STUDIES ON SESAME PHYLLODY INCITED BY *Phytoplasma*

By

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B.Sc. (Ag.)

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I, **Ms. K. PRASINDHU**, hereby declare that the thesis entitled "**STUDIES ON SESAME PHYLLODY INCITED BY *Phytoplasma***" submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

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This is to certify that the thesis entitled **"STUDIES ON SESAME PHYLLODY INCITED BY *Phytoplasma*"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** of the Acharya N.G. Ranga Agricultural University, Guntur, is a record of the bonafide original research work carried out by **Ms. K. PRASINDHU,** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
A260/A280	:	Absorbance ratio
ANGRAU	:	Acharya N.G Ranga Agricultural University
Bp	:	Base pairs
cDNA	:	Complementary Deoxyribose nucleic acid
Cm	:	Centimetre(s)
Conc.	:	Concentration
CTAB	:	Cetyl Trimethyl Ammonium Bromide
DAI	:	Days After Inoculation(S)
DAS	:	Days After Sowing
DMRT	:	Duncan's Multiple Range Test
DNA	:	Deoxyribo Nucleic Acid
dNTPs	:	Deoxy Nucleotide Triphosphates
EDTA	:	Ethylene Diamine Tetra Acetic Acid
et al.,	:	And other people
etc	:	And so, on
Fig.	:	Figure
g	:	Grams
g-1	:	Per gram
ha	:	Hectares
hr	:	Hour(s)
i.e	:	That is
Kb	:	Kilo base
Kbp	:	Kilo base pair(s)
Kg	:	Kilogram(s)
M	:	Million
M	:	Molar
M ha	:	Million hectares
mg	:	Milli gram(s)
$Mgcl_2$	:	Magnesium dichloride
Min	:	Minute(s)
mm	:	Milli metre(s)
mM	:	Millimolar
MT	:	Million tonnes

MW	:	Molecular weight
ng	:	Nanogram(s)
nm	:	Nanometer
^{o}C	:	Degree Celsius
PCR	:	Polymerase Chain Reaction
pH	:	-log10[H]
r	:	Correlation coefficient
RARS	:	Regional Agricultural Research Station
RH	:	Relative humidity
RNA	:	Ribo nucleic acid
Rpm	:	Revolutions per minute
S. No	:	Serial number
Sec	:	Second(s)
T	:	Tons
TAE	:	Tris Acetic acid EDTA
Taq	:	*Thermus aquaticus*
U	:	Unit(s)
UV	:	Ultra violet
V	:	Volts
v/v	:	Volume per volume
viz.,	:	Namely
µ	:	Micro (10^{-6x})
µg	:	Microgram
µl	:	Micro litre
µM	:	Micro molar

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ABSTRACT

Sesame (*Sesamum indicum* L.) is one of the important oilseed crops grown in tropics as well as sub tropics. The phyllody in sesame is reported to be the most devastating disease, caused by *Candidatus* Phytoplasma which is transmitted by phloem feeding insects such as planthoppers, psyllids and leafhoppers. In addition to the insect vectors, the disease can be transmitted by the parasitic dodder and grafting of infected material to a healthy plant. The sesame phyllody became epidemic in India, majorly in the peninsular regions, it causes considerable yield reduction even upto 80 per cent depending on the stage of crop it affected. Identification of resistant varieties is the most desired approach to alleviate the problem. With this premise the present study was done to molecular confirmation of phytoplasma, to identify the resistant genotypes to phyllody and identify the prominent method to transmit the disease to screen the genotypes under artificial conditions.

The sesame plants showing characteristic phyllody symptoms such as phyllody, virescence, shoot proliferation, malformation of capsules and cracking of capsules were collected from research fields of IIOR. Total DNA was isolated and amplified by direct and nested PCR with the universal primer pairs P1/P7 and R16F2n/ R16R2 and got 1.8 kb and 1.2 kb fragments of 16S rRNA gene respectively. About 1200 bp product was purified, sequenced and length of sequence was determined with good quality as 986 bp. The BLAST analysis of 16S rDNA partial sequence with reference phytoplasmal gene sequence retrieved from GenBank NCBI shared 100 per cent sequence identity with *Candidatus* Phytoplasma aurantifolia.

Phyllody disease was successfully transmitted from diseased to healthy test plants via grafting, cuscuta and the leafhopper *Orosius albicinctus* and the disease was not transmitted by either of the methods of sap abrasion and sap injection to sesame. The grafting method can successfully transmitted

phyllody of 90, 80 and 100 per cent in CUMS-17, JCS-1020 of sesame and Periwinkle respectively. The dodder transmission reported only 60, 40 and 80 per cent in CUMS-17 and JCS-1020 of sesame from infected sesame to healthy sesame and from infected periwinkle to healthy periwinkle respectively. The leafhopper *O. albicinctus* transmitted an average 75, 35, 100 and 80 per cent of disease from the infected sesame to healthy CUMS-17, JCS-1020 of sesame, periwinkle and sunhemp respectively. Among all these methods the graft method of transmission reports best method of transmission in these studies.

Among 60 sesame genotypes screened under field conditions, one genotype was found to be resistant, three genotypes showed moderately resistant reaction, nine genotypes showed tolerant reaction, 15 genotypes were found to be moderately susceptible reaction, 21 genotypes showed susceptible reaction and 11 genotypes were found to be highly susceptible based on per cent disease incidence of phyllody in correlation to the leafhopper nymphs per plant.

Chapter –I

Introduction

Chapter –I

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the important oilseed crop grown in many parts of the world. It is a diploid species with 2n = 2x = 26 chromosomes, belongs to the family of Pedaliaceae of *Sesamum* genus and most commonly cultivated edible oilseed crop species are all over 30 species in this genus (Kobayashi *et al*., 1990). It is also known as the queen of oilseeds. In India the crop is generally known as Till. It is rich source of antioxidants such as sesamin, sesamol and sesamoline (Shyu and Hwang, 2002). The sesame seeds are rich source of edible oil (50 %), protein (20 %), oleic acid (47 %) and linolenic acid (39 %) (Uzun *et al.*, 2008). The long shelf life of sesame seed oil is due to the presence of sesamol.

Globally the annual sesame seeds production was 3.84 MMT. After China, India ranks in second place by producing around 0.81 MMT (26 %) (FAO, 2018). In India, the area of sesame is 1.6 M ha with the production of 0.75 MT and productivity of 478 kg ha^{-1} (Ministry of Agriculture, Government of India, 2018).

Sesame is vulnerable to infection by a number of diseases *viz.,* charcoal rot/ root rot/ stem rot, bacterial leaf spot, leaf blight, powdery mildew, wilt, leafspot, phyllody and stem blight that cause considerable yield losses. Among the major diseases, economically important diseases affecting sesame are phyllody, root/stem rot and powdery mildew. The sesame phyllody became epidemic in India, majorly in the peninsular regions with an incidence of approximately 75 per cent (Manjunatha *et al*., 2012). The sesame phyllody causes a yield loss upto 80 per cent (Salehi *et al*., 2017). The cause of disease was assumed to be a virus, which was later confirmed as ‘mycoplasma like organisms’ (MLO) and termed as phytoplasmas (Das and Mitra, 1998) which takes a leading role resulting in significant yield losses. About one per cent

increase in disease intensity decreases its yield by 8.36 kg ha^{-1} (Maiti *et al*., 1988).

The phytoplasmas are specialized bacteria which do not have cell wall, non helical, obligate, intracellular parasites belonging to the class Mollicutes and resides in the phloem of the plants (Doi *et al*., 1967). In cross section of affected plant parts, phytoplasma appear as rounded pleomorphic bodies with an average diameter of 200 to 800 µm (Lee *et al*., 2000). The affected plants exhibit a wide range of symptoms like shoot proliferation, smaller leaves, virescence, abnormal floral organs, sterility of flowers, necrosis of phloem and plant decline (Bertaccini and Duduk, 2009), sometimes these symptoms were accompanied with yellowing, cracking of seed capsule, vivipary and formation of dark exudates on the foliage (Akhtar *et al.,* 2009). The phytoplasmas were transmitted by the phloem feeding insects such as planthoppers, psyllids and leafhoppers. In addition to the insect vectors, the disease can be transmitted by the parasitic dodder and grafting of infected material to healthy plant, but it is not transmitted through sap and seed (Gogoi *et al.*, 2017).

The sesame phyllody, is a serious threat in regions where aster yellows (16SrI), peanut witches' broom (16SrII), pigeon pea witches' broom (16SrIX) and clover proliferation (16SrVI) phytoplasmas are present. Although four different phytoplasma groups have been reported in sesame so far, the majority (around 77 %) of identified sesame phytoplasma have been known to belong to the 16SrII and 16SrIX groups (Khan *et al.*, 2008c and Win *et al*., 2010).

In phytoplasma the 16S rRNA gene was highly conserved and present in two copies, making it is easy to amplify. So, the universal phytoplasma specific primers were used for molecular identification (Seemuller *et al*., 1998). The phytoplasma cannot be cultured *in vitro*, rRNA gene sequence revealed that the phytoplamsa were helical, wall less prokaryotes colonize in sieve elements of phloem and insects. Comparison of 16S rRNA gene

sequences among members of the class Mollicutes and several prokaryotes by Weisburg *et al.* (1989) indicated that the Mollicutes arose from a gram positive *Clostridium* like bacterial ancestor of the genus *Lactobacillus*, whose genome has low guanine plus cytosine (G+C) content. Whereas, the G+C content of phytoplasmal DNA is estimated to be between 23-29 mol per cent based on estimates from buoyant density centrifugation (Kollar and seemuller, 1989).

Similar to other members of the Mollicutes, the phytoplasma contain one circular double stranded DNA molecule (Niemark and Kirkpatrick, 1993). Using chromosomal DNA illuminated by gamma irradiation and pulse field gel electrophoresis for DNA separation, Neimark and Kirkpatrick (1993) and Marcone *et al.* (1999) reported that the size of phytoplasma genome varies considerably, ranging from 530 to 1350 bp. The genome of the Bermuda grass white leaf phytoplasma represented the smallest genome size (530 bp) found in phytoplasmas till date.

The identification of new species in the class Mollicutes requires descriptions of the species in pure culture, but phytoplasmas cannot be cultured *in vitro* and the phenotypic features used to describe mollicute species are inaccessible for uncultured phytoplasmas. Therefore, a provisional taxonomy system using the *Candidatus* category has been accommodated for phytoplasmas. Five *Candidatus* species names have been published till date (Davis *et al*., 1997; Griffiths *et al*., 1999 and Sawayanagi, 1999).

The 16S rRNA sequence similarities among members of a subgroup of given phytoplasma range from 95 to more than 99 per cent. Whereas the similarities between two distinct 16S rRNA groups range from 88 to 94 per cent (Lee *et al*., 1994). Recently, Seemuller *et al.* (1998) proposed 20 diverse phytoplasma groups derived from 57 phytoplasma strains on the basis of phylogenetic analysis of 16S rRNA gene sequences.

Phytoplasmas have been associated with diseases in several hundred plant species together with many important food, vegetable, fruit crops, ornamental plants, timber and shade trees (Athrens *et al*., 1993). The phytoplasma do not have a homogeneous distribution and many are confined to one continent or a specific geographical location. The clover proliferation group (16SrVI), the ash yellows group (16SrVII) and most of the X-disease group (16SrIII) of phytoplasmas shown to be restricted to the American continent or western hemisphere, while the rice yellow dwarf group (16SrXI) and peanut witches' broom group (16SrII) phytoplasmas are confined to the Southeast Asian region, whereas the apple proliferation group (16SrX), stolbur subgroup (16SrXII-A) are limited to the European continent (Lee *et al*., 1992). The geographical isolation of some phytoplasmas seems to be associated with the distribution of their host plants and the insect vectors that are subject in to the particular region. For example, maize bushy stunt phytoplasma (16SrI-B) is limited to Central and South America and part of North America due to the insect vectors *Dalbulus madis* and *D. elimatus* were prominent in these regions (Davis *et al*., 1988 and Harrison, 1996).

In recent years, the incidence of phyllody tremoundously increasing in sesame fields of the Andhra Pradesh. Since there is lack of much information regarding the phytoplasma associated with the sesame phyllody, the present work has been undertaken with the following objectives.

OBJECTIVES:

1. Molecular identification of phytoplasma from phyllody infected plants with PCR and nested PCR.
2. To study the transmission of sesame phyllody carried by different methods.
3. Screening of sesame genotypes against phyllody under field conditions.

Chapter – II

Review of Literature

Chapter-Ⅱ

REVIEW OF LITERATURE

A brief review of available literature pertaining to phyllody disease of sesame and various aspects related to the present study on symptomotology, detection of phytoplasma through molecular, nature of transmission and screening of genotypes have been presented in this chapter.

2.1 ECONOMIC IMPORTANCE OF PHYTOPLASMAL DISEASES

Phytoplasmal infections were primary limiting factors for production of many crops all over the world. The aster yellows phytoplasma contributed to the major economic loss of many vegetable crops (*e.g*., carrot, lettuce, celery) and ornamental plants (*e.g*., gladiolus, hydrangea) in Europe. Peach yellows to the loss of peach and cherry crops in the United States, rice yellow dwarf to the loss of rice crops in some parts of South East Asia, maize bushy stunt and potato witches' broom to the loss of corn and potato crops in South and Central America respectively. Cassava witches' broom to the loss of cassava crops in South America, sweet potato witches' broom and related diseases to the loss of sweet potato crops in Asia and Australia, grapevine yellows to the loss of grapevine production in Europe and Australia, pear decline, apple proliferation and other fruit declines to the loss of fruit production in the United States and Europe, legume diseases such as peanut witches' broom, sesame and soybean phyllody to the loss of oilseed crops in Asia and paulownia witches' broom, elm yellows, mulberry dwarf and coconut lethal yellowing to the loss of plantation crops on different continents (Lee *et al*., 1992 and McCoy *et al*., 1989). Because of these diseases, the movement of many plant species were restricted by quarantine regulations internationally.

2.2 GEOLOGICAL DISTRIBUTION AND LOSSES

2.2.1 Distribution of Phytoplasma among Worldwide:

The occurrence of phytoplasma was worldwide and they have been reported in at least 85 countries (McCoy *et al*., 1989). Phytoplasma do not have a uniform distribution (Lee *et al*., 2000) many were restricted to one continent or to a specific geographical location.

Table 2.1. Global distribution of phytoplasmal diseases of plants

Phytoplasma Group and Subgroup	Disease(S)	Geographical Distribution
Aster yellows group (16Sr□)		
16SrI-A	Aster yellows, lettuce yellows	North America
16SrI-B	Aster yellows, onion virescence	Worldwide
16SrI-C	Clover phyllody	North America
16SrI-D	Paulownia witches' broom	Asia
16SrI-E	Blueberry stunt	North America
16SrI-F	Apricot chlorotic leaf roll	Spain
Peanut witches' broom group (16SrII)		
16SrII-A	Peanut witches' broom	Asia
16SrII-B	Lime witches' broom	Arabian peninsula
16SrII-C	Faba bean phyllody and cotton phyllody	Africa, Asia
16SrII-D	Sweet potato little leaf	Australia
X-disease group (16SrIII)		
16SrIII-A	Peach, Cherry X-disease	North America
16SrIII-B	Clover yellow edge,	America, Asia
16SrIII-C	Pecan bunch	USA
16SrIII-D	Golden rod yellows	USA
16SrIII-E	Spirea stunt	USA
16SrIII-F	Milkweed yellows	North America
16SrIII-G	Walnut witches' broom	USA
16SrIII-H	Poinsettia branching inducing	Worldwide

Contd..

Table 2.1 (Contd…)

Coconut lethal yellows group (16SrIV)		
16SrIV-A	Coconut lethal yellows	Florida
16SrIV-B	Tanzanian coconut lethal decline	Africa
Elm yellows group (16SrV)		
16SrV-A	Elm yellows, Rubus stunt and alder yellows	North America,
16SrV-B	Cherry lethal yellows	China
16SrV-C	Jujube witches' broom	Asia
16SrV-D	Flavescence dor (Grapevine)	Europe
Clover proliferation group (16SrVI)		
16SrVI-A	Clover proliferation	North America
16SrVI-B	Strawberry multiplier	North America
Ash yellows group (16SrVII)		
16SrVII-A	Ash yellows and lilac witches' broom	North America
Loofah witches' broom group (16SrVIII)		
16SrVIII-A	Loofah witches' broom	Taiwan
Pigeon pea witches' broom group(16SrIX)		
16SrIX-A	Pigeon pea witches' broom	North America
Unclassified	Echium vulgare yellows	Italy
Apple proliferation group (16SrX)		
16SrX-A	Apple proliferation	Europe
16SrX-B	Apricot chlorotic leaf roll	Europe
16SrX-C	Pear decline, peach yellow leaf roll	Europe
Rice yellow dwarf group (16SrXI)		
16SrXI-A	Rice yellow dwarf	Asia
16SrXI-B	Sugarcane white leaf	Asia
Unclassified	Leafhopper borne (BVK)	Germany
Stolbur group (16SrXII)		
16SrXII-A	Stolbur (pepper and tomato)	Europe, Italy, Spain

Contd..

Table 2.1 (Contd…)

16SrXII-B (*Candidatus* Phytoplasma australiense)	Papaya dieback	Australia
Mexican periwinkle virescence group (16SrXIII)		
16SrXIII-A	Mexican periwinkle virescence	Mexico
16SrXIII-B	Strawberry green petal	Florida
Bermuda grass white leaf group (16srXIV)		
16SrXIV-A	Bermuda grass white leaf	Asia, Italy
Other undesignated groups		
Italian bindweed stolbur group	Italian bindweed stolbur	Italy
Buckthorn witches' broom group	Buckthorn witches' broom	Germany

Lee *et al*. (1994) and Seemuller *et al.* (1998).

2.2.2 Distribution of Phytoplasma in India

Phytoplasma caused diseases in several plant species in India and resulted in serious threat to the affected crop as a source of alternative host for the spread of phytoplasmas to other economically important plants and thereby chances of causing severe losses to a maximum level. So far, 34 phytoplasmas belonging to seven groups have been identified on different plant species.

Table 2.2. Distribution of phytoplasmal diseases of plants in India

Diseases groups	Ribosomal Subgroup	Geographic distribution (Place/State)	References
Aster yellows (16SrI)			
Cannabis witches' broom	16SrI-A	Lucknow and Gorakhpur (UP)	Raj *et al*. (2008a); Mall (2009)
Parthenium virescence and witches' broom	16SrI-B	Lucknow (UP)	Raj *et al*. (2008b)

Contd..

