Original Research Article

STUDIES ON TRANSMISSION OF PHYTOPLASMA IN SESAME

ABSTRACT

An experiment was conducted to artificially transmit phytoplasma, the causal organism of sesame phyllody by mechanical sap inoculation and with insect vector *i.e.*, *Orosius albicinctus* and *Hishimonus phycitis*. The experimental findings revealed that the phytoplasma could not be transmitted by sap inoculation. The mean transmission rate was found to be 54.67 per cent, with the highest rate of phyllody transmission (93.33%) observed in treatments with 3 insects/plant + 7 DAF+ 5 DIF and 3 insects/plant + 7 DAF+ 7 DIF, respectively and the lowest in treatment 3 insects/plant + 3 DAF+ 3 DIF with 13.3 per cent phyllody transmission from infected to healthy sesame plants. All the treatments were found to be statistically significant over control, however, the treatments T_8 and T_9 with three insects per plant and an acquisition feeding period of 7 days and inoculation feeding of 5 and 7 days, respectively were found to be at par with each other statistically.

Keywords: Sesame, Leafhoppers, Sap inoculation, Vector transmission

1. Introduction

Sesame (*Sesamum indicum* L.) commonly known as gingelly (nuvvulu in Telugu; *til* in Hindi, Punjabi, Bengali; tal in Gujarati; ellu in Tamil, Malayalam; rasi in Odia; beeja in Kannada) is one of the important oilseed crops grown worldwide. Sesame yield is relatively low due to non availability of high yielding and resistant varieties (biotic and abiotic stresses), its cultivation in marginal and sub-marginal lands with poor crop management, low harvest index, seed shattering and indeterminate growth habit (Chauhan *et al.*, 2016). Sesame phyllody is one of the major diseases caused by phytoplasma and is transmitted by leafhopper, *Orosius albicinctus* Distant (Thangjam and Vastrad, 2015). The rate of transmission of phyllody disease from infected to healthy seedlings depends on number of insect population, life stage, gender, flight behavior, quality of the host plant, movement between the plants, time spent on plants and mode of transmission of pathogen which ultimately influence the transmission efficiency of the disease (Bosco and Marzachi, 2016). It is also transmitted from infected to healthy plants *via* dodder (*Cuscuta* sp.), grafting and cuttings but cannot be transmitted through mechanical sap inoculation (Gogoi *et al.*, 2017). Hence, keeping in view the present investigation was carried out to conduct transmission studies of phytoplasma by sap inoculation and insect vector.

Material and methods

1. Mechanical sap transmission

Phyllody infected leaf samples collected from the field were used for infectivity studies. Sesame leaves showing clear phyllody symptoms from the infected plants were collected, washed in running water thoroughly to remove dirt and were blot dried. The inoculum was extracted by macerating the infected leaves in the presence of pre-chilled 0.05M phosphate buffer (pH 7.0), (9 ml of potassium phosphate buffer per 1 g of leaf sample) in pre-chilled mortar and pestle. The resultant inoculum was squeezed through two folds of sterile muslin cloth, before using for mechanical transmission. Healthy, well established, actively growing three week old young sesame plants raised in insect proof cages in greenhouse, Insectary were used for mechanical transmission studies. Prior to inoculation, a pinch of celite was added to the filtrate. A sterile cotton pad immersed in the inoculum was gently rubbed over the leaves in single direction to cause mild injury. Immediately after inoculation, the leaves were washed with a jet of sterile distilled water to remove the excess inoculum and abrasive celite. The inoculated plants were labeled and kept in insect proof cages in glasshouse for symptom expression. Healthy seedlings of sesame plants were kept as control.

