *et al*., 2013). Overexpression of gibberellin 2 oxidase in witches broom disease of acidlime (Mardi *et al*., 2015) and overabundance of glycine rich protein in paulownia witches' broom (Cao *et al*., 2017) inactivates bioactive GAs.

It is also found that GA stimulated the production of Ribulose 1,5-diphosphate carboxylase in leaves and thereby an increase in photosynthesis. The reduced GA content in root (wilt) affected coconut palms hence result in enhanced senescence (Cherian, 1983). Gibberellins are involved in retardation of senescence in intact leaves due to increase in the rate of chlorophyll synthesis over disintegration (Pearce *et al*., 2015).

Impaired water uptake might result in increased ABA in root (wilt) affected coconut palms (Cherian, 1983). High sugar concentrations in infected mulberry yellow dwarf leaves may cause an increase in intracellular ABA levels in infected leaves compared to healthy leaves. The senescence of infected leaves and restriction of shoot development could be due to a high concentration of ABA (Gai *et al*., 2014).

Genes encoding ACC oxidase and ACC synthase grapevine flavescence doree disease (Gambino *et al*., 2013) and in paulownia witches' broom (Fan *et al*., 2015) were significantly upregulated in infected plants, suggesting that pathogens may induce increased concentrations of ethylene which can relate to the activation of defense response connected to ethylene signaling.

Expression level of linoleate 13S-lipoxygenase 2-like protein was six times lower in jujube witches' broom infected plants than control (Ye *et al*., 2017). Increase in jasmonate O-methyl transferase catalyzing methylation of jasmonate to methyl jasmonate and downregulation of synthase gene in witches' broom disease of acid lime results in the downregulation of JA biosynthesis which increase the fitness of insect vectors. Asters yellows witches broom protein 11 (SAP11) effector downregulates JA biosynthesis, modulating plant defense to the advantage of insect vector (Tomkin *et al*., 2018).

Plant-pathogen interactions can result in the activation of numerous mechanisms of local and systemic defenses. PR proteins are generally considered to be defense proteins that prevent or limit the invasion or spreading of the pathogens in plants. Over-expression of genes involved in the response of apple plants to Apple Proliferation infection confirms that these proteins are not pathogen-specific, but are determined by the reaction of the host plants. Increased PR1, PR 5 and PR 8 were observed in recovered apple plants compared to healthy. This gives reason for the activation of plant defenses and could explain the fact that recovered plants are more resistant than healthy plants to new phytoplasma infections (Musetti *et al*., 2013).

Although the grapevine's response to phytoplasma infection is mediated by salicylates, full activation of this response does not appear to be efficient in

establishing resistance to the Bois noir disease. By either failing to activate or suppressing the expression of some jasmonate responsive genes that act downstream of jasmonate biosynthesis, as well as the first events of the jasmonate signalling pathway, activation of the salicylate signalling pathway appears to antagonise the jasmonate mediated defence response. The activation of the whole jasmonate signalling pathway in recovered plants, on the other hand, implies that jasmonate-regulated defences are important in preventing Bois noir phytoplasma infections and disease progression. By inhibiting the activation of defence genes connected to salicylate signalling and activating jasmonate signalling, recovery could be accomplished and maintained over time (Paolacci *et al*., 2017).

2.4 MOLECULAR DETECTION, CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF PHYTOLASMA

Phytoplasmas (Mycoplasma-like organisms) are obligatory parasitic bacteria that lack a cell wall and are present in plant sieve elements and in some insect vectors. Yellow plant disease, formerly thought to be caused by virus, was not visible in infected plants. Later, phytoplasma that cause yellow disease was described by Japanese scientists (Doi *et al*., 1967). As it could not be grown in vitro, the rRNA gene sequence revealed that they were non-spiroplasma, which seem to be wall-less prokaryotes that invade plant phloem and insects, and belong to the Mollicutes class. It is proposed to include phytoplasma in the *Candidatus* phytoplasma genus. Phytoplasma have a single-unit membrane surrounding them, and a pleomorphic form with an average diameter of 0.2-0.8 µm (Doi *et al*., 1967; McCoy *et al*., 1989).

Phytoplasma diseases, on the other hand, have roughly 150-year history in India. The root (wilt) disease of coconut was the first identified, in 1874 in South Kerala and afterwards it spreads throughout Kerala. After that, in the early nineteenth century sandal spike disease was first described in the Coorg district of Karnataka. Since then, hundreds of phytoplasma infections have been found and confirmed on a variety of plant species, with over 172 plant species known to be hosts of 10 different phytoplasma families in India to far (Rao, 2021).

Lee *et al*. (1998) developed a phylogenetic classification scheme based on RFLP analysis of phytoplasma 16S rDNA sequences. They found the association of two or more distinct phytoplasmas with a given disease of the same plant host showing similar symptomatology in different regions, and also a given type of phytoplasma can cause diverse diseases in different plant hosts. A large number of new phytoplasmal strains on several crops have been reported in India during last decade, which revealed the more diverse nature of phytoplasmas than the earlier thought. The most prevalent and widespread group of phytoplasma in India is aster yellows group (16Srl) followed by 16Sr11, 16SrV1, 16SrX1 and 16SrX1V groups (Rao, 2021).

Phyllody and witches' broom symptoms on sesame plants grown in different countries were found to belong to different 16Sr groups and subgroups, including II-D in Turkey, Pakistan, and Oman (Al-Sakeiti *et al*., 2005; Akhtar *et al*., 2009; Ikten *et al*., 2014), VI-A and IX-C in Turkey (Sertkaya *et al*., 2007; Catal *et al*., 2013) and I-B in Myanmar (Nang *et al*., 2010).

Madhupriya *et al*. (2015) reported the subgroup level classification of phytoplasma strains from sesame in India for the first time. The infected samples were analysed by Polymerase Chain Reaction using phytoplasma specific primers, which amplifies 16 SrRNA and secA gene. The phylogenic analyses classified them into aster yellows (16Sr1) and peanut witches' broom (16Sr11) phytoplasma groups. RFLP analysis results in classification into 16Sr1-B, 16Sr11-C and 16Sr11-D subgroups, 16Sr11-C subgroup was observed in Uttar Pradesh, Madhya Pradesh, Chhattisgarh and Rajasthan whereas '*Candidatus* Phytoplasma asteris' of subgroup 16Sr1-B was present in West Bengal and Uttar Pradesh, 16Sr11-D subgroup was present in Delhi, Gujarat, Rajasthan, Maharashtra and Tamil Nadu.

Manjunatha *et al*. (2012) first reported the presence of phytoplasma in sesame from South India (Karnataka) which was detected by molecular techniques. In surveyed regions, an incidence 17-75 per cent was observed. The infected plants showed pale green and bushy appearance. Infection after flowering resulted in reduced pod size. From diseased plants, PCR products of 1.8 kb was amplified using

universal primer P1/P7 and reamplified in nested PCR, using primers R16F2n/R16R2. The analysis indicates a close relation to ribosomal group 16Sr1 with more than 99 per cent nucleotide identity.

Singh *et al*. (2016) found variations in the infecting phytoplasma subgroup in yearly collected phyllody-affected sesame samples from Delhi for four years (2007-2010), with 16SrII-D phytoplasma in the 2007 collection and 16SrI-B phytoplasma in the following three years. The existence of the subgroup 16SrI-B was found in samples collected in North India (Uttar Pradesh, Haryana, eastern Rajasthan, and Delhi). The presence of group 16SrII-D was found in samples taken from Punjab and western Rajasthan. Only group 16SrII phytoplasmas were detected in South Indian sesamum collections. 16SrII-A, II-C, and II-D were identified as subgroups, with 16SrII-D being prevalent in all five southern states surveyed, but primarily in Tamil Nadu. Andhra Pradesh, Maharashtra, and Karnataka were all found to have the subgroup 16SrII-A. The subgroup 16SrII-C was detected in only one sesame sample from Raichur (on the border of Karnataka and Andhra Pradesh).

The sesamum phyllody and witches broom disease etiology were studied from samples collected from different fields of Delhi, Uttar Pradesh and Bihar. The BLAST analysis and phylogenetic analysis of 16Sr RNA and sec A gene sequences revealed that phytoplasma associated belonged to *Candidatus* Phytoplasma asteris (16Sr1) and *Candidatus* phytoplasma aurantifolia (16Sr11) groups. The restriction enzyme digestion study classified these isolates into 16Sr 1-B and 16Sr 11-C subgroups. Isolates from Uttar Pradesh were 16Sr 1-B and 16Sr 11-C subgroups and isolates from Delhi and Bihar were 16Sr 1-B subgroup (Nabi *et al*., 2015b).

Devanna *et al*. (2020) studied the etiology of sesame phyllody disease in different cropping seasons conducted in summer (Karnataka) and kharif seasons (Telangana, Karnataka and Maharashtra). The phytoplasma associated in both summer and kharif season were belonged to peanut witches' broom group (16Sr11). The summer isolates were caused by 16Sr11-D subgroup whereas Kharif season isolates were caused by 16Sr11-A and 16Sr11-D subgroups.

Detection by Southern hybridization using specific DNA probes was also conducted. Hybridization assay using the chromosomal DNA fragment of the sesamum phyllody phytoplasmas, SP28, as a DNA probe showed that the phytoplasmas were distributed not only in flowers showing symptoms but also in symptomless leaves and stems of phytoplasma infected sesame plants (Nakashima *et al*., 1999). Sesamum, eggplant and an annual weed *Richardia scabra* exhibiting phytoplasma disease like symptoms were observed in National Bureau of Agricultural Insect Resources Research Farm in Bengaluru. The nucleotide sequence showed cent per cent identity to *Candidatus* Phytoplasma trifolii isolate Tirupati. RFLP pattern analysis indicated a close relation to the reference pattern of the 16Sr V1-D subgroup (Kumar, 2021).

In Turkey, sesame phyllody phytoplasma showed RFLP patterns closely related to strains belonging to clover proliferation group (16SrVI-A) (*Ca*. P. trifolii). The same phytoplasma detected in sesame has also been found in pepper and brinjal plants, confirming that a phytoplasma closely related to '*Ca*. P. trifolii' can be associated with stolbur-like disease of pepper and other solanaceous plants (Sertkaya *et al*., 2007).

The association of chickpea phyllody phytoplasma with *Candidatus* Phytoplasma aurantifolia"-related strain belonging to subgroup 16SrII-E subgroup from northern Karnataka (Balol *et al*., 2021) and 16SrII-D from Tamil Nadu (Latha *et al*., 2021) were reported.

The association of distant phytoplasma groups has been reported from Kerala. Association of 16SrI (Aster yellows phytoplasma) with phyllody of *Piper nigrum* from Calicut (Bhat *et al*., 2006), 16SrXI-B (*Ca*. P. oryzae) with Root (wilt) disease of *Cocos nucifera* from Kayamkulam (Manimekalai *et al*., 2010), 16SrII (Peanut witches' broom phytoplasma) with phyllody of *Pedalium murex* from Kayamkulam (Merin *et al*., 2014), 16SrXIV (Bermuda grass white leaf phytoplasma) with Bermuda grass white leaf from Alappuzha (Merin *et al*., 2014), 16SrXV-A (*Ca*. P cynodontis) with stunting and spear rot disease in *Elaeis guineensis* from Kollam and 16SrXI-B (*Ca*. P. oryzae) with spear rot disease in *Elaeis guineensis* from Wayanand

(Sumi *et al*., 2015), *Ca*. phytoplasma trifolii with little disease of brinjal (Saranya, 2015), 16SrXIV with white leaf disease of *Eleusine indica* from Kasargod (Mall *et al*., 2015), 16SrXIV-A (*Ca*. P. cynodontis) with flat stem of *Vigna unguiculate* from Idukki (Rao *et al*., 2017b), 16SrII-D (*Ca*. P. australasia) with shoot proliferation disease of *Elettaria cardamomum* from Idukki (Mishra *et al*., 2019), 16SrXI-B (*Ca*. P. oryzae) with witches' broom and little leaf disease of *Garcinia gummigutta* from Kottayam (Mitra *et al*., 2019a), 16SrI-B (*Ca*. P. asteris) (Kirdat *et al*., 2019) and 16SrXI-B (Rice yellow dwarf phytoplasma) (Sundararaj *et al*., 2020) with Sandal spike disease as reported from Marayoor, 16SrXIV-A with flat stem and witches' broom disease of *Manilkara zapota* from Thiruvananthapuram (Rao *et al*., 2020) were reported.

2.5 EVALUTION OF *P. indica* AGAINST PHYTOPLASMA IN SESAMUM

P. indica is a novel root colonizing endophytic fungus isolated from the rhizosphere soils of xerophytic woody shrubs in sandy desert soils of Rajasthan. It lacks host specificity and is found to be colonizing endophytically in roots of many agricultural, floricultural, horticultural, agroforestry and medicinal plants leading to their growth promotion (Qiang *et al*., 2012). It resembles arbuscular mycorrhizal fungi (AMF) in promoting plant growth and inducing resistance against many biotic and abiotic stresses in colonized plants and in contrast to AMF, *P. indica* can grow axenically (Varma *et al*., 2012).

The repeated subculturing on semisynthetic medium adversely affects the root colonization efficiency of the fungus. To maintain this, the fungus should be periodically inoculated to the roots of host plants and then re-isolated from the internally colonized roots. *P. indica* culture were maintained by placing colonized root pieces on Kaefer Medium plates and incubated in the dark at 22-24^{0}C for 5-7 days. Then it should be subcultured by taking single hyphal tip from the initiated fungal growth to fresh Kaefer Medium plate (Johnson *et al*., 2013).

Comparing with Hill-Kaefer medium which produced 12-15 g l^{-1} of the fungal biomass, the medium containing Jaggery produced 16-18 g l^{-1} of the fungal biomass

and also obtained optimum chlamydospore production (Varma *et al*., 2014). Nassimi and Taheri (2017) maintained the culture in potato dextrose agar medium and were incubated at 28^0C in dark for 7 days. Liquid culture was maintained by placing three *P. indica* plugs of 0.5 cm diameter in 100 ml potato dextrose broth (PDB) medium and then shaking at 200 rpm under 28^0C in the dark for 3 days before application. The *P. indica* suspension contained about 60 g mycelia mass L^{-1} and $1x10^5$ chlamydospores ml^{-1} (Cheng *et al*., 2020).

Inoculation of sesame roots with an endophytic fungus *Piriformospora indica* could promote the growth of sesame seedlings, boostened the drought resistance and improves the yield of sesame. Before and after drought treatment, the difference in leaf length, leaf width and plant height were highly significant and the number of expanded leaves, basal stem diameter, chlorophyll concentration and root collar diameter were significant between the inoculated and uninoculated plants. For yield traits of grain weight per plant, the difference was significant both under field and green house conditions (Zhang *et al*., 2014). Anith *et al*. (2018) observed black pepper plants inoculated with *P. indica* produced a greater number of leaves and leaf area per plant compared to the control plants. In terms of chlorophyll a and total chlorophyll concentration of leaf tissues, there was a marked difference between inoculated and uninoculated plants. Plants treated with *P. indica* showed early flowering and spike setting. The fresh and dry weights of berries harvested from *P. indica* infected plants were substantially higher than those harvested from control plants. The fungus also enhanced the overall oleoresin and piperine content in the berries upon inoculation.

For evaluating the disease incidence and severity of sesamum phyllody in endophyte treated and non-treated plants, phytoplasma need to be mechanically inoculated. Ravindar (2017) sap inoculated twenty sesamum plants and none has showed a positive reaction in PCR assay, whereas seventeen grafts out of twenty graft inoculated plants have showed positive reaction. Hence, seed and sap transmission has not been reported, and graft transmission was found successful. Sesame phyllody was successfully transmitted from infected plants to healthy plants through grafting

and unlike viruses they are not transmitted mechanically by inoculation with phytoplasma containing sap from affected plants (Vamshi *et al*., 2019).

In south India, the crop is primarily grown as a summer crop in low-nutrient rice fallows. Because of the low nutrient availability in sesame soils and the sparse development of the root system, the plant relies heavily on root invading endosymbionts such as arbuscular mycorrhizal fungi (AMF) for nutrient absorption (Gahoonia *et al*., 2005). Apart from the cultivated sesame (*Sesamum indicum* L.), three wild sesame species have been documented in Kerala, *S. malabaricum*, *S. mulayanum* and *S. radiatum*. A study was conducted to investigate the extent of arbuscular mycorrhizal fungus colonisation in cultivated and wild sesame species. The amount of arbuscular mycorrhizal fungus infection in different sesame species was found to differ. The wild species *S. malabaricum* has the largest percentage of arbuscular mycorrhial fungal colonisation in its roots, which enhances drought tolerance (John *et al*., 2005).

Harikumar (2013) investigated the reaction of indigenous AMF isolates from rice fallows to sesame, a crop that is grown as a follow-up to rice. Inoculation with indigenous AMF modified growth attributes such as rootlet number, shoot length, leaf number and leaf area significantly. The ability of isolates to improve these parameters, however, differed. *Acaulospora lacunosa* inoculated plants had more rootlets and shoot length, while *Glomus dimorphicum* inoculated plants had the highest leaf number and area. The plants inoculated with indigenous AMF significantly improved yield components like capsule number, capsule weight, and seed number. Inoculation with *G. dimorphicum* significantly increased the yield components in sesame among the various AMF examined. An enhancement in plant height and root length was observed in sesamum plants inoculated with *Pseudomonas fluorescens* and Arbuscular mycorrhizal fungi (AMF). Application of mycorrhiza with other bioinoculants (*P. fluorescens*) did have much effect on AM spore count and the root colonization efficiency. An increased percent of seed protein, oil content and other nutrients (K, Ca, Mg, Fe) were also recorded compared to control (Yadav *et al*., 2021).

The fungus also influences the phytohormone level by interfering the biosynthetic pathways, modifications or degradation or in its signaling pathways (Oelmuller *et al*., 2009). It manipulates the plant defense by recruiting phytohormones and thus imparts induced systemic resistance in *P. indica* colonized plants against a number of foliar pathogens and hence utilized for many roots and foliar disease management (Johnson *et al*., 2014). Increased activity of pathogenesis related proteins and defense enzymes like polyphenol oxidase in *P. indica*-colonized plants resulted in reduced vulnerability index of *Blackeye cowpea mosaic virus* in cowpea (Chandran *et al.*, 2021). Chippy (2020) revealed the effect *P. indica*-colonization on bhindi yellow vein mosaic virus infected plants. The *P. indica* colonized-BYVMV inoculated okra plants showed remission of symptoms, which would be attributed by enhanced defense related enzymes, soluble proteins and chlorophyll.

P. indica provides protection against root parasite *Fusarium verticillioides* in maize plants (Kumar *et al*., 2009). This fungus inhibits the colonization by *F. verticilliodes*. Antioxidant enzyme for example catalase activity was also found to be significantly high in *F. verticilliodes*-*P. indica* colonized roots. *P. indica* markedly induced resistance to a susceptible cultivar T07-1 against *Tomato yellow leaf curl virus*. Disease incidence and severity were proved to be reduced by 26 per cent and 1.25 respectively. The phytohormone signaling pathways might be involved in the interaction of plant, endophyte and pathogen (Wang *et al*., 2015). In wheat, it is found to be beneficial as a biocontrol agent against pathogenic fungus *Pseudocercosporella herpotrichoides* (Sharma and Varma, 2021).

