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SESAMUM PHYLLODY DISEASE

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Abstract

Sesamum Phytoplasma is a systemic disease of the sesamum plant that mainly affects the floral organs. The floral organs have been modified into leaf-like structures. This disease is caused by two types of bugs: Orosius aalbicinctus, which eats sesamum flowers, and Hishimonous phycitis, which eats leaves. This has had a significant impact on the yield of the plant, with the disease being reported in 52 districts of Northeastern states between 2015 and 2017. The incidence of the disease ranged from 2 to 29 percent in different locations of this region. 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. In Assam, a total of 38 alternative hosts were identified, belonging to three distinct phytotlasma classes: aster yellows in 16SrI, clover proliferation in 16SrVI, and stolburs in 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

Oil is a fundamental component of human nutrition, and with the ever-evolving diet, a wide variety of oilseeds is being grown. Of the various types of oilseeds grown in North East India, Sesamum occupies a particularly important place. It is the oldest oilseed crop ever cultivated by humans, and is a member of the Pedaliaceae family, which includes sixteen genera and sixty species, of which the sole cultivar, Sesamorum indicum, is cultivated. (See 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 seed is a highly nutritious food source, containing a wide range of essential fatty acids, including linoleic acid. It is rich in protein, edible oil and oleic acid, as well as linolenic acid. The oil content of sesamum seeds ranges from 46 to 52 per cent in various varieties, as reported by Pathak (2011). It has a high proportion of essential fatty acids (39 per cent), and is highly stable due to its natural antioxidants, including sesamoline and sesamin, as well as sesamol (Akhtar *et al.,* 2009). Sesamum has a wide range of medicinal properties, including bone health, blood pressure, headache and migraine prevention, stress reduction, diabetes prevention, heart health, oral health, skin and hair health, and more. As a result, it is commonly referred to as the "queen of oilseeds".

Sesame is mainly grown in many tropical and subtropical countries. In Assam, it is grown in an area of 12,128 hectares with a production of 54,86,598 tons and yield of 674 kg per hectare (Anon., 2021). Unlike other sesame-producing states of India, where it is grown mainly for oil extraction, in Assam the product is used to make a variety of sweets during festivals and social and religious functions as well as for oil exploitation. Although sesame has higher yield potential, the harvest index is reduced due to various biotic and abiotic stresses. Among all, sesame phyllodic disease caused by phytoplasma is the most devastating. The disease causes yield losses of 5-15% globally, 10-100% in India (Sridhar *et al.,* 2013) and 57.20-100% in Assam (Gogoi *et al.,* 2017b). The disease was first reported in Burma (McGibbon, 1924), where it was thought to be of viral origin, but the causative agent was later confirmed to be mycoplasma-like organisms (MLO) or phytoplasmas (Das and Mitra, 1998). Later it was also discovered in India (Kashiram, 1930). Phytoplasma are specialized bacteria that are microscopic (200-800 µm), pleomorphic, obligate, have no cell wall and are enclosed by a unit membrane. These obligate parasites are found in the sieve elements of plants and in the bodies of some insect vectors. Phytoplasmas are mainly spread by insects of the families Cicadellidae (leaf hoppers), Fulgoridae (leaf hoppers) and Psyllidae (hoppers), which feed on the phloem tissues of infected plants. (Bertaccini and Duduk, 2009). Orosius albicinctus (District) has been recognized as a vector of sesame disease in various parts of the world as well as in India (Akhtar *et al.,* 2009; Thangjam and Vastrad, 2017; Gogoi *et al.,* 2017a). Phytoplasmas can overwinter in insect vectors or in perennial plants and interact in a variety of ways with insect hosts (Christensen *et al.,* 2005). This disease was named "blue bloom disease" (Robertson, 1929). Molecular characterization of Sesamum Phyllody phytoplasma has been carried out in various countries and reported as a member of three different phytoplasma groups such as 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 conducted in five districts of Assam (Jorhat, Biswanath, Sonitpur, Nagaon and Karbi Anglong, respectively) in 2014-2015 revealed varying prevalence of sesame leaf spot. The highest prevalence of sesame weed was observed in Karbi Anglong district (23.22%), followed by Biswanath (20.23%), Sonitpur (19.91%), Jorhat (18.38%) and Nagaon (17.46%). (Gogoi et al., 2017b) The presence of sesame Phyllody was detected using molecular methods in different states of northeast India during a mobile survey conducted between 2015 and 2017. The states include Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura, comprising 52 districts. The incidence of sesamoidosis ranges from 2 to 29% in Assam, which includes 30 districts, and the highest incidence is recorded in Sonitpur district. Similarly, other states in Northeast India also have a prevalence of the disease 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).