The phytoplams was artificially transmitted with the help of two leafhopper species that were found in sesame field *i.e*., *Orosius albicinctus* and *Hishimonus phycitis*. 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 leafhoppers. The leafhoppers were collected from sesame field by gently turning the leaves upwards and sucking with an aspirator. The leafhoppers were released on sesame plants maintained in insect proof cages for rearing. Infected sesame twigs showing phyllody symptoms were maintained in acquisition cages. The leafhoppers were collected from the rearing cages using an aspirator and were released into acquisition cages for acquaintance of phytoplasma inoculum. The hoppers were given different acquisition feeding periods (3, 5 and 7 days) as detailed in Table 1. Sesame seeds were sown in pots and seedlings were maintained in insect proof cages for inoculation studies. After giving prescribed acquisition feeding, the leafhoppers were removed carefully from the acquisition cages and transferred to inoculation cages as approved (3, 5 and 7 days) for transferring the inoculum from infected plants to healthy seedlings. The cages were kept in greenhouse for symptom expression. After prescribed period

of inoculation feeding, the insects were killed using imidacloprid @ 0.3 ml l^{-1}. Data was recorded on rate of per cent phyllody transmission and number of days taken for development of phyllody symptoms.

Table 1 Treatment details of insect transmission studies

Treatment No	Treatments
T_1	3 insects/plant + 3 DAF+ 3 DIF
T_2	3 insects/plant + 3 DAF+ 5 DIF
T_3	3 insects/plant + 3 DAF+ 7 DIF
T_4	3 insects/plant + 5 DAF+ 3 DIF
T_5	3 insects/plant + 5 DAF+ 5 DIF
T_6	3 insects/plant + 5 DAF+ 7 DIF
T_7	3 insects/plant + 7 DAF+ 3 DIF
T_8	3 insects/plant + 7 DAF+ 5 DIF
T_9	3 insects/plant + 7 DAF+ 7 DIF
T_{10}	No inoculation (Control)

DAF- Days of Acquisition feeding; DIF- Days of Inoculation feeding

3. Results and discussion

Mechanical Sap Transmission

Mechanical inoculation of sap collected from phyllody infected leaves into the young plants of sesame (variety Gowri) at three weeks stage induced no mechanical dissemination of phytoplasma. The plants that were sap inoclulated and maintained in insect proof cages did not produce any phyllody symptoms during the period of observation. The results clearly indicated that there was no mechanical transmission of phytoplasma into healthy plants (Table 2).

Transmission with leafhopper, *Orosius albicinctus*

An experiment was designed to artificially transmit sesame phyllody phytoplasma through leafhopper and the results were presented in Table 3. The sesame phyllody disease caused by phytoplasma was successfully transmitted by leafhopper, *Orosius albicinctus.* The results revealed that the per cent phyllody transmission by leafhooper was in the range of 13.3 to 93.3 per cent in the treatments and with zero per cent transmission rate in control. As insects

were not released in the control pots, there were no findings of phyllody transmission and symptom expression in control pots that were maintained in insect proof cages.

From the treatments with three insects per plant and acquisition feeding period (DAF) of 3 days, the highest per cent phyllody transmission was achieved in treatment where insects were provided 7 days inoculation feeding period (DIF) (46.7%), followed by five days inoculation feeding period (26.7%) and three days inoculation feeding period (13.3%). The per cent phyllody transmission was increased when the acquisition feeding periods were increased to five and seven days when compared to three DAF. At five DAF, the highest per cent phyllody transmission was observed in pots with 3 insects/plant and acquisition feeding of 5 DAF and inoculation feeding of 7 DIF (86.7%), followed by 5 DIF and 3 DIF with 60.0 and 53.3 per cent transmission, respectively. At 7 DAF, the treatment with 7 DAF+5 DAF and 7 DAF +7 DIF recorded the highest per cent phyllody transmission of 93.3 per cent, respectively, followed by 7 DAF+3 DAF with 73.3 per cent successful transmission of phyllody to healthy sesame plants. From the transmission study, it was concluded that the mean transmission rate of phyllody transmission was 54.67 per cent. In all the treatments with 3, 5 and 7 days of DAF and DIF, the number of days taken for first appearance of phyllody symptoms was in the range of 29-41 days. All the treatments were found to be statistically significant over control, however, the treatments T_8 and T_9 with three insects per plant while given an acquisition feeding of 7 days and inoculation feeding of 5 and 7 days, respectively were found to be at par with each other statistically in recording highest per cent phyllody transmission from infected to healthy sesame plants.