Table 2.2 (Contd…)

Catharanthus phyllody	16SrI-C	Gorakhpur (UP)	Chaturvedi *et al*. (2009b)
Rosa little leaf	16SrI-D	Gorakhpur (UP)	Chaturvedi *et al*. (2009a)
Adenium little leaf	16SrI-E	Lucknow (UP)	Raj *et al*. (2007b)
Hibiscus yellow leaf	16SrI-F	Gorakhpur (UP)	Chaturvedi *et al*. (2010)
Chrysanthemum little leaf	16SrI-G	Lucknow (UP)	Raj *et al*. (2007a)
Achyranthes yellows	16SrI-H	Lucknow (UP)	Raj *et al*. (2009b)
Gladiolus yellowing and malformation of flower spikes	16SrI-I	Lucknow (UP)	Raj *et al*. (2009a)
Chilli little leaf	16SrI-J	Bahraich (UP)	Khan and Raj (2006)
Crotalaria witches' broom	16SrI-K	Meghalaya (Assam)	Baiswar *et al*. (2010)
Sandal witches' broom	16SrI-L	Gorakhpur (UP)	Khan *et al*. (2008a)
Sesame witches' broom	16SrI-M	Lucknow (UP)	Khan *et al*. (2007)
Pigeon pea little leaf	16SrI-N	Lucknow (UP)	Raj *et al*. (2006)
Piper Yellows	16SrI-O	Karnataka	Adkar-Purushothama *et al*. (2009)
Peanut witches' broom (16SrII)			
Amaranthus white leaf	16SrII-A	Pantnagar (UC)	Arocha *et al*. (2008)
Lactuca sativa yellow leaf	16SrII-B	Pantnagar (UC)	Arocha *et al*. (2008)
Daucus carota white leaf	16SrII-C	Pantnagar (UC)	Arocha *et al*. (2008)
Phaseolus vulgaris little leaf	16SrII-D	Pantnagar (UC)	Arocha *et al*. (2008)
Citrus aurantifolia witches' broom	16SrII-E	Nagpur (Maharashtra)	Ghosh *et al*. (1999)
Chickpea witches' broom	16SrII-F	Bahraich (UP)	Ghanekar *et al*. (1988)
Marigold yellows	16SrII-G	Pantnagar (UC)	Arocha *et al*. (2008)

Contd..

Table 2.2 (Contd…)

X-disease group (16SrIII)			
Peach yellow leaf	16SrIII	Meghalaya (Assam)	Thakur *et al*. (1998)
Clover proliferation 16SrVI			
Araucaria little leaf	16SrVI-A	Lucknow (UP)	Gupta *et al*. (2010)
Brinjal little leaf	16SrVI-B	Karnataka	Schneider *et al*. (1995)
Withania little leaf	16SrVI-C	Pantnagar (UP)	Zaim and Samad (1995); Samad *et al*. (2006)
Portulaca little leaf	16SrVI-D	Lucknow (UP)	Samad *et al*. (2008)
Datura little leaf	16SrVI-E	Ambedkar nagar (UP)	Raj *et al*. (2009b)
Sugarcane white leaf (16SrXI)			
Sugarcane grassy shoot	16SrXI	Gorakhpur (UP)	Rao *et al*. (2007b)
Areca palm yellow leaf disease	16SrXI	Sullia Dist, Kerala	Manimekalai *et al*. (2010a)
Coconut root wilt	16SrXI	Kasaragod, Kerala	Manimekalai *et al*. (2010b)
Sugarcane yellow leaf (16SrXII)			
Sugarcane yellow leaf	16SrXII	Lucknow (UP)	Gaur *et al*. (2008)
Bermuda white leaf (16SrXIV)			
Bermuda grass white leaf	16SrXIV	Gorakhpur and Lucknow (UP)	Rao *et al*. (2007a)
Dichanthium white leaf	16SrXIV	Gorakhpur (UP)	Rao *et al*. (2009)
Digitaria white leaf	16SrXIV	Delhi	Rao *et al*. (2010)
Oplismenus white leaf	16SrXIV	Gorakhpur (UP)	Rao *et al*. (2010)
Pigeon pea witches' broom 16SrIX			
Toria phyllody (*Brassica campestris* var *toria*)	16SrIX	New Delhi	Azadvar *et al*. (2009)

Sesame phytoplasma is a major disease that infects the plant and destroying the entire plant under severe condition. The phyllody sometimes named as “leaf curl” which was traced 1908 in Mirpurkhas area of India (now in Pakistan) (Sahambi, 1970). Occurrence of disease was first reported from Burma (McGibbon, 1924) and later in India in 1930 (Kashiram, 1930). The disease is now reported in several countries *viz.*, Iran, Iraq, Israel (Klein, 1977), Sierra Leone, Tanzania (Deighton, 1932), Thailand (Kulthongkham, 1948) Sudan (Mazzani and Malaguti, 1952) and Turkey (Turkmenoglu and Ari, 1959).

In Burma, 90 per cent incidence was reported in sagang and lower chin districts (Robertson, 1928). According to survey in Thailand during 1969 and 1970, the phyllody incidence was so severe in Northern Thailand so that, the farmers decreased the sown area of sesame crop (Choopanya, 1973). The plants infected with disease showed virescence, phyllody, yellowing, flower sterility and stem proliferation disease caused yield loss up to 70 per cent during 2003-05 in Yazd provenience (Hosseini *et al.*, 2007).

The infected plants partially or completely sterile resulting in total loss in yield, the disease incidence recorded as much as 10-100 per cent in India (Sahambi, 1970). Abraham *et al.* (1977b) reported the plants infected with phytoplasma caused a yield loss of 33.9 per cent. It was estimated that one per cent increase in phyllody incidence decreases the sesame yield by 8.4 kg ha^{-1} under Coimbatore conditions (Ravindar, 2017), Vyas (1981) reported due to infection of disease the losses in plant yield, germination and oil content of sesame seeds were noticed as 93.66, 37.77 and 25.92 per cent respectively. Borkar and Krishna (2000) mentioned depending on weather condition and vector population field incidence of the phyllody disease ranged from 1-100 per cent.

2.3 CAUSAL ORGANISM

The causal agent of sesame phyllody was phytoplasma (mycoplasma like organism) the phytoplasmas were bacterial plant pathogens that caused devastating yield losses in diverse low and high value crops worldwide (Bertaccini, 2007 and Lee *et al*., 2000). The phytoplasmas were wall less, non helical, obligate, intracellular parasites belonging to the class Mollicutes and resides in the phloem of the plants (Doi *et al*., 1967). They were obligate symbionts of plants and insects and in most cases need both hosts for dispersal in nature. In plants, they were mainly restricted to the phloem tissue (Doi *et al*., 1967) and spread throughout the plant by moving through the pores of the sieve plates that divide the phloem sieve tubes.

Because of the difficulty in culturing phytoplasma *in vitro,* the organism remain one of the most poorly characterized groups of plant pathogens. In the last decade, however molecular, biology methods have made it possible to determine the phylogenetic and taxonomic relations between phytoplasmas and other prokaryotes. Currently, the classification of phytoplasma is mainly based on the nucleotide sequences of the 16S rRNA gene (Gundersen and Lee, 1996 and Seemuller *et al*., 1994).

Phytoplasma were prokaryotes lacking cell wall, gram positive, low G+C content currently classified in the division Firmicutes, class Mollicutes, order Acholeplasmatales, genus *Candidatus* Phytoplasma (Bertaccini, 2007). Phytoplasmas were mainly transmitted by phloem feeders of the insect vectors of order Hemiptera, mostly leafhoppers (Cicadellidae), planthoppers (Fulgoridae) and psyllids (Psyllidae) (Weintraub and Beanland, 2006).

In insects, phytoplasmas invaded the gut and salivary glands and many other tissues where they can accumulate at great numbers inside and outside cells (Ammar and Hogenhout, 2006). Phytoplasmas have to traverse the gut and salivary gland cells in order to reach the saliva for subsequent introduction into the phloem during insect feeding (Lefol *et al*., 1994;

Nakashima and Hayashi, 1995). The latent period, i.e., the time between initial acquisition of the phytoplasmas by the insect vector from plants and the ability for the insect to introduce phytoplasmas back into plants, can vary between 7 to 80 days (Raygoza and Nault, 1998 and Murral *et al*., 1996). In plants, symptoms can develop at 7 days after introduction of the phytoplasma by the insect vector but can take much longer (6-24 months), depending on the phytoplasma and plant species. Phytoplasmas may overwinter in infected vectors, as well as in perennial plants that serves as reservoirs of phytoplasmas that were spread in the following spring.

Thus far, there has been no substantial evidence to indicate that phytoplasmal diseases were seed borne. However, phytoplasmas can be spread by vegetative propagation through cuttings, storage tubers, rhizomes or bulbs (Lee and Davis, 1992). Phytoplasmas that caused many ornamental and fruit tree diseases were spread by vegetative propagation. Phytoplasmas can be transmitted through grafts but they cannot transmitted mechanically by inoculation with phytoplasma containing sap.

2.4 SYMPTOMOTOLOGY

The disease is characterized by the transformation of floral parts into green leaf like structure followed by abundant vegetative growth. The sepals become leaf like but were smaller in size. The veins of sepals and petals in phyllody flowers were usually thick and prominent. The carpels get transformed into leaf like outgrowth which forms a pseudosyncarpous ovary by their fusion at the margins. The false ovary becomes very much enlarged and flattened with a soft texture and a wrinkled surface due to the veins of the capillary wall. Small petiole like out growth which replaced the ovules, grow and burst through the wall of the ovary producing small shoots. These shoots continue to grow and produced more leaves and phyllody flowers resulting in flower pedicel grown through the flower with unlimited growth (Kolte, 1985).

The sesame infected plants expressed symptoms depending on the stage of the crop growth and time of infection. A plant infected in its early growth stage remains stunted to about two thirds of a normal plant and the entire plant got affected. The entire inflorescence is replaced by a growth consisting of short, twisted leaves, closely arranged on a stem with short internodes. However when infection taken place in later stages, normal capsules were formed on the lower portion of the plant and phyllody flowers were present on the top of the main branches (Vasudeva and Sahambi, 1955 and Klein, 1977).

Different types of disease symptoms were reported to be associated with sesame phyllody. The major symptoms were phyllody (production of leafy structures of floral parts), floral virescence (color change to green), witches' broom, shoot tip fasciation, flattening of the shoot apex, intense leaf, flower bud proliferation and cracking of seed capsules (Abraham *et al*., 1977a and Madhupriya *et al*., 2015). The affected plants were stunted (Abraham *et al*., 1977a and Selvanarayanan and Selvamuthukumaran, 2000) with the floral parts being modified into leafy structures. The crown region become bushy with shortened internodes, tiny reduced leaves, phylloid flowers, flower buds and aborted capsules (Selvanarayanan and Selvamuthukumaran, 2000). Leaves on the lower parts of infected plants, stems and roots usually did not exhibit symptoms. Sometimes vivipary was also recorded where germination of seeds in the capsules occurs (Akhtar *et al.*, 2009 and Vamshi *et al.*, 2018).

Choopanya (1973) observed the symptoms of disease as stunted growth, extreme proliferation of growing tip, numerous small leaves resulted in witches' broom appearance and part or whole floral parts were transformed into green leaf like structure.

Salehi and Izadpanah (1992) and Sarwar and Akhtar (2009) observed floral virescence, phyllody, proliferation, yellowing, cracking of seed capsules, germination of seeds in capsules and formation of dark exudates on the foliage were reported from regions of Iran and Pakistan. Al-Sakeiti *et al.*

(2005) observed the excessive development of short shoots and internodes resulted in witches' broom appearance in Oman region.

2.5 MOLECULAR IDENTIFICATION OF PHYTOPLASMA FROM PHYLLODY INFECTED PLANTS WITH PCR AND NESTED PCR

Sertkaya *et al.* (2007) carried a survey during 2002-04 in Turkey and they collected phytoplasma infected samples of sesame, periwinkle, eggplant, pepper and tomato, leafhoppers of *Empoasca* spp. and *Orosius orientalis* from infected fields and carried molecular characterization to these samples and they observed that phytoplasmas detected in sesame, periwinkle, pepper, eggplant and *O. orientalis* belonged to Clover proliferation group (16SrVI-A) and phytoplasma associated with tomato big bud belonged to the Stolbur phytoplasma group (16SrXII-A).

Win *et al*. (2010) investigated etiological agent associated with sesame phyllody in Myanmar, they detected phytoplasma from the phyllody symptoms by using Polymerase Chain Reaction (PCR) and amplified phytoplasmal 16S rDNA fragments (1.8 kb and 1.2 kb) from the examined samples by a phytoplasma universal primer set. They revealed that the sesame plants showing phyllody symptoms in Yezin area, Myanmar were infected by a phytoplasma designated SP-MYAN strain. Based on RFLP profiles with six restriction enzymes, the phytoplasma associated with phyllody disease in sesame plants was classified into 16SrI-B group sequence analysis confirmed that SP-MYAN was a member of '*Candidatus* Phytoplasma asteris'.

Tabatabaei *et al.* (2011) studied 27 sesame accessions it includes 13 Iranian genotypes from seven different locations and 14 exotic genotypes, variation among these population was characterized by 24 morphological, phenological and reproductive traits by Random Amplified Polymorphic DNA (RAPD) markers. They observed that these genetic markers underscored the high variability present in Iranian genotypes (0.39-0.92) compared with

exotic (0.40-0.81) genotypes, they concluded that RAPD markers can effectively evaluate the genetic variation in the sesame germplasm.

Manjunatha *et al*. (2012) identified phytoplasma based on sympto-motology and 16S rDNA amplification in nested PCR followed by RFLP analysis and sequencing. PCR products of 1.8 kb were amplified using universal primer P1/P7 pair from diseased sesame but not from healthy plants. The samples were reamplified in nested PCR using primers R16F2n/R16R2 and a DNA fragment of 1.2 kb was obtained. The 1.2 kb amplicon obtained from nested PCR was cloned and sequenced. The BLAST analysis indicated a close relationship with several phytoplasmas belonging to ribosomal group 16SrI and showed more than 99 per cent nucleotide identity.

Ikten *et al*. (2014) performed the molecular identification and transmission of phytoplasma associated with sesame phyllody by using phytoplasma specific universal primers P1/P7 and R16F2n/ R16R2 for amplification of the 16S rRNA gene through direct and nested PCR respectively. They observed that phytoplasma belonged to the 16SrIX-C and 16SrII-D group were the causal agent of sesame phyllody and *Orosius orientalis* was the vector associated with the disease.

Hosseini *et al.* (2015) carried the survey in sesame fields in Yazd province during 2008-10. They observed that phyllody occurred in all the sesame growing fields in Yazd and whole infection of field was observed. On the basis of RFLP analysis, they revealed that the phytoplasma was identified as a member of Peanut witches' broom (16SrII) group.

Nabi *et al.* (2015a) surveyed phyllody infected fields in three states *viz*., Delhi, Uttar Pradesh and Bihar in India. With the help of direct and nested PCR by using phytoplasma specific primers (P1/P6 and R16F2n/R16R2) they confirmed that sesame phyllody and witches' broom isolates from Delhi and Bihar belonged to 16SrI-B subgroup and isolates from Uttar Pradesh belonged to the 16SrI-B and 16SrII-C subgroups. Similarly, Madhupriya *et al*. (2015) identified the 16Sr I-B, II-C and II-D subgroups were found in North India

Nabi *et al.* (2015b) studied the molecular characterization of phytoplasma associated with sesame phyllody and identification of its natural vector at three different locations. They confirmed that phytoplasma was associated with phyllody by amplification of phytoplasma 16S rDNA through direct and nested PCR. Out of the four different leafhoppers were collected from these locations only *Hishimonas phycitis* was the vector associated with phytoplasma by using universal primer pair R16F2n/R16R2 in nested PCR.

Singh *et al*. (2016) collected phyllody affected sesame plants from nine different states of India which were found to be infected by phytoplasmas belonged to two 16Sr groups, namely 16SrI and 16SrII. In which 16SrI-B and 16SrII-D were prevalent in symptomatic samples collected from North India, whereas phytoplasma of only 16SrII group was found in South India.

Junior *et al*. (2017) investigated the association between affected plants of phyllody and phytoplasma using molecular and phylogenetic methods. They extracted total DNA from asymptomatic and symptomatic plants. They used nested PCR assays with primers R16SN910601/R16SN011119 and R16F2n/R16R2, the amplified products revealed the presence of phytoplasma in all diseased plants. The phytoplasmas nucleotide sequences shared 99 per cent similarity with phytoplasmas belonged to group 16SrI.

Thangjam and Vastrad (2017) collected symptomatic and asymptomatic plants from the phyllody infested field. The total genomic DNA was extracted and amplified by using the primer pair P1/P7 and R16F2n/R16R2 in first and second round PCR and they got the band size of 1.8 kb and 1.2 kb respectively. The results clearly revealed that the sesame phyllody disease was caused by phytoplasma.

Venkataravanappa *et al*. (2017) confirmed the phytoplasma in phyllody affected sesame plants by using PCR with universal primers of 16S rRNA R16F2n/R16R2 and secY gene (secYF2 and secYR1) respectively. They observed that the amplified products shared highest nucleotide identity of 16S

rRNA (97.9-99.9 %) and SecY (95.8 to 96.3 %) with subgroup 16SrII-D, the Peanut witches' broom group and they concluded that phytoplasma belonged to 16Sr II-D subgroup of Peanut witches' broom group was the potential threat infecting to sesame in North India.

Al-Subhi *et al*. (2018) collected the symptoms of phytoplasma infection from symptomatic and asymptomatic plants from 11 crops of Oman region. Amplification and sequencing of 16S ribosomal RNA, secA, tuf, imp and sap11 genes, revealed that these sequences share more than 99 per cent similarities with the Peanut Witches' broom subgroup (16Sr□-D).

Rao *et al*. (2018) amplified 1.3 kb DNA product in all symptomatic plants of cowpea, pigeon pea, lentil and mung bean by using 3Far/3Rev primer pair in nested PCR assays. No amplification was observed in any of the non symptomatic samples of different legume species tested. Virtual RFLP analysis of 16S rDNA sequences confirmed the mung bean witches' broom at Delhi was identified to be associated with strain related to '*Candidatus* Phytoplasma aurantifolia' (16SrII-D), pigeon pea little leaf at Faizabad, UP with strain related to '*Candidatus* Phytoplasma phoenicium' (16SrIX-C), lentil witches' broom at Faizabad, UP with '*Candidatus* Phytoplasma trifoli' (16SrVI-D) and cowpea flat stem disease at Kerala with '*Candidatus* Phytoplasma cynodontis' (16SrXIV-A).

Singh *et al*. (2018) extracted the total genomic DNA from both symptomatic sesame and cannabis plants and subjected to nested PCR assays using two sets of universal phytoplasma specific primers P1/P7 followed by R16F2n/R16R2 and 3Far/3Rev to amplify the 16S rDNA fragments. The amplified products indicated the presence of phytoplasma in all diseased plants. The phytoplasma sequences of sesame and cannabis strains revealed the association of '*Candidatus* Phytoplasma asteris' (16SrI group).

Bandakkanavara *et al*. (2019) noticed phyllody disease on niger at Main Research Station, Hebbal, Karnataka and carried investigations during *kharif* 2016. They observed etiological agent of phyllody based on symptoms, amplification of 16S rDNA of the phytoplasma was done by Polymerase Chain Reaction (PCR) from infected samples and they got the amplified PCR product of 1.2 kb by using nested PCR phytoplasma specific universal primers R16F2n/R16FR2. The 16S rDNA sequence of niger phyllody phytoplasma had maximum nucleotide identity of 90 per cent with the 16S rDNA sequence of '*Cymbopogon citratus*' white leaf phytoplasma, alfalfa witches' broom phytoplasma, *Sesamum indicum* phyllody phytoplasma and *Vigna radiata* phyllody phytoplasma.

