SESAMUM PHYLLODY DISEASE

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Abstract

Sesamum phyllody is a very important disease of sesamum caused by phytoplasma which affects mostly the floral organs of the plant. The floral organs are modified into leaf like structures and hence the yield is directly affected by this disease. Sesamum phyllody is a systemic disease and is transmitted by leafhoppers, *Orosius albicinctus* (Dist.) and *Hishimonus phycitis* (Dist.). This disease has been reported from 52 districts of North Eastern States during 2015 to 2017 and the disease incidence varied from 2 to 29 per cent in different locations. Epidemiological data in Assam shows that the incidence of sesamum phyllody disease was higher during the *kharif* season (22.16 %) as compared to the summer season (17.60 %) and is highly correlated to the vector population. It was also observed that maximum and minimum temperatures along with relative humidity were directly correlated with the disease incidence of sesamum phyllody. The reduction in pod per plant in the diseased plant over healthy plant was observed to be 72.82- 100 %. As this disease affects the yield of sesamum, not only the quantity of yield is affected but also the quality of sesamum oil also gets deteriorated to a great extent as the saponification value decreases and free fatty acid and iodine value in oil increases. A total of 38 alternate hosts were detected in Assam which belonged to three different groups of phytoplasma *viz.*, aster yellows (16SrI), clover proliferation (16SrVI) and stolbur phytoplasma (16SrXII). On the other hand, molecular characterization and RFLP analysis revealed that there is no genetic difference between the isolates of sesamum phyllody disease collected from all the six agro-climatic zones of Assam as they belonged to 16SrI-B group.

Introduction

An essential part of human diet includes oil with the ever changing diet. Hence a wide range of oilseeds are being cultivated. Amongst the different oilseeds cultivated in North east India, sesamum holds a very crucial position. Sesamum is the most ancient oilseed crop cultivated by human. It belongs to the family Pedaliaceae, which included 16 genera and 60 species, of which only *Sesamum indicum* is cultivated. (Weiss, 1983) It is an annual herbaceous plant, maturing in about 70 to 140 days and the fruit is known as capsule or pod containing small ovate seeds (Vaughan, 1970). Sesamum seeds are rich source of protein, edible oil, oleic acid and linolenic acid (Shyu and Hwang, 2002). Oil content in sesamum seed ranges from 46-52 per cent in different varieties (Pathak, 2011). It has high amount of essential fatty acid such as linoleic acid (39 per cent) and is excellently stable due to the presence of natural antioxidants such as sesamoline, sesamin and sesamol (Akhtar *et al.*, 2009). Sesamum also possesses many medicinal values and helps in promoting bone health, lowering blood pressure, preventing headache and migraine, reducing stress, preventing diabetes, promoting heart health, boosting oral health, promoting healthy skin and hair, etc. Due to all these goodness, it is known as the 'Queen of Oilseeds'.

Sesamum is mainly grown in many tropical and sub-tropical countries. In Assam it is grown in an area of 12,128 ha with production of 54,86,598 tonnes and productivity of 674 kg per hectare (Anon., 2021). Unlike the other sesamum growing states of India where it is mainly grown for oil extraction, in Assam the produce is used for making various confectioneries during festivals and other social and religious functions along with extraction of oil. Although sesamum has a higher potential of yield, yet the harvest index decreases due to the various biotic and abiotic stress. Amongst all, sesamum phyllody disease caused by phytoplasma is the most destructive. The disease causes 5-15 % loss in yield worldwide, 10-100 % in India (Sridhar *et al.*, 2013) and 57.20-100 % in Assam (Gogoi *et al.*, 2017b). This disease was first reported from Burma (McGibbon, 1924) which was thought to be of viral origin but later on the causal agent was confirmed as mycoplasma like organisms (MLOs) or phytoplasmas (Das and Mitra, 1998). Later it was also detected in India (Kashiram, 1930). Phytoplasmas are specialized bacteria of minute size (200-800 μm), pleomorphic, obligate which lacks cell wall and is surrounded by a single-unit membrane. These obligate parasites are found in sieve elements of plants and in the body of some insect vector. Phytoplasmas are mainly spread by insects belonging to the family Cicadellidae (leafhoppers), Fulgoridae (planthoppers), and Psyllidae (psyllids), which feed on the phloem tissues of infected plants