Musetti *et al*. (2011) studied the interaction between *Candidatus* Phytoplasma mali and the apple endophyte *Epicoccum nigrum* in experimental host *Catharanthus roseus.* Endophyte treated apple proliferation grafted plants showed only leaf yellowing whereas endophyte untreated – grafted plants showed malformed leaves, enlarged veins, leaf discolorations, diffuse yellowing and little flowers.

Materials and Methods

3. MATERIALS AND METHODS

The research work entitled “Prevalence of sesamum phyllody in Onattukara tract and evaluation of fungal root endophyte *Piriformospora indica* for its management” with objectives of symptomatology, molecular detection and characterization of phytoplasma inciting sesamum phyllody disease in AEU 3 (Onattukara tract); and evaluation of fungal root endophyte *P. indica* for its management was conducted in Department of Plant Pathology, College of Agriculture, Vellayani and Onattukara Regional Agricultural Research Station, Kayamkulum during 2019-2021. The materials and methods followed during the course of study are presented in this chapter.

3.1 COLLECTION OF PHYTOPLASMA INFECTED SESAMUM PLANTS, WEED HOSTS SHOWING THE SYMPTOM AND ASSOCIATED INSECTS FROM AEU 3

Phytoplasma infected sesamum plants were collected from different sesamum growing areas viz., Karthikapally, ORARS Kayamkulum D block and F block (Plate 1). Disease incidence and characteristic symptoms observed at each location were recorded. Insects associated with sesamum phyllody had been collected from phytoplasma infected sesamum fields using yellow sticky traps and sweep nets. The collected insects were identified at Division of Entomology, IARI NewDelhi. The weeds expressing symptoms resembling phyllody were also collected from the phytoplasma infected sesamum fields of Onattukara.

3.2 SYMPTOMATOLOGY

3.2.1 Symptomatology

Characteristic symptoms expressed in sesamum due to phytoplasmal infection were studied and documented under field conditions. Disease incidence and vulnerability index of disease at surveyed locations were calculated based on the disease scoring scale (score 0-5) developed by Lovely *et al*. (2021) (Table 1, Plate 2).

Per cent disease incidence and vulnerability index (Bos, 1982) were calculated using the given formula.

- Percent disease incidence = $\frac{\text{No of plants infected}}{\text{Total number of plants}} \times 100$

- Vulnerability index = $\frac{(0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5)}{n_t\,(n_c - 1)} \times 100$

$n_0, n_1 \ldots\ldots\ldots n_5$ - no of plants in category 0,1,2,3,4,5

n_c - no of categories

n_t - total no of plants

Table 1. Disease score chart of sesamum phyllody disease

Score	Symptom
0	No symptom
1	Slight vein clearing on young leaves
2	Reduction in leaf size
3	Floral parts converted into leafy structures
4	Floral proliferation and little leaf production
5	Shoot apex fasciation

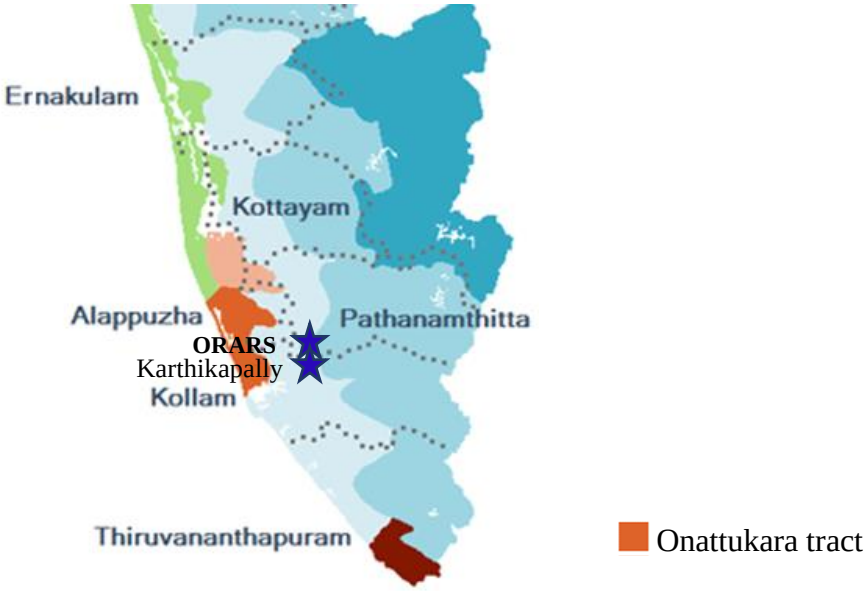
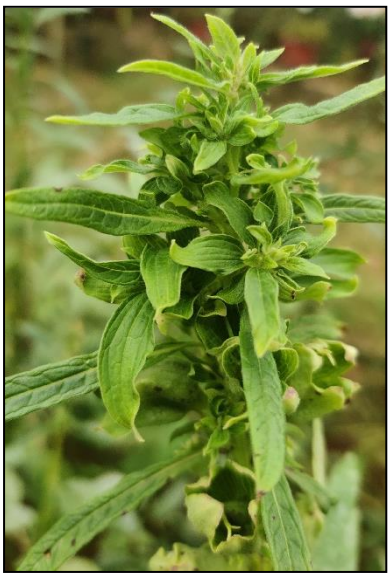


Plate 1. Different sesamum growing areas surveyed for the collection of phytoplasma infected sesamum samples

0-No symptom

1-Slight vein clearing on young leaves

2-Reduction in leaf size



3-Floral parts converted into leafy structures

4-Floral proliferation and little leaf production

(5- was not observed in the field)

Plate 2. Disease score chart of sesamum phyllody disease ranging 0-5

3.2.2 Microscopic examination of healthy and diseased tissues

Microscopic techniques would generally use as preliminary methods to assess and analyze the presence of phytoplasma and its distribution in the phloem tissues of infected host.

Samples were collected from sesamum fields in rice fallows in the Onattukara tract 50 days after sowing. The leaves and stem tissues from healthy and infected samples were taken with five replications each. From the infected samples, leaves at the axil region of phylloid branches and stem portion in between the nodes were taken for staining. The tissues were washed in sterile distilled water and one inch piece of each sample were taken for microtome sectioning.

Microtome sections of phytoplasma infected and healthy leaf and stem of sesamum were stained with 4,6-diamidino-2-phenylindole (DAPI) stain, and observed under fluorescence microscope (ZEISS Axiocam).

The steps followed for microtome sectioning and DAPI staining were

a. One inch piece of tissue sample was fixed for 24 hours in a fixative solution comprising of 5 ml glacial acetic acid, 10 ml formaldehyde and 85 ml of 50 per cent ethanol.

b. Dehydration was done by treating the samples in graded series of 5 per cent ethanol for 30 minutes followed by 10 per cent ethanol for 1 h, 20 per cent, 35 per cent, 50 per cent, 65 per cent, 75 per cent and 85per cent each for 30 minutes, 95 per cent for 1 h and 100 per cent for 1 h or overnight.

c. Fifty per cent xylene in absolute ethanol was prepared for cleaning and treated the dehydrated tissues for 30 min followed by treating two times in absolute xylene each for 1 h. Finally, the tissues were embedded in paraffin wax.

Microscopic slides were smeared with Haupt’s gelatin adhesive with finger until a thin coat of the substance remained on the slide. The paraffin ribbon was prepared

by microtome sectioning and placed on the smeared microscopic slides. Then the paraffin ribbon was made to stick onto the slide by placing on the water bath.

d. For de-waxing and de-staining, the slides were treated in absolute xylene two times each for 1 h followed by treating in graded series of ethanol at 100 per cent, 85 per cent, 75 per cent, 65 per cent, 50 per cent, 35 per cent, 20 per cent and 10 per cent each for 30 min. Then the slides were placed in phosphate buffer saline overnight.

In a dark chamber, DAPI stain 1 µl was diluted in 10 ml PBS buffer and added into the wells created by DPX mountant on the slides, kept for 10 min. Then de-stained the slides using PBS buffer to remove the excess stain and then poured glycerol onto the slides and placed the coverslips. The slides were observed under fluorescence microscope (ZEISS Axiocam).

3.3 HORMONAL VARIATION IN DISEASED AND HEALTHY PLANTS

Samples were collected from sesamum fields in rice fallows of Onattukara tract at 55 days after sowing. The leaves and flowers from healthy and infected samples were taken with five replications each. From the infected samples, leaves at the axil region of phylloid branches and flowers showing virescence symptoms were taken for hormonal analysis. The samples were analysed for the following growth hormone levels.

1. Indole-3-acetic acid (Gordon and Weber, 1950)
2. Gibberellic acid (Holbrook *et al*., 1961)

3.3.1 Estimation of IAA using spectrophotometric method

A calibration graph was prepared from the known aliquots of pure indole-3-acetic acid and a linear relationship was constructed to estimate the IAA content of the samples in reference to the calibration graph (Fig. 1).

For preparing calibration graph, standard IAA (10 ppm) was prepared. Transferred 2, 4, 6, 8 and 10ml aliquots to each of test tubes and added absolute alcohol to 10 ml. From this solution, 1ml aliquot was taken and 2 ml of $FeCl_3$-$HClO_4$

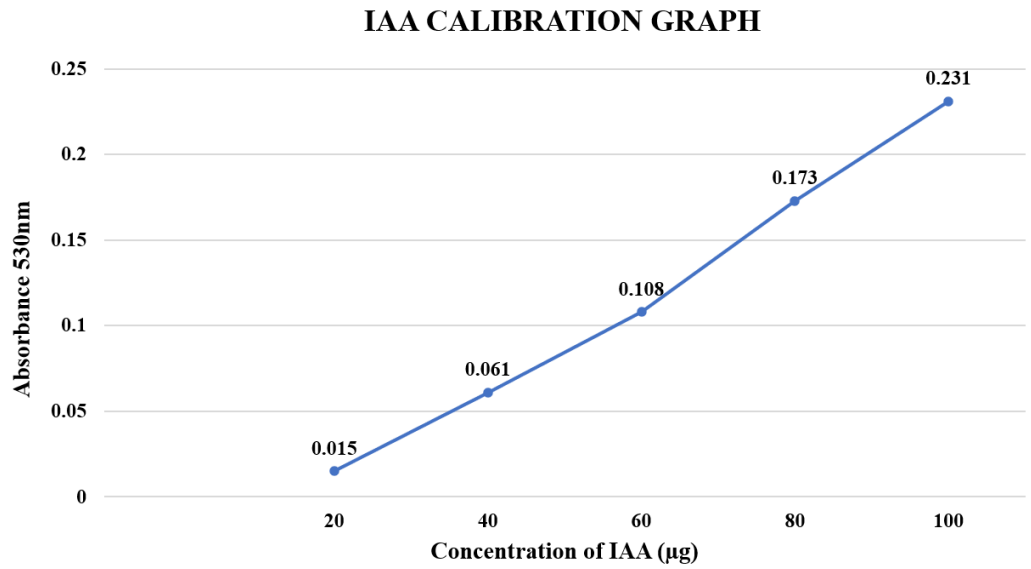


Fig. 1 IAA calibration graph

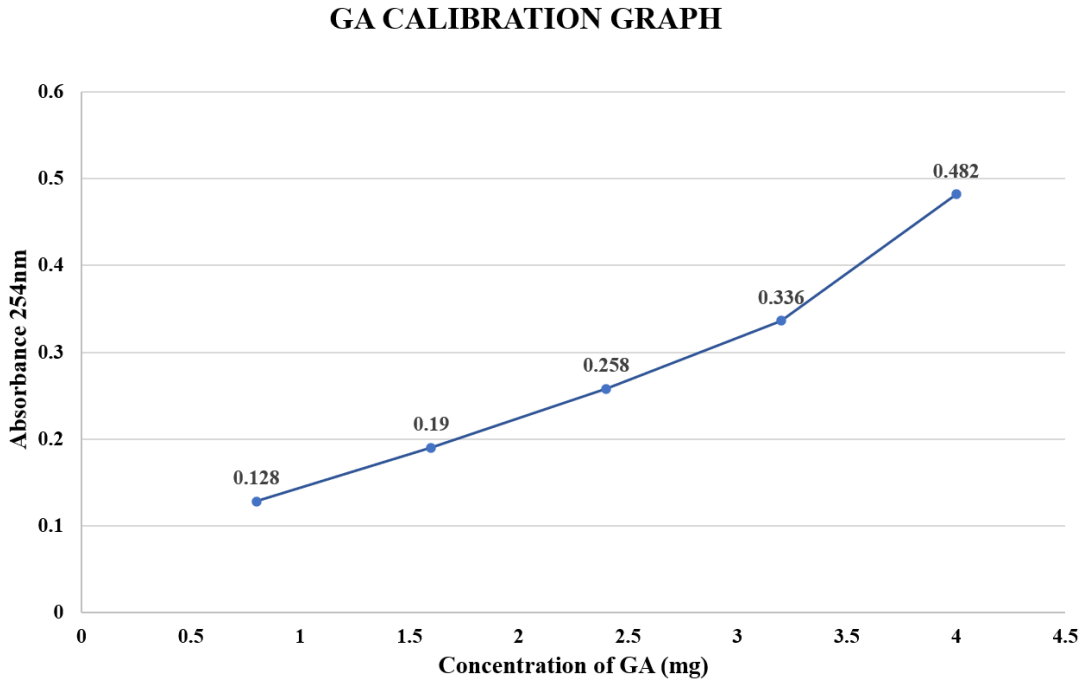


Fig. 2 GA calibration graph

reagent was added into it. The absorbance was measured at 530 nm after 25 minutes using bio-spectrophotometer (Eppendorf, Germany).

For treatment of samples, dissolved 1 g of sample in absolute alcohol and diluted to 10 ml with absolute alcohol. To a 1 ml aliquot, added 2 ml of $FeCl_3$-$HClO_4$ reagent. Kept for 25 minutes and measured the absorbance at 530nm.

3.3.2 Estimation of GA using spectrophotometric method

A calibration graph was prepared from the known aliquots of pure gibberellic acid, and a linear relationship was constructed to estimate the gibberellic acid content of the samples in reference to the calibration graph (Fig. 2).

For construction of calibration graph, dissolved 40 mg of pure gibberellic acid in absolute alcohol and then dilute it to 100 ml in a volumetric flask with absolute alcohol (1 ml = 0.4 mg). 2, 4, 6, 8 and 10 ml aliquots of standard gibberellic acid solution were transferred to 100 ml volumetric flasks and brought the volume in each flask to 10 ml by adding absolute alcohol. Placed 10 ml of absolute alcohol in sixth flask to provide a reagent blank. It was further diluted to volume with 30 per cent dilute hydrochloric acid, commencing with the flask containing smallest aliquot. The flasks were placed in water bath at 20 ± 1°C after mixing the contents. Repeated the procedure for remaining flasks at 3-minute intervals. Each flask was retained in water bath for 75 minutes, and immediately followed by measuring the absorbance of the contents in a bio-spectrophotometer (Eppendorf, Germany) against water in blank cell at 254 nm. For constructing a calibration graph, the reagent blank reading was subtracted from the obtained reading.

For treatment of samples, dissolved 50-60mg of accurately weighed quantity of sample in absolute alcohol and dilute with absolute alcohol to 100ml in a volumetric flask (Solution A). To each of two 100ml volumetric flasks transferred 5 ml aliquots of solution A and added 5 ml of absolute alcohol. Diluted the contents of one flask with 30 per cent dilute hydrochloric acid to 100ml, and allowed to stand in a water bath at 20 ± 1°C for 75 minutes exactly and measure the absorbance at 254nm against water. To the second flask add 35ml of 5 per cent dilute hydrochloric acid, diluted to

volume with water, mixed well and measured the absorbance immediately at 254nm against water. Subtracted the blank reading from sample reading and attain gibberellic acid content with reference to the obtained calibration graph.

3.4 MOLECULAR DETECTION, CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF THE PHYTOPLASMA

3.4.1 Isolation of Genomic DNA

DNA isolation was done using DNeasy plant mini kit (Qiagen, India: Cat no: 69104) following the manufacturer's protocol. 100 mg of healthy and phyllody infected sesame leaves were weighed and disrupted using liquid nitrogen using a pre-chilled pestle and mortar. 400 µl of AP1 buffer and 4 µl of RNase were added into it and mixed well. The samples were transferred into autoclaved 2 ml centrifuge tubes and vortexed. Incubate it at 65°C for 10 min on a heating block and during incubation tubes were inverted 2-3 times. To the samples 130 µl of buffer P3 was added, mixed well and incubated on ice for 5 min and then centrifuged at 14000 rpm for 5min. The supernatant was transferred into a QIA shredder spin column which is placed on a 2 ml collection tube, centrifuged at 14,000 rpm for 2 min. The flowthrough was collected and were transferred into a new collection tube without disturbing the pellet. 1.5 volumes of buffer AW1 was added into it and mixed well by pipetting. DNeasy minispin column was placed in a 2 ml collection tube and transferred 650 µl of the mixture into it. Then centrifuged at 8000 rpm for 1 min and discarded the flowthrough. This step was repeated using the remaining samples. Then 500 µl of buffer AW2 was added into the spin column placed in a new collection tube and centrifuged at 14000 rpm for 2 min. The spin column was carefully taken from the collection tube so that the column does not come into contact with the flowthrough. It was transferred into a new 2 ml micro-centrifuge tube and 100 µl of AE buffer was added into it for elution. Then it was kept at room temperature for incubation for 5 min and centrifuged at 8000 rpm for 1min. The elution step was repeated and finally the isolated DNA was stored at temperature of -20°C.

The quality and quantity of isolated total DNA was evaluated using biospectrophotometer. 5µl of isolated DNA sample was diluted in 2 ml distilled water

and the absorbance was read at 260 and 280nm. The quality was obtained by calculating the ratio of absorbance value at 260 to absorbance value at 280. For high quality DNA, the ratio was found to be 0.8. The quantity of isolated DNA was calculated as, absorbance at 260 nm multiplied by 50 ng μl^{-1} and the dilution factor.

3.4.2 Agarose gel electrophoresis

One per cent agarose gel was prepared in 1X TAE buffer with 3 µl ethidium bromide, casted in a horizontal gel electrophoresis unit (Bio-Rad, USA) and the presence of DNA was confirmed by running the samples. 6µl of DNA was mixed with 2µl of 6X loading dye and loaded into the wells. The gel was run at 60Vcm^{-1} using the powerpack unit (Bio-Rad, USA). After completion of the run, the gel was visualized in UV trans-illuminator system (Bio-Rad, USA) and documented in Gel Doc System (Gel DOC TM XR^{+}, Bio-Rad, USA).