2.6 TO STUDY THE TRANSMISSION OF SESAME PHYLLODY CARRIED BY DIFFERENT METHODS

2.6.1 Transmission of Sesame Phyllody through Sap Inoculation

Akhtar *et al*. (2009) studied sap transmission of the infectious agent could not be achieved under greenhouse conditions, which indicates that sesame phyllody was not mechanically transmissible, however, the phytoplasma that caused phyllody disease was successfully transmitted from infected to healthy plants via grafting, dodder and the leafhopper *Orosius albicinctus.*

Gogoi *et al*. (2017) studied the sesame phyllody phytoplasma could not be transmitted by seed or sap inoculation method, they observed that plants inoculated with sap extracted from sesame phyllody infected plants were remained free from infection and also the plants raised from seed collected from sesame phyllody infected plant did not showed any symptoms.

Rajput and Raghuvanshi (2017) investigated on the technique of screening by ratoon infector rows disease transmission. They observed that the phytoplasma was restored in the cell sap of previously grown identified infected sesame plants which was translocated in the newly emerged shoots

i.e., ratoon and later transmitted from plant to plant by sap sucking insects such as leafhoppers in which they were also able to replicate.

Ravindar (2017) concluded that the sesame phyllody cannot be transmitted through sap. While he carried sap inoculation to twenty healthy plants, no symptoms were observed on inoculated plants. Out of twenty sap inoculated plants, no one is showed positive reaction in PCR assay.

2.6.2 Transmission of Sesame Phyllody through Grafting

Pal and Nath (1935) reported the causal agent of the disease in sesame can be easily transmitted by grafting. Salehi and Izadpanah (1992) reported transmission of phytoplasma by veneer grafting in 6-8 week old sesame plants resulted in development of characteristic symptoms virescence, phyllody and witches' broom after 30-60 days.

Sertkaya (1999) reported sesame phyllody incidence on three plants out of twenty seven plants which were grafted by side grafting. Akhtar *et al.* (2009) reported transmission of sesame phytoplasma in Pakistan from diseased to healthy sesame by grafting. When four week old healthy sesame plants were grafted with scion from diseased sesame, symptoms were developed within 25-35 days after inoculation.

Gogoi *et al*. (2017) reported that sesame phyllody disease was successfully transmitted from an infected plant to the healthy sesame plant by grafting and observed symptoms started appearing from 30th day of grafting and continued up to 40th day.

Ravindar (2017) revealed that sesame phyllody was transmitted successfully from infected plants to healthy plants through grafting. When grafting was done to twenty healthy plants, only seventeen inoculated plants were showed phyllody symptoms at 45 DAI. Out of the twenty inoculated plants, only seventeen graft inoculated were showed positive reaction to the sesame phyllody in PCR and concluded that 85 per cent of disease was

transmitted into the graft inoculated plants and inoculated plants showed characteristic symptoms of phyllody, floral virescence, yellowing of leaves and stunted growth.

2.6.3 Transmission of Sesame Phyllody through Dodder.

Marcone *et al*. (1999) reported dodder transmission of pear decline, European stone fruit yellows, rubus stunt, picris echioides yellows and cotton phyllody phytoplasma to periwinkle. The reports showed transmission depends on both of phytoplasma and vector i.e., *Cuscuta campestris*. The rubus stunt and cotton phyllody phytoplasma were transmitted at high efficiency by *Cuscuta europia* and *Cuscuta campestris* respectively. Whereas the other phytoplasmas were transmitted only at a low rate.

Sertkaya (1999) reported transmission of sesame phyllody phytoplasma from diseased to healthy sesame plant by dodder (*Cuscuta campestris*), they carried transmission of phytoplasma through dodder for 20 plants and they observed that eighteen out of twenty plants showed symptoms.

Akhtar *et al*. (2009) studied sesame phyllody was successfully transmitted by dodder from diseased to healthy sesame, when five week old healthy sesame plants were in contact with diseased sesame through dodder bridge, later, the plants were free of dodder and symptoms were developed within four weeks after inoculation.

Salehi *et al*. (2009) reported transmission of safflower phyllody phytoplasma from diseased safflower to healthy safflower and periwinkle by dodder. They carried transmission of phytoplasma through dodder for five safflower and six periwinkle plants and they observed that after five weeks after inoculation, two out of five safflower and three out of six periwinkle plants showed disease symptoms.

Gogoi *et al*. (2017) studied dodder (*Cuscuta campestris*) successfully transmitted the sesame phyllody phytoplasma from infected plant to healthy

sesame plants under net house conditions and they recorded that the dodder transmitted sesame plants took 35 to 45 days to express the symptoms after transmission.

2.6.4 Transmission of Sesame Phyllody through Leafhopper

Insect vectors primarily leafhoppers, planthoppers and psyllids were one of the sources of natural phytoplasma transmission they have a profound effect on creating epidemics, hence their role in phytoplasma transmission and spread is of much concerned.

The disease was successfully transmitted in nature by the leafhopper vector, *Orosius albicinctus* distant (Vasudeva and Sahambi, 1955 and Hosseini *et al*., 2007), Pakistan (Akhtar *et al*., 2009). *Orasius cellulosus* in Upper Volta (Desmidts and Laboucheix, 1974 and Kolte, 1985), in Fars provinence of Iran (Salehi and Izadpanah, 1992) and Turkey (Kersting, 1993).

Sahambi (1970) reported phytoplasma successfully transmitted by vector when minimum acquisition access period, inoculation feeding period and incubation period in the vector and plants were found to be 4 hr, 30 min, 15 to 23 days and 13 to 25 days respectively.

Hosseini *et al*. (2007) reported the disease development under green house condition by providing 30 days acquisition access period on diseased plants and 21 days of inoculation access period on healthy plants, the plants showed the symptoms of virescence, phyllody, yellowing, flower sterility and stem proliferation**.**

Akhtar *et al*. (2009) reported sesame phyllody was successfully transmitted by leafhopper transmission. When leafhoppers were released on four week old healthy sesame plants for inoculation period of seven days, symptoms were developed on 60 per cent of inoculated plants.

Thangjam and Vastrad (2015) collected four species of leafhoppers from the phyllody infested field and their total genomic DNA was extracted

and it was amplified by using the primer pair P1/P7 and R16F2n/R16R2 in first and second round PCR respectively and they got the bands size 1.8 kb and 1.2 kb were from vector *Orosius albicinctus*. They confirmed that *O. albicinctus* was the vector associated with sesame phyllody.

Gogoi *et al*. (2017) studied sesame phyllody phytoplasma was successfully transmitted from infected sesame plants to healthy sesame plants by the leafhopper vector *O. albicinctus* distant under net house condition and they recorded that highest disease transmission (84.26 %) was observed in plants inoculated with three leafhopper/plant.

Salehi *et al*. (2017) conducted surveys in the major sesame growing areas of Iran, during 2010-14 and collected leafhoppers of *Circulifer haematoceps* and *Orosius albicinctus* from the phyllody infested fields. They carried transmission studies to identify the transmission of sesame phyllody strains, *C. haematoceps* leafhopper transmitted 16SrII-D, 16SrVI-A and 16SrIX-C phytoplasmas from affected sesame plants to healthy sesame and periwinkle plants. *O. albicinctus* only transmitted 16SrII-D phytoplasma from affected sesame plants to healthy sesame and periwinkle plants.

2.7 SCREENING OF SESAME GENOTYPES AGAINST PHYLLODY UNDER FIELD CONDITIONS

Saravanan and Nandarajan (2005) had taken up screening for resistance against phyllody under field conditions on eight cultivated sesame genotypes of *Sesamum indicum* and three other wild relatives. The results revealed that the cultivar SVPR 1 and wild relatives such as *Sesamum alatum*, *Sesamum malabaricum* and *Sesamum yanaimalaiensis* showed resistance to phyllody disease and can be utilized in breeding against phyllody disease.

Sarwar and Haq (2006) screened 106 genotypes of sesame collected from different parts of the world for resistance against phyllody in Pakistan. They found maximum disease in genotypes NS 3103 (20 %), TS-3 (3.80 %) and no disease symptom was observed in variety Til-89.

Sertkaya *et al*. (2007) carried survey during 2002-04 in Turkey to screen for phytoplasmas in sesame, periwinkle and solanaceous crops like eggplant, pepper and tomato. They observed that sesame and periwinkle plants shown phyllody symptoms, in pepper with stolbur symptoms, in eggplant with little, yellow leaves and in tomato with big bud symptoms.

Manjunatha *et al*. (2012) conducted survey during 2009-10 on incidence of phyllody disease associated with sesame in southern parts of Karnataka. They observed sesame plants infected with phyllody were pale green and bushy due to severe reduction in leaf size, reduced internodal length, excessive axillary proliferation and the pods in infected plants after flowering were very small. They recorded disease incidence ranged from 17 to 75 per cent in surveyed regions of southern Karnataka, Highest disease incidence was recorded at Konehally (75 %), followed by Nagathihally (55 %) in Tumkur district and the lowest disease incidence was recorded at Kodihally (17 %) of Hassan district.

Akhtar *et al.* (2013) screened 133 genotypes of sesame that belonged to different regions for three successive years, of these the three genotypes namely NS 98003-04, NS 98002-04 and NS 99005-01 were ranked as highly resistant and they remained as symptomless upto the harvest of crop. While eleven genotypes namely Sumboonkkae, NS 99006-04, NS 97001-04, NS 01004-04, Ahnsankkac, NS 940051-04, NS 20005-04, Hansumkkae, NS11704, NS11504 and NS 96019-04 were ranked as resistant during the second year. Out of which only four genotypes namely NS 98002-04, NS 01004-04, NS 99005-01 and NS 98003-04 were scored as resistant during third year.

Pathak *et al*. (2013) conducted survey in Saurashtra region of Gujarat and they recorded phyllody incidence during *kharif* and *summer* seasons that was ranged from 0 to 1.5 per cent and 0 to 4.7 per cent respectively. They observed that spread of disease was very slow and disease symptoms were noticed at 40 DAS and maximum at 75 DAS.

Sridhar *et al*. (2013) conducted a survey in different parts of Karnataka to know disease severity and to identify the hot spot for sesame phyllody they noticed phyllody disease incidence was ranged from 2.62 to 55.7 per cent in all

the surveyed locations. They recorded the maximum average disease incidence (52.39 %) in Raichur district followed by Gulbarga district (50.55 %), Gadag district (39.55 %) and Haveri district (16.57 %). Whereas, the least average percent disease incidence (3.46 %) was observed in Dharwad district.

Nabi *et al*. (2015a) conducted survey in phyllody infected fields of three states, *viz*., Delhi, Uttar Pradesh and Bihar in India during July-October 2013. They observed phytoplasma suspected symptoms like virescence, phyllody and witches' broom in surveyed fields. They recorded 15-35 per cent incidence of sesame phyllody (SP) and witches' broom (WB) disease in different fields.

Nabi *et al*. (2015b) conducted survey in sesame fields at Kushinagar and Gorakhpur districts in Uttar Pradesh and Indian Agricultural Research Institute, New Delhi, India during July-October (2013). They suspected phytoplasma symptoms like virescence, phyllody and witches' broom were recorded on sesame plants. Little leaf, witches' broom and chlorosis symptoms were also observed on weed species i.e., *Sclerocarpus africanus* of family Asteraceae in sesame phyllody affected fields at Gorakhpur and they recorded phyllody incidence of 12 to 35 per cent in these locations.

Singh *et al*. (2016) evaluated 32 released varieties, 150 germplasm lines and four wild spp. of sesame under field conditions during *kharif* 2005 and recorded disease incidence of parental lines on individual plant basis. They observed that seven cultivars *viz.*, RJS 78, RJS 147, KMR 14, KMR 79, Pragati, IC 43063 and IC 43236 and two wild spp. i.e., *Sesamum alatum* and *Sesamum mulayanum* were resistant to phytoplasma with mean incidence of below 5 per cent in all the parental lines.

Salehi *et al*. (2017) conducted surveys in the major sesame growing areas of Fars, Yazd and Isfahan provinces (Iran), during 2010-14. They observed the occurrence of sesame phyllody disease in all surveyed areas and recorded disease incidence rate it approached 100 per cent. They also noticed the prevalent symptoms of disease were little leaf, yellowing, internode shortening, witches' broom, floral virescence, phyllody, proliferation, capsule cracking and germination of seeds in capsules.

Ustun *et al*. (2017) screened 542 genotypes of sesame for the disease resistance in the field by using a disease incidence scale of 1-5. In which they eliminated 304 genotypes which showed high disease intensity and only 30 genotypes scored as resistant by using the disease incidence scale. They conducted Real-time qPCR for detection and quantification of phytoplasmas to select true resistant genotypes, in which the sesame accessions ACS 38 and ACS 102 were identified as resistant to the disease after evaluation in field, greenhouse and qPCR assays.

Venkataravanappa *et al*. (2017) conducted a survey in Varanasi and Mirzapur districts of Uttar Pradesh, India during September to December, 2012-14. They collected leaf samples from sesame plants which exhibiting phyllody disease at different locations in the farmers fields. They observed that the range of phyllody incidence from 30-70 per cent indicating that it was prevalent in Uttar Pradesh.

Singh *et al*. (2018) conducted a survey during May-June 2016, they observed little leaf and phyllody symptoms on eight per cent of sesame crop in Chakbhalwal and Kotbhalwal regions of Jammu, India and phytoplasma suspected witches' broom symptoms on *Cannabis sativa* subsp. *sativa* in and around sesame fields and they observed that the incidence varied from 5 to 12 per cent in different fields.

Taye *et al*. (2019) conducted field experiment at Werer Agricultural Research Center for three successive years. During the first year (2015) two varieties namely T-85 and Argane were grouped as moderately resistant while, Mehado-80, Serakamo and Abasena grouped as moderately susceptible. Kelafo 74 and Adi were grouped as susceptible and tolerant respectively. During second year (2016) T-85 and Argane grouped as moderately resistant and Kelafo-74 and Serkamo grouped as susceptible. During last year (2017) only T-85 was ranked as resistant and Kelafo-74 and Abasena were ranked as moderately susceptible. The Combined analysis of data showed that Argane and T-85 were moderately resistant against phyllody disease and they concluded that the use of resistant varieties is an economical and durable method of controlling phyllody disease.

Chapter – III

Material and Methods

Chapter III

MATERIALS AND METHODS

The present study was conducted during 2019-20. The materials used and methods followed to conduct the present study were detailed in this chapter.

3.1 LOCATION OF WORK

The present investigation of phyllody disease of sesame (*Sesamum indicum* L.) was carried out at Department of Plant Pathology, ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad during 2019-20.

3.2 MOLECULAR IDENTIFICATION OF PHYTOPLASMA FROM PHYLLODY INFECTED PLANTS WITH PCR AND NESTED PCR

3.2.1 DNA Extraction from Host Plant

Protocol:

The total genomic DNA was isolated from leaves of healthy and phyllody affected plants by following CTAB (Cetyl Trimethyl Ammonium Bromide) method of Murray and Thomson (1980), with some modifications. The modifications were made to improve the quality of DNA.

Procedure:

- The leaf samples were grounded into a fine powder with liquid nitrogen or CTAB extraction buffer.
- Approximately 100 mg of the powder was transferred into 2 ml eppendorf tube by spatula, to this 1.5 ml of CTAB extraction buffer (Appendix C) was added and then incubated in water bath at 65 °C for 45 min with occasional mixing at every 20 min interval.

- The tubes were removed from the water bath and allowed to cool at room temperature and after incubation the tubes were centrifuged at 14000 rpm for 10 min.
- Then equal volume of Phenol: Chloroform (1:1 v/v) was added to the supernatant and mixed thoroughly by gentle inversion and centrifuged for 20 min by keeping in rotator at 13000 rpm (Eppendorf centrifuge, 5804R) at 25 °C and then the supernatant was carefully pipetted out into new 2 ml eppendorf tubes.
- To this supernatant chloroform: Iso amyl alcohol (24:1 v/v) was added then mixed thoroughly by gentle inversion and centrifuged at 12000 rpm for 15 min at 25ºC. Then supernatant was carefully pipetted out in to new 1.5 ml eppendorf tubes.
- To the supernatant, ice cold isopropanol of about 0.6 volumes (2/3rd of pipetted volume) and 50 µl of 3 M sodium acetate of pH 5.2 (Appendix C) were added. The contents were mixed gently by inversion and kept undisturbed for about 2 hours or at -20 ºC.
- Subsequently, the tubes were centrifuged at 13000 rpm for 20 min at 4 ºC temperature to pellet out DNA.
- The supernatant was discarded gently and the DNA pellet was washed with 70 % ethanol and centrifuged at 13000 rpm for 10 minutes.
- The supernatant was removed and the tubes were allowed to air dry completely until ethanol smell was lost and then the pellet was dissolved in 50 µl of autoclaved water.

3.2.1.1 Purification of the Extracted DNA

The isolated DNA was re-purified by adding 0.5 ml of autoclaved water, 1/10th volume (50 µl) of 3 M sodium acetate of pH 5.2 and 2-3 volumes of 5 % ethanol and then incubated on ice for 15 minutes. Later the DNA containing solution was centrifuged for 20 minutes at 4 ºC. The supernatant was decanted and pellet was rinsed with 70 % ethanol and centrifuged at

12000 rpm for 10 minutes at 4 ^{o}C to remove the leftover ethanol from DNA. Then the DNA pellet was air dried until ethanol smell was lost and dissolved it in 50 µl of 1X TE buffer (Appendix C). Later the dissolved DNA was stored at -20 ^{o}C for long term usage.

3.2.2 Quality and Quantity of DNA

DNA was assessed for its purity and intactness using agarose gel electrophoresis.

3.2.2.1 Quantification of DNA by 0.8 % Agarose Gel Electrophoresis

3.2.2.1.1 Preparation of 0.8 % Agarose Gel: 0.8 g of agarose was placed in a conical flask containing 100 ml 1X TAE buffer (Appendix C). The conical flask along with its contents was placed in an oven until agarose gets melted completely and clear solution was formed and then the flask was taken out from the oven and allowed to cool. 3 µl of Ethidium Bromide (10 mg ml^{-1}) was added to this 100 ml of agarose gel and mixed thoroughly. Later the solution was poured slowly into the gel casting tray which is pre-set with 0.5 mm combs, to avoid the formation of bubbles. After solidification the gel with casting tray was placed in gel tank and the comb was removed gently without disturbing the wells that formed upon solidification.

3.2.2.1.2 Electrophoresis of DNA samples: 3 µl of each of dissolved genomic DNA samples were mixed with 2 µl of 6X Gel loading dye (Appendix B) and were loaded in the 0.8 % agarose 1X TAE gel along with lambda (λ) Hind III digest (New England Biolabs, UK). Then, the gel with loaded samples was electrophoresed at 90 V at room temperature for about an hour. After that, the gel was visualized in an UV gel documentation system (Biorad Gel Doc XR^{+} Imaging System) and saved the image for further use. Later, based on the intensity and thickness of genomic DNA compared to λ DNA, the concentration of DNA in individual samples was determined.