(Bertaccini and Duduk, 2009). In various reports *Orosius albicinctus* (Dist.) have been recognized as the vector of sesamum phyllody from various regions of the world as well as in India (Akhtar *et al*., 2009; Thangjam and Vastrad, 2017; Gogoi *et al*., 2017a). Phytoplasmas may overwinter in insect vectors or in perennial plants and interact in various ways with insect hosts (Christensen *et al*., 2005). The disease has been named "Green flowering disease" (Robertson, 1929). Molecular characterization of Sesamum Phyllody phytoplasma has been done in different countries and reported as member of three different phytoplasma groups like 16Sr I (Win *et al*., 2010; Nabi *et al*., 2015), 16Sr II (Akhtar *et al*., 2009; Hosseini *et al*., 2007) and 16Sr VI (Sertkaya *et al*., 2007).

Distribution of sesamum phyllody in North east India

A roving survey carried out in five districts of Assam (Jorhat, Biswanath, Sonitpur, Nagaon and Karbi Anglong) during 2014-2015 showed varied disease incidence of sesamum phyllody disease. Highest incidence of sesamum phyllody was observed in Karbi Anglong district (23.22 %) followed by Biswanath (20.23 %), Sonitpur (19.91 %), Jorhat (18.38 %) and Nagaon (17.46 %) districts. (Gogoi et al., 2017b) The presence of sesamum phyllody has been detected through molecular methods in different states of North east India in a roving survey during 2015-2017. The states included Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura which covered 52 districts. Sesamum phyllody disease incidence was ranged between 2-29 % in Assam, covering 30 districts and the highest disease incidence was recorded in Sonitpur district. Similarly the other states of North east India showed disease incidences such as 2-12 % in Arunachal Pradesh, 4-8 % in Manipur, 6-9 % in Meghalaya, 1-6 % in Mizoram, 1-3 % in Nagaland, and 2-4 % in Tripura. (Kalita *et al*., 2018) Among the two seasons of sesamum cultivation in Assam i.e., summer and *kharif*, the disease incidence was observed to be higher in the *kharif* season (22.16 %) as compared to summer season (17.60 %). (Phookan *et al*., 2020) In case of incidence of sesamum phyllody in different cultivars, a study showed that among the most commonly cultivated cultivars of Assam, 10 were selected and its disease incidences were recorded. The cultivars included Kaliabor local, Nagaon local, Nempo karjung, Nempo soksu, Nempo charap, Punjab til 1, Nempo thepo, AST 1, Madhabi and DT1683. Among these Kaliabor local recorded the highest incidence of 23.33 % while Madhabi recorded lowest disease incidence (15.66 %). (Gogoi, 2021)

Symptomatology of sesamum phyllody disease

The symptoms of sesamum phyllody disease are very prominently observed in the floral parts of the plant. As the name phyllody says, the flowers are converted to green leafy structures. Also the growth of the plant is retarded which results in dwarfing of plants. Infection of phytoplasma can occur at different stages of the plant growth. When the plants are infected at early growth stages the symptoms mostly observed are yellowing of leaves, dwarf plants, underdeveloped leaves etc. The symptoms observed on sesamum plants when infected at later stages include floral virescence, floral proliferation, phyllody, cracking of pods in longitudinal direction. Most plants showed formation of pods but with deformities and cracking symptoms when the plants were infected at later stages. (Gogoi et al., 2017b; Kalita *et al*., 2018; Phookan *et al*., 2020)

Fig.1. Symptoms of sesamum phyllody disease, A. yellowing of leaves; B. Floral virescence; C. Floral proliferation; D. Phyllody; E. Fasciation of shoot apex; G. Witches' broom; H. cracking of pods.