3.4.3 PCR amplification

Universal primer pairs P1/P6 (Table 2) was used for the detection of pathogen from symptomatic samples. Bermuda grass white leaf (BGWL) phytoplasma served as positive control. The reactions were carried out in 25 µl reaction mixture (Table 3) under specified conditions (Table 4). All the PCR amplifications were carried out in Techne Flexigene Thermal Cycler, UK. The amplicons were resolved in 1.0 per cent agarose gel and documented in Gel Doc System (Bio-Rad, USA). PCR products amplified with P1/P6 primers were purified using QIA quick PCR purification kit (Qiagen, Germany) and sequenced at M/s AgriGenome Labs Pvt Ltd., Kochi, Kerala.

Table 2: Universal primer pair P1/P6

Primer	Primer sequence	Reference
Forward primer (P1)	5′-AAG AGT TTG ATC CTG GCT CAG GAT T-3′	Deng and Hiruki, (1991)
Reverse primer (P6)	5′-CGG TAG GGA TAC CTT GTT ACG ACT TA-3′	

Table 3: Components of reaction mixture of 25 µl for each sample

Reagents	Volume(µl)
10X Enzyme buffer	2.5
$MgCl_2$	1.5
dNTP mixture	0.5
P1 (10 µM/ µl)	0.5
P6 (10 µM/ µl)	0.5
DNA template (100ng/ µl)	1.0
Taq DNA polymerase (5U/ µl)	0.25
Sterile distilled water	19.0
Total Volume	25.0

Table 4: Thermal conditions of the PCR

Steps	Temperature	Time	Cycles
Initial denaturation	94°C	5 min	1
Denaturation	94°C	45 sec	35
Annealing	63°C	1 min	
Primer Extension	72°C	2 min	
Final primer Extension	72°C	10 min	1

3.4.4 Characterization and phylogenetic analysis of phytoplasma

A database search of sequences homologous to the 16S rDNA sequences under the study was performed by BLAST at National Center for Biotechnology Information (NCBI). In order to analyze the relatedness of the pathogen with other phytoplasmal groups, sequences were aligned using ClustalW and phylogenetic tree was constructed with 16S rDNA sequences using Mega X software by neighbour joining method with 1000 replications for bootstrap analysis. *Acholeplasma laidlawii* strain PG8 (GenBank acc. No. NR-025961.1) was used as an outgroup.

In silico RFLP analysis of 16S rDNA sequences with restriction endonucleases *Alu*I, *BamH*I, *Bfa*I, *BstU*I, *Dra*I, *EcoR*I, *Hae*III, *Hha*I, *Hinf*I,*Hpa*I, *Hpa*II, *Kpn*I, *Sau*3AI, *Mse*I, *Rsa*I, *Ssp*I and *Taq*I and virtual gel plotting were carried out using the iPhy-Classifier online tool (https://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi) as described by Zhao *et al*. (2009).

3.5 EVALUATION OF *P. indica* AGAINST PHYTOPLASMA IN SESAMUM

3.5.1 Maintenance of beneficial fungal root endophyte *P. indica*

P. indica culture obtained from Department of Plant Pathology, College of Agriculture, Vellayani (from Prof. Dr. Ralf Oelmuller, Institute of General Botany and Plant Physiology, Friedrich-Schiller University, Jena, Germany; and originally from Prof. Dr. Ajit Varma; No. INBA3202001787) was maintained by subculturing in potato dextrose agar medium. Hyphal tip from actively growing two weeks old culture was placed in Petri plates with PDA medium and PDB medium and was incubated at room temperature. It was subcultured at fortnightly intervals. The cocultivation of *P. indica* with sesamum was done as per the protocol of Jojy *et al*., 2020. The fungal mat in broth was strained and mixed with sterilized coipith: farmyard manure (1:1) mixture amended with 2 per cent gram flour for mass multiplication.

3.5.2 Root colonization of sesamum var. Thilak by *P. indica*

Sesamum seeds were sown in the *P. indica* mass multiplied medium and the roots were stained at different time intervals after the cocultivation. Roots were initially washed in running water and cut into pieces of about 1 cm length. It was transferred into freshly prepared 10 per cent KOH solution taken in test tubes. The test tubes were then placed in water bath for 5 min at 65°C. Roots were then taken out and washed thoroughly in water and placed in 1 per cent HCl for 5 min. After the treatment in HCl, washed the root bits in water and were transferred into lactophenol trypan blue stain for about 2 min for staining the chlamydospores present in roots. Then the root bits were placed on microscopic slides and observed under microscope (Leica – ICC50 HD, USA) to identify the chlamydospores produced by the fungus in the colonized root bits.

3.5.3 Standardization of wedge grafting in sesamum

Healthy sesamum plants at 30 days after germination were taken as root stock. Healthy and phytoplasma infected sesamum scions were collected for grafting. The thickness of the root stock and scion need to be similar for successful graft union. The scion was cut with a sharp blade in single motion on both the sides of the stem to form a wedge of approximately 4 cm and was placed into the cleft of 4-5 cm made at the top of the rootstock. The graft union was made intact using parafilm or grafting tape and was covered with polythene cover for maintaining the humidity for 2-3 days.

3.5.4 EVALUATION OF *P. indica* AGAINST PHYTOPLASMA IN SESAMUM

Pot experiments were conducted for evaluating the effect of beneficial fungal root endophyte *P. indica* against phytoplasma causing sesamum phyllody (Plate 3). Two experiments were laid out as follows.

Experiment: 1 Replications: 7

T1 - Seedlings grafted with phytoplasma infected scion after 30 days

T2 - *P. indica* colonization on seedlings, followed by grafting phytoplasma infected scion after 30 days

Experiment: 2 Replications: 7

T1 - Absolute control (non-colonized seedlings)

T2 - Sesamum seedlings colonized with *P. indica*

For the co-cultivation studies, *P. indica* was mass multiplied in FYM and coir pith (1:1 ratio) mixture amended with 2 per cent gram flour (20g per kg of mixture). Surface sterilized sesamum seeds were sown in portrays filled with mass multiplied mixture and in control with sterilized mixture. Three-week-old seedlings were then transferred into growbags filled with potting mixture.

After two weeks, wedge grafting was performed with phytoplasma infected scion for evaluating the effect of *P. indica* colonization against phytoplasma (experiment 1). The observations were recorded for the symptom development and other growth parameters.

Plate 3. General view of pot experiment for evaluating the bioefficacy of *P. indica* against phytoplasma in sesamum

Results

4. RESULTS

The research work entitled “Prevalence of sesamum phyllody in Onattukara tract and evaluation of fungal root endophyte *P. indica* for its management” with the objectives of symptomatology, molecular detection and characterization of phytoplasma inciting sesamum phyllody disease in AEU 3 (Onattukara tract); and evaluation of fungal root endophyte *P. indica* for its management was conducted in Department of Plant Pathology, College of Agriculture, Vellayani and Onattukara Regional Agricultural Research Station, Kayamkulum during 2019-2021. The results of the study are detailed in this chapter.

4.1 COLLECTION OF PHYTOPLASMA INFECTED SESAMUM PLANTS, WEED HOSTS SHOWING THE SYMPTOM AND ASSOCIATED INSECTS FROM AEU 3

Sesamum plants with phytoplasmal infection were collected from Onattukara Regional Agricultural Research Station D block and F block and Karthikapally. Disease incidence recorded from these areas was in the range of 25-40 per cent with the highest incidence at Karthikapally (Table 5). The varieties cultivated in these areas were Thilak and Thilarani.

Disease symptoms were observed at the stage of flowering of sesamum plants in all the sampled locations. The phytoplasma infected plants in ORARS D block (var. Thilarani) exhibited virescence and phyllody, and floral proliferation (Plate 4). Plants in ORARS F block (var. Thilak) also exhibited symptoms of virescence and phyllody at early stages of flowering, which later progressed into floral proliferation (Plate 5). In Karthikapally, the plants (var. Thilak) also showed symptoms of virescence and phyllody (39.44 per cent) (Plate 6).

Table 5. Sesamum phyllody disease incidence at different surveyed locations

Sl. No.	Location	Variety	D.I(%)
1.	ORARS (D block)	Thilarani	31.00
2.	ORARS (F block)	Thilak	26.00
3.	Karthikapally	Thilak	39.44

D.I. - Disease incidence

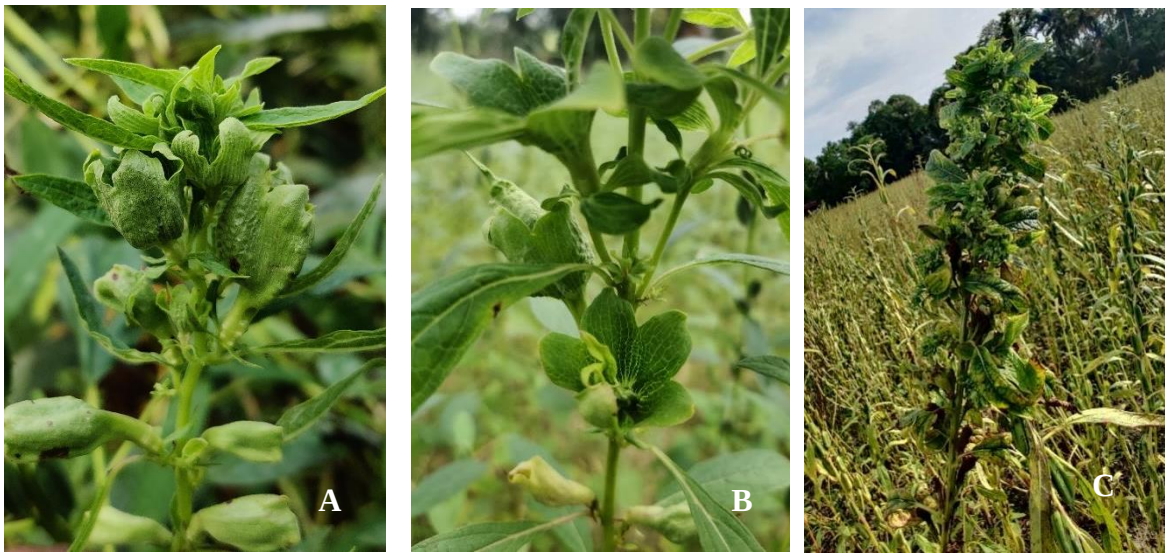


Plate 4. Field symptoms of sesamum phyllody in ORARS F block (A) virescence (B) phyllody (C) proliferation

Plate 5. Field symptoms of sesamum phyllody in ORARS D block (A) virescence (B) phyllody (C) floral proliferation

Plate 6. Field symptoms of sesamum phyllody in Karthikapally (A) virescence (B) phyllody

Weed plants and insects act as natural reservoir of these plant pathogenic phytoplasmas. Chocolate weed, *Melochia corchorifolia*, (family Sterculiaceae) was collected from the phytoplasma infected sesamum fields of Onattukara (Plate 7). The collected weed exhibited symptoms of shoot proliferation, resembling phyllody in sesamum.

Hoppers associated with phytoplasmal infection were collected from the infected fields using yellow sticky traps (Plate 8a). The collected hoppers were identified as *Orosius albicintus* (Plate 8b), *Hishimonas phycitis* (Plate 8c) and *Nephotettix* sp (Plate 8d). Population of *O. albicintus* and *H. phycitis* were higher (68 per cent) among the collected insects.

4.2 SYMPTOMATOLOGY

4.2.1 Symptomatology

Phytoplasmal infection in sesamum manifests at the stage of flowering, when greening of the floral parts (Plate 9a) and its transformation into leafy like structures occurs (Plate 9b). The flowers were sterile. In some cases, the ovary was modified into elongated structures, which resembled a shoot and it continued to grow producing more phylloid flowers (Plate 9c). Reduction in internodal length and thickening of the floral veins were also observed. In case of severe infection, the virescence and phyllody progresses to axillary shoot proliferation (Plate 9d & e) and twisted appearance (Plate 9f). Disease incidence and vulnerability index was also recorded from the surveyed areas. Highest incidence and severity of infection were recorded from Karthikapally with an incidence of 39.44 per cent and severity of 22.36 respectively (Table 6).

4.2.1 Microscopic examination of healthy and diseased tissues

DAPI stain is mostly used for the detection of phytoplasma. The presence of phytoplasmas was observed in the microtome sections of infected stem and leaf using DAPI (4,6diamidino-2-phenylindole) stain, a DNA binding fluorochrome under fluorescence microscopy. Upon staining, a diffuse fluorescence could be observed in

phloem cells of infected stem (Plate 10) and leaf (Plate 11), which was brighter than the one in the nuclei of parenchymal cells. These bright spots indicate the presence of phytoplasma.

Table 6. Sesamum phyllody incidence and its severity as vulnerability index at different locations

Sl. No.	Location	Variety	D. I. (%)	V. I.
1.	ORARS (D block)	Thilarani	31.00	20.50
2.	ORARS (F block)	Thilak	26.00	17.75
3.	Karthikapally	Thilak	39.44	22.36

*100 samples were counted at ORARS and 180 samples at Karthikapally.

Plate 7. Chocolate weed (A) Healthy plant, (B-D) Plant expressing symptoms resembling phyllody

Plate 8. Hoppers collected from phyllody infected sesamum fields (A) Yellow sticky trap with hoppers, Stereomicroscopic image (100X) of (B) *Hishimonas phycitis* (C) *Orosius albicintus* (D) *Nephotettix* sp.

Plate 9. Symptoms associated with sesamum phyllody (A) Virescence (B & C) Phyllody (D & E) Floral proliferation (F) Twisted appearance of inflorescence

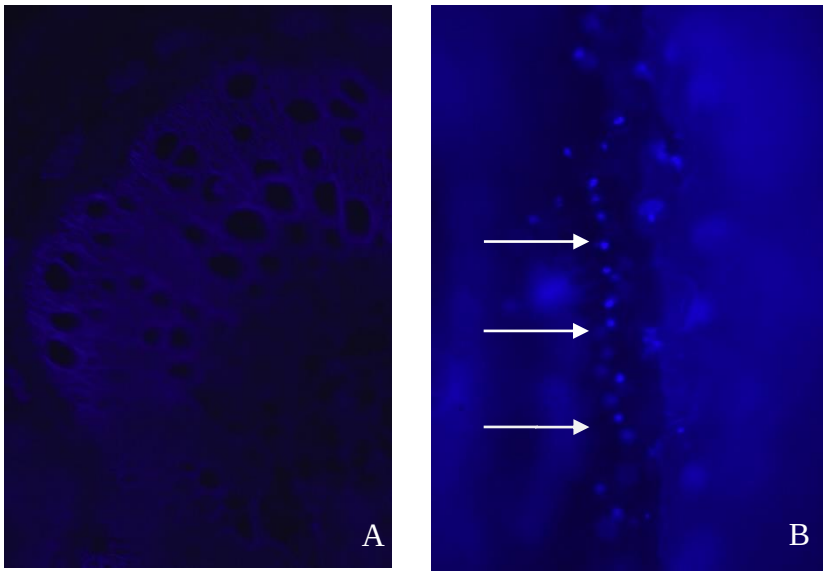


Plate 10. Microscopic view of (A) healthy and (B) infected sesamum stem under DAPI staining (400X)

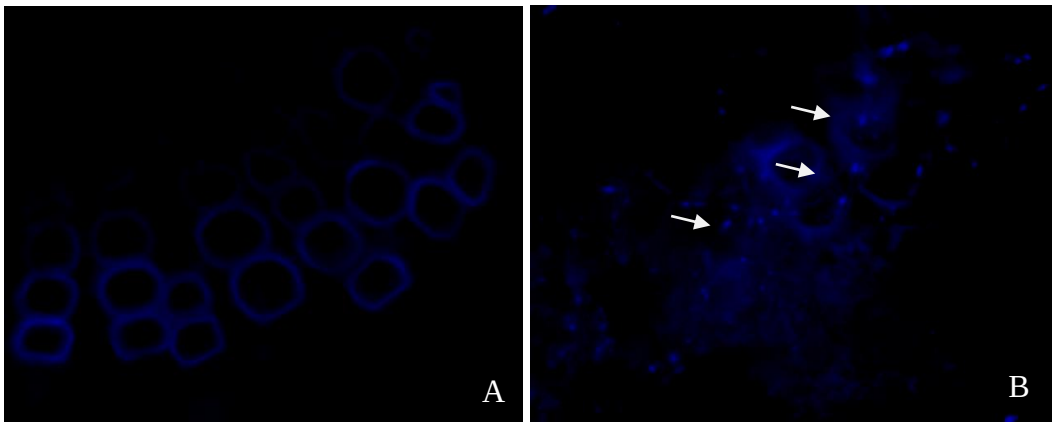


Plate 11. Microscopic view of (A) healthy and (B) infected sesamum leaf under DAPI staining (400X)

4.3 HORMONAL VARIATION IN DISEASED AND HEALTHY PLANTS

Plant hormone levels in the phytoplasma infected plants were found to be altered, when compared to the healthy plants. It can be due to the phytoplasma effect on the biosynthesis or signaling of these plant growth regulators. In this study, analysis of gibberellic acid and indole-3-acetic acid were done. A calibration graph was prepared from the known aliquots of pure gibberellic acid and indole-3-acetic acid and a linear relationship was constructed to estimate the GA and IAA contents of the samples in reference to the calibration graph.

The indole-3-acetic acid content was found to be reduced in both leaves and flowers in phytoplasma infected plants compared to healthy plants. IAA content in infected leaves was 131.33 µg g^{-1}, whereas in healthy leaves 164.74 µg g^{-1}. Hence, a reduction of about 1.25 times was recorded in phytoplasma infected leaves compared to the healthy leaves. In case of flowers, IAA content in infected flowers was only 143.16 µg g^{-1}, whereas healthy flowers contain 282.86 µg g^{-1}. Hence, 1.25 times reduction in leaves and 1.97 times reduction in flowers were observed in phytoplasma infected tissues compared to healthy tissues (Table 7).

In the phyllody infected sesamum leaves and flowers, the gibberellic acid content was found to be increased compared to the healthy samples. GA content in infected leaves was 1.39 mg g^{-1}, whereas healthy leaves contained only 0.62 mg g^{-1}. Hence, an increase of about two times was estimated in phytoplasma infected leaves compared to healthy leaves. In case of flowers, GA content in infected flowers was 0.61 mg g^{-1}, whereas healthy flowers contained only 0.06 mg g^{-1}. Hence, an increase of about ten times was recorded in phytoplasma infected tissues compared to healthy tissues (Table 8).

Table 7. IAA content (µg g^{-1}) in healthy and diseased samples of sesamum leaves and flowers

	Leaf	Flower
Healthy	164.7363	282.8573
Diseased	131.3316	143.1601
T calculated	2.420	8.835
T table (0.05)	2.306	

Table 8. GA content (mg g^{-1}) in healthy and diseased samples of sesamum leaves and flowers

	Leaf	Flower
Healthy	0.616	0.058
Diseased	1.391	0.607
T calculated	4.197	17.190
T table (0.05)	2.306	

4.4 MOLECULAR DETECTION, CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF THE PHYTOPLASMA

Total DNA from the phytoplasma infected plants was isolated using DNeasy plant mini kit (QIAGEN Cat. No. 69104). The quality and quantity of total DNA were assessed using bio-spectrophotometer (Table 9). Among the isolated DNA samples, sample from Karthikapally had the highest DNA quality with A260/A280 value of 1.21.