3.2.2.2 Quantification of DNA by NanoDrop Spectrophotometer

The NanoDrop spectrophotometer model ND1000 was used to assess the quantity and quality of DNA employing the following procedure. Before initializing the NanoDrop Reader, the pedestal was cleaned with tissue paper to remove the dust particles. Then for initializing the instrument, 1-2 µl of distilled water was placed on the lower pedestal, now closed the upper one and clicked on measure option. Then the pedestal was cleaned with tissue paper and 1.5 µl of 1X TE buffer was placed on lower pedestal and repeated the procedure for blank measurement. Later, the pedestal was cleaned and 1.5 µl of DNA sample was placed and quantity and quality of DNA was measured. The process is repeated for all the DNA samples.

The ratio of absorbance at 260 nm and 280 nm was used to assess the purity of DNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at/or near 280 nm.

3.2.3 Normalization of DNA concentration:

Normalization of DNA samples was done to equalize the concentration of all the samples to be used for PCR reaction. The purpose of normalization was to avoid erroneous analyses due to difference in the brightness of the bands obtained after electrophoresing the PCR products. Normalization was done by diluting the DNA samples with sterile distilled water to their required dilution factor which in turn depends upon the initial concentration of DNA sample (found from quantification readings) and the type of analysis done (markers used). After normalization of samples the concentration of DNA was 50 ng/ µl.

3.2.4 Polymerase Chain Reaction

PCR was used to selectively amplify a specific segment of the DNA to a billion fold (Mullis *et al*., 1986). Universal primers specific to phytoplasma

were used to detect the phytoplasma in phyllody infected samples. The primer sequences used in PCR technique were given in Table 3.1.

Table 3.1. Primers used for PCR amplification (Sertkaya *et al*., 2007)

Type of PCR	Primer name	Sequence (5'-3')	Reference
Direct PCR primer pair	P1-Forward Primer	AAGAGTTTGATCCTGGCTCAGGAT	Deng and Hiruki (1991)
	P7-Reverse Primer	CGTCCTTCATCGGCTCTT	
Nested PCR primer pair	R16F2n	TGACGGGCGGTGTGTACAAACCCCG	Gundersen and Lee (1996)
	R16R2	GAAACGACTGCTGCTAAGACTGG	

The reaction mixture was given a short spin for thorough mixing of cocktail components and then the PCR tubes with reaction mix were placed in the gradient thermal cycler (Eppendorf) and the reaction program was set as given in Table 3.2.

Table 3.2. PCR programme for amplification of 16S rDNA (first round PCR)

Steps	First round PCR								
	Sesamum			Periwinkle			Sunhemp		
	Temp. (ºC)	Time (min)	No. of cycles	Temp. (ºC)	Time (min)	No. of cycles	Temp. (ºC)	Time (min)	No. of cycles
Initial Denaturation	94	2	1	94	2	1	94	2	1
Denaturation	94	1	30	94	1	35	94	1	30
Annealing	52	1		51.5	1		51.5	1	
Extension	72	2		72	2		72	2	
Final Extension	72	5	1	72	5	1	72	5	1
Hold	4	∞	1	4	∞	1	4	∞	1

Temp- Temperature

The steps from 2-4 were repeated for amplification of targeted DNA. Annealing temperature of primer was standardized by doing PCR with the temperature range of 48-56 ˚C. The PCR products were stored at 4 ˚C for short periods and at -20 ˚C for long duration.

3.2.4.1 Amplification of DNA using Polymerase Chain Reaction

The PCR technique was standardized and the following conditions were used for the amplification of DNA from phyllody affected samples. DNA was subjected to Polymerase Chain Reaction (PCR) by using Universal primer pairs. PCR tubes of 0.2 ml were taken and 2 µl of DNA (50 ng/µl) was added. PCR reaction was performed in a 20 µl volume of mix containing the components as given in Table 3.3.

Table 3.3. Components, their concentration and volume used for the Polymerase Chain Reaction

Component	**Concentration**	**Reaction volume** (µl)		
		Sesame	**Periwinkle**	**Sunhemp**
Taq buffer (10X) with $MgCl_2$	10X	2.0	2.0	2.0
dNTPs mixture	2.5 mM	2.0	2.0	2.0
Primer P1	5 pM	1.0	1.0	1.0
Primer P7	5 pM	1.0	1.0	1.0
Taq Polymerase	3 U/µl	0.5	0.4	0.4
Template DNA	50 ng/µl	2.0	2.0	2.0
Sterile distilled water	-	11.5	11.6	11.6
	Total	20	20	20

The volume is in µl/tube. After preparing the PCR mixture, the mixture was spun briefly and inserted into the wells of a thermocycler (Eppendorf Thermocycler).

3.2.4.2 Analysis of PCR Products by Gel Electrophoresis

Three µl of the PCR product was loaded on the 3 % agarose gel (Gel was prepared as per the methodology detailed in the section 3.2.2.1.1) by mixing with 2 µl of 6X loading dye. A 100bp ladder was loaded as a standard reference marker. The gel was run at constant voltage of 90 V for about 2-3 hours, until the tracking dye migrated to the end of the gel. The banding pattern was analyzed using gel documentation system (Biorad Gel Doc XR^+ Imaging Systems) and saved the image for later use.

3.2.4.3 Nested PCR

The product from direct PCR primed with primers P1 and P7 was diluted 1:10 with sterile deionized water and 1 µl was used as template in a Nested PCR.

Table 3.4. Contents of Nested PCR reaction mixture

Reagents	Sesamum	Periwinkle	Sunhemp
Sterile distilled water (µl)	12.5	12.5	12.5
10X PCR buffer (supplied with the enzyme) (µl)	2.0	2.0	2.0
2.5 mM dNTPs mixture (µl)	2.0	2.0	2.0
Primer R16 F_2n (5pM) (µl)	1.0	1.0	1.0
Primer R16 R_2 (5pM) (µl)	1.0	1.0	1.0
Taq Polymerase (3u/µl)	0.5	0.5	0.5
Template DNA (µl)	1.0	1.0	1.0
Total	20	20	20

After preparing the PCR mixture, the mixture was spun briefly and inserted into the wells of a thermal cycler (Eppendorf Thermocycler).

Table 3.5. PCR programme for amplification of 16S rDNA (Second round PCR)

Steps	Second round PCR (Nested PCR)								
	Sesamum			Periwinkle			Sunhemp		
	Temp. (ºC)	Time (min)	No. of cycles	Temp. (ºC)	Time (min)	No. of cycles	Temp. (ºC)	Time (min)	No. of cycles
Initial Denaturation	94	2	1	94	2	1	94	2	1
Denaturation	94	1	30	94	1	30	94	1	30
Annealing	52	1		52	1		52	1	
Extension	72	2		72	2		72	2	
Final Extension	72	5	1	72	5	1	72	5	1
Hold	4ºC	∞	1	4ºC	∞	1	4ºC	∞	1

Temp- Temperature

The PCR products were stored at 4 ˚C for short periods and at -20 ˚C for long duration.

3.2.4.4 Analysis of Nested PCR Products by Gel Electrophoresis

3 µl of the PCR product was loaded on the 3 % agarose gel (Gel was prepared as per the methodology detailed in section 3.2.2.1.1) by mixing with 2 µl of 6X loading dye. A 100bp ladders weas loaded as a standard reference markers. The gel was run at constant voltage of 90 V for about 2-3 hours, until the tracking dye migrated to the end of the gel. The banding pattern was analyzed using gel documentation system (Biorad Gel Doc XR^+ Imaging Systems) and saved the image for later use.

3.3 TO STUDY THE TRANSMISSION OF SESAME PHYLLODY CARRIED BY DIFFERENT METHODS

To study the transmission of sesame phyllody through different methods, 6 genotypes (E-8, Thilothama, CUMS-17, RJR-170, Nirmala and JCS-1020) selected for sap inoculation, two genotypes JCS-1020, variety

(CUMS-17) and periwinkle plants (alternate host) were used for transmission through grafting, dodder and leafhopper methods.

3.3.1 Mechanical Transmission

3.3.1.1Preparation of Inoculums

The infected leaves were grinded in 0.05 M potassium phosphate (KH_2Po_4) buffer (pH 7.0) containing 0.02 per cent 2-mercaptoethanol in a pre-chilled pestle and mortar. The resultant inoculum was filtered separately through double covered muslin cloth, before being used for mechanical inoculation.

3.3.1.2 Method of inoculation:

I. By abrasion method:

Healthy sesame plants of 20, 25, 30 and 35 days old were inoculated by conventional leaf abrasion using cotton swab described by Akhtar *et al*. (2009). Corborundum powder was used as an abrasive and it sprinkled on leaves before inoculation, plants were rinsed with water immediately after inoculation. Healthy seedlings of sesame without inoculation were kept as control.

II. By injection method:

Healthy sesame plants of 20, 25, 30 and 35 days old were inoculated by injecting the phytoplasma sap using fine needle syringe. The phytoplasma sap was injected into the stem near the third leaf of plant. Healthy seedlings of sesame injected with distilled water were kept as control.

All the inoculated plants were maintained in insect proof net house and were regularly observed for development of symptoms. Observations with respect to the incubation period, sequence of symptom development and level of PLO’s transmission were recorded.

3.3.2 Graft transmission

3.3.2.1 Donor plants

Infected plants of sesame maintained in field under natural conditions were used as donor or source plants for the study.

3.3.2.2 Method of grafting

Side grafting method was employed for graft transmission. The disease scions from sesame infected plants were cut on both sides to form into 'V' shaped structure. The scions were inserted into the slanting cut of same shape made on the healthy stock plants of sesame and periwinkle. The grafted portion was tied tightly with a parafilm. The inoculated plants were kept in insect proof net house for symptom development. Observations were recorded on number of plants showing phyllody symptoms and time taken for symptom development.

3.3.3 Dodder transmission:

The soaked dodder seeds were sown in earthen pots containing sesame plants and they were germinated one month after sowing and the growing ends of the dodder (*Cuscuta campestris*) were twined on young growing shoots of infected sesame and periwinkle plants in clock wise direction. The dodder so established was allowed to grow as bridge between infected sesame and periwinkle plants to test plants such as healthy sesame and periwinkle respectively for 30 days and later it was removed, then the test plants were kept in insect proof net house for development of symptoms. Observations were recorded on number of plants showing symptoms and time taken for symptom development.

3.3.4 Insect transmission

A wooden frame measuring 45x45x30 cm fixed with wire mesh was used for the studies. Healthy sesame, periwinkle and sunhemp plants were kept in the insect rearing cages to transmit the phytoplasma from infected sesame to healthy sesame, periwinkle and sunhemp plants.

An aspirator comprising a glass tube (10 cm length and 2 cm diameter) and a rubber tube of 15 cm length was used for the collection of nymphal leafhoppers. The nymphal leafhoppers were collected from sesame field by gently turning the leaves upwards and sucking with an aspirator during early morning and evening. The phyllody infected sesame was kept into the cage through narrow mouth and the nymphs were released into the cage and then tightly closed. About 20-25 nymphal leafhoppers were collected from infected field and released into cages in which infected plant previously inserted and allowed to feed for 15 days as acquisition feeding period. After the acquisition feeding period, the viruliferous leafhoppers were taken out and allowed to feed on healthy seedling.

About 10-15 viruliferous nympal leafhoppers collected from acquisition cages and released into inoculation cages in which healthy sesame seedlings were previously inserted and allowed to feed for 15 days as an inoculation feeding period.

The leafhoppers in the inoculated pots were killed using insecticide after required inoculation feeding period was over. Inoculated plants were continuously monitored for symptom expression. Data on percent disease incidence, time required for appearance of the symptoms were recorded.

3.4. SCREENING OF SESAME GENOTYPES AGAINST PHYLLODY UNDER FIELD CONDITIONS

Screening of sesame genotypes against phyllody caused by phytoplasma was carried out to identify the sources of resistance.

The field trial was laid out with 58 genotypes of sesame along with susceptible check *viz*., RJR- 170 and resistant check *viz*., GT- 10 against the phyllody in the farm of Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad during *kharif*, 2019-20. The experimental location was situated at an altitude of 536 m above MSL on 17°15N latitude and 78°18E longitude in the Southern Agro Ecological Zone of Telangana. The experiment was laid out in a randomized block design with two replications. The experimental

material consisted of 60 genotypes of sesame of diverse origin. Each genotype was sown in two rows of 3 m length, with a spacing of 45 cm between the rows and 10 cm between plants within the row. The crop was raised following all the recommended agronomic practices and kept free from insecticidal sprays.

Table 3.6. Genotypes used for the study

S.No	Genotype	S.No	Genotype	S.No	Genotype
1	S-2019-F6-1	21	SEL-S-2019-1013	41	IS-245
2	S-2019-F6-2	22	SEL-S-2019-1014	42	IC-500425
3	S-2019-F6-3	23	SEL-S-2019-1015	43	IC-500377
4	S-2019-F6-4	24	SEL-S-2019-1016	44	IC-500329
5	S-2019-F6-5	25	SEL-S-2019-1017	45	IC-500325
6	S-2019-F6-6	26	SEL-S-2019-1018	46	IC-500401
7	S-2019-F6-7	27	SEL-S-2019-1019	47	KIC-357
8	S-2019-F6-8	28	SEL-S-2019-1020	48	IC-500386
9	S-2019-F6-9	29	SEL-S-2019-1021	49	IC-500419
10	S-2019-F6-10	30	SEL-S-2019-3001	50	IC-500429
11	S-2019-F6-11	31	Swetha	51	IC-500431
12	GTG-30	32	SEL-S-2018-1001	52	IC-500437
13	Piayur	33	SEL-S-2018-1002	53	IC-205601
14	Chagatham Local	34	SEL-S-2018-1003	54	IC-205629
15	SEL-S-2019-1007	35	SEL-S-2018-1004	55	IC-500477
16	SEL-S-2019-1008	36	DSK-1-A	56	IC-511014
17	SEL-S-2019-1009	37	B-24	57	IC-205699
18	SEL-S-2019-1010	38	SI-250-A	58	IS-238
19	SEL-S-2019-1011	39	S-0448	59	GT-10
20	SEL-S-2019-1012	40	IS-24-A	60	RJR-170

During the period of study, incidence of leafhopper across different genotypes was recorded during 30 days after sowing. Five plants were selected randomly in each genotype to record the incidence of leafhopper at early stage of the crop growth. At early stage of the crop, number of leafhopper nymphs per each selected plant was recorded from three leaves from top, middle and bottom of the plant. Average of two replications was worked out and the mean leafhopper/3 leaves/plant was calculated.

Data was also be recorded on per cent disease incidence of sesame phyllody by counting total number of plants and number of infected plants with in 2 rows of 3 m length for both replications starting from appearance of disease upto end of the crop season.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100$$

Based on the incidence of leafhopper nymphs and per cent disease incidence of phyllody, 60 genotypes were grouped into different categories *viz.*, Highly resistant, Resistant, Moderately resistant, Tolerant, Moderately susceptible, Susceptible and Highly susceptible as per the scale given by Akhtar *et al.* (2013).

Table 3.7. Disease scale for sesame phyllody (Akhtar *et al*., 2013).

Rating	Per cent disease incidence	Disease Reaction
0	No symptoms on any plant	Highly resistant
1	0.1-10	Resistant
2	10.1-20	Moderately resistant
3	20.1-30	Tolerant
4	30.1-40	Moderately susceptible
5	40.1-50	Susceptible
6	More than 50%	Highly susceptible

The different kinds of symptoms of sesame phyllody were also observed during the field screening and the diseased samples were collected tested for phytoplasma by PCR protocols as mentioned in 3.2.1.

3.4.1 Statistical analysis of data:

The data on incidence of phyllody disease in different dates of sowing and vector population at an early stage were analyzed with the statistical package SPSS (SPSS, 2016).

Chapter – IV

Results & Discussion

Chapter IV

RESULTS AND DISCUSSION

The present investigation on “Studies on sesame phyllody incited by *Phytoplasma*” was conducted with an aim to molecular confirmation of phytoplasma from phyllody infected plants, the transmission studies of phyllody through different methods and screening of sesame genotypes against phyllody under field conditions. The results obtained and data analysed were presented and discussed detailed in this chapter.

4.1 MOLECULAR IDENTIFICATION OF PHYTOPLASMA FROM PHYLLODY INFECTED PLANTS WITH PCR AND NESTED PCR

4.1.1 Symptomotology

Different types of phyllody symptoms were observed on sesame plants under field conditions. The prominent disease symptoms were floral virescence (Plate 4.1a), phyllody, proliferation (Plate 4.1b), short internodes (Plate 4.1c), stem bending (Plate 4.1d) and witches broom (Plate 4.1h). Additionally, capsule cracking (Plate 4.1f), seeds germination in capsules (Plate 4.1g), formation of dark exudates on foliage and sometimes yellowing also accompanied with the disease. On occasion shoot apex fasciation was also observed (Plate 4.1e). The Phyllody infected sesame plants exhibited symptoms varied according to growth stage and time of infection. Infection at an early stage of growth resulted reduction in leaf size, cessation of internode elongation and stunting. Infections that occurred later in the season caused characteristic symptoms such as virescence, phyllody and witches broom. Symptoms and the disease incidence were increased significantly during flowering and extended rapidly to the older leaves.

The expression of phyllody disease is generally varied for different plant species and within the same species different genotypes express symptoms at different stages of crop growth. Plants infected by phytoplasma

exhibit a wide range of symptoms. Symptoms of diseased plants may differ with the phytoplasma, stage of the disease, host plant, age of the host plant at the time of infection and environmental conditions (Lee *et al*., 2000 and Seemuller *et al*., 2002).

Rao *et al.* (1991) noticed that sesame variety Madhavi exhibited the initial symptoms at 50 days after sowing and the symptoms attained maximum at 71 days after sowing. Vamshi *et al*. (2018) studied the symptomatology of phytoplasma associated with phyllody disease of sesame reported that out of the 20 genotypes that were assessed for per cent disease incidence, none of the genotypes expressed symptoms at 30 DAS, it was only after 45 DAS symptoms started expressing themselves. They also noticed that some of the varieties, the symptoms were not exhibited until 60 DAS and the authors suggested that though the disease is prevailing in tolerant varieties the symptoms were expressed at further stages of the crop growth while the load of phytoplasma is more within phloem vessels.

4.1.2 Isolation of Total DNA

The sesame plants showing typical phyllody symptoms such as phyllody, virescence, shoot proliferation, reduced leaf size, green modified flowers and deformed capsules without seeds were collected from research fields of IIOR, Rajendranagar during *kharif* 2019 and total DNA was isolated by CTAB method as described earlier.