Transmission of sesamum phyllody disease

Sesamum phyllody is a systemic disease and is transmitted by leafhoppers. A total of 7 different genus of leafhoppers were found in the fields of sesamum where sesamum phyllody disease symptoms were observed. The transmission tests showed that the leafhoppers responsible for transmission of sesamum phyllody in Assam included *Orosius albicinctus* and *Hishimonus phycitis* which were also later confirmed by PCR assays. In the BLAST analysis the sequences of the nested PCR products showed high sequence similarities among each other and in the phylogenetic analysis, the phytoplasma detected in *H. phycitis* clustered together with a phytoplasma strain from *Brassica oleracea* from Italy (GenBank accession number JQ181539) classified as aster yellows (Gogoi *et al*. 2017a; Phookan *et al*., 2019) This disease also showed successful transmission by grafting (80%) and also dodder (90%).

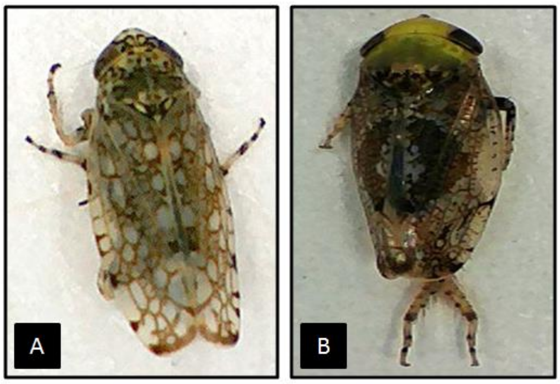

Fig 2. Leafhopper vectors associated with sesamum phyllody disease, A. Orosius albicinctus; B. Hishimonus phycitis

Detection and diversity of sesamum phyllody

The presence of sesamum phyllody disease could be observed from the symptoms expressed by the plants in the sesamum fields. Further detection of this disease requires various molecular tools. Sesamum phyllody disease in the states of North east India was detected through nested PCR analysis of two genes viz. 16Sr DNA and secA. (Kalita *et al*., 2018) The gel electrophoresis results of both the genes are shown in Fig.3 below.

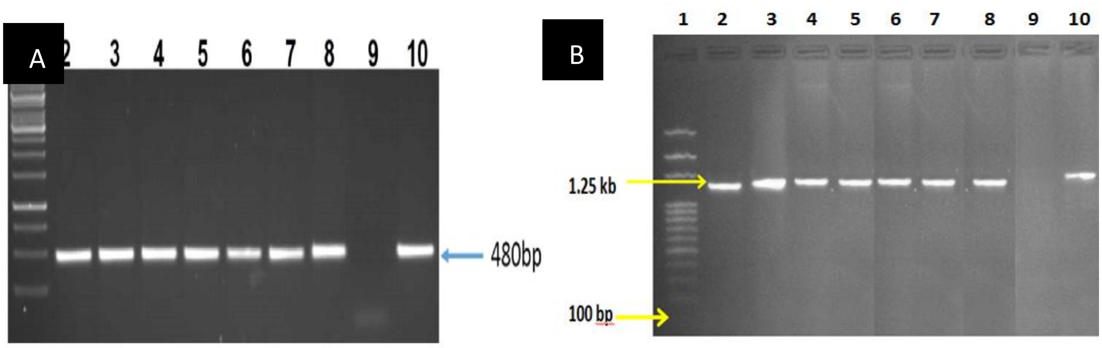

Fig. 3. Gel electrophoresis image of A. 16S rDNA(P1/P6; R16F2n/R16R2) and B. secA (SecAfor1/SecArev3; SecAfor2/ SecArev3) gene results of phytoplasma DNA amplification from sesamum phyllody plants; lane 1: marker 1kb DNA ladder; lane 2: SPP, Assam; lane 3: SPP, Tripura; lane 4: SPP, Arunachal Pradesh; lane 5: SPP, Nagaland; lane 6: SPP, Meghalaya; lane 7: SPP, Mizoram; lane 8: SPP, Manipur; lane 9: negative control asymptomatic plant sample and lane 10: positive control (BLL).