In direct PCR with P1/P6 primers, the phytoplasma infected samples from ORARS lowland (S1, S2), Karthikapally (S3), ORARS upland (S4), and Vellayani (S5) showed amplification at approximately 1.4 kb confirming the association of phytoplasma with the disease (Plate 12). None of the primer pairs produced amplification in any of the samples from asymptomatic plants. The consensus sequences of the amplicons obtained using P1/P6 primers were deposited in NCBI GenBank (Accession No. OK597186, OK625449, OK625583, OK625584, OK625585) (Table 10). BLAST analysis of the 16S rDNA gene sequence of sesamum phyllody phytoplasma showed that all the five isolates shared more than 99.0 per cent sequence similarity with that of the '*Candidatus* Phytoplasma aurantifolia' strains in GenBank. In the phylogenetic tree constructed using the 16S rDNA sequences, the sesamum phyllody phytoplasma under the study clustered with the 16Sr II group phytoplasmas causing sesamum phyllody in various regions (Plate 13). The sesamum phyllody phytoplasma belonging to 16Sr I has already been reported from some parts in India formed a separate cluster. Thus, the phytoplasma causing sesamum phyllody in Onattukara tract is identified as a '*Candidatus* Phytoplasma aurantifolia' related strain.

The virtual RFLP pattern derived from 16S rDNA fragment of sesamum phyllody phytoplasma generated by *i*PhyClassifier was found to be identical (similarity coefficient 1.00) to the reference pattern of 16Sr group II, subgroup D (GenBank accession: Y10097) (Plate 14).

Based on the results, the phytoplasma causing sesamum phyllody in Onattukara tract was identified as "*Candidatus* Phytoplasma aurantifolia"-related strain belonging to subgroup 16SrII-D.

Table 9. Quality and quantity of DNA of sesamum phyllody samples

Sl. No.	Isolate	OD at 260 nm	OD at 280 nm	260/280 value	Quantity of DNA (µg µl^{-1})
1.	ORARS lowland (S1, S2)	0.092	0.081	1.13	1.84
2.	Karthikapally (S3)	0.053	0.041	1.21	1.06
3.	ORARS upland (S4)	0.249	0.226	1.10	4.98
4.	Vellayani (S5)	0.041	0.034	1.20	2.10

Table 10. Accession numbers for sesamum phyllody phytoplasma sequences submitted to GenBank

Sample	GenBank accession number
S1	OK597186
S2	OK625449
S3	OK625583
S4	OK625584
S5	OK625585

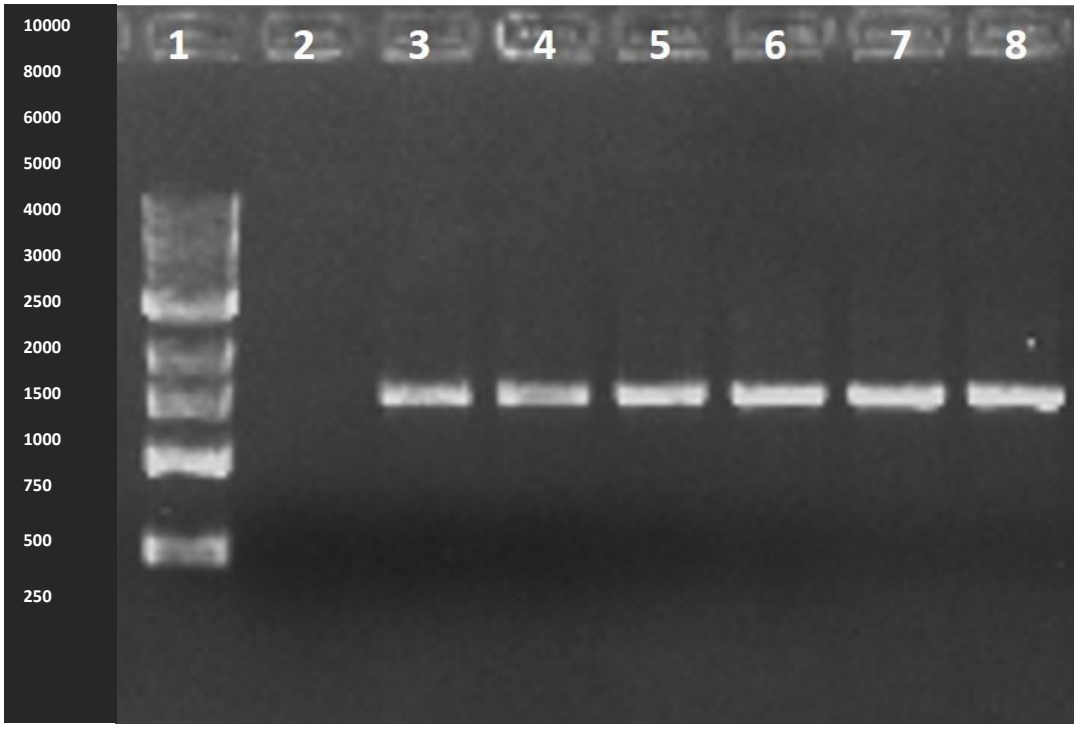


Plate 12. Amplified products (1.4kb) of sesamum phyllody phytoplasma using P1/P6 primers Lane1:1 kb DNA ladder, 2:NTC (No template control, 3:S1, 4:S2, 5:S3, 6:S4, 7:S5, 8:Bermuda grass white leaf phytoplasma (positive control)

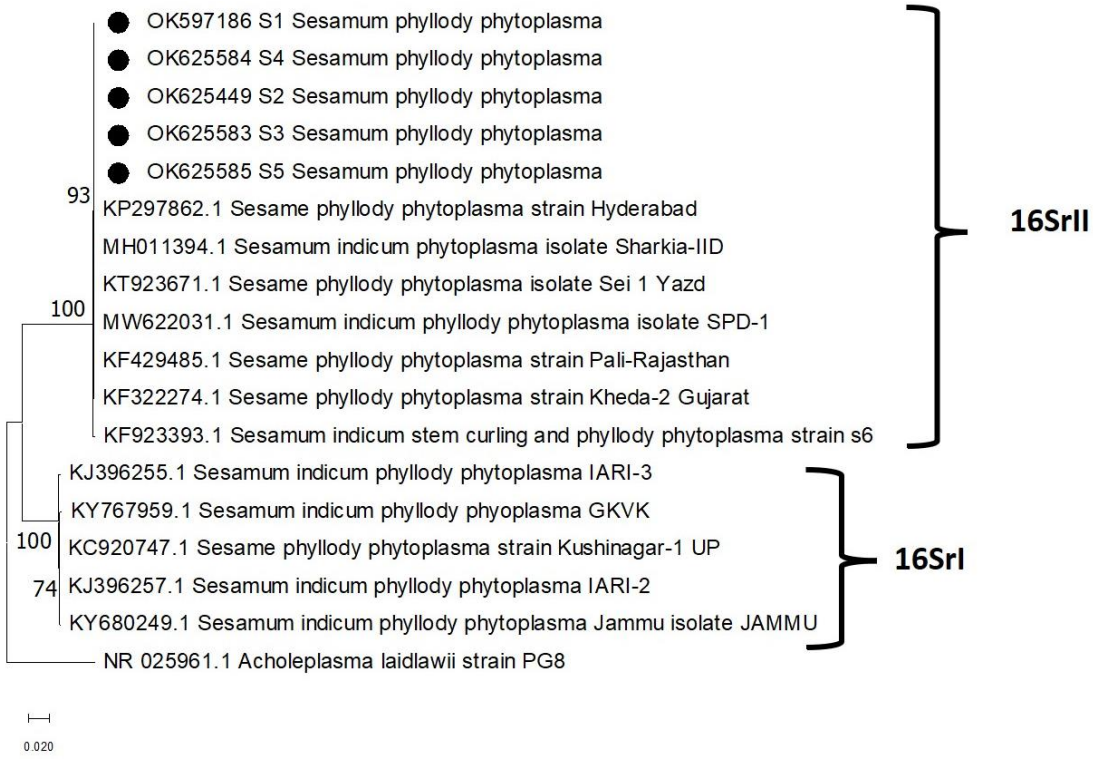


Plate 13. Phylogenetic tree comparing with the selected phytoplasmas from GenBank

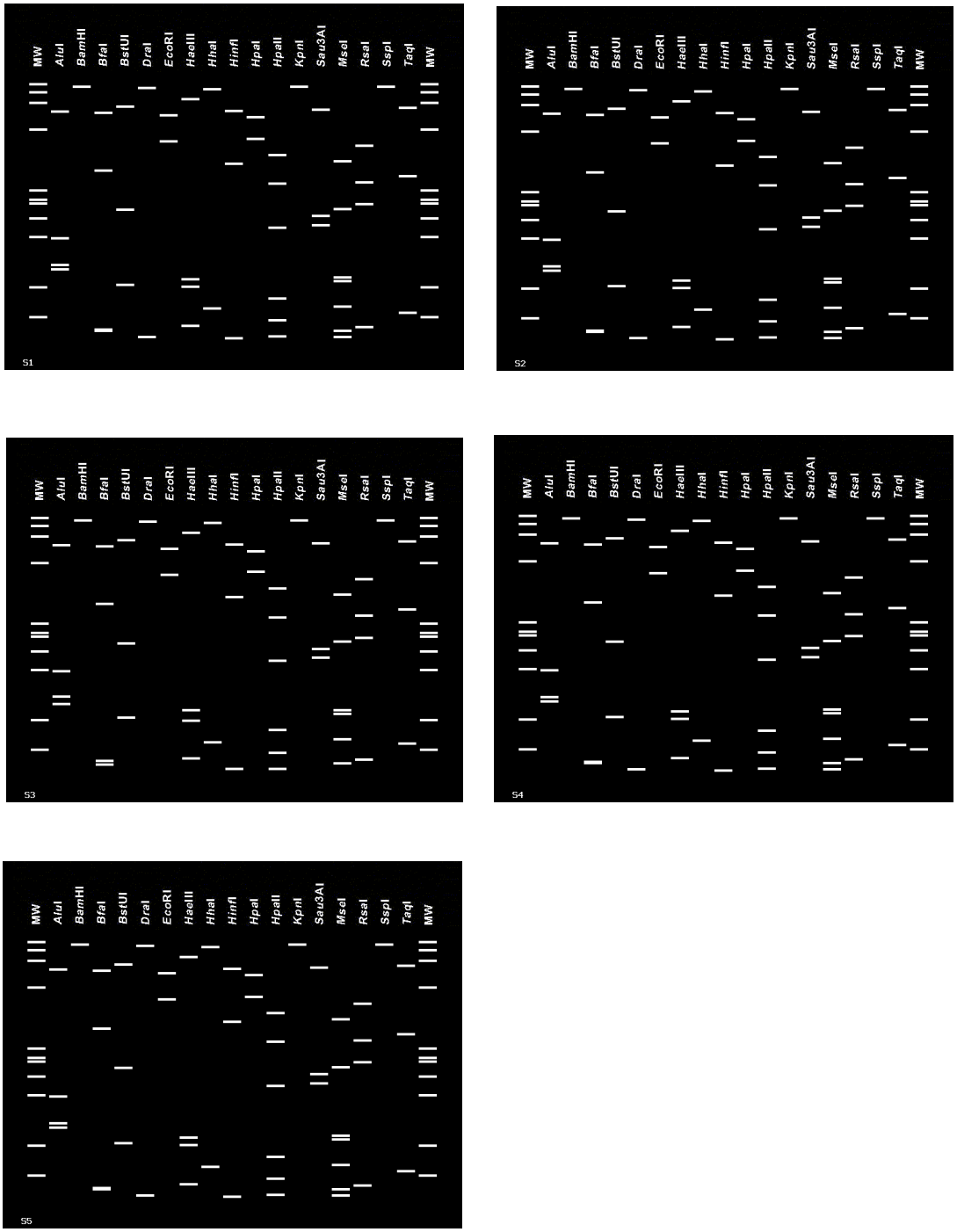


Plate 14. Virtual RFLP gels constructed based on 16SrDNA sequences of sesamum phyllody phytoplasma using iphyclassifier

4.5 EVALUATION OF *P. indica* AGAINST PHYTOPLASMA IN SESAMUM

P. indica obtained from Department of Plant Pathology, College of Agriculture, Vellayani was maintained in potato dextrose agar (PDA) by subculturing and cultured in potato dextrose broth (PDB), and mass multiplied in sterilized coir pith: FYM mixture (1:1) amended with 2 per cent gram flour (Plate 15). Sesamum seeds were sown in the mass multiplied medium. *P. indica* colonization in sesamum seedlings was observed at 7 days after germination. Microscopic examination of roots of colonized plants at different intervals of 7, 15 and 45 days after co-cultivation revealed the presence of chlamydospores in roots (Plate 16). Wedge grafting was standardized in sesamum 30 days after germination. Active growth of wedge grafted healthy scion indicates the successful graft union formation (Plate 17). Plants grafted with infected scion 30 days after germination exhibited virescence symptom at 25 days after grafting (Plate 18).

P. indica developed symbiotic relationship with plants and offers benefits such as plant development, as well as playing a key role in the control of plant diseases caused by numerous pathogens. From the pot experiment conducted, it was found that *P. indica* colonization could significantly reduce the disease incidence and severity of infection compared to the non-colonized plants (Table 11). After 30 days of grafting, an incidence of 20 per cent and severity of five was observed in the colonized plants grafted with infected scion, whereas an incidence of 60 per cent and severity of 45 was observed in non-colonized plants grafted with infected scion. The colonized plants did not express any phytoplasmal symptoms at 30 days after grafting, even with the active grafted proliferated scion, whereas the non-colonized plants expressed virescence symptoms (Plate 19).

After 45 days of grafting, an incidence of 60 per cent and severity of 50 were observed in the colonized plants grafted with infected scion, whereas an incidence of 80 per cent and severity of 75 were observed in non-colonized plants grafted with infected scion. The non-colonized plants expressed floral proliferation symptoms at 45 days after grafting, whereas among the colonized plants, 60 per cent of colonized plants expressed symptoms after a period of active pod development and 40 per cent

plants did not express any of the phytoplasmal symptoms (Plate 21). Hence, the expression of symptoms was found to be delayed in the colonized plants compared to non-colonized plants.

Table 11. Disease incidence and vulnerability index at 30 and 45 DAG'

Treatments	Disease incidence (%)		Vulnerability index		Days taken for symptom development
	30 DAG'	45 DAG'	30 DAG'	45 DAG'	
P. indica + phyllody	20	60	5	50	30-45 DAG'
Phyllody alone	60	80	45	75	25-32 DAG'

*DAG'- Days after grafting

P. indica-colonized plants grafted with infected scion recorded an enhancement in shoot length at 30 and 45 days after grafting and also earliness in flowering compared to non-colonized plants. Shoot length was 36.8 cm and 47.2 cm in the colonized plants, whereas 31.2 cm and 38.8 cm in non-colonized plants at 30 and 45 days after grafting respectively. Number of leaves were 43.6 in the colonized plants, whereas 37.2 in non-colonized plants at 30 days after grafting. Colonized plants took 18-23 days for flowering, whereas non-colonized grafted plants failed to flower (Table 12).

P. indica-colonized plants exhibited increased shoot and root length compared to the non-colonized plants at 30 and 55 days after germination (Plate 22). Shoot length was 10.2 cm and 38.2 cm in the colonized plants, whereas 7.6 cm and 32.8 cm in non-colonized plants at 30 and 55 days after germination. Number of leaves were 14.6 in the colonized plants, whereas 10.8 in non-colonized plants at 30 days after germination. Leaf area was 13.40 in colonized plants, whereas 11.10 in non-colonized plants at 30 days after germination. Colonized plants flowered at 47-53 days after germination, whereas non-colonized plants flowered only at 50-59 days after germination.

Table 12. Effect of *P. indica* colonization on growth parameters of sesamum plants inoculated with the phytoplasma by grafting

Treatments	Height (cm)		No of leaves		Leaf area (cm^2)		Days taken for flowering
	30DAG'	45DAG'	30DAG'	45DAG'	30DAG'	45DAG'	
P. indica + phytoplasma	36.80 ±3.42	47.20 ±3.56	43.60 ±3.57	53.00 ±5.24	5.08 ±0.57	2.80 ±0.09	18-23
Phytoplasma alone	31.20 ±3.96	38.80 ±5.76	37.20 ±2.58	59.40 ±2.30	4.90 ±0.72	2.33 ±0.14	-
T calculated	2.392	2.772	3.241	2.499	0.437	6.098	
T table (0.05)	2.306						

Values are mean of 7 replications ± standard deviation

*DAG'- Days after grafting

Table 13. Effect of *P. indica* colonization on growth parameters in sesamum plants

Treatments	Height		No of leaves		Leaf area (cm^{-2})		Days taken for flowering
	30DAG	55DAG	30DAG	55DAG	30DAG	55DAG	
P. indica colonized	10.20 ±1.30	38.20 ±2.86	14.60 ±2.79	22.20 ±1.48	13.40 ±1.14	6.06 ±0.48	47-53DAG
Absolute control	7.60 ±1.14	32.80 ±2.16	10.80 ±0.83	25.20 ±0.83	11.10 ±0.89	7.12 ±0.28	50-59DAG
T calculated	3.357	3.362	2.914	3.939	3.549	4.19	
T table (0.05)	2.306						

Values are mean of 7 replications ± standard deviation

*DAG- Days after germination

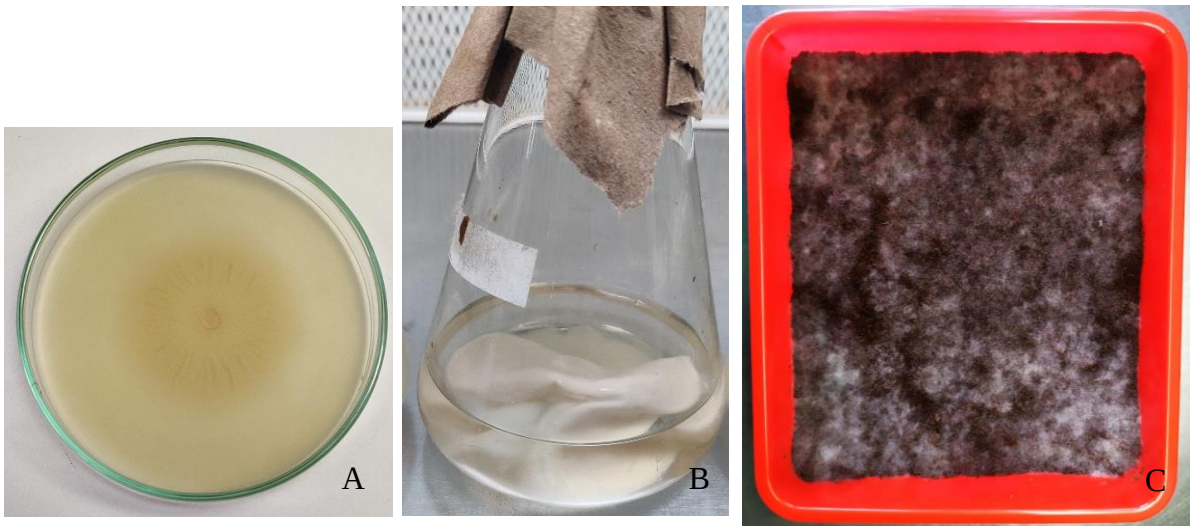


Plate 15. *P. indica* culture in (A) PDA (B) PDB (C) mass multiplication in sterilized coirpith: FYM mixture (1:1) amended with 2 per cent gram flour.