Transmission with leafhopper, *Hishimonus phycitis*

Attempts were made to transmit sesame phyllody caused by phytoplasma with the help of another leafhopper, *Hishimonus phycitis* (Table 4). No single plant developed typical phyllody symptoms after introduction of insects during the period of observation. The results from the transmission studies with leafhopper, *H. phycitis* revealed that the leafhopper could not be able to disseminate phytoplasmsa from infected sesame plants to healthy seedlings; thereby we can

conclude that *H. phycitis* cannot be considered as insect vector responsible for transmission of sesame phyllody disease caused by phytoplasma.

The results from the current experiment were in accordance with Akhtar *et al.* (2009b) who reported that sap transmission of chickpea phyllody was not achieved indicating that phyllody is not mechanically transmissible. The research findings of Gogoi *et al*. (2017), Karra (2017) and Yadav *et al.* (2021) did not induce any typical phyllody symptoms when sap was inoculated mechanically onto healthy sesame seedlings which further confirm the results obtained during the present study. Akhtar *et al.* (2009a) reported that the leafhopper, *Orosius albicinctus* transmitted 60 per cent phyllody disease from phyllody infected sesame plants to healthy plants by showing symptoms of floral virescence and proliferation with acquisition and inoculation feeding of seven days, respectively. Ikten *et al*. (2014), Gogoi *et al*. (2017), Omidi *et al*. (2010) and Prasindhu *et al.* (2020) reported that phytoplasma was successfully transmitted by leafhoppers which confirm the results obtained during the present study. The present findings were in accordance with Jayashree *et al.* (1999) who noticed increase in per cent disease transmission of pumpkin yellow vein mosaic virus and decrease in number of days required for symptom expression with an increase in acquisition feeding period and inoculation feeding period. Similar results were reported by Gogoi *et al*. (2017) where an increase in sesame phyllody disease transmission was observed when the acquisition feeding (AFP) increased from 3 days (49.38%) to 5 days (64.57%). The findings were in close agreement with Akhtar *et al.* (2009b) who noticed successful transmission of chickpea phyllody from infected to healthy chickpea plants by insect vector, *Orosius orientalis* with transmission rate of 70 per cent and Akhtar *et al.* (2012) who reported 70-80 per cent of phyllody transmission by leafhoppers from infected to healthy mungbean plants, while, per cent phyllody transmission by leafhoppers from infected sesame plants to healthy periwinkle plants and days taken for symptom expression were 100.00 per cent and 55-60 days, respectively as given by Prasindhu *et al.* (2020) were in close association with the current results. The infected plants showed symptoms of reduction of leaf size, yellowing and stunted growth in periwinkle. However, the results from the present study were in contradiction to Jutimala *et al*. (2019) who reported that *H. phycitis* was found to transmit sesame phyllody phytoplasma with a transmission rate of 83.33 per cent to healthy sesame plants. The presence of phytoplasma in *H. phycitis* was confirmed by nested PCR assays

and concluded that *H. phycitis* can be considered as a new vector of phyllody from North East region of India in addition with *Orosius albicinctus.*

Leafhopper species belonging to cicadellidae were reported as the major insect vectors for transmitting sesamum phyllody of which, *Orosius orientalis* was considered as a major vector transmitting the phyllody disease in India, Iran, Turkey and other Asian countries (Esmailzadeh *et al.*, 2007; Akhtar *et al.*, 2009a and Pathak *et al*., 2013) and *O. albicinctus* in India (Gogoi *et al.*, 2017) and Iran (Esmailzadeh *et al.*, 2007). However recent reports have suggested another species *Hishimonas* also acting as vector of sesamum phyllody that is acquiring and transmitting the phyllody from infected to healthy plants (Nabi *et al.*, 2015 and Jutimala *et al*., 2019).