During the two sesame growing seasons in Assam, i.e. summer and kharif, the incidence was observed to be higher during kharif season (22.16%) than during summer (17.60 %) (Phookan *et al.,* 2020). In the case of sesame weevil occurrence in different cultivars, a study has shown that among the most popular cultivars in Assam, 10 cultivars were selected and their incidence of them has been recorded. Cultivars include Kaliabor local, Nagaon local, Nempo karjung, Nempo soksu, Nempo charap, Punjab til 1, Nempo thepo, AST 1, Madhabi and DT1683. Among these residents, Kaliabor recorded the highest prevalence of the disease at 23.33%, while Madhabi recorded the lowest prevalence of 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 plants are infected in the early stages of growth, the most noticeable symptoms are yellow leaves, stunted plants, underdeveloped leaves, etc. Symptoms observed on sesame plants when infected in later stages include virogenic flowers, flower overgrowth, bud growth, peel cracking in the longitudinal direction. direction. Most trees show fruit formation but symptoms of deformation and cracking occur when the tree is infected at a later stage. (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 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%).

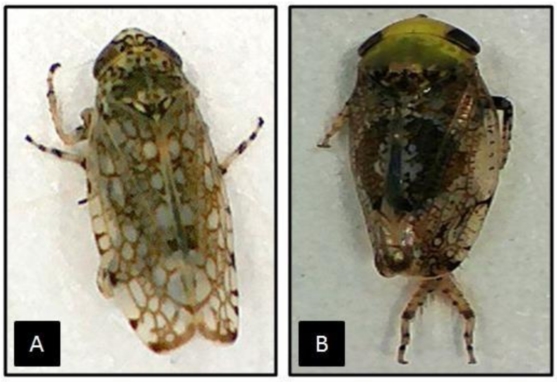

Fig 2. Leafhopper vectors associated with sesamum phyllody disease, A. Orosius albicinctus; B. Hishimonus phycitis

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.

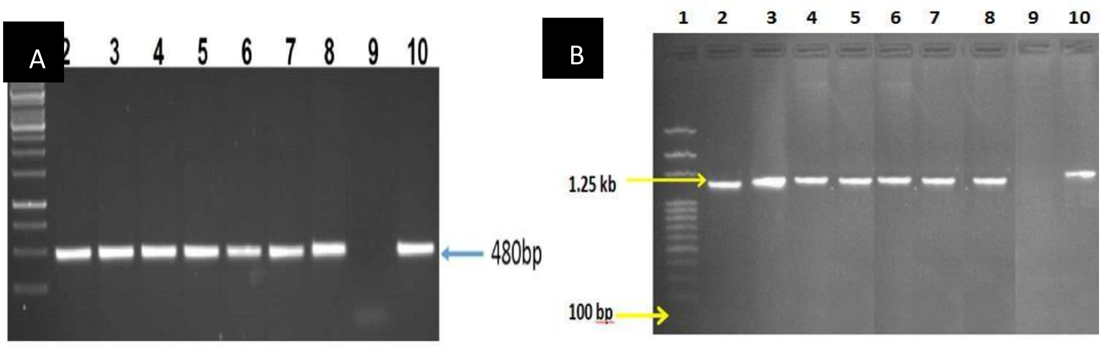

Fig. 3. Gel electrophoresis image of A. 16S rDNA(P1/P6; R16F2n/R16R2) and B. secA (SecAfor1/SecArev3; SecAfor2/ SecArev3) gene results of phytoplasma DNA amplification from sesamum phyllody plants; lane 1: marker 1kb DNA ladder; lane 2: SPP, Assam; lane 3: SPP, Tripura; lane 4: SPP, Arunachal Pradesh; 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).