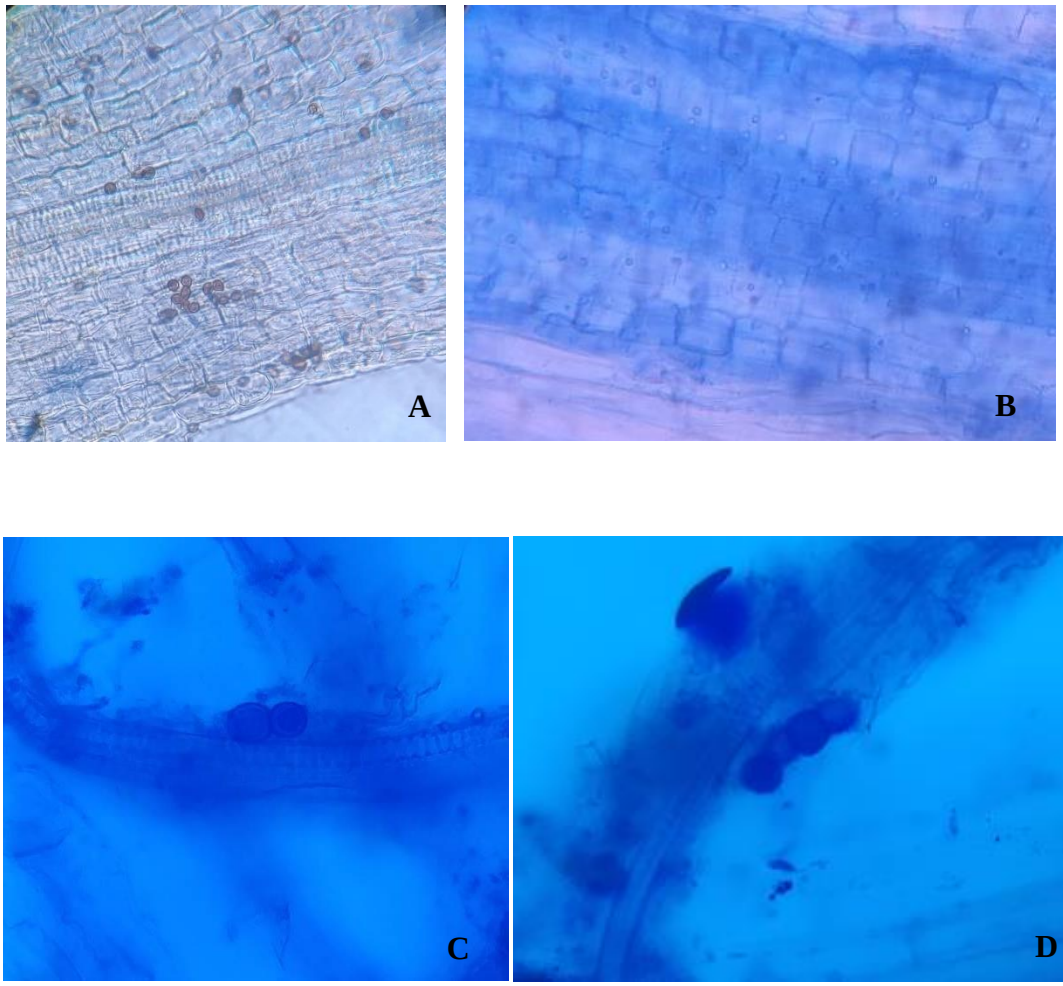


Plate 16. Sesamum roots colonized with *P. indica* (A)7DAG (B)15DAG (C & D) 45 DAG (400X)

***DAG – Days after germination**

Plate 17. Wedge grafting in sesamum (A) Healthy sesamum plant grafted with healthy scion 30 days after germination (B) Grafted plant with actively growing scion at 10 days after grafting

Plate 18. Grafted sesamum plant with infected scion showing virescence symptom after 25 days of grafting

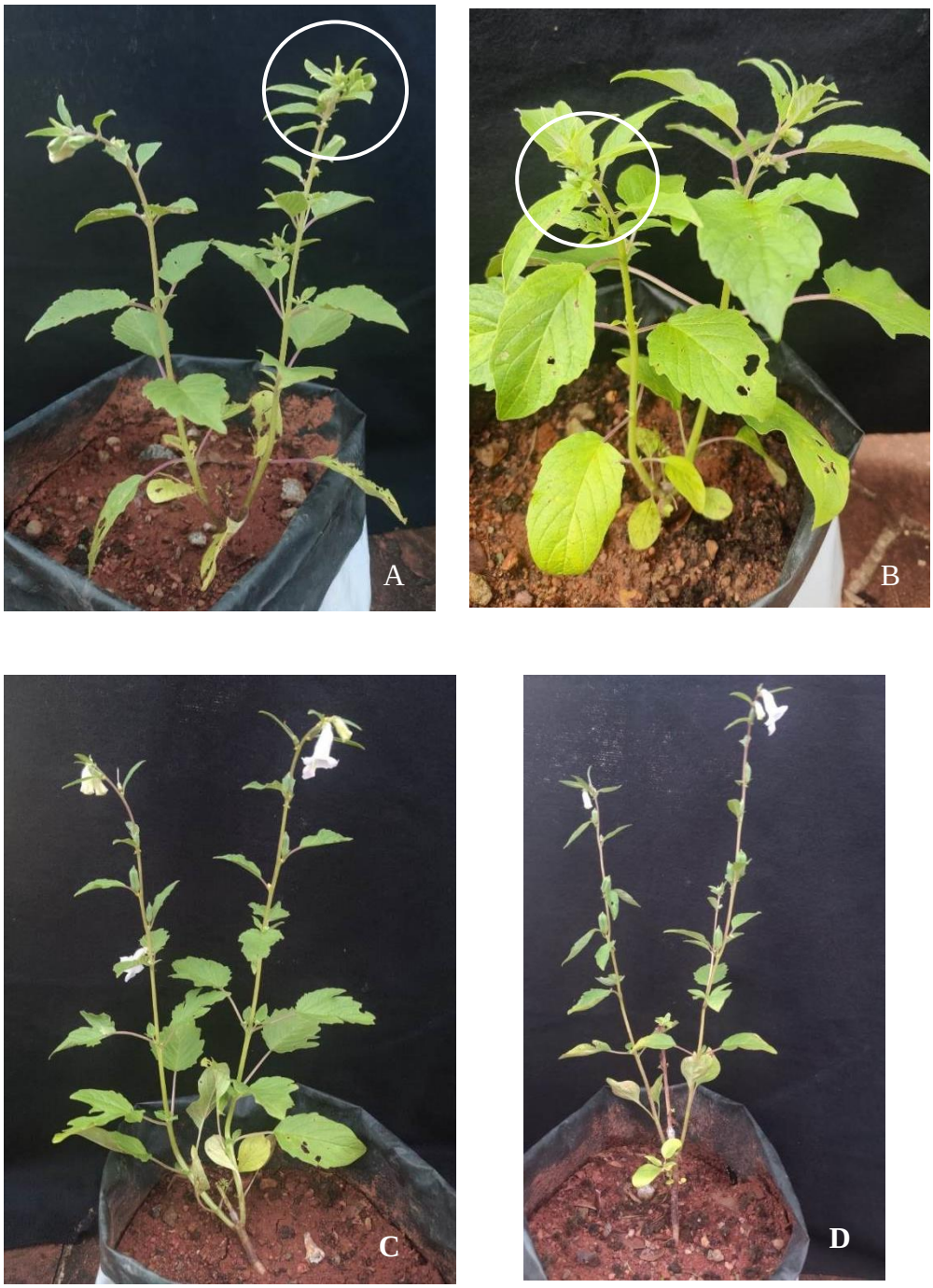


Plate 19. Sesamum plants(A&B) non-colonized showing virescence symptom and (C&D) *P. indica* colonized at 30 days after grafting

Plate 20. Sesamum plants (A) non-colonized with *P. indica* showing proliferation symptom (B) colonized with *P. indica* showing virescence symptom (C) colonized with *P. indica* with active pod development at 35 days after grafting

Plate 21. Sesamum plants (A) non-colonized with *P. indica* showing proliferation symptom (B) colonized plant with active pod development and (C) colonized plant with delayed expression of symptom at 45 days after grafting

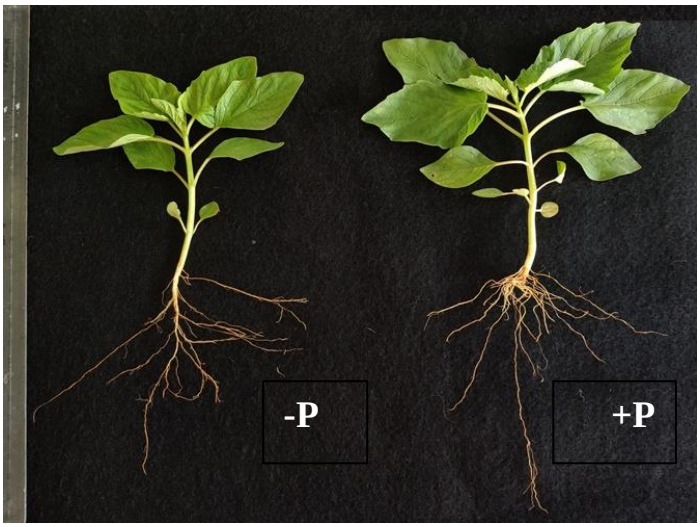


Plate 22. Growth promotion in sesamum var. Thilak colonized by *P. indica* 30 and 55 days after germination

Discussion

5. DISCUSSION

Sesamum (*Sesamum indicum* L.) is one of the oldest cultivated oilseed crops. In view of its high-quality oil and protein content, it is regarded as the Queen of oilseeds. Onattukara is the traditional sesamum cultivating region of Kerala practicing a multiple cropping system with cropping sequence of paddy-paddy-sesamum. In the summer rice fallows of Onattukara tract, sesamum is mostly raised. The crop thrives in this area because of the residual moisture found in rice fields. Waterlogging, pests and diseases can impede long-term output. Pests and diseases that harm seedlings, foliage, flowers, pods, and stems cause 25 per cent yield loss on average around the world. Sesamum phyllody disease, caused by phytoplasmas, is one of the most serious diseases, resulting in yield losses of 5-15 per cent worldwide, in severe cases can cause yield loss upto cent per cent. The relationship of different phytoplasma groups with sesamum growing in different parts of the world was reported by phylogenetic analysis. Insecticides, roguing, and production of phytoplasma-free propagation materials are the mainstays of traditional management approaches. *P. indica*, a beneficial root colonising endophytic fungus, forms symbiotic relationship with plants and offers benefits such as growth development, as well as playing a key role in the control of plant diseases caused by numerous pathogens. This chapter discusses the findings of the study on 'Prevalence of sesamum phyllody in Onattukara tract and evaluation of fungal root endophyte *P. indica* for its management.'

5.1 COLLECTION OF PHYTOPLASMA INFECTED SESAMUM PLANTS, WEED HOSTS SHOWING THE SYMPTOM AND ASSOCIATED INSECTS FROM AEU 3

Survey was conducted to collect phyllody infected samples from different sesamum growing areas from December 2020 to May 2021. Onattukara Regional Agricultural Research Station D block and F block and Karthikapally were the regions surveyed. In the surveyed areas, disease incidence was found ranging from 25 per cent to 40 per cent with highest incidence at Karthikapally (39.44 per cent). In Onattukara tract, the crop is mostly raising in the summer season and we could observe prolific phyllody disease incidence after intermittent rains during the

prolonged summer, which may be due to the increase in vector population. Reports of disease incidence indicate a wide occurrence of sesamum phyllody in cultivated states of India. Madhupriya *et al.* (2015) recorded 7-55 per cent incidence of sesame phyllody and witches' broom symptoms in sesame-growing regions in nine Indian states viz., Uttar Pradesh, West Bengal, Madhya Pradesh, Chhattisgarh, Delhi, Rajasthan, Gujarat, Tamil Nadu, and Maharashtra during 2011 - 2013. In sesame fields of nine Indian states surveyed, both phyllody (conversion of floral components into leaf-like structure) and witches' broom (dense mass of younger shoots growing from a single point) symptoms were observed. From the sesamum fields of Delhi (NBPGR), Singh *et al.* (2016) reported 3 per cent of plants with fasciation symptoms, with normal flower and fruit development. A false capitulum-like structure with whorled leaves and flowers at the shoot apical region and shortening of internodes were noted in such plants. The size of leaves and flowers, were severely reduced, indicating little leaf symptoms. Almost 10 per cent of the plants had displayed these symptoms.

Pathak *et al.* (2013) evaluated the disease incidence in Gujarat districts during kharif and summer seasons of 2008-09. During kharif season, the incidence was 0-1.5 per cent, while during the summer season, it was 0-4.5 per cent. The greater incidence during the summer season would be due to the shorter incubation period of disease agent in both the host plant and the insect vector. In the Coimbatore conditions of India, Chattopadhyay *et al.* (2015) found that one per cent rise in disease incidence lowered the yield by 8.36 kg ha^{-1}. Devanna *et al.* (2020) assessed the phyllody incidence at different locations of Karnataka, where average disease incidence of 18.0-31.6 per cent was observed in summer season (April to June) crop, whereas an incidence of 24.8-38.0 per cent was recorded in the same fields during followed kharif season (June to September). The difference in the incidence of sesamum phyllody in two subsequent growing seasons might be due to increased vector population due to the continuous host cultivation, which could carry the inoculum from the preceding season crop.

Phookan *et al.* (2020) identified the relationship between meteorological parameters and vector population with the sesamum phyllody incidence in Assam.

During summer season with increased temperature, the vector population and disease incidence were found to be higher, whereas during the kharif season, the vector population and incidence were higher only when maximum day and night temperatures are at higher level and decreased total rainfall. Our results are in concurrence with Madhupriya *et al.* (2015), Pathak *et al.* (2013), Singh *et al.* (2016), Devanna *et al.* (2020) and Phookan *et al.* (2020).

Certain weed plants may serve as natural reservoir for phytoplasmas that cause plant disease. In the sesamum growing fields of Onattukara, chocolate weed, *Melochia corchorifolia*, Sterculiaceae family, was found to be exhibiting indications of shoot proliferation resembling phyllody. Our results are in accordance with Chen *et al.* (2017) and Yu *et al.* (2021), wherein *Melochia corchorifolia* showing symptoms of witches' broom, virescence and phyllody was reported from China. Here 16Sr I-B and 16Sr II-V group phytoplasmas were found to be associated. (Chen *et al.*, 2017; Yu *et al.*, 2021).

Rao *et al.* (2017a) found *Ocimum canum* plants with little leaf disease in phyllody-infected sesamum fields of Gorakhpur district, with an incidence of 5 per cent. The *O. canum* little leaf disease was linked to 16 SrI group phytoplasma, and the same phytoplasma was detected in affected sesame plants. In the sesamum growing districts of Jammu, Singh *et al.* (2018) noticed the phytoplasma suspected witches' broom symptoms in a natural weed *Cannabis sativa* subsp. *sativa* with an incidence of 5-12 per cent. The association of *Ca.* Phytoplasma asteris' (16Sr I group) and 16SrI-B subgroup with the symptomatic plants was observed. Along with our results, the findings of Rao *et al.* (2017a), Chen *et al.* (2017), Singh *et al.* (2018) and Yu *et al.* (2021) imply that weeds could act as natural reservoir of phytoplasmas, contributing to the spreading of related phytoplasmas.

Presence of hoppers including *H. phycitis*, *O. albicintus* and *Nephotettix* sp. were identified from the collected insects from phytoplasma infected fields of Onattukara. In a blast analysis of 16S rDNA sequences from infected sesamum plants, weed (*Sclerocarpus africanus*), and the leaf hopper (*H. phycitis*), Nabi *et al.* (2015a) found 99 per cent sequence identity, revealing the importance of particular weeds and insects in secondary spread of phytoplasmal diseases. From phyllody-infected

sesamum fields in Assam, Phookan *et al.* (2019) had also found higher population of *H. phycitis* and *Exitianus indicus*. *H. phycitis* was found to transmit sesamum phyllody phytoplasma (83.33 per cent) to healthy sesamum plants in the transmission studies. BLAST analysis had confirmed its association with the aster yellows phytoplasmas. Hence, our study is in consistent with that of Nabi *et al.* (2015a) and Phookan *et al.* (2019), wherein they observed higher population of *H. phycitis* in the phyllody infected sesamum fields.

5.2 SYMPTOMATOLOGY

The phytoplasma infection is most noticeable during the flowering stage, when the floral parts turn green and convert into leafy-like structures. The ovary has been replaced by elongated structures resembling a shoot, which continues to grow and produce new phylloid flowers, and the flowers have become sterile. Thickening of the floral veins, reduced internodal length, and floral proliferation has also been observed. These are in conformity with the symptoms observed by Markad *et al.* (2018) and Yadav *et al.* (2021), wherein floral virescence, drying of the phylloid flowers, conversion of flowers into leafy structures, floral proliferation, witches' broom and stunted growth of plants were documented.

Nakashima *et al.* (1999) noticed similar symptoms, in which the pistils united at the edges and changed into a pseudosyncarpous ovary. Here, instead of ovules, the ovary had few petiole-like outgrowths that grew through the wall of false ovary, producing miniature shoots. As the shoot grew, it produced additional phylloid flowers at the axillae. As a result, phyllody-infected plants were sterile, resulting in complete yield loss. According to Cagirgan *et al.* (2013), the infection hinders filament function, resulting in 'pin type' flowers. Gogoi *et al.* (2017) observed the conversion of sepals and petals into green leaf-like structures with thick veins and reduced size. Majority of the stamens kept their original shape, but some of the anthers morphed into green leaf-like structures and flattened. Namba (2019) reviewed that the resultant symptoms of phytoplasmal infection would be a strategy employed by phytoplasmas to make it more desirable for the insect vectors that feed on the plant sap, resulting in increased transmission efficiency and survival of the pathogen. Phookan *et al.* (2020) noted the infection at early growth stages resulted in yellowing

of leaves, reduction in leaf size and stunting, whereas later stage infection developed symptoms of virescence, phyllody, floral proliferation and cracking of pods.