4.1.3 Optimization of PCR Protocol for Detection of Sesame Phyllody *Phytoplasma*

Polymerase Chain Reaction (PCR) was employed to detect the presence of phytoplasma through amplification of sesame phyllody phytoplasma specific allele corresponding to 16S rDNA of its genome. The PCR protocol was slightly modified by altering the PCR conditions as the phytoplasma DNA was not amplified by following the standard PCR protocol suggested by Lee *et al.* (1994). No amplification of the phytoplasma specific PCR product was observed with the reported annealing temperature of 48 ^{o}C

Plate 4.1a. Floaral virescence

Plate 4.1b.Floral proliferation

Plate.4.1c. Short internodes with reduced leaves

Plate 4.1d. Stem bending

Plate 4.1e.Shoot apex fasciation

Plate 4.1f. Capsule cracking

Plate 4.1g. Vivipary inside the capsule

Plate 4.1h. Witches broom

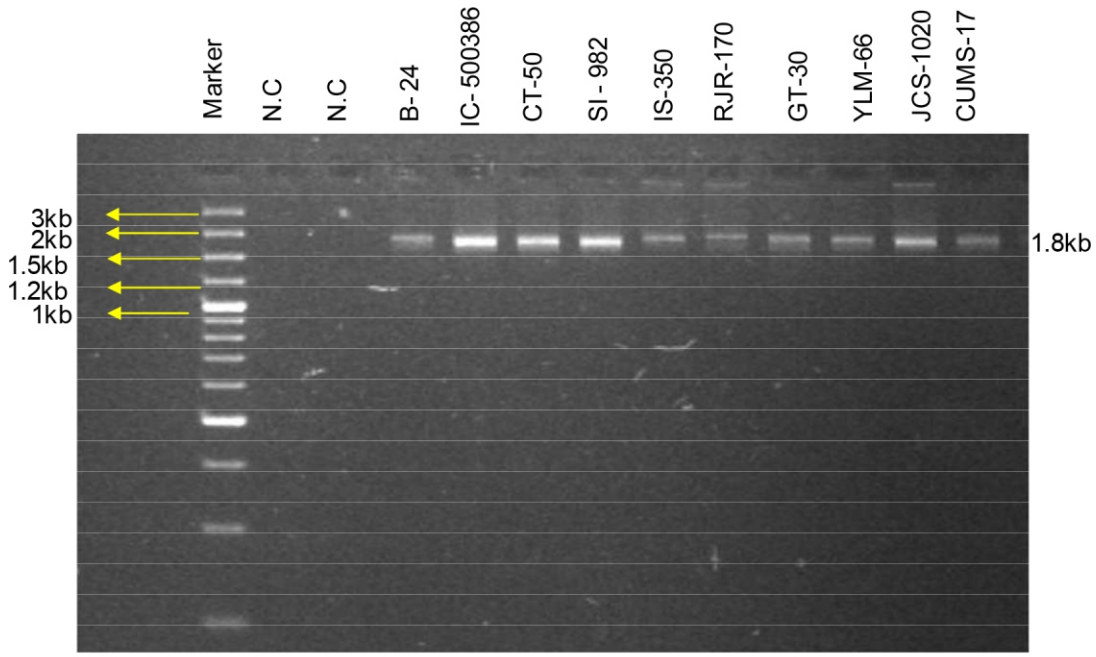

Plate 4.2. Amplification with P1/ P7 primer

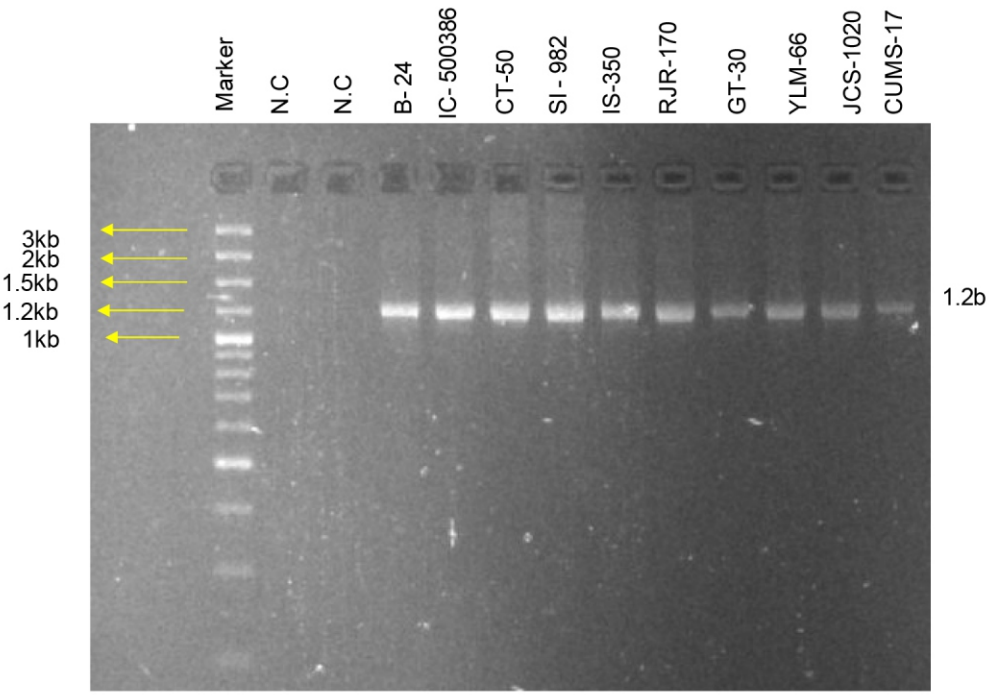

Plate 4.3. Amplification with R16F2n/R16R2

as suggested by the various workers. Annealing temperature of 52 ^{o}C for one minute was found suitable for amplifying sesame phyllody phytoplasmal specific allele of 1.8 kb by using phytoplasma specific universal primer pair P1/P7 designed for 16S rDNA.

The PCR amplicons of expected size of 1.8 kb fragment was obtained in all the 10 samples collected from phyllody symptomatic sesame plants by using the universal primer pair P1/P7 in direct PCR (Plate 4.2). No such product was obtained with DNA from healthy plant samples.

4.1.4 Nested PCR Analysis

The single step direct PCR reaction occasionally failed to amplify or resulted in weak amplification of the target phytoplasma DNA. However, nested PCR amplification consistently yielded phytoplasma specific PCR amplicons of 1.2 kb from all symptomatic sesame plants using phytoplasma specific universal primer pair R16F2n/ R16R2.

Nested PCR analysis of 1.8 kb from all the 10 symptomatic sesame plants were carried out by using phytoplasma specific universal primer pair R16F2n/ R16R2 (Plate 4.3) with the annealing temperature of 52 ^{o}C for one minute. The results clearly indicated that the sesame phyllody was caused by phytoplasma. The results of current study is in-line with the reports of Sertkaya *et al*. (2007), Akhtar *et al*. (2009), Ikten *et al*. (2014) and Singh *et al*. (2016) who used the same primer pair in their studies on sesame phyllody.

4.1.5 Sequence Analysis of 16s rDNA Amplicons

Amplicons of 1.2 kb that were obtained through nested PCR were sequenced in both the directions (5'-3' and 3'-5') and aligned with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo) from both ends and length of phytoplasma sequence determined with good quality as 986 bp. The sequence was compared with those of phytoplasma species accessible in the GenBank using BLAST similarity search tool. The BLAST analysis of 16S rDNA partial sequence of sesame phyllody isolates revealed 100 per cent sequence identity among themselves and shares 100 per cent identity with

strains of *Candidatus* Phytoplasma australasia (='*Candidatus* Phytoplasma aurantifolia') reference strain (NCBI accession No. KT265706) of 16Sr group II (Fig. 4.1). Therefore, the sesame phyllody phytoplasma at Hyderabad was identified as the strain related to '*Candidatus* Phytoplasma aurantifolia' of 16Sr II-D subgroup.

The above results are in-line with the Singh *et al*. (2016) wherein they collected the phyllody affected sesame plants from nine different states of India and observed that two 16Sr groups, namely 16Sr I and II were associated with sesame phyllody and noticed that 16Sr I-B and 16Sr II-D subgroups were prevalent in North India, whereas phytoplasma of only the 16Sr II group of different subgroups (16Sr II-A, II-C and II-D) were found in South India.

In India, only 16Sr I group was associated with sesame phyllody (Khan *et al*., 2007c and Manjunatha *et al*., 2012). But Nabi *et al*. (2015a) observed the association of two phytoplasma subgroups (16Sr I-B and II-C) with sesame phyllody and witches broom isolates from states of Uttar Pradesh, Bihar and Delhi and they confirmed that wide occurrence of 16Sr I-B subgroup phytoplasma infecting sesame crops in Uttar Pradesh, Bihar and Delhi. However, 16Sr II-C subgroup was reported only from Uttar Pradesh. Madhupriya *et al*. (2015) identified the 16Sr I-B, II-C and II-D subgroups from Delhi, Bihar, Uttar Pradesh, West Bengal, Madhya Pradesh, Chhattisgarh, Rajasthan, Gujarat, Maharashtra and Tamil Nadu, whereas, phytoplasma of only the 16Sr II group of different subgroups (16Sr II-A, II-C and II-D) were found in South India (Singh *et al*., 2018).

In India, 16Sr II group of phytoplasmas are most important group and has been associated with several phytoplasma diseases of plants (Rao *et al*., 2010). Phyllody phytoplasma isolates causing diseases on different field crops like sesame, chick pea, cowpea, mung bean, pigeon pea and vegetable crops like carrot, faba bean, field pea, alfa alfa, spinach, eggplant and tomato were reported by different researchers (Sertakaya *et al.*, 2007).

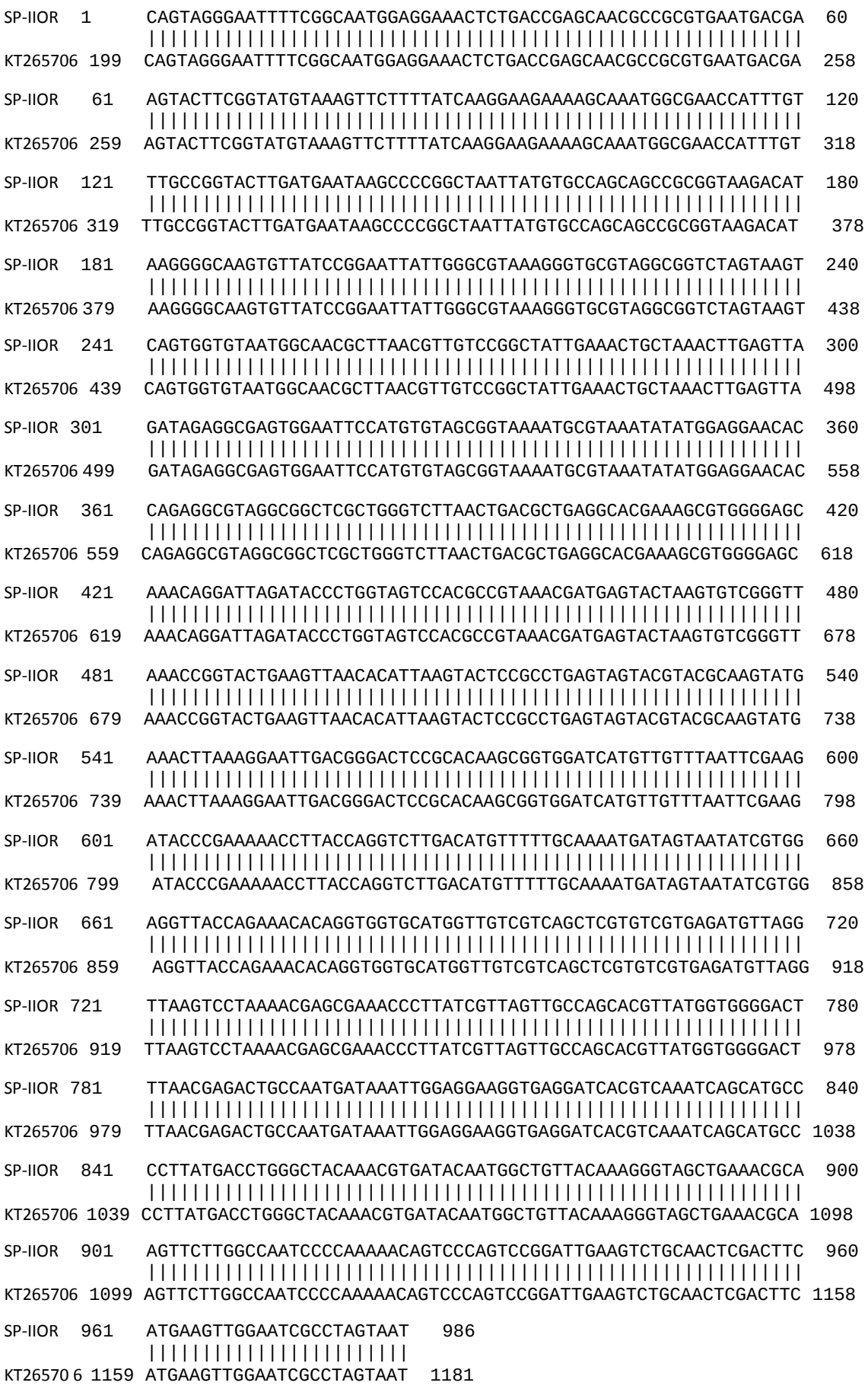

Fig.1. NCBI-BLAST alignment of 986bp obtained in the current study with KT265706, a phyllody phytoplamsa isolate' sequence (*Candidatus* Phytoplasma aurantifolia)

4.2 TO STUDY THE TRANSMISSION OF SESAME PHYLLODY CARRIED BY DIFFERENT METHODS

The transmission of sesame phyllody was conducted through different methods by selecting six genotypes (E-8, Thilothama, CUMS-17, RJR- 170, Nirmala and JCS-1020) for sap Transmission. Genotypes namely JCS-1020, CUMS-17 and periwinkle plants were used for transmission through grafting, cuscuta and leafhopper methods. Both percent of disease incidence and time taken for the appearance of symptoms were recorded.

4.2.1 Transmission of Sesame Phyllody through Sap Inoculation

In the present investigation, the sesame phyllody phytoplasma could not be transmitted by sap inoculation by both abrasion and injection methods (Plate 4.4a, b and 4.5a, b). The plants inoculated with sap extracted from sesame phyllody infected plants were remained free from infection. Out of the 12 plants of 3 replicates inoculated at 20, 25, 30, 35 days old with sap by abrasion and by injection method on each of 6 genotypes symptoms were not observed in inoculated plants by neither of these methods. The present findings were in conformity with the earlier reports (Akhtar *et al.,* 2009 and Pathak *et al.*, 2012).

Plate 4.4a. *Phytoplasma* sap inoculated to sesame by abrasion method

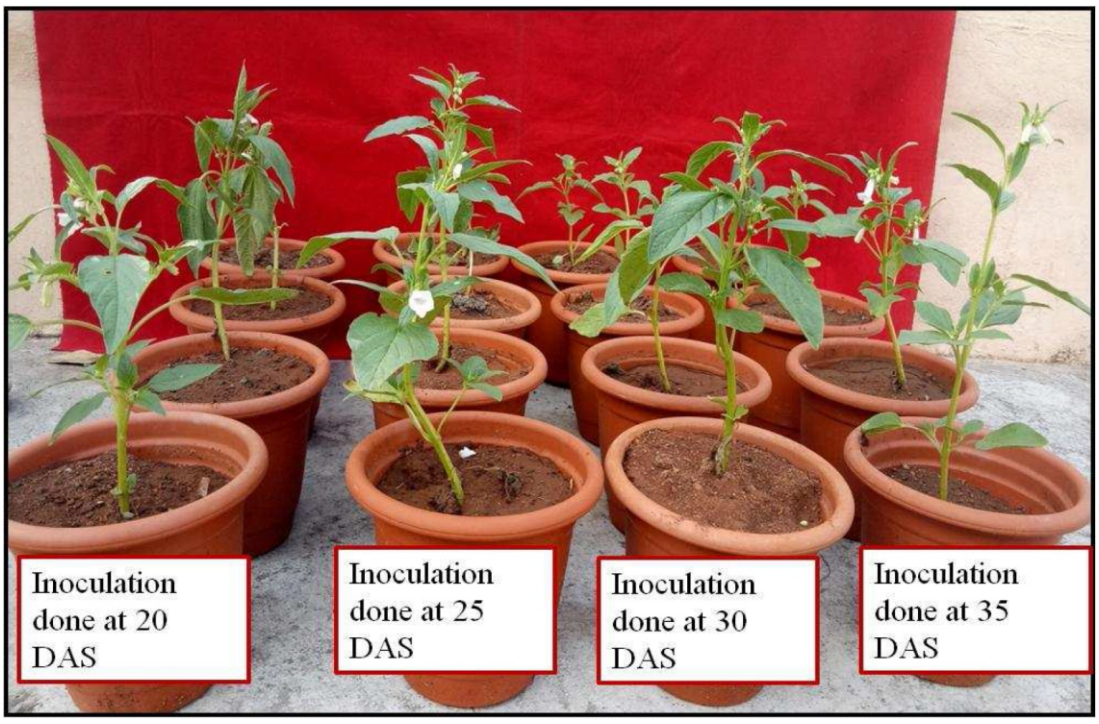

Plate 4.4b. No expression of symptoms on sap inoculated plants by abrasion method

4.2.2 Transmission of Sesame Phyllody through Grafting

Studies on transmission of sesame phytoplasma from infected sesame plant to healthy sesame and periwinkle plants by side grafting were carried out as explained in 3.3.2 and the results were presented in Table 4.1.

Sesame phyllody was successfully transmitted from infected sesame plants to healthy test plants through grafting (Plate 4.6a, b and c). In sesame, CUMS-17 and JCS -1020 genotypes were selected. Out of the 20 graft inoculated plants of each genotype, 18 plants of CUMS-17 and 16 plants of JCS-1020 showed characteristic symptoms of phyllody such as yellowing of leaves and stunted growth at 25-28 and at 32-36 Days after inoculations respectively. While no symptoms were developed in the control plants. These infected plants showed positive and control plants showed negative reaction to sesame phyllody in both direct and nested PCR. The results clearly indicated, by graft inoculation 90 and 80 per cent of disease transmission was observed in CUMS-17 and JCS -1020 respectively.

In periwinkle, out of the 10 graft inoculated plants, all the inoculated plants were showed characteristic symptoms of phyllody such as small leaves and yellowing of leaves (Plate 4.7a and b) at 45-50 DAI and the infected plants were showed positive reaction to the sesame phyllody in both direct and nested PCR (Plate 4.14 and 4.15). The results clearly indicated that the 100 per cent of disease was transmitted into the graft inoculated periwinkle plants (Table 4.1). Similar observations were obtained by Salehi *et al.* (2009) and Pathak *et al.* (2012).

The Phytoplasmas move within the plants through phloem from source to sink and they efficiently passed through sieve tube elements in phloem (Christensen *et al.,* 2004). Due to this nature the phyllody phytoplasma consistently transmitted through the process of grafting.

Akhtar *et al*. (2009) reported transmission of sesame phytoplasma from diseased to healthy sesame by grafting and they observed symptoms within

25-35 DAI. Ravindar (2017) reported 85 per cent of phyllody disease transmitted into the graft inoculated plants and Ikten *et al*. (2014) observed characteristic symptoms of phyllody, floral virescence, yellowing of leaves and stunted growth when transmitted through graft inoculation.

While Salehi *et al*. (2009) reported the successfully transmission of safflower phyllody phytoplasma through grafting to healthy safflower and observed characteristic symptoms such as very small leaves, phyllody, floral virescence, yellowing of leaves and stunted growth within 45 to 70 DAI.