In all our field experiments at S.V. Agricultural College, Tirupati *Orosius albicinctus* was found to be most abundant in addition to other species such as *H. phycitis* which were less abundant. An attempt was made in the present investigations to demonstrate the ability of both the species of leafhopper *i.e., O. albicinctus* and *H. phycitis* to acquire and transmit the disease from infected to healthy sesame plants. The results revealed that *O. albicinctus* was the main vector in transmitting the disease from infected plant to healthy plants and did produce the phyllody symptoms at around 30 days after 7 days of active inoculation period. However, studies with *H. phycitis* have not produced any conclusive results on appearance of symptoms even after 40 days of inoculation, where the insect was given a maximum period of 7 days of acquisition and 7 days of inoculation feeding period. This probably due to the occurrence and abundance of different leafhopper species across various geographical locations, along with differences in genetic variation among the population of same species at different geographical locations, that could affect the acquisition and transmission of the phyllody by the insect vector. Among the leafhopper species that were found to be vectors of sesamum phyllody, *Hishimonus* sp. was found to be more abundant in Northern part of India (Nabi *et al*., 2015) and was able to tramsit the disease into healthy plants, whereas, *Orosius* spp. was found to be more prevalent in Southern India (Manjunatha *et al*., 2012 and Madhupriya *et al*., 2015). Additionally, the genetic differentiations among the *H. phycitis* populations, at different geographical location could also affect the vectoring capability of the vector and thus the appearance of the symptoms (Hemmati *et al*., 2018). As of variation in geographical areas, the abiotic factors also vary and accordingly

can influence the acquisition and transmission of phyllody by the insect vector and the expression of symptom (Chellappan *et al*., 2005; Anhalt and Almeida, 2008). The abiotic factors prevailing during the period of experimentation with *Hishimonus* at Tirupati may be different from the experimental conditions prevailed by the work of Nabi *et al*. (2015) at IARI, New Delhi , which might have contributed to non appearance of phyllody symptoms when *Hishimonus* was used as vector for disease transmission in the present investigations. Also mere acquisition of phytoplasm in the insect vector doesn't mean that the insect can transmit the disease successfully in to a healthy plant and the success of the transmission by the vector also depends on the quantity or titer load of the pathogen during the acquisition period (Alma *et al.*, 2019).

It has also been demonstrated that the latent period *i.e*. the period between acquisition of phytoplasma (AAP) and the ability of the insect vector to transmit the phytoplasm into a healthy plant ranges from 7 to 80 days, which is again temperature dependent (Nagaich *et al*., 1974; Murral *et al*., 1996 and Moya-Raygoza and Nault, 1998). In our experiments we have given an maximum of 7 days as inoculation period for the phyllody infected insect vector (after a maximum of 7 days of acquisition period on phyllody infected plant) which could also be one of the reasons for absence of appearance of any phyllody symptoms when *Hishimonus* was used as vector in the present transmission studies. However the studies on insect vector transmission with species of leafhoppers other than *O. albicinctus* is still in infancy stage and further studies on identification of all the available leafhopper species at different agro climatic zones have to be undertaken by taxonomic keys as well as molecular identification and the acquisition, inoculation, latent period of phyllody in the insect vector and the presence of phyllody and the titer of load in the insect vector as well as presence and expression of symptoms in the healthy plants have to confirmed with insect vector transmission studies as well as molecular confirmation with the help of specific primers.

4. Conclusion

The transmission studies conducted with two leafhopper species, *O. albicinctus* and *H. phycitis* confirmed that leafhopper, *O. albicinctus* was the potential insect vector responsible for dissemination of phytoplasma, the causal organism of sesame phyllody from infected plants to healthy sesame seedlings. The findings revealed that leafhoppers play a major role in

transmission of phytoplasma under field conditions. The sap inoculated plants did not produce any significant phyllody symptoms during the period of observation.