Under fluorescence microscopy, the presence of phytoplasmas was detected in microtome cut sections of infected tissues using DAPI (4,6 diamidino 2 phenylindole) stain, a DNA binding fluorochrome. When stained, phloem cells from infected tissues showed a diffuse inflorescence that is brighter than that seen in the nuclei of parenchymal cells. The presence of phytoplasmas, which is lacking in healthy tissues, is shown by these bright patches. Microscopy techniques such as Transmission Electron Microscopy (TEM), light microscopy and DAPI (DNA-specific-6-diaminido-2-phenylindole) fluorescence microscopy technique were previously used in India to diagnose phytoplasma disease. The fluorochrome dye DAPI (4'6 diamidino-2-pheilindole) binds to AT-rich dsDNA in phytoplasma cells (Deeley *et al.*, 1979). Similar results were observed by Musetti and Favali (2004) using two DNA-binding fluorochromes, 4'-6-diamidino-2-phenylindole (DAPI) and the benzimidole derivate Hoechst 33258, under fluorescence microscope and established the presence of phytoplasmas in frozen microtome slices of phytoplasma infected tissues. Rao *et al.* (2011) stained young tissues (petioles of young leaves, phloem tissues, shoots, branches, and roots) with DAPI solution and examined under a fluorescence microscope in thin sections (20–30um). The presence of phytoplasmas was indicated as accumulations of a bluish-white fluorescence in sieve elements of tissues.

5.3 HORMONAL VARIATION IN DISEASED AND HEALTHY PLANTS

When plant hormone levels of phytoplasma infected plants were compared to healthy plants, it was found to be altered. It could be owing to the phytoplasmal effect on production or signaling of this plant growth regulators.

The IAA content was found to be decreased in both leaves and flowers of phyllody infected samples compared to healthy samples (Fig. 3). During the hormonal study, a reduction of 1.25 times in leaves and 1.97 times in flowers were detected. Any imbalance in IAA and cytokinin content might lead to abnormal growth pattern of infected plants. Hence altered auxin-cytokinin ratio might be partly responsible for

the proliferation symptoms. These findings are in accordance with Chang (1998), Perica (2008), Zafari *et al.* (2012) and Minato *et al.* (2014). Chang (1998) theorised the phytoplasma dependency on mevalonic acid (MVA) and isopentenyl pyrophosphate (IPP), the common precursors of plant sterols and IAA. The persistent demand for MVA for sterols would upset the balance of IAA in the infected plants. Perica (2008) found that prolonged treatment of '*Ca* Phytoplasma asteris' and '*Ca* Phytoplasma solani' infected shoots with added IAA in subsequent sub cultures resulted in symptom remission as well as improved elongation in treated shoots. The phytohormone IAA was shown to be considerably lower in phytoplasma-infected lime leaves than in healthy ones, according to Zafari *et al.* (2012). Minato *et al.* (2014) discovered that when TENGU, a small secreted phytoplasma protein produced in *Nicotiana benthamiana* plants, it caused witches' broom and dwarfism symptoms. TENGU inhibits flower maturation by reducing endogenous levels of IAA by repressing ARF6 and ARF8.

The gibberellic acid level of phyllody infected sesamum leaves and flowers was found to be higher than in healthy samples (Fig. 4). There was a two-fold rise in leaves and a ten-fold increase in flowers. This is in conformity with the results of Youssef *et al.* (2018) and Mardi *et al.* (2015). Youssef *et al.* (2018) found a considerable increase in gibberellin levels in phyllody-infected sesamum plants compared to control, concluding that it plays a role in plant defence through altering plant defence responses. According to Mardi *et al.* (2015), increased vulnerability to biotrophic infections is frequently associated with increased gibberellic acid levels. GA promotes disease vulnerability by inducing the expression of expansins, which are involved in cell wall loosening.

Our result is in contradiction to the results of Bahar and Alfadhal (2020), wherein the HPLC results of phytoplasma infected sesame, brinjal, tomato and vinca showed higher concentrations of IAA, GA and abscisic acid in the infected plants compared to healthy samples. This might be in consistent with the expressed symptoms, wherein an increased vegetative growth was in way with the increased growth hormone levels.

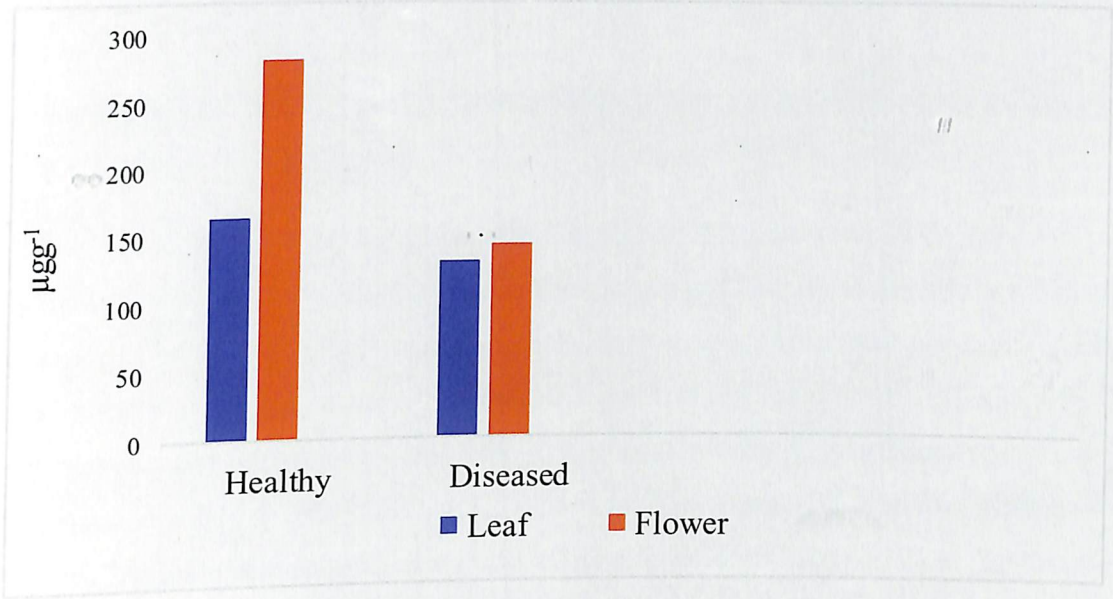


Fig. 3 IAA content in healthy and diseased samples

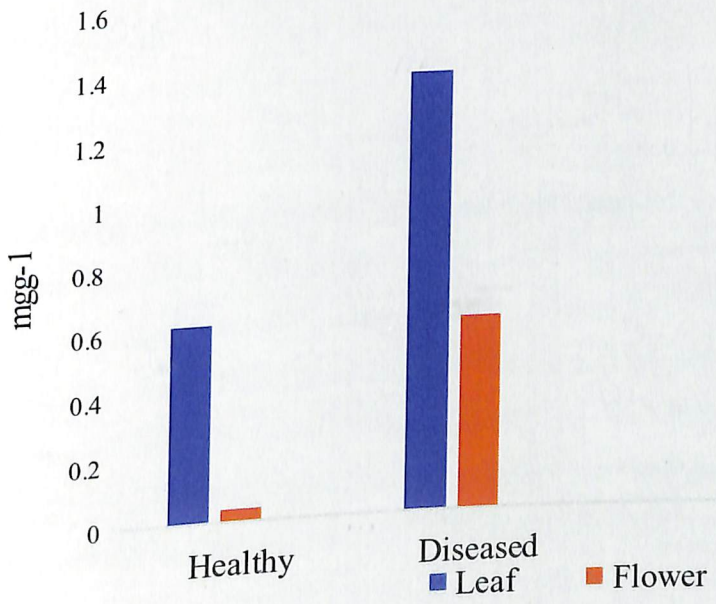


Fig. 4 GA content in healthy and diseased samples

4.4 MOLECULAR DETECTION, CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF THE PHYTOPLASMA

Amplification of approximately 1.4 kb amplicon in direct PCR with universal primers P1/P6 confirmed the association of phytoplasma with the disease. BLAST analysis of the 16S rDNA gene sequence showed more than 99.0 per cent sequence similarity with '*Candidatus* Phytoplasma aurantifolia' strains in GenBank. In the phylogenetic tree constructed, the sesamum phyllody phytoplasma under study clustered with the 16Sr II group phytoplasmas. The virtual RFLP pattern generated by *i*PhyClassifier was found to be identical (similarity coefficient 1.00) to the reference pattern of 16Sr group II, subgroup D.

Phylogenetic analysis revealed the association of distinct phytoplasmas with sesamum grown in various parts of the world. Four phytoplasma groups 16SrI, 16SrII, 16SrVI and 16SrIX are reported to be associated with sesame phyllody disease (Rao *et al.*, 2015). Rao (2021) observed the most prevalent and widespread group of phytoplasma in India is aster yellows group (16SrI) followed by 16SrII, 16SrVI, 16SrXI and 16SrXIV groups.

Our results are in conformity with Singh *et al.* (2016), detected only group 16SrII phytoplasmas in South Indian sesamum collections. 16SrII-A, II-C, and II-D were identified as subgroups, with 16SrII-D being prevalent in all five southern states surveyed, but primarily in Tamil Nadu. Andhra Pradesh, Maharashtra, and Karnataka were all found to have the subgroup 16SrII-A. The subgroup 16SrII-C was detected in only one sesame sample from Raichur (on the border of Karnataka and Andhra Pradesh). The group 16SrII-D in sesamum phyllody samples taken from Punjab and western Rajasthan were detected. They found variations in the infecting phytoplasma subgroup in yearly collected phyllody-affected sesame samples from Delhi for four years (2007-2010), with 16SrII-D phytoplasma in the 2007 collection and 16SrI-B phytoplasma in the following three years. In contradiction to our results, the association of the subgroup 16SrI-B was found in samples collected in North India (Uttar Pradesh, Haryana, eastern Rajasthan and Delhi).

Our results are in concurrence with Devanna *et al.* (2020), where 16Sr II group were found to be associated. They reported the association of distinct phytoplasma strains in two subsequent growing seasons (Summer and Kharif) of Karnataka. The isolates from summer crop (April-June) were belonged to 16Sr II-D subgroup whereas isolates from Kharif crop (June-September) were belonged to 16Sr II-A and 16Sr II-D subgroups. It could be due to the absence of vectors specific to 16Sr II-A strains of phytoplasma or due to the lesser sample size in summer crop.

In contradiction to our results, Manjunatha *et al.* (2012) and Nabi *et al.* (2015b) found close relation of phytoplasma infected sesamum plants from Karnataka, Delhi, Uttar Pradesh and Bihar to ribosomal group 16Sr1. Manjunatha *et al.* (2012) observed isolates from Karnataka with more than 99 per cent nucleotide identity with 16Sr1 group. The sesamum phyllody and witches broom disease etiology were studied by Nabi *et al.* (2015b) from samples collected from different fields of Delhi, Uttar Pradesh and Bihar. The BLAST analysis and phylogenetic analysis of 16Sr DNA and sec A gene sequences revealed the isolates from Uttar Pradesh was 16Sr I-B and 16Sr II-C subgroups, and isolates from Delhi and Bihar were 16Sr 1-B subgroup.

4.5 EVALUATION OF *P. indica* AGAINST PHYTOPLASMA IN SESAMUM

P. indica colonization in sesamum seedlings was examined at 7 days after cocultivation. Microscopic examination of roots of the colonized plants at different intervals of 7, 15 and 45 days after co-cultivation revealed the presence of chlamydospores in roots. Zhang *et al.* (2014) observed colonization of *P. indica* in sesamum roots with colonization efficiency of 75 per cent. Chandran (2019) observed the presence of chlamydospores in vegetable cowpea roots at 7 days after cocultivation with *P. indica.*

Wedge grafting was standardized in sesamum 30 days after germination. Active growth of grafted healthy scion indicates the successful graft union formation. Plants grafted with infected scion exhibited virescence symptom at 25 days after grafting. This is in agreement with the result obtained by Ravinder (2017) and Vamshi *et al.* (2019), wherein sesamum phyllody was successfully transmitted from

infected plants to healthy plants through side grafting, whereas it cannot transmit through seed and sap.

P. indica forms symbiotic relationship with plants and promoting the plant growth parameters and helping to control plant diseases caused by different pathogens. When compared to non-colonized plants, *P. indica* colonization could significantly reduce the disease incidence (Fig. 5) and severity of infection (Fig. 6). After 30 days of grafting, the disease incidence of 20 per cent and severity of five was observed in the colonized plants grafted with infected scion, whereas an incidence of 60 per cent and severity of 45 was observed in non-colonized plants grafted with infected scion. The colonized plants did not express any phytoplasmal symptoms at 30 days after graft-transmission of the phytoplasma and even with the active grafted proliferated scion, whereas the non-colonized plants expressed virescence symptoms.

After 45 days of grafting, an incidence of 60 per cent and severity of 50 was observed in the colonized plants grafted with infected scion, whereas an incidence of 80 per cent and severity of 75 were observed in non-colonized plants grafted with infected scion. The non-colonized plants expressed floral proliferation symptoms at 45 days after grafting, whereas among the colonized plants, 60 per cent of colonized plants expressed symptoms after a period of active pod development and 40 per cent plants did not express any of the phytoplasmal symptoms

P. indica colonized plants exhibited increased shoot and root length compared to the non-colonized plants at 30 and 55 days after germination. Shoot length was 10.2 cm and 38.2 cm in the colonized plants, whereas 7.6 cm and 32.8 cm in non-colonized plants at 30 and 55 days after germination. Number of leaves were 14.6 in colonized plants, whereas 10.8 in non-colonized plants at 30 days after germination. Leaf area was 13.40 in colonized plants, whereas 11.10 in non-colonized plants at 30 days after germination. Colonized plants flowered at 47-53 days after germination, whereas non-colonized plants flowered only at 50-59 days after germination.

According to Zhang *et al.* (2014), inoculation of sesamum roots with the endophytic fungus *P. indica* promoted the growth of seedlings, improved drought

resistance, and increased sesamum yield. It also affects phytohormone levels through interfering with biosynthesis, modification, and degradation pathways, as well as signalling pathways (Oelmuller *et al.*, 2009). Anith *et al.* (2018) observed black pepper plants inoculated with *P. indica* produced a greater number of leaves and leaf area per plant compared to the control plants. Plants colonized with *P. indica* showed early flowering and spike setting. The fresh and dry weights of berries harvested from *P. indica* colonized plants were substantially higher than those harvested from control plants. The fungus also boosted the overall oleoresin and piperine content in the berries upon inoculation.

Harikumar (2013) reported the reaction of indigenous AMF isolates from rice fallows to sesame, a crop that is grown as a follow-up to rice in Kerala. Inoculation with indigenous AMF modified growth attributes such as rootlet number, shoot length, leaf number, leaf area and yield components like capsule number, capsule weight, and seed number significantly. *Acaulospora lacunosa* inoculated plants had more rootlets and shoot length, while *Glomus dimorphicum* inoculated plants had the highest leaf number and area significantly increased the yield components in sesame. Our results are in accordance with Oelmuller *et al.* (2009), Harikumar (2013), Zhang *et al.* (2014), Anith *et al.* (2018) and Chandran *et al.* (2021), where endophyte colonization showed significant effect on several growth parameters.

P. indica protects maize plants from the root parasitic fungus *Fusarium verticillioides* by inhibiting its colonization. Antioxidant enzyme catalase activity was also found to be significantly high in *F. verticillioides-P. indica* colonized roots (Kumar *et al.* 2009). *P. indica* significantly induced resistance to Tomato yellow leaf curl virus in a susceptible cultivar T07-1. The incidence of disease and disease index were proved to be lowered by 26 per cent and 1.25 per cent and here according to Wang *et al.* (2015) the phytohormone signaling pathways might be involved in the interaction of plant, endophyte and pathogen. Chandran (2019) reported greater activity of pathogenesis-related proteins and defence enzymes such as polyphenol oxidase in *P. indica*-colonized plants resulted in a lower vulnerability index of Blackeye cowpea mosaic virus in cowpea. Chippy (2020) revealed the effect

P. indica colonization on bhindi yellow vein mosaic virus infected plants. The *P. indica* colonized-BYVMV inoculated okra plants showed remission of symptoms, which would be attributed by enhanced defense related enzymes, soluble proteins and chlorophyll.

In the experimental host *Catharanthus roseus*, Musetti *et al.* (2011) investigated the relationships between *Candidatus* Phytoplasma mali and the apple endophyte *Epicoccum nigrum*. Endophyte-treated apple proliferation grafted plants had showed only leaf yellowing, whereas untreated apple proliferation grafted plants had deformed leaves, enlarged veins, leaf discolorations, diffuse yellowing and little flowers. *P. indica* is similar to arbuscular mycorrhizal fungus (AMF) as it promotes plant development and induces resistance to a variety of biotic and abiotic stressors in colonised plants, but unlike AMF, it may grow axenically (Varma *et al.*, 2012). Our results are in concurrence with Kumar *et al.* (2009), Musetti *et al.* (2011), Wang *et al.* (2015), Chippy (2020) and Chandran *et al.* (2021) where endophyte treatment showed significant effect on imparting resistance to biotic and abiotic stresses.