Table 4.1. Transmission of sesame phyllody disease by graft transmission method

Tested plant	Total no.of plants		Symptoms observed	Days to express the symptoms	% of infection
	Inocualted	Infected			
Sesame I.CUMS-17	20	18	Yellowing of leaves and stunted growth	25- 28	90
II.JCS-1020	20	16	Yellowing of leaves and stunted growth	32-36	80
Periwinkle	10	10	Small leaves and yellowing of leaves	45-50	100

Plate 4.5a. ***Phytoplasma*** **sap inoculated to sesame by injection method**

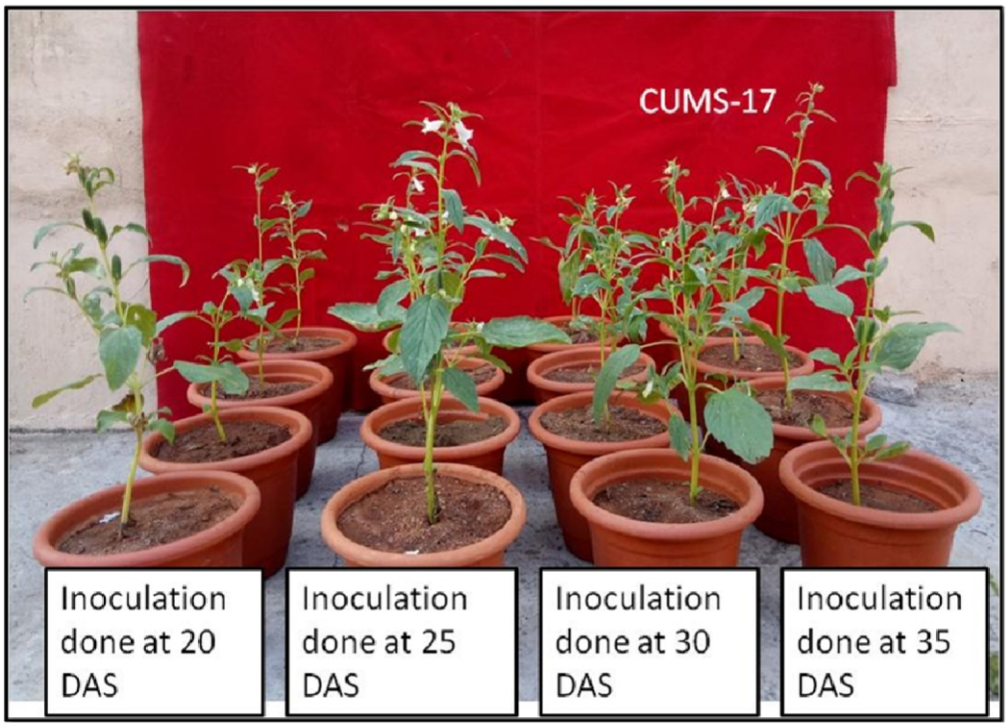

Plate 4.5b. No expression of symptoms on sap inoculated plants by injection method

Plate 4.6a. Side grafting of infected sesame scion on healthy sesame stock

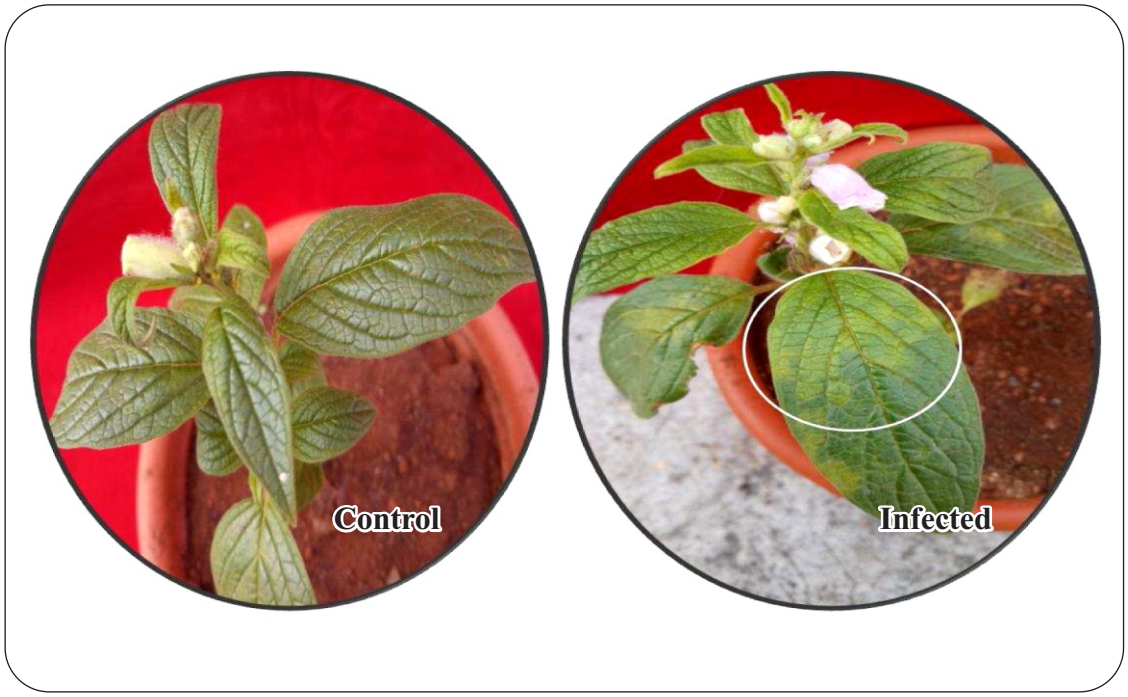

Plate 4.6b. Yellowing symptom on graft inoculated sesame plant

Plate 4.6c. Phyllody Symptoms on graft inoculated sesame plants

Plate 4.7a. Side grafting of infected sesame scion on healthy periwinkle stock

Plate 4.7b. Yellowing symptom on graft inoculated periwinkle plant

4.2.3 Transmission of Sesame Phyllody through Dodder

Studies on dodder transmission of the phytoplasma were carried out as described in 3.3.3 and the data presented in Table 4.2.

Dodder (*Cuscuta campestris*) successfully transmitted the phyllody phytoplasma from infected sesame plants to healthy sesame and from infected periwinkle to healthy periwinkle plants under green house conditions (Plate 4.8a).

The water imbibed dodder seeds were sown in 40 pots of two varieties of sesame plants. Out of 40 dodder were germinated only in 5 pots of each variety and these plants were used for transmission of phyllody phytoplasma through dodder.

In sesame, CUMS-17 and JCS-1020 genotypes out of 5 plants used for dodder transmission on each genotype, 3 and 2 plants exhibited typical phyllody symptom such as floral virescence, yellowing of leaves and floral proliferation (Plate 4.8b) within 40 to 45 and 45 to 50 DAI respectively. No symptoms were observed in the control plants. In the PCR assay infected plants showed positive and control plants showed negative reaction to the sesame phyllody in both direct and nested PCR (Plate 4.13 and 4.14). The results indicated 60 and 40 per cent of disease transmitted through dodder in CUMS-17 and JCS-1020 respectively.

In periwinkle out of the 5 inoculated plants, 4 inoculated plants exhibited the characteristic symptoms of phyllody such as small leaves, yellowing of leaves and stunted growth (Plate 4.9a and b) at 60-65 DAI. While no symptoms were developed in the control plants. In PCR assay the infected plants showed positive reaction to the sesame phyllody in both direct and nested PCR. The results clearly indicated that the dodder (*Cuscuta campestris*) successfully transmitted the phytoplasma from infected periwinkle plants to 80 per cent of healthy periwinkle plants.

These results clearly indicated that dodder acts as an efficient means in natural transmission of the disease in the field. Earlier, Abraham *et al.* (1977a) reported that dodder (*Cuscuta campestris*) played role for transmission, donor as well as reservoir of sesame phyllody phytoplasma.

Similarly many other workers also could successfully transmitted the disease from infected sesame to healthy sesame with the help of dodder (Salehi and Izadpanah, 1992; Akhtar *et al.*, 2009 and Pathak *et al.*, 2012). While Marcone *et al.* (1997) noticed successful dodder transmission of alder yellows phytoplasma to the experimental host *Vinca rosea* (periwinkle). Similarly, Salehi *et al.* (2009) could successfully transmitted Safflower phyllody phytoplasma by dodder.

Table 4.2. Transmission of sesame phyllody disease by dodder transmission method

Tested plant	Total no.of plants		Symptoms observed	Days to express the symptoms	% of infection
	Inoculated	Infected			
Sesame . CUMS-17	5	3	Virescence, yellowing of leaves and floral proliferation	40-45	60
. JCS-1020	5	2	Virescence, phyllody and floral proliferation	45-50	40
Periwinkle	5	4	Small leaves, yellowing of leaves and stunted growth	60-65	80

Plate 4.8a. Establishment of dodder on infected sesame and periwinkle plants

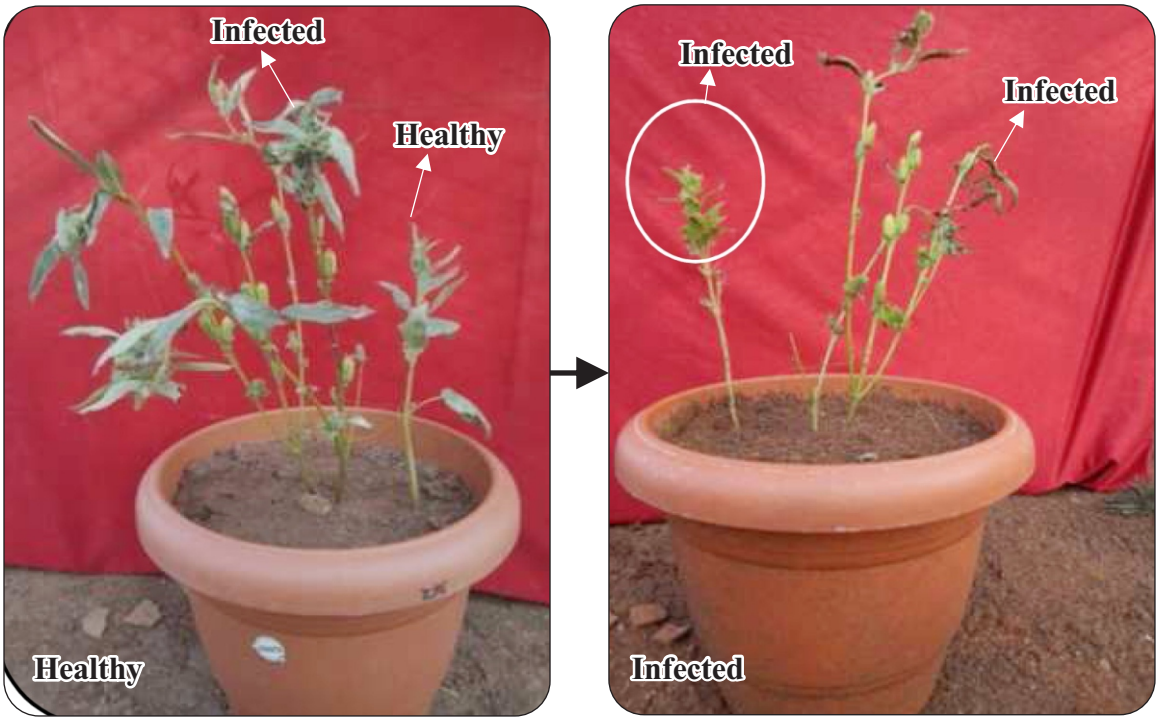

Plate 4.8b. Floral virescence symptom on dodder infected sesame plant

Plate 4.9a. Dodder transmission of phyllody to periwinkle

Plate 4.9b. Yellowing symptom on dodder inoculated periwinkle plant

4.2.4 Transmission of Sesame Phyllody through Leafhopper

Attempts were made to transmit the phytoplasma causing sesame phyllody disease through leafhoppers were detailed in 3.3.4 and the results were presented in Table 4.3. The phytoplasma that causes phyllody disease was successfully transmitted from infected sesame to healthy sesame (Plate 4.10a, b and c), sunhemp (Plate 4.11) and periwinkle plants (Plate 4.12) via the leafhopper, *Orosius albicinctus*.

In sesame, CUMS-17 and JCS-1020 genotypes were taken for study. Out of the 20 inoculated plants of each genotype, 15 of CUMS-17 and 7 of JCS-1020 produced disease symptoms such as transformation of floral parts into green leaf like structures, virescence, phyllody and floral proliferation within 35-40 and 45-50 DAI respectively by leafhopper transmission. These infected plants were showed positive reaction to the sesame phyllody in both PCRs. While plants in the control cage with no insect infestation did not exhibit any disease symptoms and were negative for the phytoplasma in PCR assays. The results clearly indicated that the leafhopper *O. albicinctus* successfully transmitted the phytoplasma to 75 and 35 per cent of healthy test sesame plants.

In periwinkle, out of the 10 leafhopper inoculated plants, all the inoculated plants showed phyllody symptoms at 50-60 DAI. The infected plants showed characteristic symptoms such as small leaves, yellowing of leaves and stunted growth (Plate 4.12) and these plants shown positive reaction to the sesame phyllody in both direct and nested PCR (Plate 4.13 and 4.14). No symptom was appeared in plants kept in control cage. These results clearly indicated that the leafhopper *O. albicinctus* successfully transmitted the phytoplasma from infected sesame plants to 100 per cent of healthy periwinkle plants.

In sunhemp, out of the 10 leafhopper inoculated plants, 8 inoculated plants were showed phyllody symptoms at 45-50 DAI. The infected plants

showed characteristic symptoms such as small leaves, yellowing of leaves and stunted growth (Plate 4.11). While no symptoms were developed in the control plants. The infected plants were showed positive reaction to the sesame phyllody in both direct and nested PCR. The results clearly indicated that the leafhopper *O. albicinctus* successfully transmitted the phytoplasma from infected sesame plants to 80 per cent of healthy plants. Similarly, sesame phyllody disease could be transmitted to sunhemp, chickpea and berseem through *Orosius* spp. under natural conditions (Vasudeva and Sahambi, 1955; 1959).

The results of transmission assay confirmed that the leafhopper *O. albicinctus* played a major role as a natural vector of phytoplasma associated with sesame phyllody disease. This indicated that leafhopper played major role in natural transmission of the phyllody disease in field conditions.

Table 4.3. Transmission of sesame phyllody disease by insect transmission method

Tested plant	No. of plants		Symptoms observed	Days to express the symptoms	% of infection
	Inoculated	Infected			
Sesame □.CUMS-17	20	15	Virescence, phyllody and floral proliferation	35-45	75
II. JCS-1020	20	7	Virescence, phyllody and floral proliferation	45-50	35
Periwinkle	10	10	Small leaves, yellowing of leaves and stunted growth	50-60	100
Sunhemp	10	8	Little leaves, yellowing of leaves and stunted growth	45-50	80

Nymphs collected through aspirator

Nymphs reared on infected plant (Acquisition feeding period)