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Table 2 Transmission of sesame phyllody disease by mechanical sap inoculation

Test plant	No. of plants taken	No. of plants inoculated	No. of plants showing phyllody symptoms	Days taken for appearance of phyllody symptoms	Per cent phyllody transmission

Sesame	150	150	0	0	0.00

Table 3 Transmission of sesame phyllody disease by insect vector, *Orosius albicinctus*

Treatment No	Treatments	Days required for appearance of phyllody symptoms	Per cent phyllody transmission
T_1	3 insects/plant + 3 DAF+ 3 DIF	38-41	13.3^{h}
T_2	3 insects/plant + 3 DAF+ 5 DIF	33-36	26.7^{g}
T_3	3 insects/plant + 3 DAF+ 7 DIF	33-34	46.7^{f}
T_4	3 insects/plant + 5 DAF+ 3 DIF	32-38	53.3^{e}
T_5	3 insects/plant + 5 DAF+ 5 DIF	32-36	60.0^{d}
T_6	3 insects/plant + 5 DAF+ 7 DIF	29-33	86.7^{b}
T_7	3 insects/plant + 7 DAF+ 3 DIF	31-35	73.3^{c}
T_8	3 insects/plant + 7 DAF+ 5 DIF	31-34	93.3^{a}
T_9	3 insects/plant + 7 DAF+ 7 DIF	29-31	93.3^{a}
T_{10}	No inoculation (Control)	0	0.00^{i}
Mean			**54.67**
F-test			**Sig.**
SEm±			**1.37**
CD (P=0.05)			**4.05**
CV (%)			**4.38**

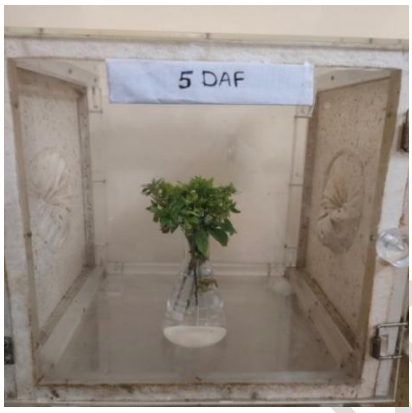
DAF – Days of Acquisition Feeding DIF – Days of Inoculation Feeding
Sig – Significant at 5 per cent level of significance

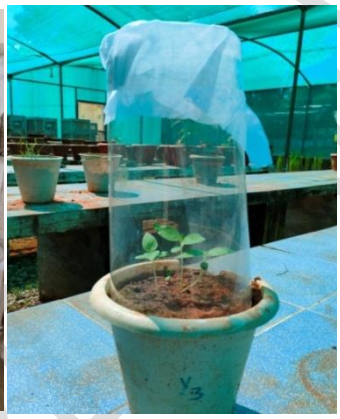
Table 4 Transmission of sesame phyllody disease by insect vector, *Hishimonus phycitis*

Treatment No	Treatments	Days required for appearance of phyllody symptoms	Per cent phyllody transmission
T_1	3 insects/plant + 3 DAF+ 3 DIF	0	0.00
T_2	3 insects/plant + 3 DAF+ 5 DIF	0	0.00
T_3	3 insects/plant + 3 DAF+ 7 DIF	0	0.00
T_4	3 insects/plant + 5 DAF+ 3 DIF	0	0.00
T_5	3 insects/plant + 5 DAF+ 5 DIF	0	0.00
T_6	3 insects/plant + 5 DAF+ 7 DIF	0	0.00
T_7	3 insects/plant + 7 DAF+ 3 DIF	0	0.00
T_8	3 insects/plant + 7 DAF+ 5 DIF	0	0.00
T_9	3 insects/plant + 7 DAF+ 7 DIF	0	0.00
T_{10}	No inoculation (Control)	0	0.00
Mean			**0.00**
F-test			**-**
SEm±			**-**
CD (P=0.05)			**-**
CV (%)			**-**

DAF – Days of Acquisition Feeding DIF – Days of Inoculation Feeding

(a)





(b) **(c)** **(d)**

Plate 1: Phytoplasma transmission studies in green house

(a) Pots maintained in insect proof cages for mechanical sap inoculation

(b) Acquisition feeding cage (c) Inoculation feeding cage

(d) Symptoms of phyllody in insect transmitted pots