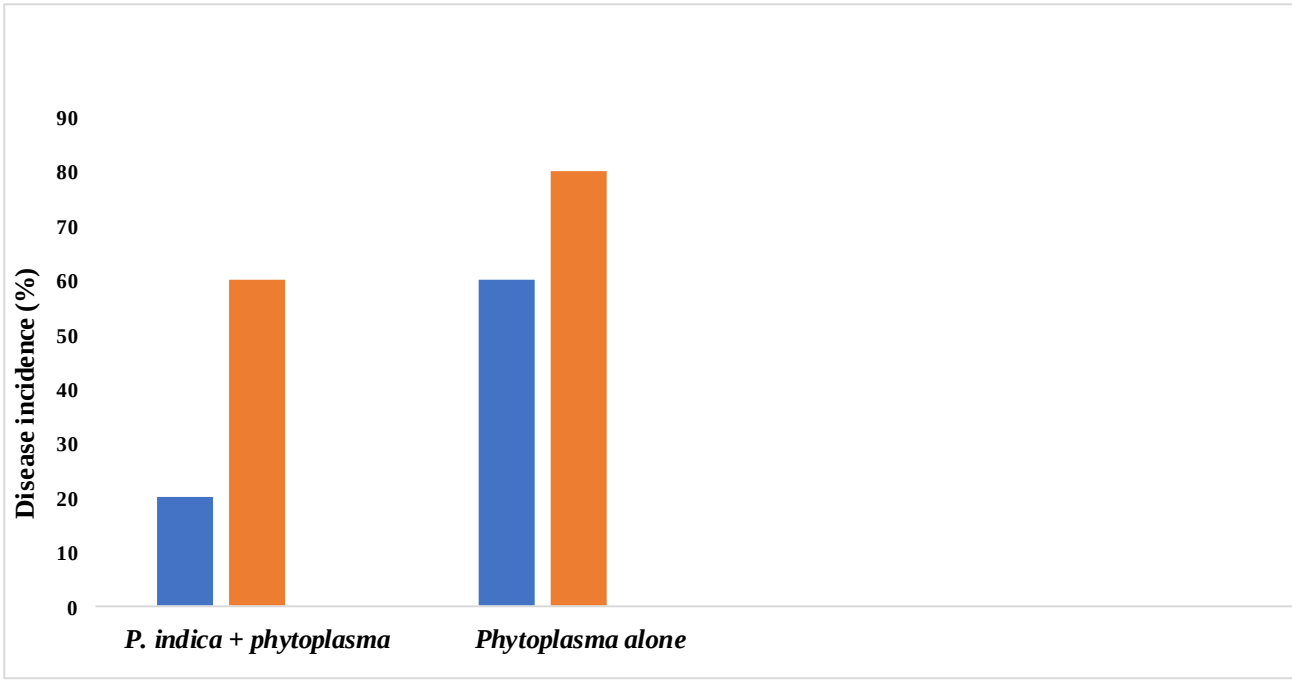


Fig. 5 Disease incidence of plants colonized and non-colonized with *P. indica* at 30 and 45 days after grafting

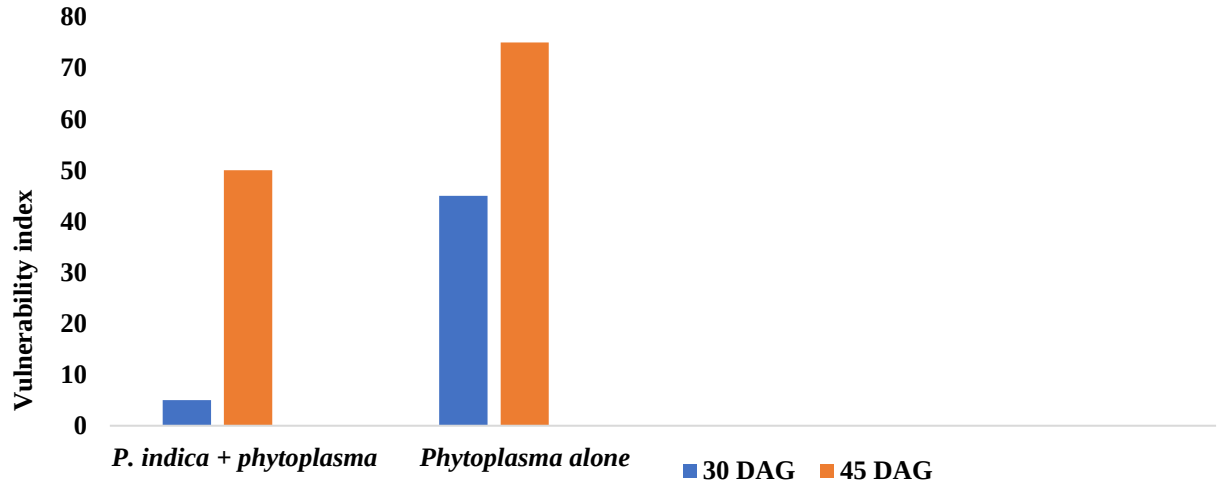


Fig. 6 Vulnerability index of plants colonized and non-colonized with *P. indica* at 30 and 45 days after grafting

Summary

6. SUMMARY

The research work entitled ‘Prevalence of sesamum phyllody in Onattukara tract and evaluation of fungal root endophyte *Piriformospora indica* for its management’ was conducted during 2019-21 at College of Agriculture, Vellayani and Onattukara Regional Agricultural Research Station with the objectives to study the symptomatology, molecular detection and characterization of phytoplasma inciting sesamum phyllody disease in AEU 3 (Onattukara tract); and evaluation of fungal root endophyte *Piriformospora indica* for its management.

Survey results indicated the highest incidence and severity of infection in Karthikapally. It has been found that certain weed plants and insects act as natural reservoirs for these plant pathogenic phytoplasmas. In the surveyed fields, Chocolate weed, *Melochia corchorifolia*, Sterculiaceae family, showed symptoms of shoot proliferation resembling phyllody and the presence of *Hishimonas phycitis*, *Orosius albicintus* and *Nephotettix sp.* were recorded from the infected fields.

The infection was evident during flowering stage, when the floral portions turned green and converted into leafy-like structures. The flowers had indeed been sterile. The ovary has been replaced in certain cases by elongated structures that resemble a stalk, which continue to grow and produced new phylloid flowers. There had also been a noticeable thickening of the floral veins. The presence of phytoplasmas was detected under fluorescence microscopy in microtome sections of infected tissues using DAPI stain. Phloem cells from infected tissues showed a diffuse fluorescence which is brighter than that seen in the parenchymal cells.

When phytoplasma-infected plants were compared to healthy plants, plant hormone levels were found to be altered. In the phyllody infected sesamum leaves and flowers, the gibberellic acid content was found to be increased and IAA content was found to be decreased compared to the healthy samples.

In direct PCR with P1/P6 primers, the phytoplasma infected samples from surveyed locations showed amplification at approximately 1.4 kb confirming the association of phytoplasma with the disease. BLAST analysis showed that all the isolates shared more than 99.0 per cent sequence similarity with that of the

'*Candidatus* Phytoplasma aurantifolia' strains in GenBank. In the phylogenetic tree constructed, the sesamum phyllody phytoplasma under study clustered with the 16Sr II group phytoplasmas causing sesamum phyllody in various regions. The sesamum phyllody phytoplasma belonging to 16Sr I has already been reported from some parts in India formed a separate cluster. The virtual RFLP pattern derived from 16S rDNA fragment of sesamum phyllody phytoplasma generated by *i*PhyClassifier was found to be identical (similarity coefficient 1.00) to the reference pattern of 16Sr group II, subgroup D (GenBank accession: Y10097). Hence, the phytoplasma causing sesamum phyllody in Onattukara tract was identified as "*Candidatus* Phytoplasma aurantifolia"-related strain belonging to subgroup 16SrII-D.

P. indica colonization in sesamum seedlings were observed at 7 days after germination. Wedge grafting was standardized in sesamum 30 days after germination. A pot experiment had been conducted to evaluate the effect of beneficial fungal root endophyte *P. indica* against phytoplasma causing sesamum phyllody. Similar to the mycorrhizal fungi, it develops symbiotic relationship with plants and promotes plant growth, yield, early flowering and increases resistance to biotic and abiotic stresses. When compared to non-colonized plants, it was found that *P. indica*-colonization could markedly lower the disease incidence and severity of infection. Colonized plants exhibited increased shoot and root length at 30 and 55 days after germination and also earliness in flowering compared to the non-colonized plants.

As a result, compared to non-colonized plants, the development of symptoms in colonised plants was shown to be delayed. In colonized plants, enhanced shoot and root length at 30 and 55 days after germination were recorded and earliness to flowering compared to non-colonized plants.

Thus, the present study revealed the prevalence of sesamum phyllody in Onattukara tract, which has been recorded with virescence, phyllody and floral proliferation symptoms and association of *Candidatus* Phytoplasma aurantifolia-related strain belonging to subgroup 16SrII-D with sesamum phyllody in the tract. The attempts on management using *P. indica* showed delay in symptom expression in treated plants and enhancement in growth attributes of the crop which need to be further examined.

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Appendix

APPENDIX – I

Reagents for microtome sectioning and DAPI staining

Haupt's gelatin adhesive

High grade gelatin: 1.00g

Distilled water : 100ml

Phenol crystals : 2.00g

Pure glycerine : 15.0ml

Dissolve gelatin in distilled water at 30°C. Add 2g phenol crystals and 15ml of pure glycerin. Stir well and filter.

1X Phosphate buffer saline (pH-7.4)

NaCl : 8.00g

$Na_2HPO_4.2H_2O$: 1.44g

Na_2HPO_4 :1.50g

K_2HPO_4 :0.20g

KCl :0.20g

Distilled water :1.00L

APPENDIX – II

Buffers for hormone analysis

$FeCl_3$-$HClO_4$ reagent

1ml of 0.5M $FeCl_3$: 1ml

50ml 35% $HClO_4$: 50ml

APPENDIX –III

Buffers used in molecular analysis

10X TAE buffer (Tris Acetate EDTA) (pH:8.5)

Tris HCl : 6.304g (pH:8)

Acetic acid : 1.142ml

0.5M EDTA : 2ml

Add distilled water to a final volume of 100ml

APPENDIX – IV

Potato Dextrose Agar (PDA) Medium

Potato : 200 g

Dextrose : 20 g

Agar : 20 g

Distilled water : 1 L

APPENDIX – V

16S rDNA sequences of sesamum phyllody phytoplasma

16S rDNA sequences of sesamum phyllody phytoplasma collected from ORARS lowland (GenBank accession number: OK597186)

AAGACTGGATAGGAAGATAAAAGGCATCTTTTATCTTTTAAAAGACCTAG
TAATAGGTATGCTTTAGGAGGGGCTTGCGCCATATTAGTTAGTTGGTAGG
GTAATGGCCTACCAAGACGATGATGTGTAGCTGGACTGAGAGGTTGAACA
GCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGT
AGGGAATTTTCGGCAATGGAGGAAACTCTGACCGAGCAACGCCGCGTGA
ATGACGAAGTACTTCGGTATGTAAAGTTCTTTTATCAAGGAAGAAAAGCA
AATGGCGAACCATTTGTTTGCCGGTACTTGATGAATAAGCCCCGGCTAATT
ATGTGCCAGCAGCCGCGGTAAGACATAAGGGGCAAGTGTTATCCGGAATT
ATTGGGCGTAAAGGGTGCGTAGGCGGTCTAGTAAGTCAGTGGTGTAATGG
CAACGCTTAACGTTGTCCGGCTATTGAAACTGCTAAACTTGAGTTAGATA
GAGGCGAGTGGAATTCCATGTGTAGCGGTAAAATGCGTAAATATATGGAG
GAACACCAGAGGCGTAGGCGGCTCGCTGGGTCTTAACTGACGCTGAGGCA
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGAGTACTAAGTGTCGGGTTAAACCGGTACTGAAGTTAACACAT
TAAGTACTCCGCCTGAGTAGTACGTACGCAAGTATGAAACTTAAAGGAAT
TGACGGGACTCCGCACAAGCGGTGGATCATGTTGTTTAATTCGAAGATAC
CCGAAAAACCTTACCAGGTCTTGACATGTTTTTGCAAAATGATAGTAATAT
CGTGGAGGTTACCAGAAACACAGGTGGTGCATGGTTGTCGTCAGCTCGTG
TCGTGAGATGTTAGGTTAAGTCCTAAAACGAGCGAAACCCTTATCGTTAG
TTGCCAGCACGTTATGGTGGGGACTTTAACGAGACTGCCAATGATAAATT
GGAGGAAGGTGAGGATCACGTCAAATCAGCATGCCCCTTATGACCTGGGC
TACAAACGTGATACAATGGCTGTTACAAAGGGTAGCTGAAACGCAAGTTC
TTGGCCAATCCCCAAAAACAGTCCCAGTCCGGATTGAAGTCTGCAACTCG
ACTTCATGAAGTTGGAATCGCTAGTAATCGCGAATCAGCATGTCGCGGTG
AATACGTTCTCGGGGTTTGTAC

16S rDNA sequences of sesamum phyllody phytoplasma collected from ORARS lowland (GenBank accession number: OK625449)

TAAGACTGGATAGGAAGATAAAAGGCATCTTTTATCTTTTAAAAGACCTA
GTAATAGGTATGCTTTAGGAGGGGCTTGCGCCATATTAGTTAGTTGGTAG
GGTAATGGCCTACCAAGACGATGATGTGTAGCTGGACTGAGAGGTTGAAC
AGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAG
TAGGGAATTTTCGGCAATGGAGGAAACTCTGACCGAGCAACGCCGCGTGA
ATGACGAAGTACTTCGGTATGTAAAGTTCTTTTATCAAGGAAGAAAAGCA
AATGGCGAACCATTTGTTTGCCGGTACTTGATGAATAAGCCCCGGCTAATT
ATGTGCCAGCAGCCGCGGTAAGACATAAGGGGCAAGTGTTATCCGGAATT
ATTGGGCGTAAAGGGTGCGTAGGCGGTCTAGTAAGTCAGTGGTGTAATGG
CAACGCTTAACGTTGTCCGGCTATTGAAACTGCTAAACTTGAGTTAGATA
GAGGCGAGTGGAATTCCATGTGTAGCGGTAAAATGCGTAAATATATGGAG
GAACACCAGAGGCGTAGGCGGCTCGCTGGGTCTTAACTGACGCTGAGGCA
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGAGTACTAAGTGTCGGGTTAAACCGGTACTGAAGTTAACACAT
TAAGTACTCCGCCTGAGTAGTACGTACGCAAGTATGAAACTTAAAGGAAT
TGACGGGACTCCGCACAAGCGGTGGATCATGTTGTTTAATTCGAAGATAC
CCGAAAAACCTTACCAGGTCTTGACATGTTTTTGCAAAATGATAGTAATAT
CGTGGAGGTTACCAGAAACACAGGTGGTGCATGGTTGTCGTCAGCTCGTG
TCGTGAGATGTTAGGTTAAGTCCTAAAACGAGCGAAACCCTTATCGTTAG
TTGCCAGCACGTTATGGTGGGGACTTTAACGAGACTGCCAATGATAAATT
GGAGGAAGGTGAGGATCACGTCAAATCAGCATGCCCCTTATGACCTGGGC
TACAAACGTGATACAATGGCTGTTACAAAGGGTAGCTGAAACGCAAGTTC
TTGGCCAATCCCCAAAAACAGTCCCAGTCCGGATTGAAGTCTGCAACTCG
ACTTCATGAAGTTGGAATCGCTAGTAATCGCGAATCAGCATGTCGCGGTG
AATACGTTCTCGGGGTTTGTACACACCGCCCGTCA

16S rDNA sequences of sesamum phyllody phytoplasma collected from Karthikapally (GenBank accession number: OK625583)

AACAGTGGCTAAGACTGGATAGGAAGATAAAAGGCATCTTTTATCTTTTA
AAAGACCTAGTAATAGGTATGCTTTAGGAGGGGCTTGCGCCATATTAGTT
AGTTGGTAGGGTAATGGCCTACCAAGACGATGATGTGTAGCTGGACTGAG
AGGTTGAACAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGA
GGCAGCAGTAGGGAATTTTCGGCAATGGAGGAAACTCTGACCGAGCAAC
GCCGCGTGAATGACGAAGTACTTCGGTATGTAAAGTTCTTTTATCAAGGA
AGAAAAGCAAATGGCGAACCATTTGTTTGCCGGTACTTGATGAATAAGCC
CCGGCTAATTATGTGCCAGCAGCCGCGGTAAGACATAAGGGGCAAGTGTT
ATCCGGAATTATTGGGCGTAAAGGGTGCGTAGGCGGTCTAGTAAGTCAGT
GGTGTAATGGCAACGCTTAACGTTGTCCGGCTATTGAAACTGCTAAACTT
GAGTTAGATAGAGGCGAGTGGAATTCCATGTGTAGCGGTAAAATGCGTAA
ATATATGGAGGAACACCAGAGGCGTAGGCGGCTCGCTGGGTCTTAACTGA
CGCTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA
GTCCACGCCGTAAACGATGAGTACTAAGTGTCGGGTTAAACCGGTACTGA
AGTTAACACATTAAGTACTCCGCCTGAGTAGTACGTACGCAAGTATGAAA
CTTAAAGGAATTGACGGGACTCCGCACAAGCGGTGGATCATGTTGTTTAA
TTCGAAGATACCCGAAAAACCTTACCAGGTCTTGACATGTTTTTGCAAAAT
GATAGTAATATCGTGGAGGTTACCAGAAACACAGGTGGTGCATGGTTGTC
GTCAGCTCGTGTCGTGAGATGTTAGGTTAAGTCCTAAAACGAGCGAAACC
CTTATCGTTAGTTGCCAGCACGTTATGGTGGGGACTTTAACGAGACTGCCA
ATGATAAATTGGAGGAAGGTGAGGATCACGTCAAATCAGCATGCCCCTTA
TGACCTGGGCTACAAACGTGATACAATGGCTGTTACAAAGGGTAGCTGAA
ACGCAAGTTCTTGGCCAATCCCCAAAAACAGTCCCAGTCCGGATTGAAGT
CTGCAACTCGACTTCATGAAGTTGGAATCGCTAGTAATCGCGAATCAGCA
TGTCGCGGTGAATACGTTCTCGGGGTTTGTACACACCGCCCGTCA

16S rDNA sequences of sesamum phyllody phytoplasma collected from ORARS upland (GenBank accession number: OK625584)

ACCCTAAAGACGAGGATAACCATTGGAAACAGTGGCTAAGACTGGATAG
GAAGATAAAAGGCATCTTTTATCTTTTAAAAGACCTAGTAATAGGTATGCT
TTAGGAGGGGCTTGCGCCATATTAGTTAGTTGGTAGGGTAATGGCCTACC
AAGACGATGATGTGTAGCTGGACTGAGAGGTTGAACAGCCACATTGGGAC
TGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATTTTCGGC
AATGGAGGAAACTCTGACCGAGCAACGCCGCGTGAATGACGAAGTACTTC
GGTATGTAAAGTTCTTTTATCAAGGAAGAAAAGCAAATGGCGAACCATTT
GTTTGCCGGTACTTGATGAATAAGCCCCGGCTAATTATGTGCCAGCAGCC
GCGGTAAGACATAAGGGGCAAGTGTTATCCGGAATTATTGGGCGTAAAGG
GTGCGTAGGCGGTCTAGTAAGTCAGTGGTGTAATGGCAACGCTTAACGTT
GTCCGGCTATTGAAACTGCTAAACTTGAGTTAGATAGAGGCGAGTGGAAT
TCCATGTGTAGCGGTAAAATGCGTAAATATATGGAGGAACACCAGAGGCG
TAGGCGGCTCGCTGGGTCTTAACTGACGCTGAGGCACGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTACT
AAGTGTCGGGTTAAACCGGTACTGAAGTTAACACATTAAGTACTCCGCCT
GAGTAGTACGTACGCAAGTATGAAACTTAAAGGAATTGACGGGACTCCGC
ACAAGCGGTGGATCATGTTGTTTAATTCGAAGATACCCGAAAAACCTTAC
CAGGTCTTGACATGTTTTTGCAAAATGATAGTAATATCGTGGAGGTTACCA
GAAACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTAG
GTTAAGTCCTAAAACGAGCGAAACCCTTATCGTTAGTTGCCAGCACGTTA
TGGTGGGGACTTTAACGAGACTGCCAATGATAAATTGGAGGAAGGTGAGG
ATCACGTCAAATCAGCATGCCCCTTATGACCTGGGCTACAAACGTGATAC
AATGGCTGTTACAAAGGGTAGCTGAAACGCAAGTTCTTGGCCAATCCCCA
AAAACAGTCCCAGTCCGGATTGAAGTCTGCAACTCGACTTCATGAAGTTG
GAATCGCTAGTAATCGCGAATCAGCATGTCGCGGTGAATACGTTCTCGGG
GTTTGTACACACC

16S rDNA sequences of sesamum phyllody phytoplasma collected from Vellayani (GenBank accession number: OK625585)

ACCCTAAAGACGAGGATAACCATTGGAAACAGTGGCTAAGACTGGATAG
GAAGATAAAAGGCATCTTTTATCTTTTAAAAGACCTAGTAATAGGTATGCT
TTAGGAGGGGCTTGCGCCATATTAGTTAGTTGGTAGGGTAATGGCCTACC
AAGACGATGATGTGTAGCTGGACTGAGAGGTTGAACAGCCACATTGGGAC
TGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATTTTCGGC
AATGGAGGAAACTCTGACCGAGCAACGCCGCGTGAATGACGAAGTACTTC
GGTATGTAAAGTTCTTTTATCAAGGAAGAAAAGCAAATGGCGAACCATTT
GTTTGCCGGTACTTGATGAATAAGCCCCGGCTAATTATGTGCCAGCAGCC
GCGGTAAGACATAAGGGGCAAGTGTTATCCGGAATTATTGGGCGTAAAGG
GTGCGTAGGCGGTCTAGTAAGTCAGTGGTGTAATGGCAACGCTTAACGTT
GTCCGGCTATTGAAACTGCTAAACTTGAGTTAGATAGAGGCGAGTGGAAT
TCCATGTGTAGCGGTAAAATGCGTAAATATATGGAGGAACACCAGAGGCG
TAGGCGGCTCGCTGGGTCTTAACTGACGCTGAGGCACGAAAGCGTGGGGA
GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTACT
AAGTGTCGGGTTAAACCGGTACTGAAGTTAACACATTAAGTACTCCGCCT
GAGTAGTACGTACGCAAGTATGAAACTTAAAGGAATTGACGGGACTCCGC
ACAAGCGGTGGATCATGTTGTTTAATTCGAAGATACCCGAAAAACCTTAC
CAGGTCTTGACATGTTTTTGCAAAATGATAGTAATATCGTGGAGGTTACCA
GAAACACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTAG
GTTAAGTCCTAAAACGAGCGAAACCCTTATCGTTAGTTGCCAGCACGTTA
TGGTGGGGACTTTAACGAGACTGCCAATGATAAATTGGAGGAAGGTGAGG
ATCACGTCAAATCAGCATGCCCCTTATGACCTGGGCTACAAACGTGATAC
AATGGCTGTTACAAAGGGTAGCTGAAACGCAAGTTCTTGGCCAATCCCCA
AAAACAGTCCCAGTCCGGATTGAAGTCTGCAACTCGACTTCATGAAGTTG
GAATCGCTAGTAATCGCGAATCAGCATGTCGCGGTGAATACGTTCTCGGG
GTTTGTAC

Abstract

PREVALENCE OF SESAMUM PHYLLODY IN ONATTUKARA TRACT AND EVALUATION OF FUNGAL ROOT ENDOPHYTE *Piriformospora indica* FOR ITS MANAGEMENT

by

GIFTY K. J.