The samples were further analysed phylogenetically. Comparison of the 1.2 kb of 3F/3R primed sequence of 16S rDNA of SP from symptomatic sesamum showed 98–99 % identity with aster yellows phytoplasma (Acc. No. AY180957), sesamum phyllody phytoplasma

Kushinagar (Acc. No. KF728954), sesamum phyllody phytoplasma Gopalganj (Acc. No. KF28956) and *Malvastrum coromandelianum* phyllody phytoplasma (Acc. No. MF490802). Phylogenetic analysis also supported the above results and all the SP strains clustered together with strains classified under the 'Candidatus Phytoplasma asteris' clade. The pairwise sequence comparison of the sesamun phyllody phytoplasma strains secA gene partial sequences revealed 99% identity with sequences of phytoplasmas classified in the 16SrI group. (Kalita *et al*., 2018) Sequencing and virtual RFLP of 16S rDNA (primer primed R16F2n/R16R2) using *i*PhyClassifier online tool and actual RFLP patterns with BamHI, EcoRI, RsaI showed that the six SP phytoplasma strains collected from different zones of Assam were completely identical and belonged to subgroup B of 16SrI group. (Gogoi, 2021)

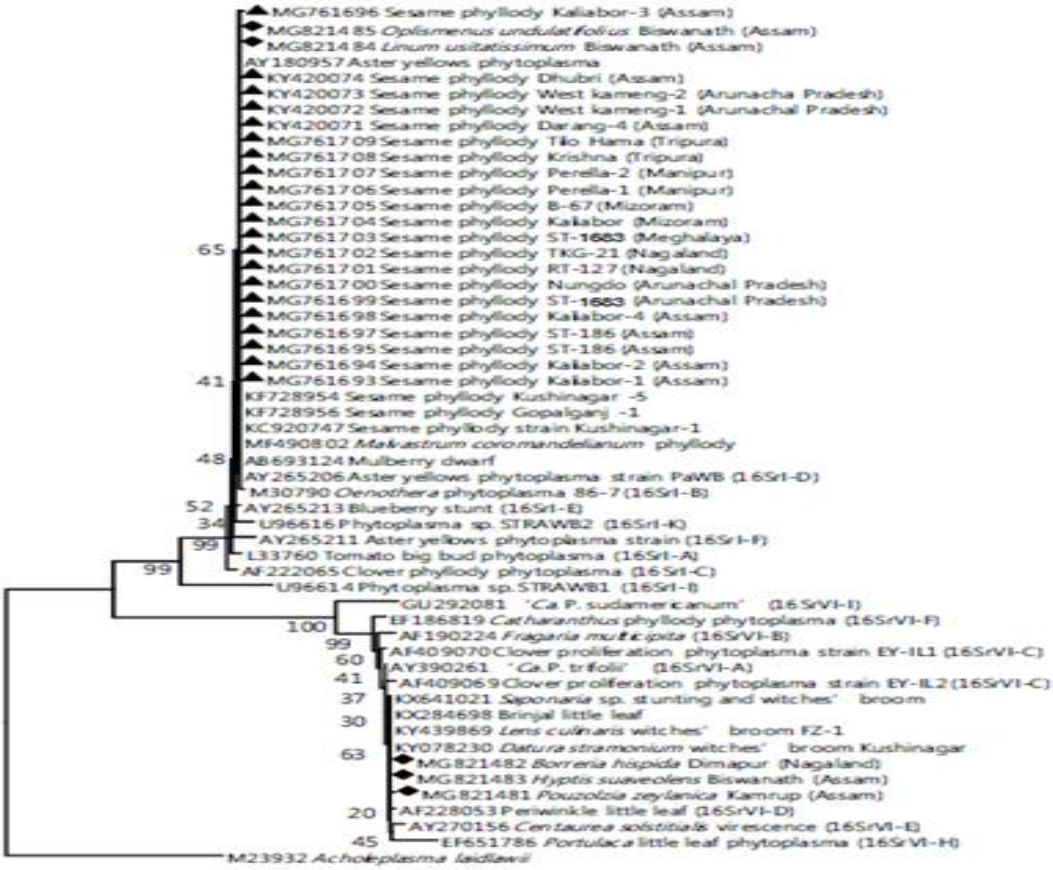

Fig. 4. Phylogenetic tree constructed by neighbor-joining method showing the relationships among sesamum phyllody strains and selected phytoplasma strains on 16S rDNA sequences.