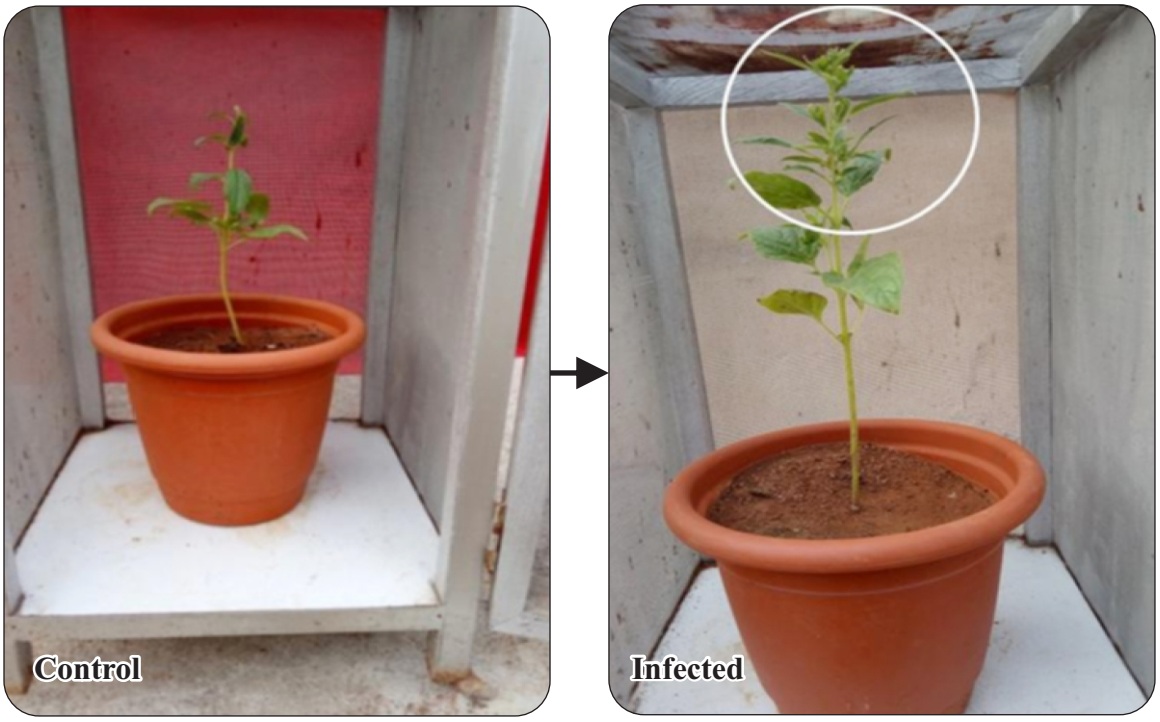

Plate 4.10a. Leafhopper transmission of sesame phyllody

Plate 4.10b. Floral virescene symptom on leafhopper inoculated sesame plant

Plate 4.10c. Phyllody symptom on insect transmitted sesame plants

Plate 4.11. Yellowing symptom on leafhopper inoculated sunhemp plant

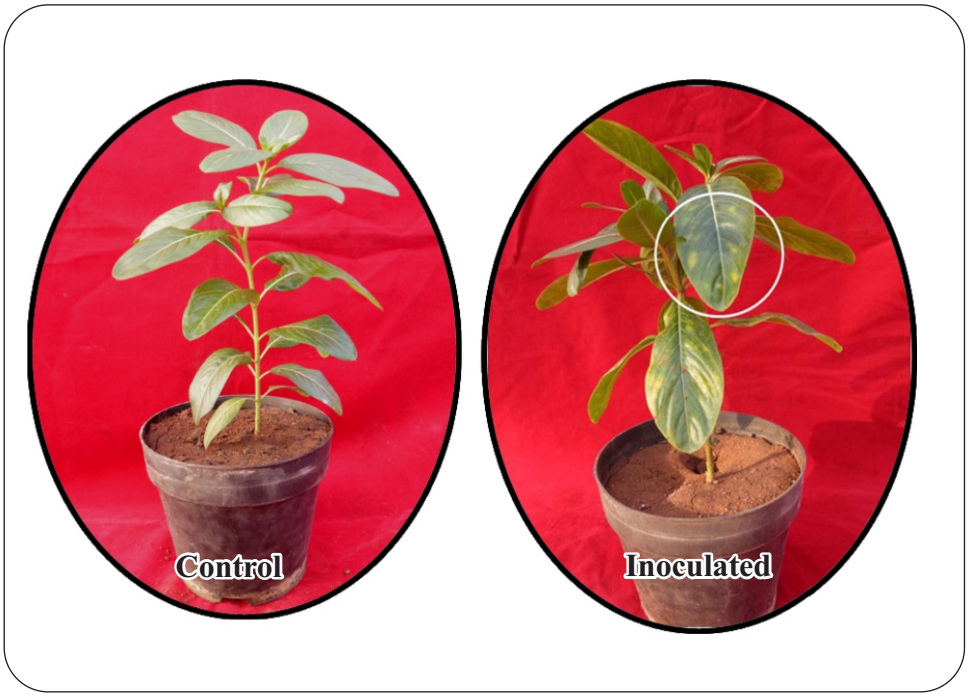

Plate 4.12. Yellowing symptom on leafhopper inoculated periwinkle plant

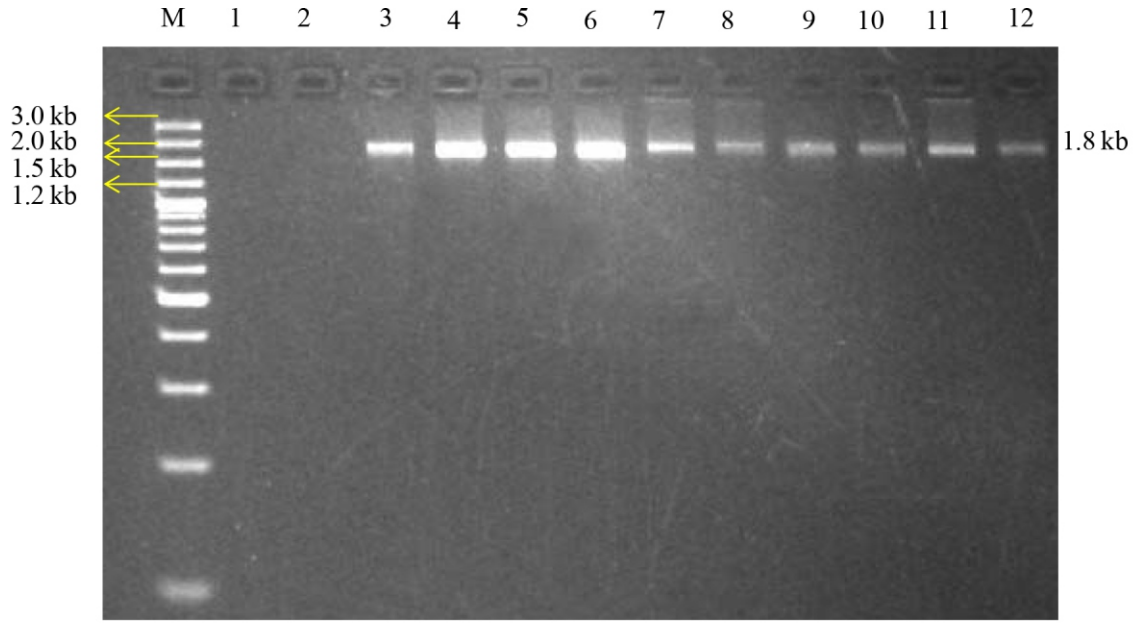

Plate 4.13. **Amplification with P1/ P7 primer**

M- Marker

1. Negative control (healthy leaf sample)

2. Negative control (distilled water)

3. CUMS-17 graft inoculated

4. JCS-1020- graft inoculated

5. Periwinkle-graft inoculated

6. CUMS-17 inoculated through dodder

7. JCS-1020 inoculated through dodder

8. Periwinkle inoculated through dodder

9. CUMS-17 inoculated through leaf hopper

10. JCS-1020 inoculated through leaf hopper

11. Sunhemp inoculated through leaf hopper

12 Periwinkle inoculated through leaf hopper

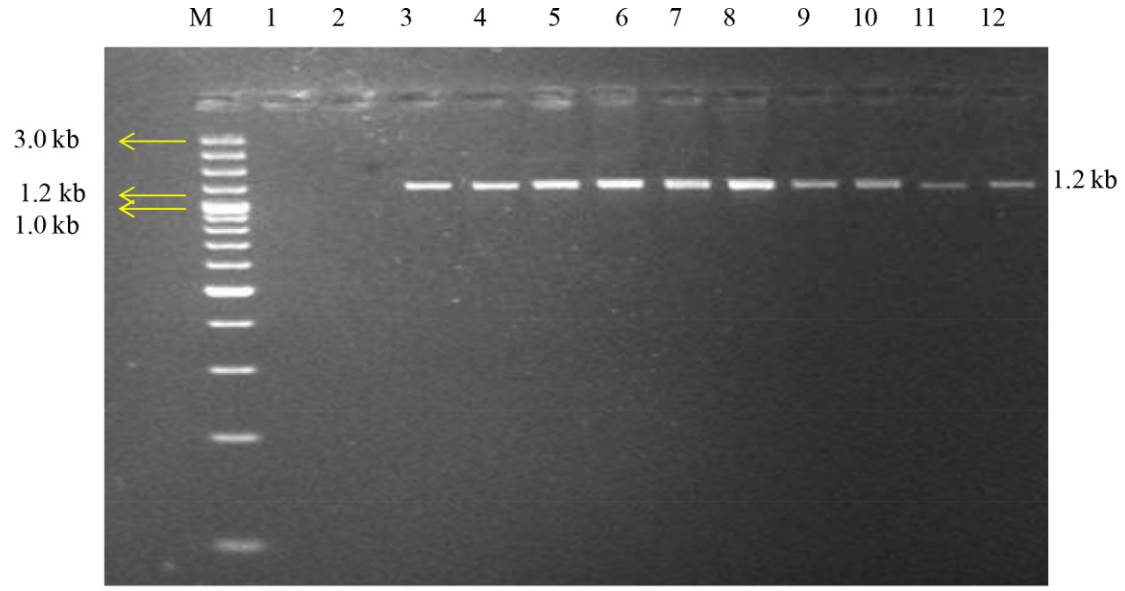

Plate 4.14. Amplification with R16F2n/R16R2

M- Marker

1. Negative control (healthy leaf sample)

2. Negative control (distilled water)

3. CUMS-17 graft inoculated

4. JCS-1020- graft inoculated

5. Periwinkle-graft inoculated

6. CUMS-17 inoculated through dodder

7. JCS-1020 inoculated through dodder

8. Periwinkle inoculated through dodder

9. CUMS-17 inoculated through leaf hopper

10. JCS-1020 inoculated through leaf hopper

11. Sunhemp inoculated through leaf hopper

12 Periwinkle inoculated through leaf hopper

In above all transmission methods, in sesame genotypes CUMS-17 expressed symptoms earlier compared to JCS -1020, whereas in JCS-1020 the expression of symptoms is lately might be due to its moderately resistant nature.

4.4 SCREENING OF SESAME GENOTYPES AGAINST PHYLLODY UNDER FIELD CONDITIONS

The experiment was conducted at ICAR-Indian Institute of Oilseeds Research experimental fields by raising 60 genotypes in Randomized block design with two replications by adopting a spacing of 45X10 cm (Plate 4.15). The data related to different kinds of symptoms and categorization of different genotypes based on percent disease incidence of phyllody were recorded.

4.4.1 Screening of Sesame Genotypes

60 genotypes of sesame, comprising of released varieties, promising advanced lines and germplasm accessions were screened against phyllody disease under field conditions at Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad. GT-10 was used as a resistant check and RJR-170 was used as susceptible check (Plate 4.16a).

Phyllody disease started appearing from 45 days after sowing and recorded disease incidence from 50 DAS. From 50 DAS till 80 DAS, all the genotypes expressed different reactions for phyllody disease incidence and they were grouped as Resistant, Moderately resistant, Tolerant, Moderately susceptible, Susceptible and Highly susceptible genotypes based on PDI values.

The per cent disease incidence of phyllody at 50 DAS ranged from 1.25 to 26.52 per cent, at 60 DAS it was 2.27 to 40.77 per cent, at 70 DAS it was 4.55 to 68.01 per cent and at 80 DAS it was 4.55 to 69.58 per cent.

At 50 DAS, among sesame genotypes were screened against phyllody disease under field conditions, 34, 21 and 5 genotypes showed resistant, moderately resistant and tolerant reactions. Symptoms appeared during 50 DAS were virescence, shortening of internodes and stunting of plants.

Symptoms appeared during 60 DAS were virescence, floral proliferation, shortening of internodes and stunting of plants. At 60 DAS, 12, 16, 20, 10 and one genotypes showed resistant, moderately resistant, tolerant, moderately susceptible and susceptible reactions to phyllody.

Among 60 sesame genotypes screened against phyllody disease under field conditions, at 70 DAS, 4, 8, 9, 21, 13 and 5 genotypes showed resistant, moderately resistant, tolerant, moderately susceptible, susceptible and highly susceptible reactions to phyllody. Symptoms appeared during 70 DAS were conversion of floral parts into leaf like structure, floral proliferation and shortening of internodes

At 80 DAS, on the basis of per cent disease incidence of phyllody disease a total of 60 genotypes were grouped into different categories. Among them 1 genotype showed resistant, 3 genotypes showed moderately resistant, 5 genotypes showed tolerant, 15 genotypes showed moderately susceptible, 23 genotypes showed susceptible and 11 genotypes showed highly susceptible reactions in final observation (Plate 4.16b and c).

Several workers had formerly reported about the resistance sources against phyllody disease of sesame. Palanna *et al*. (2015) reported GT-1 and DS-9 as resistant to phyllody and Manjunatha (2010) reported that IVT-09-1, IVT-09-2, IVT-09-14, IVT-09-19 and Kanakapura-1 (local variety) showed resistant reaction. Similarly, 42 sesame genotypes were categorised for their reaction to phyllody by Gopal *et al.* (1998) and observed that six genotypes showed resistant reaction *viz*., AT66, AT69, BT892, TC359, RT108, TC359 and TNAU12.

While most of the cultivated germplasm are susceptible, exploitation of wild relatives as source of resistance genes could be a further feasible option. Tandon and Banerjee (1968) reported moderate resistance to phyllody in several Indian sesame cultivars. Varietal resistance both to the disease and the vector was the finest way for managing this disease (Beech, 1981). The resistance to phyllody disease was noticed in the wild species *Sesamum alatum* (Srinivasulu and Narayanaswamy, 1995 and Singh *et al*., 2007).

Plate 4.15. Field view of experimental plot

GT-10

RJR-170

Plate 4.16a. Resistant check and susceptible check

GTG - 30

SEL-S-2019-1019

Plate 4.16b Varieties shown resistance to phyllody

S-2019-F6-2

S-2019-F6-5

S-2019-F6-9

IC-500437

Plate 4.16c. Varieties shown susceptible to phyllody

Table 4.4. Reaction of sesame genotypes to phyllody disease under field conditions during *kharif* 2019.

S. No	Varieties	Total plant stand	PDI of phyllody (Mean) at 50 DAS	Response	PDI of phyllody (Mean) at 60 DAS	Response
Advanced breeding lines						
1	S-2019-F6-1	52	13.88^{abcde}	MR	26.10^{abcdefghijk}	T
2	S-2019-F6-2	45	7.08^{bcde}	R	25.40^{abcdefghijk}	T
3	S-2019-F6-3	47	8.23^{bcde}	R	17.53^{abcdefghijk}	MR
4	S-2019-F6-4	32	5.00^{bcde}	R	38.33 ^{abc}	MS
5	S-2019-F6-5	45	20.39^{abc}	T	29.94^{abcdefg}	T
6	S-2019-F6-6	46	14.83^{abcde}	MR	33.96^{abcde}	MS
7	S-2019-F6-7	42	10.80^{abcde}	MR	30.22^{abcdefg}	MS
8	S-2019-F6-8	45	4.17^{cde}	R	20.42^{abcdefghijk}	T
9	S-2019-F6-9	50	15.33^{abcde}	MR	36.04^{abcd}	MS
10	S-2019-F6-10	39	4.55^{bcde}	R	15.72^{abcdefghijk}	MR
11	S-2019-F6-11	62	1.25^{e}	R	17.11^{abcdefghijk}	MR
12	SEL-S-2019-1007	66	13.90^{abcde}	MR	12.43^{defghijk}	MR
13	SEL-S-2019-1008	35	17.97^{abcde}	MR	24.05^{abcdefghijk}	T
14	SEL-S-2019-1009	52	5.33^{bcde}	R	9.90^{efghijk}	R
15	SEL-S-2019-1010	50	1.68^{e}	R	11.45^{efghijk}	MR
16	SEL-S-2019-1011	44	3.13^{de}	R	31.50^{abcdefg}	MS
17	SEL-S-2019-1012	58	26.52^{a}	T	29.78^{abcdefgh}	T
18	SEL-S-2019-1013	60	9.03^{bcde}	R	26.26^{abcdefghijk}	T
19	SEL-S-2019-1014	61	7.13^{bcde}	R	20.85^{abcdefghijk}	T
20	SEL-S-2019-1015	52	11.07^{abcde}	MR	18.91^{abcdefghijk}	MR
21	SEL-S-2019-1016	54	3.74^{cde}	R	3.74 ^{jk}	R
22	SEL-S-2019-1017	47	11.25^{abcde}	MR	16.65^{bcdefghijk}	MR
23	SEL-S-2019-1018	60	1.25^{e}	R	9.16^{fghijk}	R
24	SEL-S-2019-1019	35	2.78^{de}	R	5.05^{ijk}	R
25	SEL-S-2019-1020	53	10.53^{abcde}	MR	12.00^{defghijk}	MR
26	SEL-S-2019-1021	65	6.07^{bcde}	R	23.57^{abcdefghijk}	T
27	SEL-S-2019-3001	70	7.08 ^{bcde}	R	20.56^{abcdefghijk}	T
28	Swetha	56	3.03^{de}	R	7.20^{ghijk}	R
29	SEL-S-2018-1001	62	4.80^{dcde}	R	18.51^{abcdefghijk}	MR
30	SEL-S-2018-1002	68	18.13^{abcde}	MR	40.77 ^{a}	S
31	SEL-S-2018-1003	71	20.46^{abc}	T	37.96^{abc}	MS
32	SEL-S-2018-1004	59	12.81^{abcde}	MR	27.60^{abcdeghij}	T
33	IC-500425	51	14.02^{abcde}	MR	23.84^{abcdefghijk}	T
34	IC-500377	54	12.07^{abcde}	MR	20.54^{abcdefghijk}	T
35	IC-500329	38	15.66^{abcde}	MR	22.10^{abcdefghijk}	T
36	IC-500325	54	11.11^{abcde}	MR	11.11^{efghijk}	MR
37	IC-500401	50	6.44^{bcde}	R	16.83^{abcdefghijk}	MR
38	KIC-357	23	18.5^{cde}	MR	23.5^{abcdefghijk}	MS
39	IC-500386	43	6.98^{bcde}	R	17.38^{abcdefghijk}	MR
40	IC-500419	53	15.91^{abcde}	MR	41.64^{abcdef}	MS
41	IC-500429	63	5.88^{bcde}	R	29.06^{abcdefghi}	T
42	IC-500431	58	9.79^{abcde}	R	20.63^{abcdefghijk}	T
43	IC-500437	45	12.13^{abcde}	MR	39.38^{ab}	MS
44	IC-205601	35	6.07 ^{bcde}	R	16.45^{abcde}	MR
45	IC-205629	52	19.00^{abcd}	MR	29.73^{abcdefgh}	T

Contd…

Table 4.4 (Contd..)

46	IC-500477	60	12.37 ^{abcde}	MR	21.09^{abcdefghijk}	T
47	IC-511014	59	3.35 ^{abcde}	R	3.35^{abcdefghi}	R
48	IC-205699	51	2.27 ^{dcde}	R	2.27 ^{efghijk}	R
Varieties						
49	IS-238	43	3.57 ^{cde}	R	5.65^{k}	R
50	GTG-30	61	2.86^{de}	R	5.80^{hijk}	R
51	Piayur	55	3.56^{cde}	R	14.82^{cdefghijk}	MR
52	Chagatham Local	55	4.00 ^{bcde}	R	8.29^{ghijk}	R
53	DSK-1-A	47	8.96^{bcde}	R	14.79^{cdefghijk}	MR
54	B-24	53	11.79^{abcde}	MR	23.69^{abcdefghijk}	T
55	SI-250-A	64	6.53^{bcde}	R	28.37^{abcdefghi}	T
56	S-0448	63	5.00^{bcde}	R	17.18^{abcdefghijk}	MR
57	IS-24-A	31	11.79 ^{bcde}	MR	20.13^{abcdefghijk}	T
58	IS-245	54	21.16^{ab}	T	38.99^{ab}	MS
Checks						
59	GT-10	72	4.16^{e}	R	5.55^{k}	R
60	RJR-170	64	23.47 ^{abcde}	T	41.37^{bcdefghijk}	S
		P value	0.001*	Sig	0.001*	Sig

S. No	varieties	Total plant stand	PDI of phyllody (Mean) at at 70 DAS	Response	PDI of phyllody (Mean) at 80 DAS	Response
Advanced breeding lines						
1	S-2019-F6-1	52	34.44^{abcdefghijklm}	MS	42.50^{abcdefghijkl}	S
2	S-2019-F6-2	45	34.13^{abcdefghijklm}	MS	51.55^{abcdefgh}	HS
3	S-2019-F6-3	47	32.47^{abcdefghijklmn}	MS	48.13^{abcdefghi}	S
4	S-2019-F6-4	32	58.86 ^{a}	HS	68.03^{a}	HS
5	S-2019-F6-5	45	51.98^{abc}	HS	64.48^{ab}	HS
6	S-2019-F6-6	46	47.51^{abcdef}	S	59.37^{abcde}	HS
7	S-2019-F6-7	42	54.19^{ab}	HS	45.86^{abcdefghij}	S
8	S-2019-F6-8	45	38.75^{abcdefghij}	MS	38.75^{abcdefghijklm}	MS
9	S-2019-F6-9	50	59.53 ^{a}	HS	62.86^{abc}	HS
10	S-2019-F6-10	39	28.94^{bcdefghijklmn}	T	37.06^{bcdefghijklm}	MS
11	S-2019-F6-11	62	32.68^{abcdefghijklmn}	MS	35.71^{bcdefghijklm}	MS
12	SEL-S-2019-1007	66	21.77^{defghijklmn}	MR	31.91^{defghijklmn}	MS
13	SEL-S-2019-1008	35	35.67^{abcdefghijkl}	MS	44.01^{abcdefghijkl}	S
14	SEL-S-2019-1009	52	18.04^{ghijklmn}	MR	25.12^{hijklmn}	T
15	SEL-S-2019-1010	50	26.67^{bcdefghijklmn}	T	33.96^{cdefghijklmn}	MS
16	SEL-S-2019-1011	44	42.76^{abcdefgh}	S	55.45^{abcdefg}	HS
17	SEL-S-2019-1012	58	43.66^{abcdefg}	S	49.70^{abcdefghi}	S
18	SEL-S-2019-1013	60	36.67^{abcdefghijkl}	MS	36.67^{bcdefghijklm}	MS
19	SEL-S-2019-1014	61	36.73^{abcdefghijkl}	MS	40.76^{abcdefghijkl}	S
20	SEL-S-2019-1015	52	33.54^{abcdefghijklm}	MS	46.75^{abcdefghij}	S
21	SEL-S-2019-1016	54	9.27^{klmn}	R	14.12^{lmn}	MR
22	SEL-S-2019-1017	47	22.27^{defghijklmn}	T	29.55^{efghijklmn}	T
23	SEL-S-2019-1018	60	16.43^{ghijklmn}	MR	33.56^{cdefghijklmn}	MS
24	SEL-S-2019-1019	35	8.62^{lmn}	R	20.37^{ijklmn}	T
25	SEL-S-2019-1020	53	21.60^{defghijklmn}	T	27.22^{fghijklmn}	T
26	SEL-S-2019-1021	65	31.37^{abcdefghijklmn}	MS	34.35^{bcdefghijklmn}	MS
27	SEL-S-2019-3001	70	30.82^{abcdefghijklmn}	MS	34.92^{bcdefghijklm}	MS

Contd…

Table 4.4 (Contd..)