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Abstract of the Thesis

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2022

ABSTRACT

Prevalence of sesamum phyllody in Onattukara tract and evaluation of fungal root endophyte *Piriformospora indica* for its management

The research work entitled 'Prevalence of sesamum phyllody in Onattukara tract and evaluation of fungal root endophyte *Piriformospora indica* for its management' was conducted during 2019-21 at Department of Plant Pathology, College of Agriculture, Vellayani and Onattukara Regional Agricultural Research Station with the objectives to study the symptomatology, molecular detection and characterization of phytoplasma inciting sesamum phyllody disease in AEU 3 (Onattukara tract); and evaluation of fungal root endophyte *P. indica* for its management.

Phytoplasma infected sesamum samples were collected from D and F blocks of Onattukara Regional Agricultural Research Station and Karthikapally. Karthikapally recorded highest disease incidence (39.44 per cent) and vulnerability index (23.75). Chocolate weed, *Melochia corchorifolia*, was found to be exhibiting symptoms of shoot proliferation. Hoppers collected from the infected fields were identified as *Orosius albicintus*, *Hishimonas phycitis* and *Nephotettix* sp.

Disease symptoms were observed at the stage of flowering of sesamum plants in all the sampled locations. The associated symptoms were reduction in internodal length of stem, axillary bud proliferation, thickening of the floral veins, phyllody and floral proliferation. Microtome sections of infected and healthy leaf, stem of sesamum stained with 4,6-diamidino-2-phenylindole (DAPI) stain, and observed under fluorescence microscope emitted diffuse fluorescence from the infected tissues, which was brighter than the one from the parenchymal cells indicating the presence of phytoplasma in the infected tissues.

Studies on variations in the level of gibberellic acid (GA) and indole-3-acetic acid (IAA) in phyllody infected and healthy sesamum was undertaken. GA content was increased by 2.25 times and 10.46 times, and IAA content was decreased by 1.25 times and 1.97 times in leaves and flowers of infected samples compared to the healthy samples.

Molecular characterization of sesamum phyllody was performed with leaf samples collected from ORARS lowland, ORARS upland, Vellayani and Karthikapally. Amplicons of 1.4kb was obtained by amplifying with universal primers P1/P6 for detection of phytoplasma. The sequences obtained were subjected to BLAST analysis and the 16S rDNA gene sequence showed that all the isolates shared more than 99 per cent similarity with that of the '*Candidatus* phytoplasma aurantifolia' strains in GenBank data base. In the phylogenetic tree constructed, the sesamum phyllody phytoplasma under study clustered with the 16SrII group (*Candidatus* Phytoplasma aurantifolia) phytoplasmas causing sesamum phyllody in various regions. The virtual RFLP pattern generated by *i*PhyClassifier, derived from 16S rDNA fragment was found to be identical to the reference pattern of 16Sr group II, subgroup D (GenBank accession: Y10097). Based on the results obtained from sequence analysis and virtual RFLP pattern, the phytoplasma associated with sesamum phyllody was identified as "*Candidatus* Phytoplasma aurantifolia"-related strain belonging to subgroup 16SrII-D.

P. indica obtained from Department of Plant Pathology, College of Agriculture, Vellayani was mass multiplied in sterilized coir pith: FYM mixture (1:1) amended with 2 per cent gram flour and sesamum seeds were sown. Colonization was observed seven days after germination. Wedge grafting was standardized in sesamum at 30 days after germination. Pot culture experiment was conducted to evaluate the effect of *P. indica* against phytoplasma causing sesamum phyllody, by grafting the colonized and non-colonized plants with infected scion. *P. indica* colonization could significantly reduce the incidence and severity of infection. After 30 and 45 days of grafting, an incidence of 20 and 60 per cent, and severity of 5 and 50 were recorded in the colonized plants grafted with infected scion, whereas an incidence of 60 and 80 per cent and severity of 45 and 75 were recorded in non-colonized plants grafted with infected scion. In colonized plants, enhanced shoot and root length at 30 and 55 days after germination were recorded and also earliness in flowering compared to non-colonized plants.

Hence the associated symptoms of phytoplasma infection in sesamum are virescence, thickening of floral veins, phyllody and floral proliferation. The study revealed the association of *Candidatus* phytoplasma aurantifolia group with sesamum

phyllody prevalent in Onattukara tract. The evaluation of beneficial fungal root endophyte *P. indica* against phytoplasma revealed delayed expression of symptoms in the colonized plants.

സംഗ്രഹം

'ഓണാട്ടുകര മേഖലയിലെ എള്ളിലെ ഫില്ലോഡിയുടെ വ്യാപനവും അതിന്റെ പരിപാലനത്തിനായി മിത്ര-കുമിളയായ പിരിഫോർമോസ്പോറ ഇൻഡിക്കയുടെ മൂല്യനിർണ്ണയവും' എന്ന ശീർഷകത്തിൽ ഗവേഷണ പ്രവർത്തനങ്ങൾ 2019-21 കാലയളവിൽ കാർഷിക കോളേജ്, വെള്ളായണി, ഓണാട്ടുകര റീജിയണൽ അഗ്രികൾച്ചറൽ റിസർച്ച് സ്റ്റേഷൻ എന്നിവിടങ്ങളിൽ നടന്നു.

പഠനത്തിന്റെ ഭാഗമായി ഓണാട്ടുകര പ്രാദേശിക കാർഷിക ഗവേഷണ കേന്ദ്രത്തിൽനിന്നും കാർത്തികപ്പള്ളിയിൽനിന്നും ഫൈറ്റോപ്ലാസ്മ ബാധിച്ച എള്ള് സാമ്പിളുകൾ ശേഖരിച്ചു. കാർത്തികപ്പള്ളിയിലാണ് ഏറ്റവും കൂടുതൽ രോഗബാധയും (39.44 ശതമാനം), ദുർബലത സൂചികയും (23.75) രേഖപ്പെടുത്തിയത്. എള്ളു പാടങ്ങളിൽ കാണുന്ന കളയായ ഊർപ്പം ചിനപ്പുപൊട്ടലിന്റെ ലക്ഷണങ്ങൾ പ്രകടിപ്പിക്കുന്നതായി കണ്ടെത്തി. രോഗം ബാധിച്ച പാടങ്ങളിൽ നിന്ന് ശേഖരിച്ച ഹോപ്പറുകൾ ഒറോസിയസ് ആൽബിസിന്റസ്, ഹിഷിമോനാസ് ഫൈസിറ്റിസ്, നെഫോട്ടെറ്റിക്സ് എന്നിവയാണെന്ന് തിരിച്ചറിഞ്ഞു. സാമ്പിൾ എടുത്ത സ്ഥലങ്ങളിലെല്ലാം എള്ള് ചെടികൾ പൂക്കുന്ന ഘട്ടത്തിൽ രോഗലക്ഷണങ്ങൾ കണ്ടു. തണ്ടിന്റെ ആന്തരിക നീളം കുറയുക, കക്ഷീയ മുകുളങ്ങളുടെ വ്യാപനം, പുഷ്പ സിരകളുടെ കട്ടികൂടൽ, ഫില്ലോഡി, പുഷ്പ വ്യാപനം എന്നിവയാണ് അനുബന്ധ ലക്ഷണങ്ങൾ. രോഗബാധയുള്ളതും ആരോഗ്യമുള്ളതുമായ ഇലയുടെയും തണ്ടിന്റെയും മൈക്രോടോം ഭാഗങ്ങൾ 4,6-diamidino-2-phenylindole (DAPI) സ്റ്റെയിൻ ഉപയോഗിച്ച്, ഫ്ലൂറസെൻസ് മൈക്രോസ്കോപ്പിന് കീഴിൽ നിരീക്ഷിച്ചു. രോഗബാധിതമായ ടിഷ്യൂകളിലെ ഫൈറ്റോപ്ലാസ്മയുടെ സാന്നിധ്യം സൂചിപ്പിക്കുന്ന ഡിഫ്യൂസ് ഫ്ലൂറസെൻസ് ബാക്കി കോശങ്ങളിൽ നിന്നുള്ളതിനേക്കാൾ തിളക്കമുള്ളതായിരുന്നു.

ഫിലോഡി ബാധിച്ചതും ആരോഗ്യമുള്ളതുമായ ചെടിയിലെ ഗിബ്ബെറലിക് ആസിഡിന്റെയും (GA) ഇൻഡോൾ-3-അസറ്റിക് ആസിഡിന്റെയും (IAA) വ്യതിയാനങ്ങളെക്കുറിച്ചുള്ള പഠനങ്ങൾ നടത്തി. ആരോഗ്യമുള്ള സാമ്പിളുകളെ അപേക്ഷിച്ച് രോഗബാധിതരായ സാമ്പിളുകളുടെ ഇലകളിലും പൂക്കളിലും GA ഉള്ളടക്കം 2.25 മടങ്ങും 10.46 മടങ്ങും വർദ്ധിച്ചു, കൂടാതെ IAA ഉള്ളടക്കം 1.25 മടങ്ങും 1.97 മടങ്ങും

കുറഞ്ഞു. ഓണാട്ടുകര പ്രാദേശിക കാർഷിക ഗവേഷണ കേന്ദ്രത്തിൽനിന്നും കാർത്തികപ്പള്ളിയിൽനിന്നും ശേഖരിച്ച സാമ്പിളുകൾ ഉപയോഗിച്ചാണ് ഫൈറ്റോപ്ലാസ്മയുടെ തന്മാത്രാ സ്വഭാവം നടത്തിയത്. ഫൈറ്റോപ്ലാസ്മ കണ്ടെത്തുന്നതിനായി P1/P6 യൂണിവേഴ്സൽ പ്രൈമറുകൾ ഉപയോഗിച്ച് ആംപ്ലിഫൈ ചെയ്യുന്നതിലൂടെ 1.4kb യുടെ ആംപ്ലിക്കോണുകൾ ലഭിച്ചു. ലഭിച്ച സീക്വൻസുകൾ BLAST വിശകലനത്തിന് വിധേയമാക്കി. എല്ലാ ഐസൊലേറ്റുകളും GenBank ഡാറ്റാ ബേസിലെ 'Candidatus phytoplasma aurantifolia' സ്ട്രെയിനുകളുമായി 99 ശതമാനത്തിലധികം സാമ്യം പങ്കിടുന്നു എന്നാണ് കണ്ടെത്തിയത്. നിർമ്മിച്ച ഫൈലോജെനറ്റിക് ട്രീയിൽ, പഠനത്തിൻ കീഴിലുള്ള ഫൈറ്റോപ്ലാസ്മ വിവിധ പ്രദേശങ്ങളിൽ സെസാമം ഫൈലോഡിക്ക് കാരണമാകുന്ന 16SrII ഗ്രൂപ്പ് (കാൻഡിഡാറ്റസ് ഫൈറ്റോപ്ലാസ്മ ഔറന്റിഫോളിയ) ഫൈറ്റോപ്ലാസ്മകളോട് സാമ്യം പങ്കിടുന്നു. 16S rDNA ശകലത്തിൽ നിന്ന് ഉരുത്തിരിഞ്ഞ iPhyClassifier സൃഷ്ടിച്ച വെർച്വൽ RFLP പാറ്റേൺ, 16Sr ഗ്രൂപ്പ് II, സബ്ഗ്രൂപ്പ് D (GenBank പ്രവേശനം: Y10097) യുടെ റഫറൻസ് പാറ്റേണുമായി സാമ്യമുള്ളതായി കണ്ടെത്തി. സീക്വൻസ് അനാലിസിസ്, വെർച്വൽ RFLP പാറ്റേൺ എന്നിവയിൽ നിന്ന് ലഭിച്ച ഫലങ്ങളെ അടിസ്ഥാനമാക്കി, ഓണാട്ടുകരയിലെ സെസാമം ഫിലോഡിയുമായി ബന്ധപ്പെട്ട ഫൈറ്റോപ്ലാസ്മയെ 16SrII-D എന്ന ഗ്രൂപ്പിൽ പെടുന്ന "കാൻഡിഡാറ്റസ് ഫൈറ്റോപ്ലാസ്മ ഔറന്റിഫോളിയ"-അനുബന്ധ സ്ട്രെയിൻ ആണെന്ന് തിരിച്ചറിഞ്ഞു.

വെള്ളായണി കാർഷിക കോളേജിലെ പ്ലാന്റ് പാത്തോളജി വകുപ്പിൽ നിന്ന് ലഭിച്ച പി. ഇൻഡിക്ക അണുവിമുക്തമാക്കിയ ചകിരിച്ചോർ: എഫ്.വൈ.എം (1:1) 2 ശതമാനം കടലമാവും ചേർത്ത മിശ്രിതത്തിൽ എള്ള് വിതച്ചു. മുളച്ച് ഏഴ് ദിവസത്തിന് ശേഷം കോളനിവൽക്കരണം നിരീക്ഷിക്കപ്പെട്ടു. മുളച്ച് 30 ദിവസത്തിനുള്ളിൽ എള്ളിൽ വെഡ്ജ് ഗ്രാഫ്റ്റിംഗ് വിജയകരമായി നടത്താമെന്ന് കണ്ടെത്തി. കോളനിവൽക്കരിച്ചതും കോളനിവൽക്കരിക്കാത്തതുമായ സസ്യങ്ങളെ രോഗബാധയുള്ള സിയോണിനൊപ്പം ഒട്ടിച്ച്, സെസാമം ഫിലോഡിക്ക് കാരണമാകുന്ന ഫൈറ്റോപ്ലാസ്മയ്ക്കെതിരെ പി. ഇൻഡിക്കയുടെ പ്രഭാവം വിലയിരുത്തുന്നതിനായി പരീക്ഷണം നടത്തി. കോളനിവൽക്കരണം രോഗബാധയുടെ തീവ്രത ഗണ്യമായി കുറയ്ക്കുന്നതായി കണ്ടെത്തി. 30 ഉം

45 ഉം ദിവസങ്ങൾക്ക് ശേഷം, രോഗബാധയുള്ള സിയോൺ ഗ്രാഫ്റ്റ് ചെയ്ത കോളനിവൽക്കരിച്ച ചെടികളിൽ രോഗബാധ 20 ഉം 60 ഉം ശതമാനവും 5 ഉം 50 ഉം തീവ്രതയും രേഖപ്പെടുത്തി, അതേസമയം 60 ഉം 80 ഉം ശതമാനം രോഗബാധയും 45 ഉം 75 ഉം തീവ്രതയും ആയിരുന്നു രോഗബാധിതരായ സിയോണിനൊപ്പം ഒട്ടിച്ച കോളനിവൽക്കരിക്കാത്ത ചെടികളിൽ രേഖപ്പെടുത്തിയത്. കോളനിവൽക്കരിച്ച സസ്യങ്ങളിൽ വർധിച്ച ചിനപ്പുപൊട്ടലും വേരിന്റെ അധിക നീളവും രേഖപ്പെടുത്തി. കോളനിവൽക്കരിക്കാത്ത സസ്യങ്ങളെ അപേക്ഷിച്ച് നേരത്തെ ഇവ പൂവിടുകയും ചെയ്തു.

അതിനാൽ എള്ളിലെ ഫൈറ്റോപ്ലാസ്മ രോഗബാധയുടെ അനുബന്ധ ലക്ഷണങ്ങൾ വൈറസെൻസ്, പുഷ്പ സിരകളുടെ കട്ടിയാകൽ, ഫില്ലോഡി, പുഷ്പ വ്യാപനം എന്നിവയാണ്. കാന്ഡിഡാറ്റസ് ഫൈറ്റോപ്ലാസ്മ ഔറന്റിഫോളിയ ഗ്രൂപ്പിന് ഓണാട്ടുകരയിൽ വ്യാപകമായ എള്ളിലെ ഫില്ലോഡിയുമായി ബന്ധമുണ്ടെന്ന് പഠനം വെളിപ്പെടുത്തി. ഫൈറ്റോപ്ലാസ്മയ്ക്കെതിരെ മിത്ര കുമിളയായ പി. ഇൻഡിക്കയുടെ വിലയിരുത്തലിൽ കോളനിവൽക്കരിച്ച സസ്യങ്ങളിൽ രോഗലക്ഷണങ്ങൾക്ക് കാലതാമസം പ്രകടിപ്പിക്കുന്നതായി കണ്ടെത്തി.