A total of 38 weeds and plant species were identified for phytoplasma diseases in North east India. They include brinjal, *Cynodon dactylon*, *Synderella nodiflora*, *Pouzolzia zeylanic*, *Hyptis suaveolens*, *Linum usitatissimum*, *Oplismenus undulalifolius*, arecanut, bittergourd, bottebrush, cowpea, chilli, cockscomb, crap jasmine, jatropha, plalm grass, pineapple, toona tree, sapota, *Crotolaria* sp., cashewnut, *Solanum violaceum*, sugarcane, sarpagandha, zinnia, *Solanum torvum*, *Datura stramonium*, *Carica papaya*, *Myosotis* sp., *Gerbera jamesoni*, *Ageratum* spp., *Vigna mungo*, *Alstonia scholaris*, *Cucumis sativus*, *Solanum melongena*, *Xanthosoma* spp., *Musa* spp. and *Capsicum* sp. (Rao *et al*., 2018; Kalita *et al*., 2018; Baiswar *et al*., 2010; Mitra *et al*., 2019, Dutta *et al*., 2022b; Gogoi *et al*., 2018; Kalita *et al*., 2019) In the pairwise sequence comparison of the 16S rRNA gene partial sequences the phytoplasma strains from *P. zeylanica*, *B. hispida*, *H. suaveolens* showed a maximum identity of 98-99 % with 'Ca. *P. trifolii*' (16SrVI); *C. dactylon* and *S. nodiflora* showed maximum identity with '*Ca. P. cynodontis*' (16SrXIV), whereas *L. usitatissimum* and *O. undulatifolius* showed maximum identity with 'Ca. *P. asteris*' (16SrI). The phytoplasma strain associated with *S. violaceum* showed maximum identity with 16SrVI-D subgroup phytoplasm (Kalita *et al*., 2018) The *Solanum torvum*, *accharum officinarum* and *Ageratum* spp. isolates showed closest homology with aster yellows (16SrI) group, *Datura stramonium*, *Vigna mungo* and *Solanum melongena* isolates showed closest homology with clover proliferation (16SrVI) group and *Xanthosoma* spp.,*Musa* spp. and*Capsicum chinense* showed closest homology with stolbur (16Sr XII) group. It was verified from the phylogenetic tree that the isolates were evolutionary closest to aster yellows (16SrI), clover proliferation (16SrVI) and stolbur (16Sr XII) group of phytoplasma. (Gogoi, 2021)

Epidemiology of sesamum phyllody disease

Sesamum cultivation in Assam is cultivated in two seasons viz., summer and *kharif*. An experiment conducted in 2017 showed that the disease incidence in both the seasons went on increasing significantly with the successive sowing dates. In the correlation analysis of disease incidence with vector population and meteorological parameters it was found that during the summer season disease incidence had significant positive correlation with vector population, maximum temperature, minimum temperature, day temperature, night temperature, morning relative humidity and evening relative humidity, whereas significant negative correlation with diurnal variation and sunshine hours. During *kharif* season, disease incidence had significant positive correlation with vector population, maximum temperature and diurnal variation but significant negative correlation with wind speed, total rainfall and number of rainy days. It was observed that vector population plays an important role in the occurrence of this disease. Regression analysis for prediction of sesamum phyllody disease revealed that during the summer season, the disease could be predicted correctly up to 94 % with vector population alone and up to 97 % by addition of maximum temperature to the equation. In the *kharif* season, the disease incidence could be predicted up to 84 % with vector population alone which further enhanced up to 95 % when total rainfall was added to the equation. (Phookan *et al*., 2020)

Table 1. Regression equation for sesamum phyllody disease incidence during summer and *kharif* season, 2017

Crop season	**Regression equation** ($Y= a+b_1X_1+b_2X_2+...+b_nX_n$)	R^2
Summer, 2017	$Y = 0.237+0.201X_1$	0.94
	$Y =-108.03+0.71X_1+4.18X_2$	0.97
Kharif, 2017	$Y = 4.720+0.178X_1$	0.84
	$Y = 13.61+2.18X_1-0.92X_3$	0.95

X_1 = vector population [*Hishimonus phycitis* (Dist.)], X_2 = maximum temperature, X_3 = total rainfall