28	Swetha	56	14.27^{hijklmn}	MR	15.78^{klmn}	MR
29	SEL-S-2018-1001	62	24.53^{cdefghijklmn}	T	26.35^{fghijklmn}	T
30	SEL-S-2018-1002	68	48.27^{abcde}	S	48.27^{abcdefghi}	S
31	SEL-S-2018-1003	71	45.50^{abcdefg}	S	45.50^{abcdefghij}	S
32	SEL-S-2018-1004	59	37.80^{abcdefghijkl}	MS	40.58^{abcdefghijkl}	S
33	IC-500425	51	38.04^{abcdefghijk}	MS	45.67^{abcdefghij}	S
34	IC-500377	54	37.27^{abcdefghijkl}	MS	37.27^{bcdefghijklm}	MS
35	IC-500329	38	32.20^{abcdefghijklmn}	MS	44.89^{abcdefghijk}	S
36	IC-500325	54	35.84 ^{abcdefghijkl}	MS	39.59^{abcdefghijkl}	MS
37	IC-500401	50	27.07^{bcdefghijklmn}	MS	31.35^{defghijklmn}	MS
38	KIC-357	23	47.5^{cdefghijklmn}	S	47.5^{ijklmn}	S
39	IC-500386	43	48.54^{abcde}	S	60.36^{abcd}	HS
40	IC-500419	53	43.91^{abcdefg}	S	43.91^{abcdefghijkl}	S
41	IC-500429	63	38.60^{abcdefghij}	MS	38.60^{abcdefghijklm}	MS
42	IC-500431	58	27.99^{bcdefghijklmn}	T	35.14^{bcdefghijklm}	MS
43	IC-500437	45	49.17^{abcd}	S	56.46^{abcdef}	HS
44	IC-205601	35	68.01^{abcdefghij}	HS	69.58^{abcdefghij}	HS
45	IC-205629	52	38.14^{abcdefghijk}	MS	47.23^{abcdefghi}	S
46	IC-500477	60	45.08^{abcdefgh}	S	41.04^{abcdefghijkl}	S
47	IC-511014	59	21.0 ^{defghijklmn}	T	41.61^{abcdefghijkl}	S
48	IC-205699	51	21.3^{Jklmn}	T	25.3^{Lmn}	T
Varieties						
49	GTG-30	61	5.80^{mn}	R	16.89^{jklmn}	MR
50	Piayur	55	19.41^{efghijklmn}	MR	20.88^{ijklmn}	T
51	Chagatham Local	55	18.72^{fghijklmn}	MR	24.11^{hijklmn}	T
52	IS-238	59	10.86^{ijklmn}	MR	12.65^{mn}	MR
53	DSK-1-A	47	39.48^{abcdefghij}	MS	43.65^{abcdefghijkl}	S
54	B-24	53	27.02^{bcdefghijklmn}	T	34.05^{cdefghijklmn}	MS
55	SI-250-A	64	39.95^{abcdefghi}	MS	47.64^{abcdefghi}	S
56	S-0448	63	43.03^{abcdefgh}	S	48.03^{abcdefghi}	S
57	IS-24-A	31	48.08^{cdefghijklmn}	S	43.72^{abcdefghijklm}	S
58	IS-245	54	42.32^{abcdefgh}	S	43.54^{abcdefghijkl}	S
Checks						
59	GT-10	72	6.94^{n}	R	9.9^{n}	R
60	RJR-170	64	51.31^{defghijklmn}	HS	55.62^{ghijklmn}	HS
		P value	0.002*	Sig	0.003*	Sig

*significant at $P \leq 0.05$

Values having the same alphabet are not significantly different as per DMRT.

Sig- Significant

PDI – Per cent Disease Incidence

DAS – Days after sowing.

R- Resistant; MR- Moderately Resistant; T- Tolerant; MS- Moderately Susceptible; S- Susceptible and HS- Highly Susceptible.

Table 4.5. Grouping of sesame genotypes based on per cent disease incidence of phyllody under field conditions at differen dates of sowing.

60 DAS: Per cent disease incidence of phyllody

GRADE	REACTION	GENOTYPES
1	Resistant	GT-10, GTG-30, Chagatham Local, SEL-S-2019-1009, SEL-S-2019-1016, SEL-S-2019-1018, SEL-S-2019-1019, Swetha, IS-238, IS-17, SI-982, IS-491-4
2	Moderately Resistant	S-2019-F6-3, S-2019-F6-10, S-2019-F6-11, Piayur, SEL-S-2019-1007, SEL-S-2019-1010, SEL-S-2019-1015, SEL-S-2019-1017, SEL-S-2019-1020, SEL-S-2018-1001, DSK-1-A, S-0448, IC-500325, IC-500401, IC-500386, IC-205601
3	Tolerant	S-2019-F6-1, S-2019-F6-2, S-2019-F6-5, S-2019-F6-8, SEL-S-2019-1008, SEL-S-2019-1012, SEL-S-2019-1013, SEL-S-2019-1014, SEL-S-2019-1021, SEL-S-2019-3001, SEL-S-2018-1004, B-24, SI-250-A, IS-24-A, IC-500425, IC-500377, IC-500329, IC-500429, IC-500431, IC-205629, IC-500477
4	Moderately Susceptible	S-2019-F6-4, S-2019-F6-6, S-2019-F6-7, S-2019-F6-9, SEL-S-2019-1011, SEL-S-2018-1003, IS-245, KIC-357, IC-500419, IC-500437
5	Susceptible	SEL-S-2018-1002, RJR-170

70 DAS: Per cent disease incidence of phyllody

GRADE	REACTION	GENOTYPES
1	Resistant	GT-10, GTG-30, SEL-S-2019-1016, SEL-S-2019-1019,
2	Moderately Resistant	Piayur, Chagatham Local, SEL-S-2019-1007, SEL-S-2019-1009, SEL-S-2019-1018, Swetha, IS-238, SI-982
3	Tolerant	S-2019-F6-10, SEL-S-2019-1010, SEL-S-2019-1017, SEL-S-2019-1020, SEL-S-2018-1001, B-24, IC-500431, IS-17
4	Moderately Susceptible	S-2019-F6-1, S-2019-F6-2, S-2019-F6-3, S-2019-F6-8, S-2019-F6-11, SEL-S-2019-1008, SEL-S-2019-1013, SEL-S-2019-1014, SEL-S-2019-1015, SEL-S-2019-1021, SEL-S-2019-3001, SEL-S-2018-1004, DSK-1-A, SI-250-A, IC-500425, IC-500377, IC-500329, IC-500325, IC-500401, IC-500429, IC-205629
5	Susceptible	S-2019-F6-6, SEL-S-2019-1011, SEL-S-2019-1012, SEL-S-2018-1002, SEL-S-2018-1003, S-0448, IS-24-A, IS-245, KIC-357, IC-500386, IC-500419, IC-500437, IC-500477
6	Highly Susceptible	S-2019-F6-4, S-2019-F6-5, S-2019-F6-7, S-2019-F6-9, IC-205601, RJR-170

80 DAS: Per cent disease incidence of phyllody

GRADE	REACTION	GENOTYPES
1	Resistant	GT-10
2	Moderately Resistant	GTG-30, SEL-S-2019-1016, Swetha,
3	Tolerant	Piayur, Chagatham Local, SEL-S-2019-1009, SEL-S-2019-1017, SEL-S-2019-1019, SEL-S-2019-1020, SEL-S-2018-1001, IS-17
4	Moderately Susceptible	S-2019-F6-8, S-2019-F6-10, S-2019-F6-11, SEL-S-2019-1007, SEL-S-2019-1010, SEL-S-2019-1013, SEL-S-2019-1018, SEL-S-2019-1021, SEL-S-2019-3001, B-24, IC-500377, IC-500325, IC-500401, IC-500429, IC-500431
5	Susceptible	S-2019-F6-1, S-2019-F6-3, S-2019-F6-7, SEL-S-2019-1008, SEL-S-2019-1012, SEL-S-2019-1014, SEL-S-2019-1015, SEL-S-2018-1002, SEL-S-2018-1003, SEL-S-2018-1004, DSK-1-A, SI-250-A, S-0448, IS-24-A, IS-245, IC-500425, IC-500329, KIC-357, IC-500419, IC-205629, IC-500477
6	Highly Susceptible	S-2019-F6-2, S-2019-F6-4, S-2019-F6-5, S-2019-F6-6, S-2019-F6-9, SEL-S-2019-1011, IC-500386, IC-500437, IC-205601 and RJR-170

4.4.2 Influence of leafhopper nymphal population on per cent disease incidence of phyllody

Sesame leafhopper (*Orosius albicinctus*) population was assessed in 60 genotypes along with resistant check (GT-10) and susceptible check (RJR-170). In each genotype, leafhopper population was recorded from 3 leaves at 30 days after sowing (as mentioned in 3.4). The leafhopper population among the genotypes ranged from 0.2 (GTG-30, SEL-S-2019-1016) to 3.6 leafhoppers/3 leaves/plant (SEL-S-2018-1002). Entries SEL-S-2019-1011 and the susceptible check, RJR-170 also recorded higher leafhopper population (more than 3.0 leafhoppers/3 leaves/plant). Significant positive correlation (r=0.394) was observed between leafhopper population and phyllody incidence.

Akhtar *et al*. (2009) observed that sesame plants infected before or during flower initiation had severe symptom on the entire plant and showed

complete sterility when compared to the plants infected at later stages of the crop growth.

In the present study, the leaf hopper (*Orosius albicinctus*) population under field condition showed significant positive correlation with the per cent incidence of phyllody disease. Similar results were obtained by Pathak *et al*. (2013) and Kumar *et al*. (2019) who reported significant positive relationship between phyllody incidence and vector population.

Chapter – V

Summary & Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

The present investigation on “Studies on sesame phyllody incited by *Phytoplasma*” was conducted with an aim to molecular identification of phytoplasma from phyllody infected plants with PCR and nested PCR, to study the transmission of sesame phyllody by different methods and screening of sesame genotypes against phyllody under field conditions. The results obtained from the studies were summarized in this chapter.

The sesame phytoplasma was detected by PCR with the universal primer pairs. The PCR products were sequenced in both the directions using phytoplasma specific universal primers and aligned with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo) from both ends. The length of phytoplasma gene sequence was determined as 986bp and the sequence was compared with those of phytoplasma species available in the GenBank using BLAST similarity search tool. The analysis revealed sesame phyllody isolates showed 100 per cent sequence identity among themselves and shared 100 per cent similarity with strains of ‘*Candidatus* Phytoplasma aurantifolia’ of 16Sr II-D subgroup.

The transmission studies were conducted to know the transmission of phytoplasma to other plants or hosts through various methods. Six genotypes (E-8, Thilothama, CUMS-17, RJR- 170, Nirmala and JCS-1020) selected for sap inoculation, two genotypes (JCS-1020), variety (CUMS-17) and periwinkle plants were selected for transmission through grafting, cuscuta and leafhopper. In the present investigation, the sesame phyllody phytoplasma could not be transmitted by sap inoculation method and successfully transmitted from infected sesame to healthy test plants through grafting, dodder and the leafhopper *Orosius albicinctus*. The disease was transmitted to test plants such as CUMS-17, JCS-1020 of sesame and periwinkle through grafting showed symptoms of yellowing, mottling and stunted growth at 25- 28, 32-36 and 45-50 DAI and reported 90, 80 and 100 per cent disease transmission respectively, the transmission of disease from infected sesame to CUMS-17, JCS-1020 of sesame and from infected

periwinkle to healthy periwinkle showed symptoms of floral virescence, floral proliferation and yellowing of leaves at 40-45, 45-50 and 60-65 DAI and noticed 60, 40 and 80 per cent disease transmission respectively and the phytoplasma transmitted through leafhopper (*O. albicinctus*) reported 75, 35, 100 and 80 per cent of disease transmission from infected sesame to healthy CUMS-17, JCS-1020 of sesame, periwinkle and sunhemp exhibited virescence, phyllody and floral proliferation in sesame, little leaves, yellowing of leaves and stunted growth in periwinkle and sunhemp at 35-45, 45-50, 50-60 and 45-50 DAI respectively. Among all these methods the graft method of transmission reports best method of transmission in these studies.

Field evaluation experiment was also conducted with 60 sesame genotypes. Sown during 3rd week of July gave high phyllody incidence. Among them one genotype was found to be resistant (GT-10), three genotypes showed moderately resistant reaction (GTG-30, SEL-S-2019-1016, Swetha), nine genotypes found to be tolerant reaction (Piayur, Chagatham local, SEL-S-2019-1009, SEL-S-2019-1017, SEL-S-2019-1019, SEL-S-2019-1020, SEL-S-2018-1001, IS-17), 15 genotypes were showed moderately susceptible reaction, 21 genotypes showed susceptible reaction and 11 genotypes were found to be highly susceptible based on per cent disease incidence of phyllody in correlation to the leafhopper nymphs per plant.

During field screening the different types of phyllody symptoms such as floral virescence, proliferation, thickening of veins, twisting of stem, malformation of capsules, early drying of the plant were observed. Under severe conditions capsule cracking, germination of seeds within the capsules were also noticed.

FUTURE LINE OF WORK

1. Characterization of phytoplsama infecting other crops at 16S r DNA gene level.
2. Transmission studies from weeds to host and vice versa.
3. Determination of optimum titer of phytoplsama present in the plant at different crop growth stages.

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Appendices

APPENDIX A

EQUIPMENTS USED

- Agarose gel electrophoresis system (Genei and Biorad)
- Autoclave
- DNA thermal cycler (Eppendorf and Biorad master cycler gradient)
- Freezer -20 (Vestfrost)
- Gel documentation system (Bio-rad)
- Mini cooler of -20ºC (Tarsons)
- Magnetic stirrer (Wisd)
- Microwave oven (Samsung)
- Microcentrifuge (Eppendorf 5804R)
- Mini centrifuge (Tarsons)
- Nanodrop (ND 100)
- Pipetteman (Thermo Scientific, Eppendorf, Accupipet)
- pH meter (Eutech instruments)
- U.V Absorbance spectrophotometer (Thermo electronic corporation)
- U.V Transilluminator (Alpha Innotech)
- Water bath (Wisd and Polyscience)
- Weighing balance (Unibloc)
- Thermo mixer (Eppendorf)

APPENDIX B

LIST OF CHEMICALS

- Agarose (Genei)
- 6X loading dye (Thermo Scientific)
- Boric Acid (Avra)
- Chloroform (Fisher Scientific)
- CTAB (Himedia)
- dNTPs (Deoxy nucleotide triphosphates) (Genei)
- EDTA (Ethylene Diamino Tetra Acetic acid) (Fisher Scientific)
- Ethidium bromide (Sigma)
- Ethyl alcohol (Changshu Hongsheng Fine Chemicals)
- Isoamyl alcohol (Qualigens)
- Isopropanol (Emplura)
- NaCl (Sodium chloride) (Fisher Scientific)
- NaOH (Sodiun hydroxide) (Qualigens)
- Phenol (SRL)
- Poly vinyl pyrrolidone (SRL)
- *Taq* polymerase (Genei)
- Tris base (Himedia)
- 50bp ladder (Thermo Scientific)
- *Taq* buffer with $MgCl_2$ (Genei)
- Primers (Sigma)

APPENDIX C

PREPARATION OF BUFFERS AND STOCK SOLUTIONS

DNA Extraction Buffer

Component	Quantity
2 % (w/v) CTAB (Nalgene)	10g
100 Mm Tris HCl, pH 8.0	100 ml of 0.5 M Tris HCl
20 mM EDTA, pH 8.0	20 ml of 0.5 M EDTA
1.4 M NaCl	140 ml of 5 M NaCl
1% PVP	5g
Mercaptoethanol	290µl

All the above ingredients except CTAB were added in respective quantities and final volume was made upto 500 ml with double distilled water, the solution was autoclaved. Then it was allowed to attain room temperature and 10 g of CTAB was dissolved by intense stirring, stored at room temperature.

Tris (1M) 100 ml

To prepare 1M Tris, 12.114 g of Tris was dissolved in 100 ml of distilled water

Nacl (5M) 100 ml

Weigh 29.22 g of NaCl in 100 ml of distilled water.

EDTA (0.5M) 100 ml

Weigh 18.612g of EDTA, dissolve in 50 ml of distilled water by adding 4g of NaoH pellets. Stirr the solution by adding another 20 ml of water and allow EDTA to dissolve completely. Then check the pH and try to adjust to 8 by adding 2N NaoH drop by drop. Then make the volume to 100 ml

1 % PVP:

Add 1g of PVP was dissolved in minimal quantity of water and made upto 100ml with millipore water.

Phenol: Chloroform: Iso amyl alcohol (25:24:1)

Equal parts of equilibrated phenol and Chloroform: Isoamyl alcohol (24:1) were mixed and stored at 4^{o}C.

Absolute ethanol - Stored at 4^{0} C

70 % Ethanol

Absolute ethanol - 70 ml

Distilled water - 30 ml

90 % ethanol

Absolute ethanol - 90 ml

Distilled water - 10 ml

TAE buffer (Tris / acetate / EDTA) 50X stock solution

Tris base - 242 g

Glacial acetic acid - 57.1 ml

0.5 M EDTA (pH 8.0) -100 ml

Adjusted the pH to 8.3 with acetic acid and make up to the volume to 1 lit with distilled water. Sterilization is done by autoclaving.

TE buffer (pH 8.0)

A. 10 mM TrisHCl
B. 1 mM EDTA.

2 ml of 0.5 M TrisHCl pH 8.0 was mixed with 0.2 ml of 0.5 EDTA, make up to the vol to 100 ml with sterile distilled water.

6X Gel loading buffer

A. 0.25% (W/V) Bromophenolblue
B. 40% (W/V) sucrose in water

Dissolved 0.25g of Bromophenol blue was mixed with 40g of sucrose, make up the vol to 100ml with distilled water.

RNase preparation

RNase buffer

A. 1M Tris (pH 7.5)

B. 5M NaCl

Take 0.5ml of 1M Tris (final concentration 10mM) and 75 µl of 5M NaCl (final concentration 15mM) and make up the volume to 50 ml. Weighed 25 mg of ribonuclease H into a tube and add RNase buffer to a final volume of 5 ml (so final concentration 5mg / ml). Kept the tube in a boiling water bath for 10 min, cool and made aliquots of 1 ml in 1.5 ml Eppendorf tubes and stored at -20^{0}C.

Ethidium Bromide

Stock 20mg / ml can be prepared by dissolving 1gm of ethidium bromide in 50 ml of water.

3M Sodium acetate (pH-5.2)

Dissolve 14.6 g sodium acetate 70 ml of distilled water then adjust pH to 5.2 by using glacial acetic acid and made the volume to 100 ml by adding distilled water.