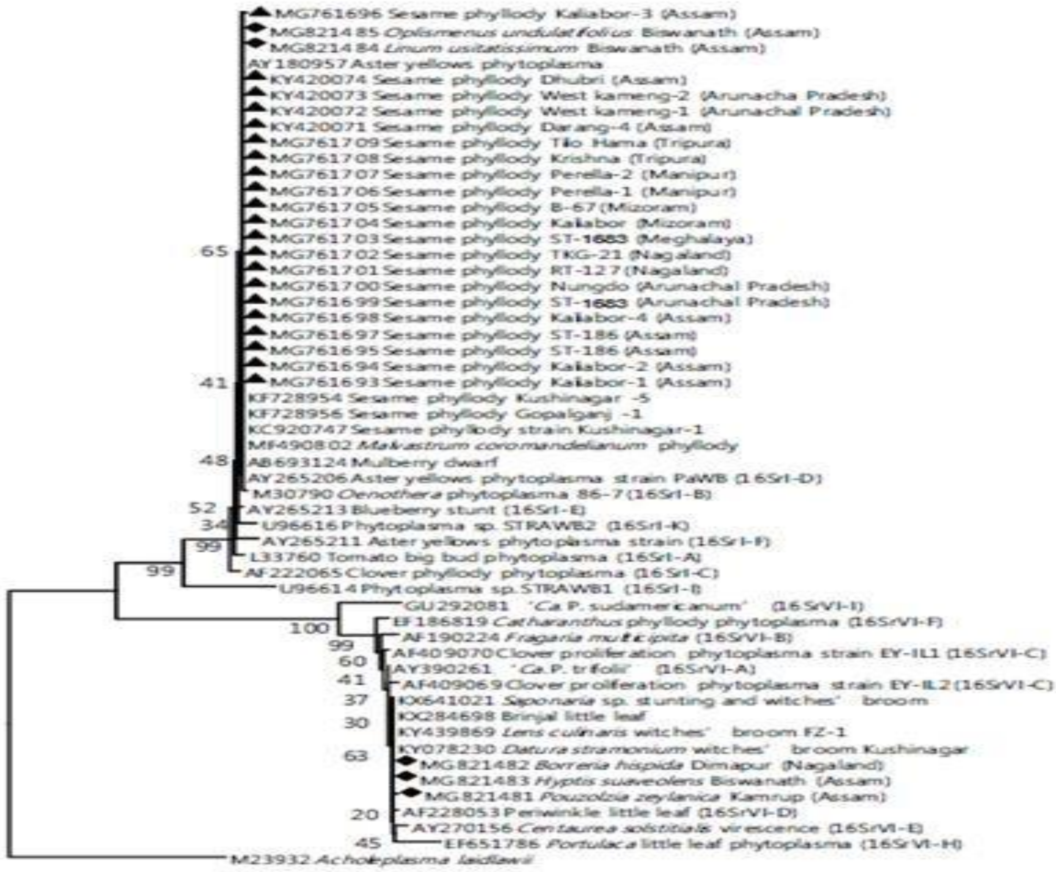

Fig. 4. Phylogenetic tree constructed by neighbor-joining method showing the relationships among sesamum phyllody strains and selected phytoplasma strains on 16S rDNA sequences.

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 amaximum 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). Thephytoplasma 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 withclover proliferation (16SrVI) group and *Xanthosoma* spp.,*Musa* spp. and*Capsicum chinense* showed closest homology with stolbur (16Sr XII) group. It was verified from thephylogenetic 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** **($Y= a+b_1X_1+b_2X_2+...+b_nX_n$)**	$\mathbf{R^2}$
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

Where 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 the oil extracted from seeds collected from diseased trees increased by 25.37% and 25%, respectively, compared with the oil extracted from seeds of healthy trees. strong. The higher acidity index of diseased seeds is associated with poor quality oil storage, i.e. the oil quality deteriorates and becomes rancid. The saponification value of oil extracted from diseased tree seeds is reduced compared to healthy tree seeds. The saponification index of the oil obtained from diseased plants was calculated to be 32.03%. Due to the breakdown of triacylglycerides, there is less space for ester bonds during saponification, so the saponification value of oil from diseased seeds decreases. Lower saponification values indicate high molecular weight with longer chain fatty acids in the oil. (Phookan *et al.,* 2020). Some other biochemical changes in diseased plants also demonstrate that sesame disease has a negative effect on the plant. Total nitrogen and crude protein (8.50-13.29%) contents as a result of infection were found to decrease, suggesting that diseased tissue may synthesize proteins at a slow rate compared to normal tissue. Phenol content increased from plants with milder symptoms to plants with more severe symptoms, from 2.24 mg/g to2.68 mg/g compared to 1.85 mg/g healthy leaves. In sesame plants infected with phyllody, a decrease in chlorophyll content was found to be positively related to disease severity. In heavily infected trees, total chlorophyll content decreased by 41.02%, while in lightly infected trees it decreased by 28.20%. After infection, the ratio of chlorophyll "a" to chlorophyll "b" gradually decreases. Phyllody infection appears to inhibit chlorophyll biosynthesis, thereby 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 betaken 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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