Effect on yield and biochemical changes due to sesamum phyllody

The phytoplasma infection on sesamum affects both the number of pod and the seed yield per plant. The reduction of pod per diseased plant varied from 72.82 % to 100 %. The reduction of seed yield per diseased plant varied from 57.20 % to 100 %. In the variety Madhavi there was the highest reduction of number of pod per infected sesamum plant (82.65-100 %) and lowest in the variety ST1683 (72.82-100%). Likewise highest reduction in the seed yield per plant was recorded in the local variety Changmi (86.15-100%) and the lowest in the variety Krishna (57.20-100%). This data also shows that when phytoplasma infection occurs at an early developmental stage of the plant highest loss of yield is observed. (Kalita *et al*., 2018). Sesamum phyllody disease also has adverse effects on the oil content. It was observed that the oil content in seeds collected from healthy plant was found to be 42 % which was reduced up to 28 % in seeds of diseased plant. To check the quality of oil extracted from sesamum phyllody infected plants some of the parameters were taken into account and the quality was checked. The free fatty acid content and iodine value of oil extracted from seeds collected from diseased plants increased by 25.37 % and 25 %, respectively as compared to oil extracted from seeds of healthy plants. The higher acid value of diseased seeds is associated with the low keeping quality of the oil i.e. the oil quality is deteriorated and turns rancid. Saponification value of oil extracted from seeds of diseased plants was decreased as compared to seeds of healthy plants. The reduction in saponification value in oil collected from diseases plants was calculated as 32.03%. Due to breakage of triacyl glycerides there was lesser space for ester linkages during the saponification process, hence there was a decrease in the saponification value of oil from diseased seeds. Lower saponification value specifies high molecular weight with longer chain fatty acid in oil. (Phookan *et al*., 2020). Some other biochemical changes in the diseased plants also prove that sesamum phyllody has adverse effect on the plant. The total nitrogen and crude protein content (8.50 to 13.29 %) due to infection were found to be decreased which shows that the diseased tissue might synthesizes the proteins at a slow rate compared to a healthy one. The phenol content increased from the plants exhibiting milder symptoms to severe symptoms as 2.24 mg/g to 2.68 mg/g compared to 1.85 mg/g of healthy leaves. In phyllody-infected sesamum plants, a decrease in chlorophyll content was found to be positively associated with the severity of the infection. In severely infected plants, the total chlorophyll content was reduced by 41.02 %, while it was reduced by 28.20 % in mildly infected plants. As a consequence of infection, the ratio between chlorophyll "a" and chlorophyll "b" gradually decreased. Phyllody infection appears to have inhibited chlorophyll biosynthesis, thus affecting photosynthetic activity. (Gogoi, 2021).

Management of sesamum phyllody disease

Management of diseases caused by virus and virus like organisms is a tricky job. As phytoplasmas are systemic in nature, once they infect a plant, the plant cannot be freed of the pathogen and the plant has to be uprooted and destroyed. Some of the management practices that can be taken up for sesamum phyllody disease are as follows.

A. **Clean cultivation-** As phytoplasma has numerous plants as hosts, hence cleaning off the weeds and other potential hosts from the cultivated area would definitely help reduction in disease incidence. Removal and destruction of the infected plants is very important as they serve as a source of inoculums.

B. **Use of tolerant/ resistant varieties-** A total of 10 cultivars of Assam was taken into account to screen the cultivars based on resistance. It was observed that the cultivar Madhavi had the local incidence of sesamum phyllody and was grouped as moderately resistant according to a scale given by Saravanan and Nadarajan (2005).

C. **Adjustment with the date of sowing-** Managing of sowing dates to escape the highest intensity of a disease can be adopted. In case of diseases transmitted by insect vectors, the time when the vector population lowers can be opted.

D. **Chemical control-** Phytoplasma could be controlled by application of tetracycline. It has to be applied at time intervals and once the application is discontinued the symptoms reappear. Tetracycline @ 500ppm could suppress the symptoms up to 13-16 days and 19-24 days having one and two sprays respectively. (Dutta *et al*., 2022a) Insecticides could also be applied to decrease the activity of the leafhopper vectors and thus reduce the transmission of sesamum phyllody disease. Seed treatment can be taken up with imidachloprid as it protects the crop from sucking pests. Spraying of Dimethoate 30EC @ 1ml/l or Spinosad @0.5ml/l could also help reduce the vector population from the field.

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